Human Reproduction vol.13 no.5 pp.1194–1199, 1998

The role of monocyte chemotactic protein-1 in intraperitoneal adhesion formation*

Hulusi B.Zeyneloglu, Levent M.Senturk, Emre Seli, Engin Oral, David L.Olive and Aydin Arici[1]

Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520–8063, USA

[1]To whom correspondence should be addressed

Abdomino-pelvic adhesions arise from infection, endometriosis, or peritoneal injury during surgery, and represent a significant source of morbidity in women of reproductive age. Monocyte chemotactic protein-1 (MCP-1) plays a role in the chemotaxis of mononuclear cells and fibroblasts in a murine wound repair model. To evaluate the role of MCP-1 in intraperitoneal adhesion formation, we investigated peritoneal fluid MCP-1 levels of women undergoing laparoscopy. Patients without endometriosis were divided into two groups: normal fertile women undergoing bilateral tubal ligation without intraperitoneal adhesions ($n = 14$) and women with pelvic adhesions ($n = 8$). Patients with endometriosis were arranged into two groups: women with ($n = 17$) and without ($n = 17$) adhesions. Peritoneal fluid MCP-1 levels were quantified using an enzyme-linked immunosorbent assay (ELISA). Peritoneal biopsy samples were immunostained for the detection of MCP-1 protein and macrophages, and were also processed for the presence of MCP-1 mRNA expression. Among women without endometriosis, the median peritoneal fluid MCP-1 level was 144 pg/ml (range 54–261) in women without adhesions and was 336 pg/ml (range 130–2494) in women with adhesions ($P = 0.01$). There was a significant correlation between adhesion scores and MCP-1 levels ($r = 0.50$; $P = 0.018$). Among women with endometriosis, peritoneal fluid MCP-1 levels significantly correlated with the stage of the disease. The presence or absence of adhesions did not significantly affect the peritoneal fluid MCP-1 levels in this group of women. In summary, we have found that women with adhesions have elevated peritoneal fluid MCP-1 levels. However, we were not able to show an incremental effect of adhesions on peritoneal fluid MCP-1 levels of patients with endometriosis. Thus, we conclude that factors besides the intraperitoneal adhesions contribute to the elevated peritoneal fluid MCP-1 levels in patients with endometriosis.

Key words: adhesions/MCP-1/pelvic adhesions/tubo-ovarian disease

Introduction

Intraperitoneal adhesion formation is a major source of morbidity among women of reproductive age (Perry *et al*., 1955; Weibel and Majno, 1973). These adhesions may lead to infertility, chronic pelvic pain, and bowel obstruction. Abdomino-pelvic surgery is a principal cause of adhesion formation, while other aetiologies include infection, endometriosis, cancer and radiation. Approximately 20% of all infertility cases are secondary to adhesions involving the uterus, Fallopian tubes or ovaries (Holtz, 1984). Adhesions are identified as the primary cause in 13–26% of women with chronic pelvic pain and in one-third of cases of bowel obstruction (Zerega and Rodgers, 1992). The economic impact of adhesion formation is huge with the cost of hospitalization for lower abdominal adhesiolysis estimated to be more than one billion US dollars each year (Ray *et al*., 1993).

Although the precise pathophysiology of adhesion formation has not been clearly outlined, it is known that infection, ischaemia, or trauma to the serosal surface of abdominal and pelvic organs are the initiators of the inflammatory reaction. After the initial insult, there is an increase in vascular permeability and the release of a fibrin-rich exudate (Milligan and Raftery, 1974; Buckman *et al*., 1976; Diamond and DeCherney, 1987). If fibrinolysis is not completed through the plasminogen–plasmin cascade, adhesions may form through collagen deposits on a framework of proteoglycan and fibronectin. Intraperitoneal adhesion formation is a complex process that involves synthesis of extracellular matrix as well as the migration and proliferation of a variety of cell types, including inflammatory cells, mesothelial cells and fibroblasts (Milligan and Raftery, 1974; Buckman *et al*., 1976; Diamond and DeCherney, 1987).

Adhesions may be viewed as an aberration of the normal wound healing cascade; normal healing of injured peritoneum would result in regeneration without adherence between intra-abdominal structures. The healing of wounds is initiated by the release of growth factors and cytokines such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-beta (TGF-β), and interleukin-1 (IL-1) from local macrophages, platelets, and endothelial cells. After the arrival of neutrophils, macrophages are recruited into the site of injury where they further secrete cytokines and growth factors that attract more macrophages and fibroblasts. The fibroblasts then proliferate and extracellular proteins such as collagen are secreted (Kovacs and DiPietro, 1994). If the initial fibrin meshwork is not completely dissolved, the entrapped fibroblasts deposit collagen which converts the fibrin meshwork into fibrous adhesion bands. It has been shown that

*Presented at the 44th Annual Meeting of Society for Gynecological Investigation, March 19–22, 1997, San Diego, California, USA

IL-1 (Hershlag *et al*., 1991) TGF-β (Chegini *et al*., 1994), and vascular endothelial growth factor (Saltzman *et al*., 1996) play a role in adhesion formation.

Monocyte chemotactic protein-1 (MCP-1) is a chemotactic and activating factor for mononuclear phagocytes (Rollins *et al*., 1991). Its murine homologue JE/MCP-1 (Yoshimura and Leonard, 1990) was first identified as a PDGF-inducible gene in fibroblasts (Cochran *et al*., 1983). MCP-1 was detected in a variety of conditions including atherosclerosis (Nelken *et al*., 1991), pulmonary fibrosis (Brieland *et al*., 1993), and dermal wound healing (DiPietro *et al*., 1995). Thus, a role for MCP-1 in the pathogenesis of these entities is suggested. The shift of cellular infiltrate to predominantly macrophages in the later stages of wound repair suggests active synthesis of factors that preferentially attract monocytes rather than neutrophils. Direct evidence for the chemoattraction of macrophages rather than neutrophils was provided in rodent wound models (Iida and Grotendorst, 1990). MCP-1 has been shown to play a role in the chemotaxis of mononuclear cells and fibroblasts in the murine wound repair model. It has also been shown that oestrogen modulates MCP-1 expression in fibroblasts (Kovacs *et al*., 1996). To evaluate the role of MCP-1 in intraperitoneal adhesion formation in the human, we measured its level in the peritoneal fluid of women with and without adhesions and looked for its presence in peritoneal adhesive bands. Since we have previously shown that peritoneal fluid MCP-1 levels correlate with the severity of endometriosis (Arici *et al*., 1997), we also investigated the relationship between the peritoneal fluid MCP-1 levels and intraperitoneal adhesions in women with endometriosis.

Materials and methods

Peritoneal fluid collection

Peritoneal fluid samples were obtained from women undergoing diagnostic laparoscopy and tubal ligation between 1993 and 1996. Informed consent was obtained from each woman prior to surgery using consent forms and protocols approved by the Human Investigation Committee of Yale University. Patients without endometriosis were divided into two groups: normal fertile women undergoing laparoscopic tubal ligation without any pelvic pathology ($n = 14$) and women with pelvic adhesions in the absence of endometriosis ($n = 8$). Adhesion scoring was done according to the revised American Fertility Society (AFS) classification (American Fertility Society, 1988). Endometriosis patients [minimal to mild endometriosis, $n = 22$; and moderate to severe endometriosis, $n = 12$, according to the revised AFS classification (American Fertility Society, 1985)] were also arranged into two groups: women with adhesions and women without adhesions. Prior to any intervention, peritoneal fluid was aspirated from the anterior and posterior cul-de-sac into a sterile syringe, centrifuged at 600 *g* for 10 min at 4°C to remove cells, aliquoted, and frozen at –80°C until assayed. Biopsies ($n = 5$) were taken from adhesions during laparoscopy and snap-frozen for immunohistochemistry and RNA extraction.

Immunohistochemical analysis of the peritoneal biopsies

Peritoneal biopsy samples for immunohistochemical analysis were frozen in OCT (Tissue Tek®; Sakura, Torrance, CA, USA). Serial 6–8 μm frozen cryosections were placed on poly-L-lysine-coated glass microscope slides and fixed in acetone for 10 min at 4°C. Sections were rinsed in phosphate buffered saline (PBS), pH = 7.4, twice for 5 min each and in permeabilization buffer (0.1% saponin in PBS) for 5 min. Slides were then incubated with 2% blocking horse serum (Vector Laboratories, Burlingame, CA, USA) for 1 h at room temperature in a humidified chamber. At the end of incubation, excess serum was drained. Primary antibodies [murine monoclonal anti-human MCP-1, type IgG_1, clone: 5D3-F7, 1.25 μg/ml (PharMingen, San Diego, CA, USA); murine monoclonal anti-human macrophage CD68 antibody, type IgG_1, clone: EBM11, 8 μg/ml (Dako, Denmark); and negative control type IgG_1 antibody, same concentrations with the primary antibodies, (Dako, Denmark)] diluted in PBS 0.1% bovine serum albumin (BSA) were added directly onto the sections. These sections were incubated with the primary antibodies overnight at 4°C in a humidified chamber. On the second day, after the slides were rinsed, endogenous peroxidase activity was quenched with 0.6% H_2O_2 in PBS for 15 min. Sections were rinsed and biotinylated horse anti-mouse antibody (1.5 mg/ml, Vector Laboratories) was added at 1/200 dilution for 45 min at room temperature. Detection of the antigen–antibody complex was achieved using a Vector avidin-biotin-peroxidase (ABC®) kit (30 min, room temperature). Diaminobenzidine (3′3 diaminobenzidine tetrahydrochloride dihydrate, Aldrich Chemical Co., Milwaukee, WI, USA): hydrogen peroxide (0.5 mg in 0.03% H_2O_2 in PBS) was used as the chromogen, and sections were counterstained with haematoxylin and mounted with Permount (Fisher Chemicals, Springfield, NJ, USA).

MCP-1 immunoassay

The immunoreactive MCP-1 levels in peritoneal fluid samples were determined by an ELISA kit using a specific monoclonal antibody that does not cross-react with other cytokines or growth factors (Quantikine kit®; R&D Systems, Minneapolis, MN, USA). The detection limit was 4.7 pg/ml. All of the peritoneal fluid samples were evaluated in a duplicate assay. The intra-assay and inter-assay coefficients of variation were 4.9% and 5.9%, respectively.

Preparation of total RNA and Northern analysis

Total RNA was extracted from peritoneal samples using Trizol (Gibco BRL, Grande Island, NY, USA). Total RNA (10 μg per lane) was size-fractionated by electrophoresis on 1% formaldehyde-agarose gels, transferred electrophoretically to Hybond-N^+ membrane (Amersham, Arlington Heights, IL, USA), and cross-linked to the membrane using ultraviolet light. Prehybridization was conducted for 5 h at 65°C in buffer composed of NaCl (0.9 M), Tris-Cl (90 mM, pH 8.3), EDTA (6 mM), 5× Denhart solution, sodium dodecyl sulphate (0.1% w/v), sodium pyrophosphate (0.1% w/v), and salmon sperm DNA (0.2 mg/ml). Hybridizations were conducted for 16 h at 65°C in a buffer that contained an oligonucleotide probe (5′-TTG GGT TTG CTT GTC CAG GTG GTC CAT GGA-3′) specific for human MCP-1 (used previously for Northern analyses by Strieter *et al*., 1989) radiolabelled with [γ-^{32}P]ATP. Thereafter, the blots were washed once with 6× standard saline citrate (SSC) and sodium dodecyl sulphate (SDS) (0.1 w/v) for 15 min at room temperature, once with 2× SSC and SDS (0.1% w/v) for 15 min at room temperature, and once for 20 min at 65°C. Autoradiography of the membrane was performed at –80°C using Kodak X-Omat AR film in each lane (Eastman Kodak, Rochester, NY, USA). Using a cDNA probe (Clontech Laboratories, Palo Alto, CA, USA), the amount of RNA in each lane was normalized by analysis of glyceraldehyde-3-phosphate dehydrogenase (G3PDH). The autoradiographic bands were quantified using a laser densitometer (Molecular Dynamics, Sunnyvale, CA, USA). Each MCP-1 band was normalized using the value for the corresponding G3PDH mRNA. Thus, any variation in the amount of RNA applied to each lane was corrected.

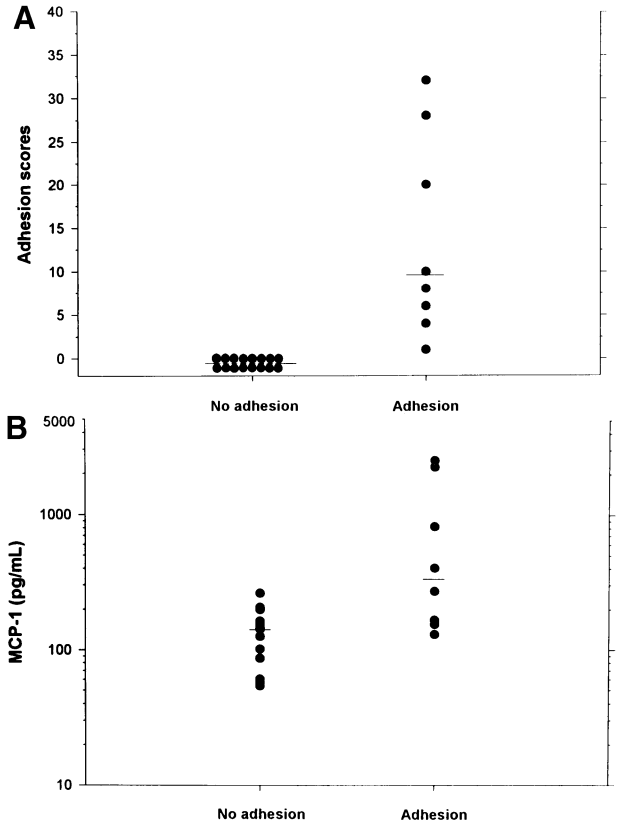

Figure 1. (**A**) Adhesion scores in women without adhesions (n = 14) and in women with adhesions (n = 8) in the absence of endometriosis ($P < 0.001$). Adhesion scoring was done according to the revised AFS classification. (**B**) Peritoneal fluid MCP-1 levels in women without adhesions (n = 14) and in women with adhesions (n = 8) in the absence of endometriosis ($P < 0.01$).

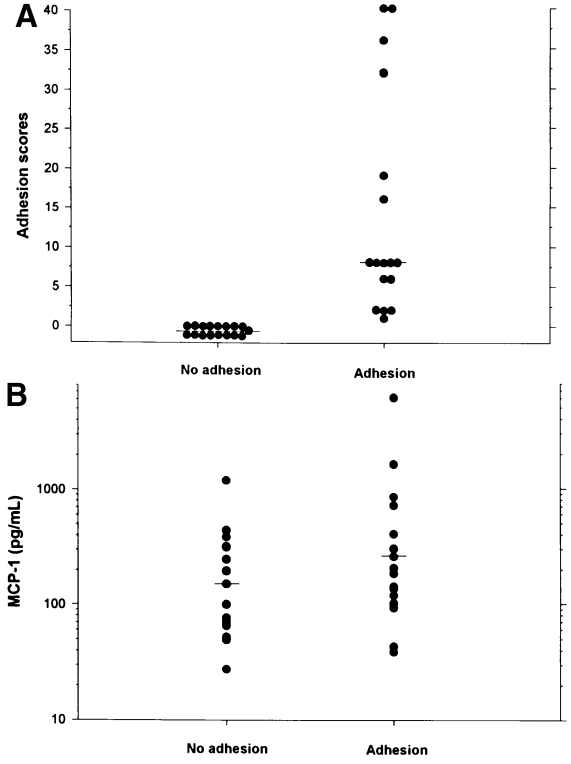

Figure 2. (**A**) Adhesion scores in women with endometriosis, grouped according to the absence (n = 17) or presence (n = 17) of adhesions ($P < 0.001$). Adhesion scoring was done according to the revised AFS classification. (**B**) Peritoneal fluid MCP-1 levels in women with endometriosis, grouped according to the absence (n = 17) or presence (n = 17) of adhesions. The difference between the two groups was not significant.

Statistical analyses

Since the peritoneal fluid MCP-1 levels were not normally distributed (as determined by Kolmogorov–Smirnov test), differences between individual groups were compared using the nonparametric Mann–Whitney rank sum test. Comparisons between multiple groups were performed using analysis of variance on ranks (Kruskal–Wallis) with post-hoc Dunn's test. For correlation, Spearman rank order correlation was used.

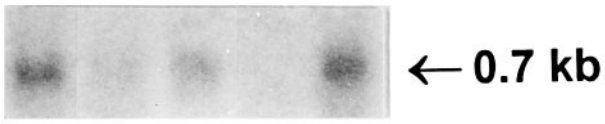

Figure 3. Expression of MCP-1 mRNA was evaluated by Northern analysis of total RNA (10 μg per lane) obtained from intraperitoneal adhesions in five women.

Results

Pelvic adhesions and peritoneal fluid MCP-1 levels

The control group consisted of women undergoing tubal ligation without any pelvic pathology and all the adhesion scores of this group were 0. In the group with pelvic adhesions without endometriosis, the median adhesion score was 9 (range 1–32; $P < 0.001$). The median peritoneal fluid MCP-1 level was 144 pg/ml (range 54–261) in women without adhesions and was 336 pg/ml in women with adhesions but without endometriosis (range 130–2494; $P < 0.01$). There was a significant correlation between adhesion scores and MCP-1 levels ($r = 0.50$; $P = 0.018$) (Figure 1A, B).

We also found a significant difference between the adhesion scores of the minimal to mild and the moderate to severe endometriosis groups. The median adhesion score was 0 (range 0–36) in women with minimal to mild endometriosis and 8 (range 0–40) in women with moderate to severe endometriosis ($P = 0.01$). The median peritoneal fluid MCP-1 levels in these groups were 138 pg/ml (range 27–1173) and 352 pg/ml (range 75–6000), respectively ($P = 0.002$). However, Spearman rank order correlation between adhesion scores and peritoneal fluid MCP-1 levels was not statistically significant. When we regrouped all patients with endometriosis according to the presence or absence of adhesions, we found adhesions in half of the patients (n = 17; total = 34). The median adhesion score was 0 in endometriosis patients without adhesions and 8 (range, 1–40) in those with adhesions ($P < 0.001$). The peritoneal fluid median MCP-1 level was 150 pg/ml (range 28–1173) in the absence of adhesions and was 204 pg/ml (range 39–6000) in the presence of adhesions, not a statistically significant difference (Figure 2A, B).

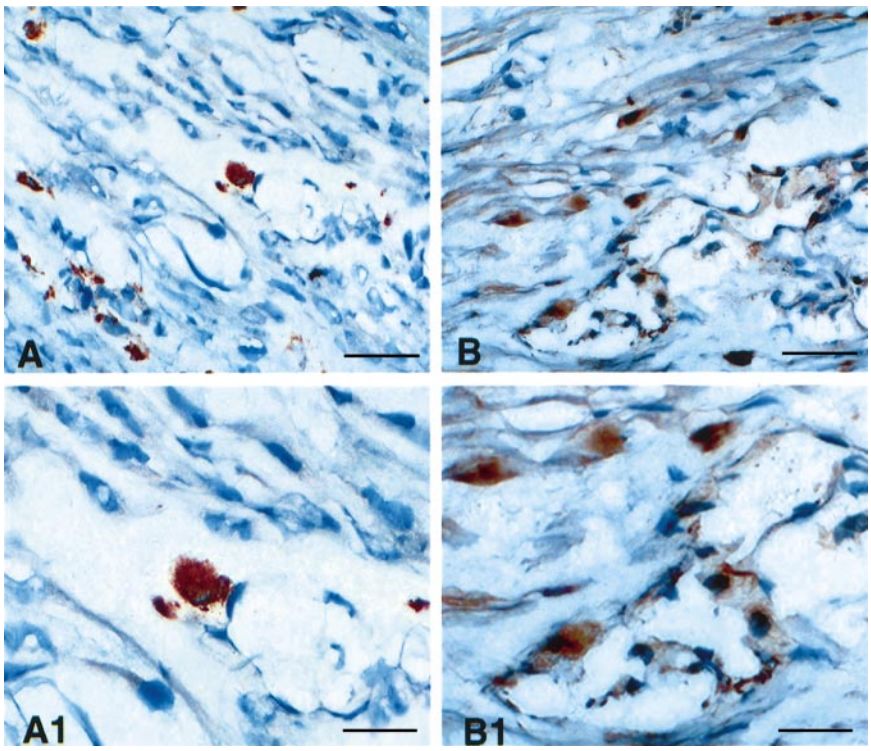

Figure 4. Immunohistochemistry depicts presence of macrophages (**A, A1**) and MCP-1 protein (**B, B1**) in fibrous adhesion bands (original magnification ×400). Bars in **A** and **B** = 30 μm; bars in **A1** and **B1** = 16 μm.

Expression of MCP-1 mRNA and protein in pelvic adhesions

Total RNA was extracted from non-endometriosis pelvic adhesion tissues (n = 5). Northern blots were hybridized with an oligo-DNA probe complementary to MCP-1 mRNA. We detected the presence of MCP-1 mRNA (~0.7 kb) in three of five peritoneal fibrous adhesive bands (Figure 3).

We performed immunohistochemistry on adhesion tissues to identify macrophages and to localize MCP-1 protein. Adhesion tissues contained vascularized areas of minute and fine capillaries immersed in a loose connective tissue. Macrophages and fibroblast-like cells were seen in these areas. Other areas contained more collagen material alternating with fascicles of numerous fibroblasts. Strong cytoplasmic staining with the CD68 antibody revealed a diffuse population of macrophages (Figure 4A, A1). MCP-1 was strongly positive in fibroblast-like cells and in endothelial cells of blood vessels in the loose connective tissue (Figure 4B, B1).

Discussion

The histopathogenesis of inflammation and the repair of mesothelium is relatively well understood (Zerega, 1990). After a peritoneal surface defect, a fibrin matrix typically develops in a process similar to coagulation. If the fibrinolysis does not occur in a timely manner, fibroblasts entrapped within the fibrin meshwork will deposit collagen and adhesions may result. Trauma (mechanical abrasion of the peritoneum such as in surgery), infection, and ischaemia activate the inflammatory system and may diminish the tissue plasminogen activator activity of mesothelium and underlying stroma, thus decreasing fibrinolysis. Macrophages and fibroblasts entrapped within the fibrin meshwork will continue to produce cytokines and growth factors such as PDGF, EGF, IL-1, and TNF-α (Kovacs and DiPietro, 1994). PDGF and TGF-β increase collagen synthesis by the fibroblasts (Kovacs, 1991). This matrix is then gradually replaced by a vascular granulation tissue containing macrophages, fibroblasts, and giant cells. Soon after, most of the fibrin disappears. A larger number of fibroblasts and associated collagen are present and macrophages are the predominant leukocyte at this stage.

The role of macrophages in adhesion formation is complex. Peritoneal macrophage-conditioned media and peritoneal fluid have been shown to stimulate cell proliferation during peritoneal tissue repair *in vitro* (Orita *et al*., 1986; Fukasawa *et al*., 1989). Macrophages are also critical in the formation of connective tissue and mesothelial syncytium (Ryan *et al*., 1973), and accumulation of the connective tissue is proportional to the number of macrophages recruited to those sites (Frazier-Jessen *et al*., 1996). In the present study, we used immunohistochemistry to demonstrate the presence of numerous macrophages in fibrous adhesive bands.

MCP-1 is a chemoattractant mainly for macrophages and fibroblasts. It is one of the chemokines that have a potential role in the premenstrual migration of macrophages into the endometrium (Jones *et al*., 1997). During an acute condition such as wound healing, infiltrated macrophages are a prominent source of MCP-1 (DiPietro *et al*., 1995). This suggests a role for MCP-1 in the attraction of monocytes and macrophages to the injury site and implies that macrophages that initially infiltrate the wound may actively recruit additional macrophages (Cushing and Fogelman, 1992). Although fibroblasts have been shown to be an active source of MCP-1 in chronic

granulomatous tissue (Lukacs *et al*., 1994), they do not appear to be a significant source of MCP-1 in an acute state like wound healing. In the present study, we used immunohistochemistry to show the presence of MCP-1 in fibroblasts of chronic adhesive bands.

We have shown that women with adhesions have significantly elevated peritoneal fluid MCP-1 levels compared to women without adhesions. In addition to demonstrating the presence of MCP-1 protein through immunohistochemistry, we also found MCP-1 mRNA within the fibrous adhesion bands. The presence of MCP-1 mRNA may indicate that adhesive bands themselves may be contributors to the level of peritoneal fluid MCP-1. In culture, mesothelial cells produce and secrete MCP-1 protein which is up-regulated by IL-1, TNF-α, and PDGF (Arici *et al*., 1997). These cytokines produced by macrophages may stimulate mesothelial cells adjacent to the adhesions to further increase MCP-1 production. In order to reveal the interaction between adhesion formation and MCP-1 protein, we have recently done a series of experiments in a mouse model. We induced adhesions surgically in mice by scraping and crushing the peritoneal sites. Forty-eight hours after the operation, an increase in MCP-1 mRNA expression and the collection of macrophages at the injury site were observed. To analyse further the role of MCP-1 in adhesion formation, we injected MCP-1 and anti-MCP-1 antibody intraperitoneally. Neutralization of MCP-1 by the antibody decreased the postoperative adhesion formation in mice by 50%. Thus, we have hypothesized that production of MCP-1 around the injury site may play a causative role in adhesion formation (Zeyneloglu *et al*., in press).

We have recently shown that women with endometriosis have increased peritoneal fluid MCP-1 levels that correlate with the severity of disease (Arici *et al*., 1997). On the other hand, women with endometriosis have higher peritoneal fluid concentrations of interleukin-13, a macrophage inhibitory factor, than those women without the disease (McLaren *et al*., 1997). Adhesions are a prominent component of endometriosis and are used in determining the stage of the disease. To investigate the role of intraperitoneal adhesions on increased peritoneal fluid MCP-1 levels in patients with endometriosis, we analysed their adhesion scores. However, we were not able to show an incremental effect of adhesions on MCP-1 levels in the peritoneal fluid of endometriosis patients. Thus, we speculate that factors besides the intraperitoneal adhesions contribute to the elevated peritoneal fluid MCP-1 levels in patients with endometriosis.

In conclusion, this is the first study to investigate the presence of MCP-1 in human peritoneal adhesions in the chronic state. A better understanding of the pathophysiology of adhesion formation will provide new approaches to therapeutic and prophylactic interventions, such as the use of specific antagonists or MCP-1 absorbent gel adhesion barriers.

References

American Fertility Society (1985) Revised American Fertility Society classification of endometriosis. *Fertil. Steril.*, **43,** 351–352.

American Fertility Society (1988) The American Fertility Society classifications of adnexal adhesions, distal tubal occlusion, tubal occlusion, secondary to tubal pregnancies, Mullerian anomalies and intrauterine adhesions. *Fertil. Steril.*, **49,** 944–955.

Arici, A., Oral, E., Attar, E. *et al.* (1997) Monocyte chemotactic protein-1 concentration in peritoneal fluid in patients with endometriosis and its modulation in human mesothelial cells. *Fertil. Steril.*, **67,** 1065–1072.

Brieland, J.K., Jones, M.L., Flory, C.M. *et al.* (1993) Expression of monocyte chemoattractant protein-1 (MCP-1) by rat alveolar macrophages during chronic lung injury. *Am. J. Resp. Cell Mol. Biol.*, **9,** 300–305.

Buckman, R.F. Jr, Buckman, P.D., Hufnagel, H.V. *et al.* (1976) A physiologic basis for the adhesion-free healing of deperitonealized surfaces. *J. Surg. Res.*, **21,** 67–76.

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

Cochran, B.H., Reffel, A.C. and Stiles, C.D. (1983) Molecular cloning of gene sequences regulated by platelet-derived growth factor. *Cell*, **33,** 939–947.

Cushing, S.D. and Fogelman, A.M. (1992) Monocytes may amplify their recruitment into inflammatory lesions by inducing monocyte chemotactic protein. *Arterioscler. Thromb.*, **12,** 78–82.

Diamond, M.P. and DeCherney, A.H. (1987) Pathogenesis of adhesion formation/reformation: application to reproductive pelvic surgery. *Microsurgery*, **8,** 103–107.

DiPietro, L.A., Polverini, P.J., Rahbe, S.M. *et al.* (1995) Modulation of JE/MCP-1 expression in dermal wound repair. *Am. J. Pathol.*, **146,** 868–875.

diZerega, G.S. (1990) The peritoneum and its response to surgical injury. *Prog. Clin. Biol. Res.*, **358,** 1–11.

diZerega, G.S. and Rodgers, K.E. (eds)(1992) Extracellular matrix. In *The Peritoneum.* Springer Verlag, New York, pp. 145–170.

Frazier-Jessen, M.R., Mott, F.J., Witte, P.L. *et al.* (1996) Estrogen suppression of connective tissue deposition in a murine model of peritoneal adhesion formation. *J. Immunol.*, **156,** 3036–3042.

Fukasawa, M., Yanagihara, D.L., Rodgers, K.E. *et al.* (1989) The mitogenic activity of peritoneal tissue repair cells: control by growth factors. *J. Surg. Res.*, **47,** 45–51.

Hershlag, A., Otterness, I.G., Bliven, M.L. *et al.* (1991) The effect of interleukin-1 on adhesion formation in the rat. *Am. J. Obstet. Gynecol.*, **165,** 771–774.

Holtz, G. (1984) Prevention and management of peritoneal adhesions. *Fertil. Steril.*, **41,** 497–507.

Iida, N. and Grotendorst, G.R. (1990) Cloning and sequencing of a new gro transcript from activated human monocytes: expression in leukocytes and wound tissue. *Mol. Cell Biol.*, **10,** 5596–5599.

Jones, R.L., Kelly, R.W. and Critchley, H.O. (1997) Chemokine and cyclooxygenase-2 expression in human endometrium coincides with leukocyte accumulation. *Hum. Reprod.*, **12,** 1300–1306.

Kovacs, E.J. (1991) Fibrogenic cytokines: the role of immune mediators in the development of scar tissue. *Immunol. Today*, **12,** 17–23.

Kovacs, E.J. and DiPietro, L.A. (1994) Fibrogenic cytokines and connective tissue production. *FASEB J.*, **8,** 854.

Kovacs, E.J., Faunce, D.E., Ramer-Quinn, D.S. *et al.* (1996) Estrogen regulation of JE/MCP-1 mRNA expression in fibroblasts. *J. Leukoc. Biol.*, **59,** 562–568.

Lukacs, N.W., Chensue, S.W., Smith, R.E. *et al.* (1994) Production of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 alpha by inflammatory granuloma fibroblasts. *Am. J. Pathol.*, **144,** 711–718.

McLaren, J., Dealtry, G., Prentice, A. *et al.* (1997) Decreased levels of the potent regulator of monocyte/macrophage activation, interleukin-13, in the peritoneal fluid of patients with endometriosis. *Hum. Reprod.*, **12,** 1307–1310.

Milligan, D.W. and Raftery, A.T. (1974) Observations on the pathogenesis of peritoneal adhesions: a light and electron microscopical study. *Br. J. Surg.*, **61,** 274–280.

Nelken, N.A., Coughlin, S.R., Gordon, D. *et al.* (1991) Monocyte chemoattractant protein-1 in human atheromatous plaques. *J. Clin. Invest.*, **88,** 1121–1127.

Orita, H., Campeau, J.D., Nakamura, R.M. *et al.* (1986) Modulation of fibroblast proliferation and transformation by activated macrophages during postoperative peritoneal reepithelization. *Am. J. Obstet. Gynecol.*, **155,** 905–911.

Perry, J.F.J., Smith, G.A. and Yonehiro, E.G. (1955) Intestinal obstruction caused by adhesions. A review of 388 cases. *Ann. Surg.*, **142,** 810–816.

Ray, N.F., Larsen, J.W. Jr, Stillman, R.J. *et al.* (1993) Economic impact of

hospitalizations for lower abdominal adhesiolysis in the United States in 1988. *Surg. Gynecol. Obstet.*, **176,** 271–276.

Rollins, B.J., Walz, A. and Baggiolini, M. (1991) Recombinant human MCP-1/JE induces chemotaxis, calcium flux, and the respiratory burst in human monocytes. *Blood*, **78,** 1112–1116.

Ryan, G.G., Grobety, J. and Majro, G. (1973) Mesothelial injury and recovery. *Am. J. Pathol.*, **71,** 93.

Saltzman, A.K., Olson, T.A., Mohanraj, D. *et al.* (1996) Prevention of postoperative adhesions by an antibody to vascular permeability factor/vascular endothelial growth factor in a murine model. *Am. J. Obstet. Gynecol.*, **174,** 1502–1506.

Strieter, R.M., Wiggins, R., Phan, S.H. *et al.* (1989) Monocyte chemotactic protein gene expression by cytokine-treated human fibroblasts and endothelial cells. *Biochem. Biophys. Res. Commun.*, **162**, 694–700.

Weibel, M.A. and Majno, G. (1973) Peritoneal adhesions and their relation to abdominal surgery. A postmortem study. *Am. J. Surg.*, **126,** 345–353.

Yoshimura, T. and Leonard, E.J. (1990) Secretion by human fibroblasts of monocyte chemoattractant protein-1, the product of JE. *J. Immunol.*, **144,** 2377–2383.

Zeyneloglu, H.B., Seli, E., Senturk, L.M. *et al.* (1998) The effect of monocyte chemotactic protein-1 in intraperitoneal adhesion formation in a mice model. *Am. J. Obstet. Gynecol.*, in press.

Received on August 22, 1997; accepted on February 4, 1998