行政院國家科學委員會專題研究計畫  成果報告

基質金屬蛋白酵素 ⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂⊂subset在子宮內膜異位症病人骨盆腔內
巨噬球的表現及調節之研究

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一、中英文摘要

宮內膜異位症是造成婦女腹部疼痛及經痛一個常見的疾病，也因此會影響婦女的生活品質。宮內膜異位症是影響在生殖年齡2~10%的婦女，儘管發生頻率高，但是造成宮內膜異位症發展的真正原因目前仍然不清楚，其中一個關鍵性因素是局部免疫系統的改變或功能不良。研究報告指出腹膜腔內巨噬球與宮內膜異位症嚴重程度有高度地相關性，從宮內膜異位症病人分離的腹膜腔巨噬球傾向有強烈地細胞動力素製造能力但是其吞噬能力不足。有許多其他的因子例如類固醇、前列腺素、及血管新生的因子也都與宮內膜異位症的病理機制有關。其中前列腺素E2是腹膜液中重要的因子，而前列腺素G/H合成酶(COX)-2是主要控制其生成的酵素，我們發現宮內膜異位症患者不分離的腹膜腔巨噬球傾向有強烈地細胞動力素製造能力但是其吞噬能力不足。

關鍵詞：子宮內膜異位症、腹膜腔巨噬球、基質金屬蛋白酵素酶、前列腺素E2
Decreased phagocytotic ability of macrophages has been reported to be associated with the severity of endometriosis, although the underlying mechanism remains uncharacterized. Expression and secretion of matrix metalloproteinase (MMP)-9 by macrophages is a means to degrade the extracellular matrix of cells that are designated for phagocytosis. Here, we describe the regulation of MMP-9 expression and activity in peritoneal macrophages of women with endometriosis. Results demonstrated that peritoneal macrophages isolated from women with endometriosis have decreased levels of protein and enzyme activity of MMP-9. Treatment of macrophages with peritoneal fluid obtained from patients with severe endometriosis inhibited MMP-9 expression and gelatinase activity. Further investigation identified prostaglandin (PG) E2 as the major factor in the peritoneal fluid that inhibited MMP-9 activity. The inhibitory effect of PGE2 was mediated via the EP2/EP4-dependent PKA pathway. Furthermore, expression of tissue inhibitor of metalloproteinase-1, tissue inhibitor of metalloproteinase-2, and RECK in macrophages was not affected by treatment with PGE2, indicating the effect of PGE2 on suppressing MMP-9 activity was not mediated by up-regulation of its inhibitor. Our results suggest that decreased phagocytotic capability of peritoneal macrophage in patients with endometriosis may be caused by PGE2-mediated decreases in MMP-9 expression.

Key Words: MMP-9, Endometriosis, Peritoneal macrophage, Prostaglandin E2

二、缘由與目的

Endometriosis is a common gynecological disorder with a complex, multifactorial etiology that causes chronic pelvic pain, dysmenorrhea, and even infertility. The prevalence of this disease is 10 to 15% among women of reproductive age. The underlying pathophysiological mechanism is still enigmatic. Although retrograde menstruation has been suggested to be the crucial constituent in the development of endometriosis, factors allowing the implantation and propagation of endometriotic lesions are primarily unclear. Aberrant production of steroids by ectopic endometriotic lesions and alteration/dysfunction of the immune system may lead to the development of endometriosis. During the development of endometriosis, immune cells are recruited into the peritoneal cavity. Among these immune cells, macrophages are the dominant cell type in the peritoneal cavity and are involved in phagocytosis and inflammation, especially in cleaning the retrograded endometrial debris. Peritoneal macrophages isolated from patients with endometriosis were found to have phenotypic and functional alterations leading to poor phagocytotic capacity, which is highly associated with severity of endometriosis. Nevertheless, the mechanism of suppressed phagocytotic capability of macrophages in endometriosis is poorly understood.

Matrix metalloproteinases (MMPs), also called matrixins, are proteinases that participate in extracellular matrix degradation. Based on substrate specificity, sequence similarity, and domain organization, vertebrate MMPs can be divided into six groups such as collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and other MMPs. Under normal physiological conditions, the activities of MMPs are precisely regulated at the level of transcription, of activation of the precursor zymogens, of interaction with specific extracellular matrix components, and of inhibition by endogenous inhibitors. Gelatinases including MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are further distinguished by the insertion of three head-to-tail cysteine-rich repeats within their catalytic domain. These inserts resemble the collagen-binding type II repeats of fibronectin and are required to bind and cleave collagen and elastin. MMP-9 is the largest and most complex family member for the remodeling of extracellular matrix
components at various physiological and pathological processes, such as development and angiogenesis. MMP-9-deficient mice were found to result in subfertility and immune dysfunction. MMP-9 is also considered as an important factor in the pathogenesis of endometriosis during the ectopic implantation and development of endometriotic tissue. Increased MMP-9 but not MMP-2 expression by eutopic and ectopic endometrial tissue in women with endometriosis was noted and was associated with the severity of endometriosis.

Macrophages can secrete MMP-2, -7, -9, and -12 to degrade elastin and have been implicated to play an important role in the pathogenesis of emphysema and aortic aneurysm. Several studies also suggested a role for MMP-9 in cell migration, leukocyte infiltration, and tissue remodeling. In addition, MMP-9 can facilitate the destruction of the type IV collagen-containing basement membrane, which separates the epithelial and stromal compartment. We hypothesize that the decreased phagocytic capability of peritoneal macrophages in women with endometriosis may be due to inhibition of MMP-9 expression and activity by unidentified factors in the peritoneal fluid (PF) of women with endometriosis. In this study, we aim to investigate the expression level and enzymatic activity of MMP-9 secreted by peritoneal macrophages derived from normal women and women with endometriosis. The effects of PF from endometriotic patients in the regulation of MMP-9 secreted by macrophages are also evaluated. Prostaglandins (PGs) are known, for many decades, to play pivotal roles in many physiological and pathological processes including modulation of immune responses. We have previously found that concentrations of PGE2 are elevated in the PF of women with endometriosis owing to aberrant expression of COX-1 and COX-2 in peritoneal macrophages and COX-2 in endometriotic stromal cells. Thus, we seek to examine whether PGE2 could be an active ingredient in endometriotic PF in suppressing MMP-9 activity.

三、研究結果與討論

Results

Expression and Enzymatic Activity of MMP-9 in Peritoneal Macrophages

Peritoneal macrophages obtained from patients of endometriosis-free (defined as normal herein, n= 7), early endometriosis (n= 8), and severe endometriosis (n=10) were purified and levels of mRNA encoding for MMP-9 were determined by RT-PCR. Macrophages derived from women with endometriosis expressed less MMP-9 mRNA than those isolated from normal controls (Figure 1A). Although the RT-PCR results were not quantitative, levels of MMP-9 mRNA tended to be inversely correlated with the severity of the disease (Figure 1A). To obtain further information, we examined levels of MMP-9 protein in peritoneal macrophages isolated from normal or endometriosis patients. Consistent with the mRNA data, Western blot analysis revealed that levels of MMP-9 protein were also decreased along with the severity of endometriosis (Figure 1, B and C). These results indicate that peritoneal macrophages in patients with endometriosis might be less active in phagocytosis as has been reported. To correlate the levels of MMP-9 expression with enzymatic activities, culture media were subjected to zymographic analysis. A time course experiment showed that MMP-9 activities secreted by peritoneal macrophages were greatly elevated during 12 to 24 hours after adherence on solid surface (Figure 2, A and B). In concordance with the mRNA and protein data, the gelatinase activity of MMP-9 in cultured medium of peritoneal macrophages collected from severe-stage endometriosis was the weakest compared with those from normal patient and early-stage endometriosis (Figure 2C). In contrast, the enzymatic activity of MMP-2 was not different among the groups (Figure 2C). On the other hand, the gelatinase activity of peripheral monocytes/macrophages was minimal and was not different among normal, early-stage
endometriosis, and severe-stage endometriosis (Figure 2D).

Suppression of MMP-9 Activity by Endometriotic PF
To explore the mechanisms responsible for inhibition of MMP-9 activity derived from peritoneal macrophages at different stages of endometriosis, pooled PF collected from patients with severe endometriosis (endometriotic PF) was used to treat peritoneal macrophages for 24 hours. Treatment of peritoneal macrophages obtained from normal women with endometriotic PF markedly suppressed MMP-9 activities (Figure 3, A and B). Suppression of MMP-9 activity was most evident by 1:1 dilution of endometriotic PF. The suppressive capability was reduced because endometriotic PF was more diluted and was totally lost when endometriotic PF was diluted 100 times (Figure 3, A and B; and data not shown). The inhibitory ability was only observed in endometriotic PF as evident by that PF collected from normal patients failed to exert such effect (Figure 3, A and C). Furthermore, the effect of PF on MMP-9 activity was not due to levels of MMP-9 present in the PF because the original PF, regardless from normal or endometriosis patients, exerted minimal or no MMP-9 activity when macrophages were omitted from the culture dish (Figure 3, A and C). Unlike MMP-9, the gelatinase activity of MMP-2 is mainly contributed by proteins present in PF and/or culture medium (Figure 3, A and B). In peritoneal macrophages obtained from early-stage endometriotic patients (defined as endometriosis in Figure 3), the basal enzymatic activity of MMP-9 is weaker than that obtained from normal women (compare the control group in Figure 3, B and D). Nevertheless, the responsiveness to endometriotic PF was the same. Treatment with endometriotic PF in 1:1 dilution markedly inhibited MMP-9 activity (Figure 3, C and D). The inhibitory effect was still evident when endometriotic PF was diluted to 1/10th (Figure 3, C and D). Similar results were also obtained when severe-stage peritoneal macrophages were used (data not shown).

Effects of PF on Inhibiting MMP-9 Expression in Peritoneal Macrophages
To characterize whether suppression of MMP-9 activity is due to an increased amount of MMP inhibitors, such as TIMP-1, TIMP-2, and RECK produced by macrophages, mRNA and/or protein levels of TIMP-1, TIMP-2, and RECK were evaluated. Western blot and RT-PCR analyses revealed no significant difference in TIMP-1, TIMP-2, and RECK levels when peritoneal macrophages of normal patients were treated with normal PF or endometriotic PF (Figure 4, A and B). In peritoneal macrophages isolated from patients with endometriosis, treatment with PF also had no substantial effect on TIMP-1, TIMP-2, or RECK expression (data not shown). To determine whether the inhibitory effect of endometriotic PF on macrophage MMP-9 activity was due to a decrease in de novo synthesis of MMP-9, the mRNA encoding for MMP-9 and levels of MMP-9 protein were evaluated. Semiquantitative RT-PCR demonstrated that expression of MMP-9 transcript was reduced by treatment with endometriotic PF irrespective of the source of peritoneal macrophages (Figure 4C). Western blot analysis revealed that MMP-9 proteins were decreased in peritoneal macrophages treated with endometriotic PF (Figure 4D). The inhibitory effect diminished when the endometriotic PF was diluted to 1/100 (Figure 4, C and D). As internal controls, there was no significant difference in GAPDH and β-actin expression in macrophages treated with either normal or endometriotic PFS (Figure 4, C and D). Because peritoneal macrophages of normal women and women with endometriosis respond to treatments with the same pattern, in terms of gene expression and MMP-9 activity, we thus performed all subsequent experiments using peritoneal macrophages obtained from women with endometriosis, unless otherwise mentioned, due to ethical concerns.
Effects of Cytokines and Proinflammatory Agents on MMP-9 Activity

To further identify the potential candidates that regulate MMP-9 expression and activity, several known cytokines and/or proinflammatory factors were administered to treat peritoneal macrophages. Results indicated that IL-1β slightly increased MMP-9 activity (Figure 5A) whereas high-dose IFN-γ partially inhibited peritoneal macrophage-derived MMP-9 activity (Figure 5A). Treatment with other cytokines such as tumor necrosis factor-α and leptin showed no substantial effects on MMP-9 activity (Figure 5A and data not shown). In contrast, treatment of peritoneal macrophages with PGE2 significantly inhibited MMP-9 activity in a dose-dependent manner (Figure 5B).

PGE2 Suppresses MMP-9 Activity by Inhibiting Its Expression

To determine whether suppression of MMP-9 activity by PGE2 could be due to an increase in MMP-9 inhibitors, expression of TIMP-1, TIMP-2, and RECK was determined by RT-PCR and/or Western blot. Treatment with PGE2 had no substantial effect on levels of mRNA encoding for TIMP-1, TIMP-2, or RECK (Figure 6A). Western blot analysis also demonstrated that TIMP-1 and TIMP-2 proteins were not affected by PGE2 (Figure 6B) suggesting the effect of PGE2 on decreased MMP-9 activity is not mediated via up-regulation of its inhibitors. We next examined whether inhibition of MMP-9 activity is due to a decrease in its expression. RT-PCR results demonstrated that PGE2 significantly inhibited MMP-9 mRNA expression (Figure 6C), which was mirrored by a decrease in cellular MMP-9 protein (Figure 6D). To further determine the mechanisms responsible for inhibition of MMP-9 expression by PGE2, selective pharmacological agonists and/or antagonists of EP receptors were used to treat peritoneal macrophages. Treatment with EP2 agonist (butaprost, 10 μmol/L) exerted a similar effect as those treated with PGE2 whereas treatment with EP3 agonist (sulprostone, 10 μmol/L) failed to inhibit MMP-9 expression (Figure 7A). Co-treatment with PGE1OH (EP2/EP4 agonist, 10 μmol/L) and AH6809 (EP1/EP2 antagonist, 80 μmol/L) also inhibited MMP-9 expression indicating that effect of PGE2 may also be mediated via EP4 receptor (Figure 7A). To test whether the lack of effect of EP3 agonist was due to lack of EP3 receptor expression, RT-PCR was used to evaluate the presence or absence of different EP receptor isoforms in peritoneal macrophages. The result demonstrated that three EP receptor isoforms, EP2, EP3, and EP4 were expressed in macrophages whereas EP1 receptor was undetectable (Figure 7B). The downstream signaling of EP2/EP4 was mediated via protein kinase A (PKA) pathway. Thus, a PKA inhibitor, H89 was used to block actions of PGE2. Pretreatment with H89 (10 μmol/L) reversed PGE2-mediated inhibition of MMP-9 expression (Figure 7C) and activity (Figure 7D). Concordantly, H89 also relieved butaprost-suppressed MMP-9 protein expression (Figure 7D).

Discussion

In the current study, we found a decrease in expression and enzymatic activity of MMP-9 in peritoneal macrophages isolated from patients with endometriosis. Decreased MMP-9 expression and activity in macrophages is due, at least in part, to exposure to factors present in the PF of women with endometriosis. The conclusion is based on results obtained in experiments in which enzymatic activity of MMP-9 is inhibited by treatment of macrophages with the PF isolated from endometriotic but not normal patients. Inhibition of MMP-9 activity by endometriotic PF is not due to increased expression of its cognate inhibitors such as TIMP-1, TIMP-2, or RECK but to down-regulation of MMP-9 protein expression. In concordance with this result, PGE2, which is aberrantly elevated in PF of women with endometriosis, potently inhibits MMP-9 expression thus suppresses its enzymatic activity. The inhibitory effect of
PGE2 is mediated via EP2/EP4-coupled PKA signaling pathway and is independent of expression levels of TIMPs. Our current findings may explain the underlying mechanism responsible for the long-standing notion that peritoneal macrophage of endometriosis patients is less active in phagocytosis compared to that of normal patients.

Endometriosis is a chronic inflammation that recruits many immune cells, especially macrophages, to the peritoneum. An increased number of active macrophages has been found in PF of patients with endometriosis. Infiltrated macrophages are supposed to function as scavengers, which help removing peritoneal debris. Nevertheless, it has been found that peritoneal macrophages isolated from patients with endometriosis have poor phagocytic capacity. It is not clear whether decreased phagocytotic capability of peritoneal macrophages in patients with endometriosis is the cause or effect of endometriosis. Regardless, dysfunction of peritoneal macrophages is a severe impairment of the defense system and an important factor leading to the development of endometriosis. In the current study, we demonstrate that expression of total and active MMP-9 in peritoneal macrophages is reduced in patients with endometriosis.

Expression and secretion of MMP-9 by macrophages is a means to degrade the extracellular matrix of cells that are designated for phagocytosis. Decreased expression and activity of MMP-9 secreted by macrophages results in reducing phagocytosis ability, which may, at least in part, account for the loss of function in peritoneal macrophages in endometriosis. Tissue inhibitors for MMPs are major cellular inhibitors of the MMP family and RECK has modest MMP inhibitory activity. It is possible that decreased MMP-9 expression and activity in peritoneal macrophage of women with endometriosis may be due to an elevation of these MMP inhibitors. However, in the current study, we found that levels of TIMPs and RECK do not contribute to the decreased phagocytosis of peritoneal macrophages in patients with endometriosis. Recently, it has been reported that concentrations of TIMP-1 are decreased in endometriotic PF over a normal control, which provides indirect evidence to support our current results that suppression of MMP-9 activity by endometriotic PF appears to be independent of the endogenous MMP inhibitors. The biochemical natures of PF between patients with and without endometriosis are quite different. Distinct activities of MMP-9 in patients with or without endometriosis may be caused by factors that are differentially present in the PF. To explore such a possibility, pooled PF collected from endometriosis-free or severe endometriosis patients were used to treat peritoneal macrophages. Our results showing that the PF from patients with endometriosis suppresses MMP-9 expression and activity provide a likely mechanism to explain the notion that macrophages isolated from women with endometriosis are less active in phagocytosis.

MMP-9 expression and activity provide a likely mechanism to explain the notion that macrophages isolated from women with endometriosis are less active in phagocytosis. It is known that several cytokines and proinflammatory agents, such as IL-1, tumor necrosis factor, leptin, and PGE2 are elevated in the PF of patients with endometriosis. These agents may act as paracrine and/or autocrine signals to regulate immune response and inflammation. For example, the immune regulatory role of leptin has been documented and numerous studies also showed that MMP-9 is regulated by many cytokines. Thus, we examined several candidate cytokines or hormones that have been demonstrated to play roles in the pathogenesis of endometriosis. Our results implicated that none of these agents except PGE2 could be the factor in endometriotic PF that exerts the inhibitory effect. At its first glance, repression of MMP-9 activity by high-dose (200 ng/ml) IFN-γ may implicate a role for IFN-γ in reducing phagocytic capability of macrophages in endometriosis patients. Nevertheless, the pathophysiological significance of IFN-γ in vivo is still uncertain because the inhibitory effect is very low and the dose for IFN-γ to exert such effect is too high. All these reasonable doubts argue against the notion
that IFN-γ could be the key player regulating the decreased phagocytic ability of macrophages in women with endometriosis. In contrast, our results clearly demonstrate that PGE2 plays a critical role in suppressing MMP-9 activity in peritoneal macrophage culture medium. The mode of actions exerted by PGE2 is similar to that by endometriotic PF in many ways. First, both inhibit MMP-9 expression rather than activation. Second, both have no effect on inducing MMP-9 inhibitors, such as TIMPs and RECK. And third, both affect only MMP-9 but not MMP-2 (compare Figures 3 and 5). Furthermore, the effective dose of PGE2 (100 nmol/L and it might be lower) is physiological (kd of PGE2 is between 30 to 100 nmol/L) and is within the concentration range of endometriotic PF. In concordance with our current result, it has recently been reported that PGE2 is able to suppress IL-1β-induced MMP-3 expression in human gingival fibroblasts. Together, these data provide evidence to support the immune modulatory role of PGE2. Obviously, PGE2 is not the only suppressor in endometriotic PF and more effort is needed to identify factors that may play important roles in suppressing MMP-9 expression and activity. There are four different receptors (EP1, EP2, EP3, and EP4) with various alternative splicing variants for PGE2. The signaling pathways coupled to these distinct EP receptors are quite different and complicated. By using pharmacological agonists and antagonist, we found that both EP2 and EP4 are important for exerting inhibitory effect of PGE2. Because the downstream signaling pathway of both EP2 and EP4 is mediated by PKA, we used H89, a selective PKA inhibitor, to block PGE2 action. Administration of H89 significantly reverses PGE2 and butaprost-inhibited MMP-9 expression and activity provides another line of evidence to support the action of PGE2 be mediated by EP2/EP4-coupled PKA signaling pathway. Further investigation is warranted to unravel mechanism responsible for PGE2-mediated MMP-9 suppression at the molecular level.

In summary, our current data provide strong evidence to support previous findings that peritoneal macrophages isolated from patients with endometriosis are less active in phagocytosis and that local immune dysfunction is a critical factor leading to the development of endometriosis. Although we have not directly demonstrated that PGE2 or endometriotic PF treatment could reduce phagocytic ability of macrophages, a plethora of evidence in the literature has implicated that MMP-9 plays a pivotal role in the macrophage’s ability to degrade basement membrane and thus its capability of phagocytosis. Considering that PGE2 can effectively suppress MMP-9 expression and activity, our current results may shed light on developing new strategies against endometriosis by trying to restore the intact phagocytic capability of peritoneal macrophages of patients with endometriosis. For example, developing more long-lasting and effective COX inhibitors cannot only reduce the pain associated with this disease but also be beneficial by inhibiting PGE2 production and thus relieving PGE2-suppressed phagocytic ability of macrophages.

四、計畫成果自評

本研究內容與原計畫相符程度及達成預期目標情況類似,發現 MMP-9 在子宮內膜異位症的重要地位。研究成果的學術價值已經於各研討會發表，並將其成果寫成論文投稿於在學術學會以及期刊發表，對於造成子宮內膜異位的致病機轉的探討，有相當重要的貢獻。同時對於臨床醫學上的診察及治療也極具參考價值。
五、图表

Figure 1. Distinct MMP-9 expression patterns in peritoneal macrophages of women with or without endometriosis. A, A representative gel picture showing RT-PCR result of levels of MMP-9 mRNA in macrophages isolated from PF of normal (N), early-stage endometriosis (EE), and severe-stage endometriosis (SE) women. GAPDH was amplified to serve as an internal control. NC, negative control omitting reverse transcriptase. B, A representative Western blot result demonstrates that expression of MMP-9 protein is decreased along with the severity of endometriosis. The band intensities of MMP-9 and β-actin were quantified using AlphaImager software and analyzed. C, The mean and SE of ratio of MMP-9 to β-actin obtained from 7 normal patients (N), 8 early-stage endometriosis (EE), and 10 severe-stage endometriosis (SE) patients. Different letters denote significant differences between groups when Tukey’s analysis yields $P < 0.05$.

Figure 2. Gelatinase activity of MMP-9 but not MMP-2 was increased in peritoneal macrophages isolated from patients with endometriosis. A and B, Analysis of the time effect on the activation of peritoneal macrophages in terms of gelatinase activity. Macrophages (2 × 10^5 cells/well) isolated from patients with endometriosis were plated in 24-well plates for the indicated time and culture media were collected for zymographic analysis. A, A representative zymographic picture revealed that the enzymatic activity of MMP-9 secreted by peritoneal macrophages was elevated along with time of culture. B, The means of relative intensity units of MMP-9 obtained from four different samples. Because the gelatinase activity proportionally increased between 12 and 24 hours after attachment, we thus empirically chose 12 hours as the time for all of the following experiments. C, A representative zymographic picture shows a decrease in MMP-9 but not MMP-2 enzymatic activity along with the severity of endometriosis. Six batches of peritoneal macrophages isolated from normal (N), early-stage endometriosis (EE), and severe-stage endometriosis (SE) women, respectively, were subjected to zymographic analyses and the results were similar. D, A representative zymographic picture shows gelatinase activity of MMP-9 as cultured media of macrophages isolated from PF and peripheral blood (PB). Three batches of macrophages isolated from peripheral blood of normal (N), early-stage endometriosis (EE), and severe-stage endometriosis (SE) women were subjected to zymographic analyses and the results were similar.

Figure 3. Endometriotic PFs suppressed gelatinase activities of MMP-9 in peritoneal macrophages. A and C, Gelatinase activity of normal and endometriotic peritoneal macrophages treated by normal peritoneal fluids (PFN) and endometriotic peritoneal fluids (PEF). Macrophages (2 × 10^5 cells/well) isolated from patients were plated in 24-well plates and treated by PWN (N) and PFPE (E) with L:1, 1/5, and 1/20 dilution. Representative zymographic pictures show that enzymatic activity of MMP-9 secreted by normal (A) or endometriotic (C) peritoneal macrophages treated with PF obtained normal (N) or endometriotic (E) fluids. B and D, Mean and SE of relative gelatinase activities of MMP-9 obtained from five and six experiments using different batches of cells obtained from normal and endometriosis patients, respectively. Asterisks denote significant difference from the control group.
Figure 4. The effect of PF on expression of MMP-9 and MMP inhibition in peritoneal macrophages. Macrophages were plated for 24 hours and then treated with peritoneal fluids from normal, PF(3), or endotoxemic; PF(E) for 24 hours as described in the Materials and Methods. A: A representative gel picture showing PF(C)gB result of levels of TIMP-1, TIMP-2, and REG1a mRNA in macrophages. This experiment was repeated four times and the results were similar. B: A representative Western blot result demonstrates the expression of TIMP-1, TIMP-2, and actin as a protein. This experiment was repeated three times and the results were similar. C: A representative gel picture showing PF(C)gB result of levels of MPA or mRNA in endotoxemic PF(3) or normal macrophages. Expression of MMP-9 mRNA was decreased by treatment with endotoxemic PF in normal (9, n = 3), endotoxemic (EE, n = 3), and peritoneal macrophages (PE, n = 3) groups, respectively. D: A representative Western blot shows a significant decrease in expression of MMP-9 protein in peritoneal macrophages obtained from normal, endotoxemic, and endotoxemic PF(3) groups. Endotoxemic PF (without macrophages) was used as a negative control to demonstrate the presence of MMP-9 that was produced by macrophage but not from PF. The bottom panel shows mean and standard error of four experiments using different batches of cells. Asterisks denote results significantly different from the control group.

Figure 5: Influence of gelatinase activity of MMP-9 by cytokines and proinflammatory agents. A: Zymographic picture showing gelatinase activity of MMP-9 secreted by peritoneal macrophages. MMP-9 activity was slightly increased by IL-1β treatment, whereas IFN-γ treatment exhibited inhibition at high dose (300 ng/ml). Treatment with leptin revealed no significant difference in MMP-9 activity. All of the experiments were repeated at least four times and the results were similar. B: A representative zymographic picture showing PGE2 treatment significantly inhibited MMP-9 activity in a dose-dependent manner. The experiment was repeated four times and the results were similar.
Figure 6: Effect of PGE₂ on expression of MMP-9 and MMP inhibition in porcine macrophages. Porcine macrophages were treated with 1 or 10 μM PGE₂. A representative gel picture showing RT-PCR bands of levels of TIMP-1, TIMP-2, and HICR mRNAs in macrophages. The experiment was repeated four times and the results were similar. B: A representative Western blot result demonstrates the expression of TIMP-1, TIMP-2, and β-actin proteins. The experiment was repeated three times and the results were similar. C: A representative gel picture showing RT-PCR result of levels of MMP-9 mRNA in PGE₂-treated macrophages. Expression of MMP-9 mRNA was decreased by treatment with 1 and 10 μM PGE₂, respectively. The experiment was repeated four times and the results were similar. D: Western blot analysis revealed a significant decrease in expression of MMP-9 protein with PGE₂ treatment in porcine macrophages. The experiment was repeated four times and the results were similar.

Figure 7: Effect of PGE₂ receptor agonist and/or antagonist treatment on gelatinase activity and expression of MMP-9 in porcine macrophages. A: A representative Western blot shows expression of MMP-9 protein in macrophages treated with PGE₂ receptor agonist and/or antagonist. Macrophages were treated with EP2 agonist (butaprost, blue), EP3 receptor agonist (造船原物, red), or a combination of EP2/EP4 receptor agonist, PGE₂-Neutral and EP1/EP3 receptor antagonists, AH109 (COPH-42), and expression of MMP-9 protein was analyzed. The bottom panel shows mean ± standard error obtained from four experiments in which different batches of cells were used. Asterisks denote significant difference from control group (P<0.05). B: A representative gel picture shows RT-PCR result of levels of EP2, EP4, and GAPDH mRNA in porcine macrophages treated at 1 to 4. C: A representative Western blot result demonstrating the expression of MMP-9 protein in macrophages treated with different EP receptor agonist and/or antagonist. This experiment was repeated three times and the results were similar. D: Zymographic gel picture shows gelatinase activity of MMP-9 secretion by porcine macrophages. Treatment with H89 reversed PGE₂- and butaprost-suppressed MMP-9 gelatinase activity. This experiment was repeated three times and the results were similar.