ン酸，還元型グルタチオン，および脂溶性ビタミンEがある。さらに，トランスフェリン，ラクトフェリン，フェリチンなどの鉄を結合する物質であり，血漿タンパク質も抗酸化物として作用すると考えられている[15]。ARDS患者や発症リスクのある患者では，抗酸化物が欠乏しているという報告がある一方，N-アセチルシステインやプロシステイン過多についての研究では矛盾する結果が示されている[15,19]。

好中球が早期肺障害において中心的な役割を果たしていることは明白であるが，好中球減少症患者でもARDSを発症する可能性があり，このことは肺胞マクロファージのような他のエフェクター細胞もまた重要であることを示唆している。肺胞マクロファージは，タンパク分解酵素，アラキドン酸代謝産物や他の炎症メディエータのみならず，活性酸素の産生，放出に関しても好中球と類似の能力を有している[20]。

c．サイトカイン

サイトカイン類は，種々の細胞から分泌される。サイトカインのうちtumor necrosis factor α（TNF-α）は，いくつかの実験モデルで，肺障害を惹起し，他のメディエータの効果を増強し，肺において微小血栓形成に関与する前凝固活性を有することが示されている[21,22]。TNF-αとインターロイキン1β（IL-1β）は共に，好中球脱顆粒，ROS産生，ライソザイム放出を増加させるのみならず，内皮の粘着性分子や好中球，単球に対する走化性因子の合成も増大させる[21]。

いくつかの研究により，早期および後期ARDS患者のBALF中TNF-αや他のサイトカインのレベルは上昇していることが示されている[23]。ARDS患者のうち，第1病日のTNF-α血漿中レベルがより高く，長期に持続的な上昇を示す症例では，発症時レベルがより低く速やかに低下するような症例と比較すると，死亡率がより高い[24]。サイトカイン類は，早期ARDSでは内皮と表皮を障害して透過性を増大させ，後期では，線維化を促進する線維芽細胞と相互に作用すると思われる[23,24]。

これらの炎症性メディエータの制御が，肺修復には重要であると考えられる。いくつかのモデルにおいて，TNF-αの効果に対抗するような作用物質が肺障害を軽減することが示されている[25]。敗血症患者における抗TNF-αモノクローナル抗体の多施設無作為化プラセボ対照試験では，敗血症ショック患者サブセット群で生存率の改善が示されている[26]。

d．エンドトキシン

敗血症において，エンドトキシンは好中球を肺に貯留し，補体と相乗的に作用し，好中球およびマクロファージの有毒物質産生を促進状態にする[20]。エンドトキシンの阻害により敗血症における死亡率が改善されるという初期の強い考えに反し，最近の多施設無作為化プラセボ対照試験では，抗エンドトキシンモノクローナル抗体HA-1Aや E5投与患者で著明な効果は示されていない[19]。

e．脂質メディエータ

敗血症患者のBALF，血清の両方で高値のホスフォリパーゼA_2（PLA_2）が認められ，その濃度はARDSのリスク増加と肺障害の重症度と相関していた。PLA_2がARDSに関与するメカニズムにはいくつかあり，特にアラキドン酸およびその代謝物や血小板活性化因子の放出が関係する[27]。アラキドン酸産生物，特にトロンボキサンA_2（TXA_2）やプロスタサイクリンは，敗血症およびエンドトキシン仲介肺障害の（シグナル伝達機構による）直接的または間接的メディエータであると言われている[28]。TXA_2は強力な平滑筋収縮物質および血小板凝集物質であり，一方，プロスタサイクリンは血管拡張物質，膜安定化作用物質，および血小板抗凝集物質である。TXA_2は炎症性細胞から，プロスタサイクリンは内皮細胞から放出される。急性肺障害の実験モデルでは，TXA_2の早期上昇は，早期障害と気管支収縮と関連している。これらのモデルにおいて，TXA_2阻害により肺高血圧の早期発症が軽減される[28]。

イブプロフェンによるシクロオキシゲナーゼの非特異的阻害は，全てではないが，いくつかのモデルにおける急性肺障害の軽減に有効であることが示されている[29]。イブプロフェンは，重症敗血症患者を対象とした2つの小規模無作為化プラセボ対照臨床試験で研究されたが，いずれも予後の

改善は認められなかった[19]。敗血症患者におけるイブプロフェン静脈内投与の大規模多施設共同無作為化プラセボ対照試験の結果がまとめられつつある。

プロスタグランジンE_1（PGE_1）に関する研究により，心拍出量の改善と血小板活性化の阻害とともに，肺および全身血管抵抗の低下が示されている[30]。初期の臨床試験では生存率の改善が認められたが，2つの対照試験（うち1試験は大規模多施設試験）では実証されなかった[30]。

3．病理学的検討

ARDSの組織学所見は，びまん性肺胞損傷である[31]。びまん性肺胞損傷の特徴は病因論的には非特異的であること，および高濃度酸素補給などのARDSのための治療や播種性血管内凝固症候群（DIC）などのARDSに関連した過程によっても，びまん性肺胞損傷の病理的変化を引き起こし得ることは理解しておくべきである[32]。これらの病理学的変化は，時間的に若干オーバーラップし，間質および肺胞の浮腫と出血が基本的特徴の滲出相，組織化と修復が起こっている増殖相，終末期病相を示す線維化相の3相に分けることができる[31, 32]。

a．滲出相

組織学的によく知られた最も早期の変化は，肺胞表面の約95%を占める内皮細胞とタイプ1肺胞上皮細胞に対する損傷である。内皮細胞が腫大し，細胞間結合部が拡大し，飲細胞小胞が増加し，毛細血管基底膜が破壊され，これらにより毛細管漏出と浮腫形成に至っている[31~33]。内皮細胞の損傷は，その修復能のために比較的目立たないこともあり，従って，間質性浮腫の程度は，内皮における比較的軽度な変化に対して不釣合いに認められることが多い[32, 33]。間質区画は伸展性で，大量の浮腫液を有することがある。しかし血管外液が正常容量の20%を超えると肺胞浮腫が起こる。血管内血栓と好中球集積が主に毛細血管内で認められる。タイプ1肺胞上皮細胞も腫大し，細胞質空胞化，嚢胞形成，それらの結果として基底膜からの脱離が認められる[31~33]。タイプ2肺胞上皮細胞は損傷に対してより抵抗性である。肺胞崩壊，ヒアリン膜も存在する[31]。

b．増殖相

増殖相は障害後早くも第3日目より始まるが，第2，3週目で極めて明確になり，タイプ2肺胞上皮細胞の再生で開始され，露出された基底膜内層を新しく覆う。これらタイプ2細胞は大型で，核異型性，膨隆核小体，層板状体異常を示す[31~33]。

増殖相では線維化が顕著となり，種々のメカニズムがその後に続く[34]。線維芽細胞と筋線維芽細胞が間質と肺胞滲出液に移動し，細胞性顆粒組織を形成する。コラーゲンが沈着し，無細胞性線維症となる。衰退しない線維増殖は末期肺胞線維症を招く。この進展は，初期の炎症反応の程度と炎症反応の遷延により決まるようである[33]。肺胞内組織は，タイプ2細胞による表層の新しい上皮形成にともなう結果として間質に取り込まれる。これは癒着による線維症として知られている過程であり，中隔あるいは壁在性線維症に帰着する[31~34]。この過程は，肺胞管，間隙および呼吸細気管支，終末細気管支に起こり，閉塞性細気管支炎に至る。

滲出相および早期増殖相では，炎症サイクルが中断されると肺の完全治癒の可能性がある。治癒にはアポトーシス過程が関与すると考えられる。アポトーシスは，プログラムされた細胞死であり，死滅細胞が食細胞作用を受けるまでの間，アポトーシス細胞毒がその死滅細胞により妨害され，その結果，さらなる炎症進展と損傷を避けることができる[35]。

肺修復時のARDS患者のBALFは，主に気腔顆粒化組織で見られる線維芽細胞および内皮細胞の両方に細胞死を誘発し得る。内皮細胞はアポトーシスにより致死する[36]。肺障害患者においてアポトーシス像を示す細胞の存在は，組織学的に確認されている[36]。動物モデルでのデータでは，タイプ2細胞による肺胞基底膜の新たな被覆が，線維芽細胞増殖と細胞外マトリクス沈着の抑制に必須であり，また線維芽細胞は肺胞タイプ2細胞の再生を刺激するマイトゲンを分泌するということが示唆されている。そしてまた，同じこれら線維芽細胞は，肺胞内皮細胞のアポトーシスを引き起こし，修復を阻害する因子も分泌する[37]。好中球のアポトーシスも同様に起こり，ラット急性肺障害

モデルにおいて，マクロファージがアポトーシスを起こしている好中球を貪食し，15～20分以内に分解したことが示されている[35]。

c．線維化相

肺線維化は損傷発生後早くも36時間後に始まり[38]，呼吸器不全の第3または第4週までに広汎な肺再構築が起こる。組織学的には，肺胞中隔と気腔壁は無細胞性および膠原性結合組織で構成されている。気腔は，小嚢腫様のもので拡張し，肺胞管線維症が認められる[32,33]。時間とともにタイプ3弾性膠原線維がタイプ1固縮膠原線維に置き換わり，ARDS非生存者では，肺コラーゲン含量は2～3倍に増加する。生検における線維化の程度は，死亡率と相関する[33,39]。ARDSのこのような後期ステージでは，15～40%の死亡が呼吸不全に直接的に起因するものである[7]。

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Correlations of surfactant protein with type II phospholipase A2 in the presence of ALI/ARDS associated with diffuse peritonitis

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Abstract : Blood levels of surfactant protein and type II phospholipase A2 (type II PLA2), in the presence of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) associated with diffuse peritonitis, were measured. Blood levels of surfactant protein-D (SP-D) and type II PLA2 were elevated at the time ALI/ARDS manifested. There was a significant correlation between SP-D and type II PLA2. The results suggest SP-D and type II PLA2 to be involved in the manifestations of ALI/ARDS.

Key words : type II phospholipase A2, SP-D, peritonitis, sepsis, ALI, ARDS

Introduction

Pulmonary surfactant is a physiologically active substance that is synthesized and secreted from alveolar type II epithelial cells, covers the inner surfaces of alveoli, and thereby prevents end-tidal alveolar collapse[1].

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) result in pulmonary microvascular hyper-permeability. It has long been accepted that the etiology of ALI/ARDS involves hemangioendothelial and alveolar epithelial cell injury, via activation of inflammatory cells such as neutrophils in response to cytokine generation[2], and the expression of adhesion factors[3].

It has recently been reported that the elevation of blood surfactant protein (SP) in the presence of ARDS is very likely to be a cause of ARDS or a defining prognostic factor[4, 5]. We have also reported the significance of SP in ARDS[6~8].

The Type II phospholipase A2 (type II PLA2) concentration is high at local sites of inflammation[9]. Vadas et al. have reported that type-II PLA2 has a strong pyogenic action[10] and that it plays a role in sepsis and septic shock[11]. We have also described the involvement of type II PLA2 in sepsis[12~14]. Some reports have recently shown type II PLA2 in respiratory disorders[15, 16]. On the other hand, tumor necrosis factor α

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(TNF-α) and interleukin 1 (IL-1) are generated by a variety of cells, and play major roles in inflammatory reactions. These inflammatory cytokines enhance type II PLA_2 generation by target cells, and type II PLA_2 promotes eicosanoid generation. This process is considered to be one of the mechanisms underlying inflammatory reactions of cytokines[17, 18].

It has also been reported that type II PLA_2 suppresses the function of surfactant[19].

In this study, we investigated blood levels of SP-A, SP-D and type II PLA_2, as well as those of inflammatory cytokines including TNF-α and interleukin 8 (IL-8), at the time of ALI/ARDS onset in sepsis patients.

Patients and Methods

Consent to participate in this study was obtained from the patients or their family members. This study was approved by the Ethics Committee of Iwate Medical University.

The patients underwent surgery on an inpatient basis at the Critical Care and Emergency Center, Iwate Medical University Hospital during calendar year 2000.

The diagnosis of sepsis conformed to the criteria established by the ACCP/SCMCC Consensus Conference Committee[20]. The diagnosis of ALI/ARDS conformed to the criteria reported by *Bernard* et al.[21].

There were 20 patients, 14 men and 6 women, with diffuse peritonitis, with which sepsis was associated. Their ages ranged from 27 to 88 years (mean, 60.0 years). ALI/ARDS were associated with the condition in 12 patients (aged 58.6 ± 17.2 years). There was no significant difference in age (64.5 ± 11.0 years) between the ALI/ARDS patients and those without ALI/ARDS.

SP-A and SP-D levels were measured by enzyme-linked immunosorbent assay (ELISA) (Teijin Institute of Biomedicine, Tokyo, Japan). The method was based on that of Shimizu and coworkers[22~24]. Cut-off levels of SP-A and SP-D were 43.8 and 109.8 ng/mL, respectively.

The blood level of type II PLA_2 was measured by immunoradiometric assay (IRMA) (Shionogi Research Institute, Osaka, Japan)[25]. The normal level was defined as being lower than 3.7pg/mL.

The blood level of TNF-α was measured by ELISA (Medogenix, Fleurus, Belgium). The measurement limit is 3 pg/mL. The blood IL-8 level was also measured by ELISA (TFB, Tokyo, Japan). The measurement limit is 3 pg/mL.

All data were expressed as the mean ± standard deviation (SD). The significance of differences was analyzed by the non-matched Wilcoxon test, and a difference at $p < 0.05$ was considered significant. Correlation coefficients were determined using Pearson's test, and correlations at $p < 0.05$ were considered significant.

Results

The SP-A level in the presence of ALI/ARDS with diffuse peritonitis was 37.6 ± 16.2 ng/mL. The maximum SP-A level during the course was 29.9 ± 8.2 ng/mL in the group without ALI/ARDS. Thus, there was no significant difference in SP-A level between the groups with and without ALI/ARDS **(Fig. 1)**.

The SP-D level in the presence of ALI/ARDS was 476.3 ± 391.2 ng/mL while the maximum SP-D level during the course was 85.9 ± 35.0 ng/mL in the group without ALI/ARDS. Thus, the level was significantly higher in the group with ALI/ARDS **(Fig. 2)**.

The Type II PLA_2 level in the presence of ALI/ARDS was 154.6 ± 102.3 ng/mL, while the maximum level during the course was 42.3 ± 30.9 ng/mL in the group without ALI/ARDS. Thus, the level was significantly higher in the group with ALI/ARDS **(Fig. 3)**.

The TNF-α level in the presence of ALI/ARDS was 209.0 ± 284.5 pg/mL, while the maximum level during the course was 48.5 ±

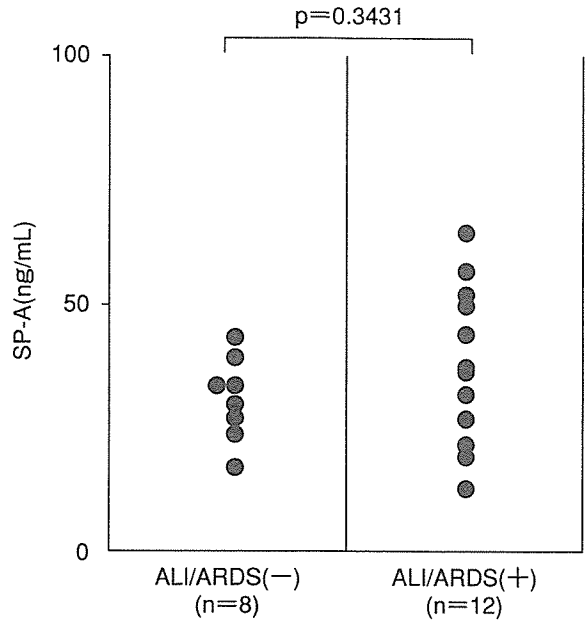

Fig. 1 The SP-A levels in patients with ARDS and without ARDS.

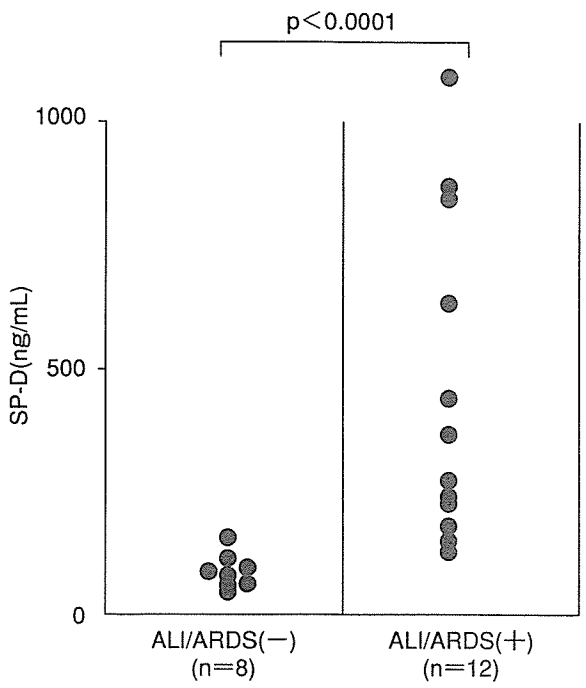

Fig. 2 The SP-D levels in patients with ARDS and without ARDS.

28.0 pg/mL in the group without ALI/ARDS. Thus, the level was significantly (p = 0.0015) higher in the group with the ALI/ARDS

The maximum blood IL-8 level during the

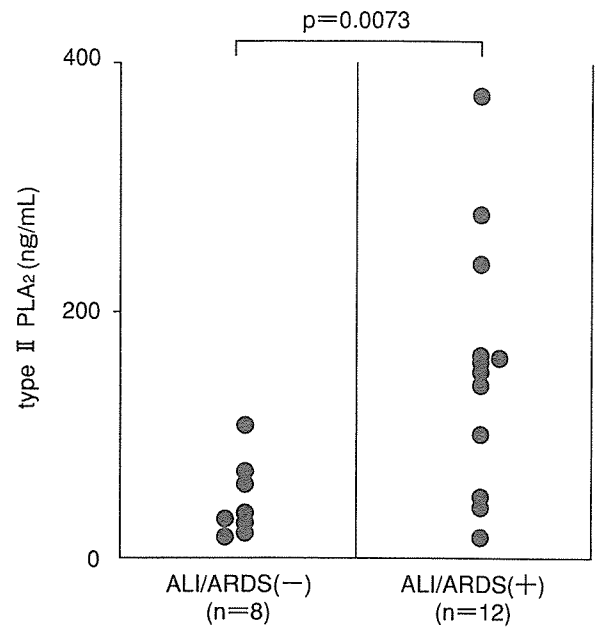

Fig. 3 The type II PLA2 levels in patients with ARDS and without ARDS.

course was 58.5 ± 30.2 pg/mL in the group without ALI/ARDS. Thus, the maximum IL-8 level was significantly (p = 0.0473) higher in the group with ALI/ARDS.

There was no significant (p = 0.1343) correlation between SP-A and SP-D levels (r = 0.3464).

There was no significant correlation between SP-D and TNF-α, whereas there was a significant (p = 0.0001) correlation between SP-D and IL-8 levels (r = 0.7967).

There was also a significant correlation between SP-D and type II PLA2 levels **(Fig. 4)**.

Discussion

Sepsis is frequently associated with the rising severity of diffuse peritonitis. Furthermore, ARDS is associated with sepsis at a frequency of 38%, and the association of ARDS has been considered an important prognostic factor[26].

In this study, SP-D and type II PLA2 were elevated in ALI/ARDS associated with diffuse peritonitis. Blood levels of inflammatory cytokines, i.e., TNF-α and IL-8, were also elevated at the time ARDS manifested. However, while

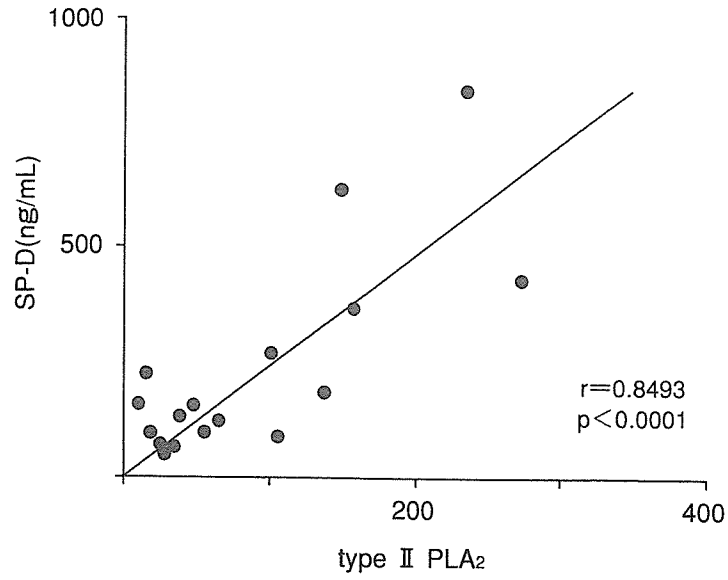

Fig. 4 The correlation between SP-D and type II PLA2 levels.

SP-A showed a tendency toward elevation in ALI/ARDS, there was no significant elevation of SP-A in the group without ALI/ARDS. SP-A is involved in pulmonary surfactant homeostasis and is closely associated with alveolar immunity and inflammation including increased phagocytosis of alveolar macrophages and suppression of cytokine release. Many details regarding the function of SP-D remain unknown, but SP-D is considered to be involved in alveolar immunological control via macrophages in a manner similar to that of SP-A.

Many details regarding the mechanisms underlying manifestations of SP-A and SP-D in blood also remain unclear. The following are considered to be involved in these mechanisms: Increased generation of type II cells, alveolar epithelial lesions or alveolar basement membrane injury, capillary hyper-permeability, etc. In cardiogenic pulmonary edema, however, with which capillary hyper-permeability is associated, the blood SP-A level is not increased, while it is increased in experimentally radiation-induced pneumonitis leading to basement membrane injury. On the basis of these observations, we speculate that increases in the generation of type II cells and alveolar epithelial lesions or alveolar basement membrane injury are the most likely mechanisms accounting for the elevations of SP-A and SP-D in blood.

A report has also shown that surfactant suppresses secretory PLA2 expression from guinea-pig alveolar macrophages[27]. Other studies have shown that respiratory disorders occur in response to inactivation of surfactant by phospholipase A2[28, 29].

The type II PLA2 elevation was consistent with the SP-D elevation, showing that SP-D was released into the bloodstream as a result of damage to pulmonary tissue by type II PLA2. This suggests that inhibition of the activity of type II PLA2 suppresses pulmonary tissue damage, thereby inhibiting the manifestations of ARDS.

The SP-D elevation in the bloodstream of an ARDS patient indicates an SP-D deficiency in pulmonary tissue, ultimately demonstrating that it would be reasonable to administer surfactant for the purpose of treating ARDS.

Elevations of TNF-α and IL-8 reflect inflammatory reactions, suggesting that both are significantly involved in type II PLA2 genera-

tion.

The present observations suggest that SP-D and type II PLA2 play major roles in the onset of ALI/ARDS in the presence of diffuse peritonitis.

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ORIGINAL ARTICLE

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Local elevation of the anti-inflammatory interleukin-10 in the pathogenesis of chronic subdural hematoma

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Abstract We investigated the relationship between inflammatory and anti-inflammatory cytokines in the pathogenesis of chronic subdural hematoma (CSDH) by measuring the plasma and subdural fluid levels of interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-10 (IL-10). The levels of IL-6, IL-8 and IL-10 were measured in the subdural fluid obtained from 34 patients with CSDH, using the enzyme-linked immunosorbent assay. The patients were classified into a high IL-10 group and a low IL-10 group according to the level of IL-10 in their subdural fluid samples. The subdural fluid levels of IL-6 and IL-8 were significantly higher in the high IL-10 group than in the low IL-10 group ($P<0.05$). A tendency for the patients in the low IL-10 group to show the separated or layer type of pattern on the CT scans was noted.

Keywords Chronic subdural hematoma · Anti-inflammatory cytokine · Interleukin-10 · Interleukin-8 · Interleukin-6

Introduction

Chronic subdural hematoma (CSDH) is well known to occur following even mild head trauma, especially in elderly patients [6, 7, 14]. Recently, local elevation of the levels of inflammatory cytokines, including those of the tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β), interleukin-6 (IL-6), and interleukin-8 (IL-8), has been shown in the subdural fluid in cases of CSDH and inflammation has been proposed as playing a leading role in the pathogenesis of CSDH [2, 20].

Interleukin-10 (IL-10) is an anti-inflammatory cytokine released by monocytes and macrophages, and suppresses the production of inflammatory cytokines by the monocytes [1, 19]. If inflammation has been proposed as playing a leading role in the pathogenesis of CSDH, local change of the anti-inflammatory IL-10 may relate with pathological features of CSDH. To evaluate the role of IL-10 in the pathogenesis of CSDH, we measured the levels of IL-10, IL-6 and IL-8 of subdural fluid in CSDH. We discussed the relationship between local elevation of the inflammatory and anti-inflammatory cytokines and radiological features of CSDH.

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Patients and methods

Thirty-four patients (29 males and five females; age range, 52–90 years; mean, 71.0 years) of CSDH were enrolled in the study. Patients with recurrent CSDH were excluded. In 29 of 34 patients, head trauma of various degrees of severity was the cause of CSDH. Nine of 34 patients received aspirin or ticlopidine as antiplatelet drugs and one patient received warfarin preoperatively. No patients presented any other risk factors for CSDH, such as chronic alcohol abuse and sever brain atrophy. CSDH was diagnosed based on computed tomography (CT), and none of the patients exhibited any signs of systemic inflammation. Blood examination was conducted in all patients on the day of the surgery, for a complete blood count and measurement of the serum level of C-reactive protein. All the patients were treated surgically by a small burr-hole craniotomy and irrigation, followed by external closed drainage, under local anesthesia. CT was performed 24 h, 7 days, 1 month, 3 months, and 6 months after the surgery, if there was no evidence of recurrent CSDH. Recurrence was considered to have occurred if there was evidence within 6 months of the surgery of increased thickness of the residual CSDH on CT images compared with that in the CT images obtained 7 days postoperatively.

We performed repeated CT at 2-week intervals for the patients who had recurrent CSDH. We considered surgical treatment when there was evidence of development of recurrent CSDH on repeated CT.

The levels of IL-6, IL-8 and IL-10 were determined by enzyme-linked immunosorbent assay. Samples of subdural fluid were collected directly from the CSDH at the time of the surgery. All the samples were centrifuged at 3,000 rpm for 10 min, and the supernatants were stored in sealed polypropylene tubes at −80°C until analysis. The all cytokines were assayed using enzyme-linked immunosorbent assay kits (R&D systems, Minneapolis, Minn.).

CSDH was classified into four types based on the pattern of the lesions on CT scans. Group 1: separated or layer type, consisting of two components of different densities, with a boundary in between; Group 2: laminar or mixed-density type, consisting of a homogenous density, with a high-density layer along the inner membrane; Group 3: trabecular type, consisting of a high-density septum between the inner and the outer membranes against a low-density to isodense background; Group 4: represented by a high-density, low-density, or isodense type of lesion, according to the classification suggested by Frati et al. [2].

For analysis of the relationship between the inflammatory cytokines IL-6 and IL-8, and the anti-inflammatory cytokine IL-10, we classified the patients into two groups: a high IL-10 group and a low IL-10 group. The differences in the levels of the inflammatory cytokines IL-6 and IL-8 between the high IL-10 group and low IL-10 group were tested by the Mann-Whitney test. The levels of these cytokines were also compared among the four groups divided according to the patterns on the CT scans, by Scheffé's F method.

Results

In all patients, blood cell count and serum level of C reactive protein were normal. Figure 1 shows the level of IL-10 in subdural fluid in all patients. Each patient was sorted into either a group with IL-10 under 60 pg/dl or a group with IL-10 over 200 pg/dl, as shown in the bar graph of Fig. 1. Consequently, we classified the patients into two groups: a high IL-10 group, in which the subdural fluid IL-10 level was over 200 pg/dl, and a low IL-10 group, in which the subdural fluid IL-10 level was under 60 pg/dl.

The subdural fluid levels of IL-6 and IL-8 were significantly higher in the high IL-10 group than in the low IL-10 group ($P<0.05$) (Table 1). The differences in the subdural fluid levels of IL-6, IL-8, and IL-10 among the four groups classified by the lesion pattern on CT were not significant, but a tendency for patients from Group 1 to show a low level of IL-10 was noted (Table 2).

Only one patient had a recurrence of CSDH in this study. This patient received aspirin pre- and postoperatively. In this patient who had CSDH classified as Group 2, the level of IL-10 in subdural fluid was 36.1 pg/dl.

Table 1 Subdural fluid levels of inflammatory cytokines in the high IL-10 vs low IL-10 group. Values represent means ± standard deviation

	IL-6 (pg/dl)	IL (pg/dl)
IL-10 over 200 pg/dl (n=5)	1,477±36.13*	776±147.8**
IL-10 under 60 pg/dl (n=29)	1,053±506.4	545.4±307.6

*Significant difference in the level of IL-6 between two groups (P=0.022)
**Significant difference in the level of IL-8 between two groups (P=0.044)

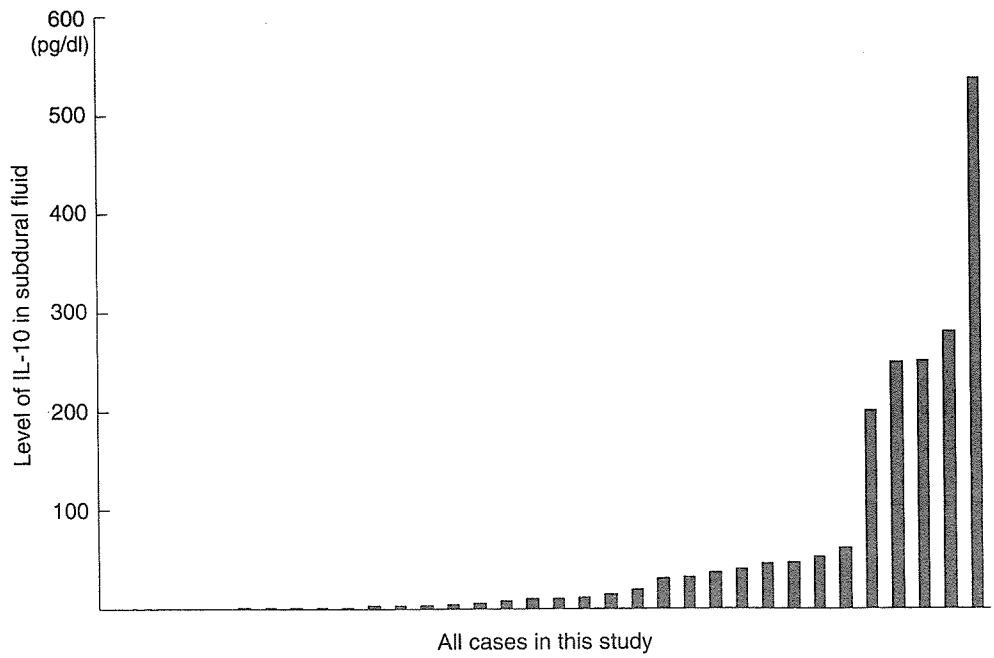

Fig. 1 Bar graph showing the level of IL-10 in the subdural fluid in CSDH in all patients. Each patient was sorted into either a group with IL-10 under 60 pg/dl or a group with IL-10 over 200 pg/dl as shown in here

Table 2 The levels of cytokines in subdural fluid by classification of CT scanning pattern. Values represent means ± standard deviation

Group[a]	IL-6 (pg/dl)	IL-8 (pg/dl)	IL-10 (pg/dl)
Group 1 (n=3)	1,138±523.2	663.0±179.4	15.5±26.1
Group 2 (n=4)	1,321±258.8	741.4±175.2	56.4±96.4
Group 3 (n=8)	1,374±197.6	575.7±308.8	57.7±80.8
Group 4 (n=19)	959.9±566.2	533.7±330.6	64.8±140.6

[a]*Group 1* the separated or layering type; *Group 2* the laminar or mixed-density type; *Group 3* the trabecular type; *Group 4* a high-density, low-density, or isodense type

Discussion

CSDH refers to a collection of blood in the space formed by cleavage of the inner dural layer (Haines and co-workers called this layer the dural border cell layer) after head trauma [8, 9, 13–15]. Recent studies have reported the local elevation of inflammatory cytokines in the cavity of the CSDH and support the hypothesis that local inflammation, angiogenesis, increased vascular permeability, and increased coagulative and fibrinolytic activity may be involved in the pathogenesis and progression of CSDH [3–5, 10–12, 16, 20, 21].

Inflammatory cytokines can stimulate the production of additional cytokines, which, together with the former, generate tissue pathology in human inflammatory diseases. A major deactivator of activated cytokine-producing cells is the anti-inflammatory cytokine IL-10 [1, 19]. We found the high IL-10 group showed significantly higher subdural fluid levels of IL-6 and IL-8 compared with the low IL-10 group. Our data on IL-10 suggest that the inflammatory process is activated during the formation of CSDH, while the balance of activation between inflammatory cytokines and anti-inflammatory cytokines is maintained. This phenomenon is similar to the inflammatory process in non-specific inflammation. Therefore, the elevation of IL-10 suggests that inflammation in the pathogenesis of CSDH may regulate it last.

Interestingly, the level of IL-10 in the subdural fluid tended to be lower in patients belonging to the Group 1 CT pattern (see Table 2). However, no significant differences in the levels of these cytokines were found among the groups divided on the basis of the lesion patterns on CT scans. Previous studies have reported that cases of CSDH showing the Group 1 CT pattern, which tend to rebleed easily, showed higher levels of inflammatory cytokines compared with other types of CSDH [2, 17, 18, 20]; therefore, elevation of the levels of inflammatory cytokines might be a risk factor for recurrence of CSDH [20]. However, our results did not support these considerations. We consider that it is difficult to explain the elevated levels of inflammatory cytokines simply by the effects of one kind of pathological event, such as bleeding into the subdural space. However, evaluation of only three patients showing the Group 1 pattern of CSDH on CT in our series may be statistically difficult. Similarly, it is difficult to evaluate the relationship between recurrence and level of IL-10, because only one patient had recurrence of CSDH in this study. We will investigate more cases with Group 1 pattern of CSDH and/or recurrent CSDH in order to demonstrate the role of inflammatory and anti-inflammatory cytokines in pathogenesis of CSDH which tend to rebleed easily.

The level of IL-10 elevated in subdural fluid of CSDH locally like inflammatory cytokines such as IL-6 or IL-8. The local activation of IL-10 in CSDH was similar to that in other non-specific inflammation. We suggest that IL-10 acts as a deactivator of inflammation in the pathogenesis of CSDH.

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高エンドトキシン血症に対する エンドトキシン吸着療法施行時の High mobility group box 1 値と肺酸素化能の検討：症例報告

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要旨

高エンドトキシン血症患者に対してpolymyxin-B immobilized fiber（PMX）による2時間のエンドトキシン吸着療法(PMX-direct hemoperfusion: PMX-DHP)を2回施行した．エンドトキシン値は速やかに低下し，High mobility group box 1（HMGB 1）値も低下し，呼吸状態は改善した．肺酸素化能の指標であるPaO_2/FIO_2比とHMGB 1値間には有意な負の相関関係がみられた（r＝－0.5243, p＝0.0176）．また，エンドトキシン値とHMGB1値間にも有意の相関関係がみられた（r＝0.5004, p＝0.0246）．呼吸障害発現におけるHMGB 1の関与の可能性，HMGB 1産生におけるエンドトキシンの関与の可能性，および高エンドトキシン血症に対するPMX-DHPの有用性が示された．

Key words *: sepsis, endotoxin, PMX-DHP, HMGB1, ARDS*

I. はじめに

グラム陰性桿菌の外膜に存在するエンドトキシンが種々の細胞を活性化してサイトカインを中心とする多くの液性因子を産生し，これらが複合して多彩な病態を惹起することを我々はこれまで報告をしてきた[1-3]．一方，High mobility group box 1（HMGB 1）はエンドトキシンショックにおいて，致死因子として作用すると報告され[4]，その後多くの病態とHMGB 1について検討されてきた[5,6]．最近，HMGB 1と急性呼吸障害との関わりについても報告されてきた[7,8]．

エンドトキシン血症の治療手段としてpolymyxin-B immobilized fiber（PMX）が開発され，臨床において用いられてきた[9]．我々は，高感度エンドトキシン測定法を用いてその有用性についてこれまで報告してきた[10, 11]．

今回，高エンドトキシン血症から敗血症性多臓器不全症候群を合併した患者に対してPMXによるエンドトキシン吸着療法（PMX-direct hemoperfusion: PMX-DHP）を施行した患者のエンドトキシン値，HMGB1値，および肺酸素化能（PaO_2/FIO_2比：P/F比）について検討したので報告する．

II. 症　例

症例：58歳，男性

既往歴：特記すべきもの無し

現病歴：4月4日午前7時30分頃交通事故で受傷し，直ちに当科に搬入された．

来院時現症：血圧76/40mmHg, 脈拍132/分，体温36.8度とショック状態にあったが，意識は清明であった．

来院時診断：骨盤骨折，両大腿骨開放骨折，両下腿骨開放粉砕骨折，出血性ショックであった．

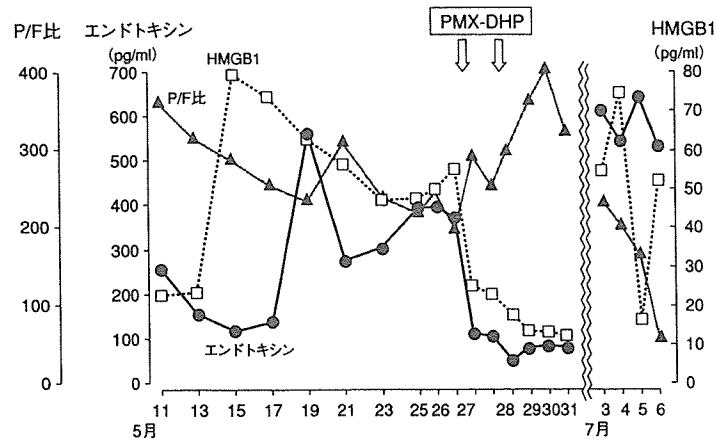

図1．エンドトキシン，HMGB1，およびP/F比の推移

臨床経過：出血性ショックに対して，輸液，輸血（濃厚赤血球，凍結人血漿）を行いながら同日午前9時より骨盤骨折に対して動脈塞栓術を施行し，バイタルサインは安定した．翌5日，両下腿の血流不良でチアノーゼが著明となったため両下腿の切断術を施行した．P/F比が186と急性呼吸促迫症候群を合併し，人工呼吸器管理とした．4月下旬頃より下腿断端部に感染を合併し，創部および血液培養で*P. aeruginosa*が，創部からMRSAが検出された．BUN（30～90mg/dL）と腎機能傷害，総ビリルビン値（35～55mg/dL）と肝機能障害，汎発性血管内凝固症候群，意識障害と敗血症性多臓器不全症候群を合併した．それぞれの病態に対する治療を行うとともに血液透析，血漿交換を適宜施行した．5月初旬頃より熱発著明（39～41度）で，エンドトキシン値は高値で推移し，5月11日に血圧が低下し（82/36mmHg），ショック状態となった．この時点でのエンドトキシン値は278pg/mlと高値を示した．敗血症性ショックに対する種々の治療を行いバイタルサインは安定したが，その後もエンドトキシン値は高値で推移した．高エンドトキシン血症に対して5月27，28日に2時間のPMX-DHPを2回施行し，エンドトキシン値の低下（374→107 pg/ml，104→52 pg/ml），およびHMGB1値の低下を認めた（55.2→24.9 ng/ml，22.7→17.6 ng/ml）（図1）．また，肺酸素化の改善もみられた（P/F比: 198→288，252→307）．本症例は，その後も，血液透析，血漿交換を含めた集学的治療を行い，一旦症状は改善したものの創部感染のコントロールが不能で7月6日に敗血症性多臓器不全症候群で死亡した．

III. 考　察

エンドトキシンは，単球やマクロファージなどの炎症細胞だけでなく，血管内皮細胞，気道上皮細胞，平滑筋細胞などの肺組織構成細胞の特異的受容体に直接作用して細胞を活性化させる．それによってtumor necrosis factor α（TNF-α），interleukin 1（IL-1），IL-6，IL-8，migration inhibiting factor（MIF），chemokines, interferon，および活性酸素種などの各種メデイエーター産生を誘導する[12]．そして，これらが急性呼吸障害の発症に重要な役割を果たしていると考えられる．

HMGB1はエンドトキシンで活性化された単球やマクロファージから産生され，エンドトキシンを投与されたマウスの血中や敗血症患者の血中に存在する[5]．HMGB1は分子量が約30Kダルトンで，DNAに結合し，ステロイドホルモン受容体やその他の転写因子がDNAに結合するときの調節をする[13]．これが

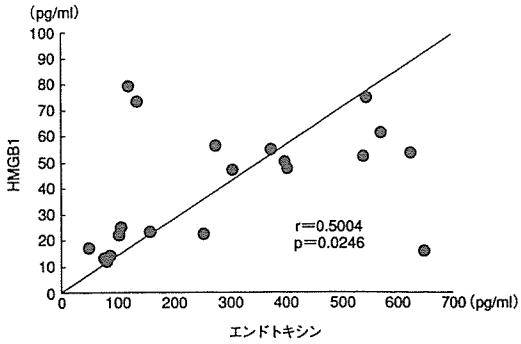

図２．経過中のエンドトキシン値とHMGR1値の相関関係（r＝0.5004, p＝0.0246）

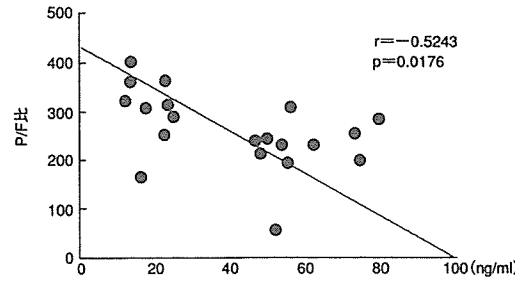

図３．経過中のHMGB1値とP/F比の相関関係（r＝ −0.5243, p＝0.0246）

多くの細胞の壊死に伴い細胞外に放出されると，receptor for advanced glycation end-products（RAGE）に作用してNF-kBを活性化し，炎症性サイトカインとして働く[14]．また，樹状細胞，マクロファージも TNFなどで刺激されるとHMGB１を分泌する．HMGB１をC3H/HeJマウスの気道内に投与すると肺に炎症を惹起する[7]．Uenoらは急性呼吸障害発現にHMGB１が重要な役割を担っていると報告している[8]．

我々は多施設共同研究において，PMX-DHPにより血中のエンドトキシン値とともにサイトカイン値も低下することを報告した[15]．しかし，PMX-DHPによりサイトカインが直接吸着されるのか，あるいはエンドトキシンが吸着されることによりエンドトキシンの刺激が軽減しサイトカインの産生が抑制されるのかは不明である．

今回の検討では，PMX-DHPにより血中のエンドトキシン値が低下し，エンドトキシン値とHMGB1値間には有意の相関関係がみられた（r＝0.5004，p＝0.0246）（図２）．経過中のHMGB1が高値であるほど肺酸素化能が低下し，HMGB１値とP/F比には有意の負の相関関係がみられ（r＝−0.5243，p＝0.0176），HMGB1の呼吸傷害に及ぼす影響を示唆するものと思われた（図３）．また，重症度の指標として用いられるSOFA スコア[16]と有意ではないが相関傾向がみられ（r＝0.3940, p＝0.0856），HMGB1が病態の重症度を反映している可能性も示唆された．１症例からの検討ではあるが，HMGB１が敗血症における病態形成と密接に関わっている可能性が窺われた．

我々は，これまでPMX-DHP施行後にエンドトキシン値が低下すると肺酸素可能が改善することを報告してきたが[16]，肺酸素化能の改善は，PMX-DHPによるエンドトキシンを吸着する直接的作用なのか，あるいはHMGB１を介した間接的な作用なのか今後症例数を重ねて検討する必要がある．

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Clinical significance of interleukin 18 in cases of multiple organ dysfunction syndrome associated with diffuse peritonitis

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Abstract

We examined the clinical significance of interleukin 18 (IL-18) in cases of the multiple organ dysfunction syndrome (MODS) associated with diffuse peritonitis, in relation to the prognosis of these patients. Forty-eight patients with diffuse peritonitis were enrolled in the study. Sepsis and MODS were diagnosed according to ACCP/SCCM Consensus Conference definitions. The SOFA score assessed the severity of MODS. Blood specimens were collected from the patients at the time of diagnosis of sepsis and at regular intervals thereafter. IL-18 was measured by ELISA. The serum IL-18 level was found to be significantly higher in patients who presented with MODS in association with diffuse peritonitis (MODS group) than in patients with diffuse peritonitis who did not develop MODS (non-MODS group). In the MODS group, the serum IL-18 level was significantly higher in the non-survivor group than in the survivor group. The serum IL-18 level showed a significant correlation with the SOFA score. There was also a significant correlation between the serum IL-18 level and the serum total bilirubin level. These findings suggest that IL-18 may induce the development of liver dysfunction, while also reflecting the severity of MODS associated with diffuse peritonitis.

Key words *: peritonitis, sepsis, IL-18, SOFA score*

I. Introduction

Sepsis triggers the release of cytokines and other mediators, which directly and/or indirectly cause a variety of pathological changes. Inflammatory cytokines or anti-inflammatory cytokines are produced whenever a strong inflammatory response, such as sepsis, occurs in the body, and we have already reported on the possibility that they are responsible for its complex pathology[1,2].

When heat-killed *Proprionibacterium acnes (P. acnes)* was administered to mice that were later given a small dose of lipopolysaccharide (LPS), an interferon-γ (IFN-γ) inducing factor that differs from interleukin 12 (IL-12) was produced in the blood. This factor is now called interleukin 18 (IL-18), and it is produced by macrophages, especially the Kupffer cells in the liver. When anti-IL-18 antibody is administered before LPS challenge a week after *P. acnes* administration, no

hepatic tissue necrosis is observed, and the AST and ALT values do not increase. We previously reported the involvement of endotoxins, cytokines and many other mediators in the pathogenesis of MODS associated with diffuse peritonitis or sepsis[3-5]. We also showed that the serum interleukin (IL-18) level reliably reflected the severity of liver dysfunction in sepsis of postsurgical cases[6-11].

This study was aimed at determining the correlation between the serum IL-18 level and the severity of MODS developing in patients with diffuse peritonitis.

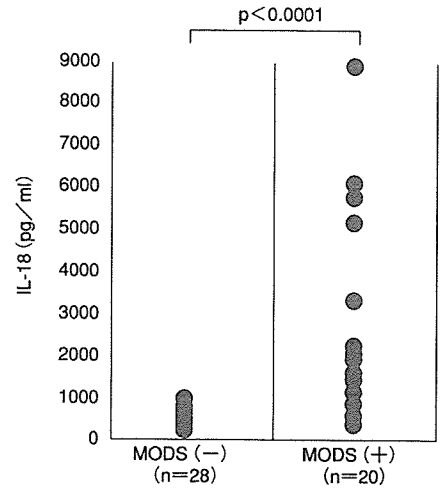

Fig. 1. IL-18 levels in patients with and without MODS

II. Subjects and Methods

Informed consent was obtained from either the patients or their family members prior to commencement of the study. The Institutional Ethics Committee of Iwate Medical University approved the study protocol. Forty-eight patients with diffuse peritonitis were enrolled in the study. The underlying conditions were perforation of the small intestine in 12 patients, appendicitis in 3 patients, mesenteric thrombosis in 7 patients, duodenal ulcer perforation in 5 patients, and colonic perforation in 21 patients. The mean age of the patients was 65±12 years, and was not significantly different between males (30 patients) and females (18 patients) (63±13 years vs. 68±10 years).

Sepsis and MODS were diagnosed according to ACCP/SCCM Consensus Conference definitions[12]. The severity of MODS was assessed by the Sequential Organ Failure Assessment (SOFA) score[13]. Blood specimens were collected from the patients at the time of diagnosis of sepsis and at regular intervals thereafter. Separated serum samples were stored at −80℃ until use. IL-18 was measured by enzyme-linked immunosorbent assay (ELISA) (MBL, Nagoya, Japan). The detection limit was 12.5 pg/ml, and the normal level was under 259.4 pg/ml. Tumor necrosis factor α (TNF-α) was also quantified by ELISA (Medgenix Diagnostics, Fleurus, Belgium), and its detection limit was 3 pg/ml. In addition, interleukin 6 (IL-6) (detection limit: 3 pg/ml) and interleukin 8 (IL-8) (10 pg/ml) were also analyzed by ELISA (TFB Inc., Tokyo). Unpaired Wilcoxon's test was used to test statistical significance.

Pearson's test was performed for analysis of correlations between the variables. $P<0.05$ was considered to denote statistical significance.

III. Results

MODS occurred in 20 of the 48 patients with diffuse peritonitis. The age and sex ratio were similar for peritonitis patients who presented with MODS (MODS group) and