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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.

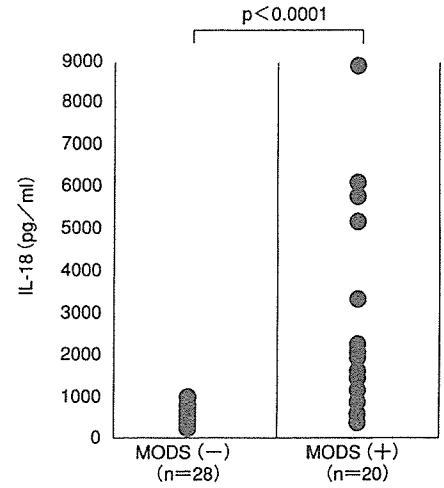

Fig. 1. IL-18 levels in patients with and without MODS

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

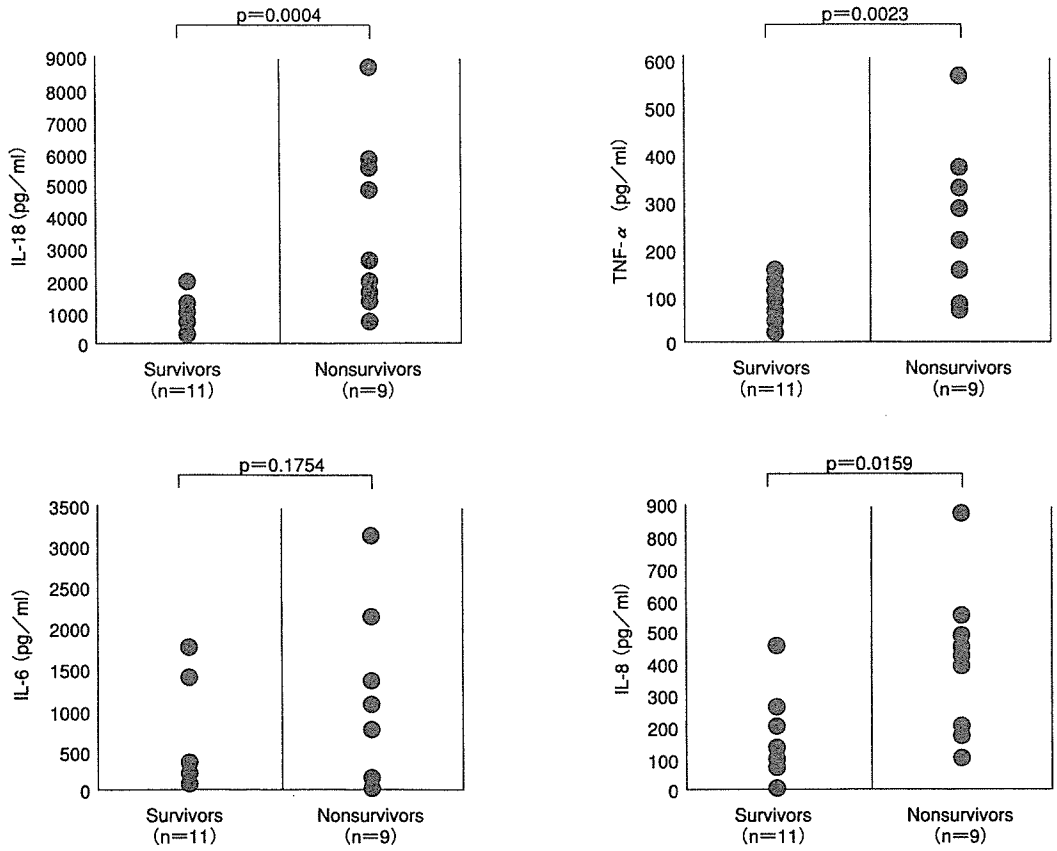

Fig. 2. IL-18, TNF-γ, IL-6, and IL-8 levels in patients who survived and died with MODS

those who did not develop MODS (non-MODS group). The maximum IL-18 level in the serum was found to be significantly higher in the MODS group than in the non-MODS group (2296±2331 pg/ml vs. 387±269 pg/ml) (Fig. 1).

All 28 patients without MODS survived. 11 patients with MODS survived, and 9 patients with MODS died. Figure 2 compares the maximum levels in the serum of IL-18, TNF-α, IL-6, and IL-8 between patients of the MODS group who survived during the study period (the survivor group) and patients of this group who died during the study period (the non-survivor group). The serum IL-18 level was significantly higher in the non-survivor group than in the survivor group (3941±2625 pg/ml vs. 950±620 pg/ml). The serum TNF-α level was also significantly higher in the non-survivor group than in the survivor group (267±141 pg/ml vs. 97±43 pg/ml). The same was the case for the serum IL-8 level, which was significantly higher in the non-survivor group than in the survivor group (379±243 pg/ml vs. 166±119 pg/ml), and the serum IL-6 level, which was also higher in the non-survivor group than in the survivor group (1050 ±1008 pg/ml vs. 389± 577 pg/ml), although the difference in this last parameter did not attain statistical significance (Fig. 2).

The maximum IL-18 level in the serum

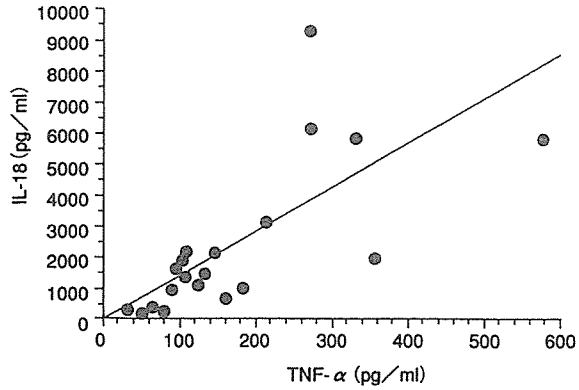

Fig. 3. The maximum IL-18 level in the serum showed a strongly significant correlation with the maximum serum TNF-α level (r=0.7283, p=0.0003)

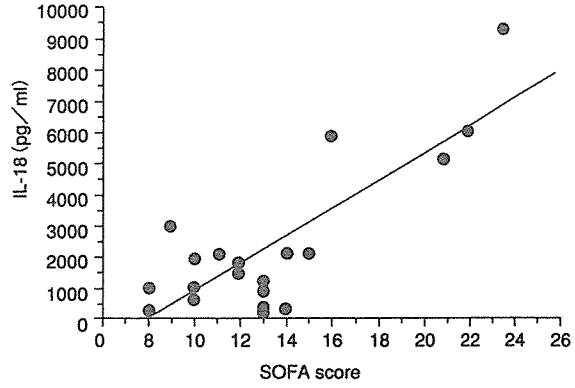

Fig. 4. The maximum SOFA score was significantly correlated with the maximum IL-18 level (r=0.4583, p=0.042)

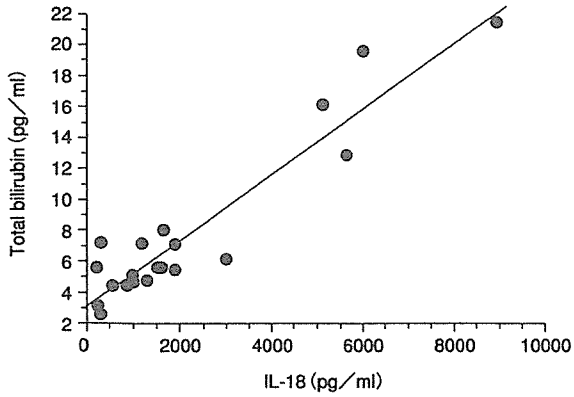

Fig. 5. A significant correlation was also observed between the maximum IL-18 level and the maximum total bilirubin level in the serum (r=0.9342, p<0.0001)

showed a strongly significant correlation with the maximum serum TNF-α level (Fig. 3). In addition, the maximum IL-18 level was also significantly correlated with the maximum serum IL-6(r = 0.7124, p = 0.0004) and maximum IL-8 (r = 0.5658, p = 0.0093) levels.

The maximum SOFA score was significantly correlated with the maximum IL-18 level (r = 0.4583, p = 0.042) (Fig. 4) and maximum TNF-α level (r = 0.5559, p = 0.0109), respectively, in the serum. A significant correlation was also observed between the maximum IL-18 level and the maximum total bilirubin level in the serum (Fig. 5).

IV. Discussion

Sepsis is a common cause of mortality and morbidity worldwide. The incidence of sepsis is estimated to be approximately 750,000 cases per year in the United States [14]. Sepsis can be viewed as the result of the activation of a cascade triggered by a microbial infection, ending in multiple organ failure and death, but the exact underlying mechanisms remain unclear.

Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, has been believed to play an important role as the initiating event of the sepsis cascade. Recent studies[15], however, have clarified that while LPS by itself is not sufficient to trigger activation of the sepsis cascade, it induces the production and release of endogenous mediators, in particular, cytokines. It has become clear that these mediators are directly and/or indirectly

involved in the pathogenesis of sepsis. Many studies have demonstrated increased levels of various cytokines in the blood of patients with sepsis. Some cytokines have even been shown to serve as biological markers of the prognosis in these patients [16, 17].

When a small amount of LPS was injected into mice pretreated with heat-inactivated bacteria of *Propionobacterium acnes (P.acnes)*, an interferon-γ (IFN-γ)-production-inducing substance, with different characteristics from interleukin 12 (IL-12), was found to be produced in the animals *in vivo*[18]. This molecule was later characterized as interleukin 18 (IL-18). IL-18 is primarily produced by macrophages, especially by those resident in the liver, namely, Kupffer cells. When anti-IL-18 antibody was injected into mice one week after the inoculation of *P. acnes*, subsequent LPS challenge did not induce hepatic necrosis or elevation of the serum AST or ALT[19].

In the present study, the serum levels of IL-18 were found to be significantly higher in patients who presented with MODS secondary to diffuse peritonitis than in those patients with peritonitis who did not develop MODS. In the MODS group, the maximum IL-18 level in the serum was significantly higher in the non-survivor subgroup than in the survivor subgroup.

These findings suggest that the serum IL-18 level is significantly correlated with the severity of MODS. In addition, the serum levels of the inflammatory cytokines TNF-α and IL-8 were also significantly higher in the non-survivor subgroup than in the survivor subgroup. On the other hand, the serum IL-6 level was not significantly different between the non-survivor group and the survivor group. This finding was consistent with the results of our previous study [14]. The MODS score, however, showed a significant correlation with the serum levels of IL-18, TNF-α, IL-6 and IL-8. Among these, the correlation between the MODS score and the serum IL-18 level was found to be the strongest.

Treatment with anti-IL-18 antibody was demonstrated to inhibit the onset of fulminant hepatitis-like symptoms in a mouse model of endotoxin-induced fulminant hepatitis-like syndrome[20]. Another study has shown that injection of anti-IL-18 antibody reduces the severity of endotoxic shock in mice. Taking into consideration these findings, along with the finding of the strong correlation between the Il-18 level and total bilirubin level in the serum in this study, it may be reasonably assumed that IL-18, which reflects the severity of MODS, may be closely involved in the pathogenesis of hepatic failure.

IL-18 has been demonstrated to facilitate the production of IL-13, a Th2 cytokine[21]. This suggests the involvement of IL-18 in a complex mechanism, in which the cytokine directly inhibits Th1 response which may result in vigorous tissue damage, while indirectly inhibiting the Th1 response via induction of a Th2 response[22].

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新しいセプシス診断　1

エンドトキシン測定の新規開発

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箱崎将規　高橋　学　八重樫泰法

えんどう しげあつ，さとう のぶひろ，すずき やすし，こじか まさひろ，はこざき まさのり，たかはし がく，やえがし やすのり：岩手医科大学救急医学

● リムルステストの原理について

エンドトキシン定量法として知られるリムルステストの名はアメリカ産カブトガニの学名 *Limulus polyphemus* から由来している。このテストは，カブトガニ血球が微量のエンドトキシンで凝固する現象が契機となり開発された[1]。カブトガニ血球の抽出液（ライセート）に存在するC因子がエンドトキシン（lipopolysaccharide：LPS）の受容体であり，これは哺乳動物の補体のC1sやC1qとの構造類似性が明らかにされている。その後，岩永らにより詳細に研究され，LPSによって活性化される系（C因子系）と，真菌の細胞壁成分であるβ-D-グルカンなどによって活性化される系（G因子系）が存在することがわかった[2]。その結果，エンドトキシン，β-D-グルカンそれぞれ特異的に反応するキットが開発された。さらに定量方法においては，比濁時間分析法[3]が開発された。これは，ゲル化に伴う濁度を透視光量値の変化として捉えて定量化している。市販のものには比濁時間

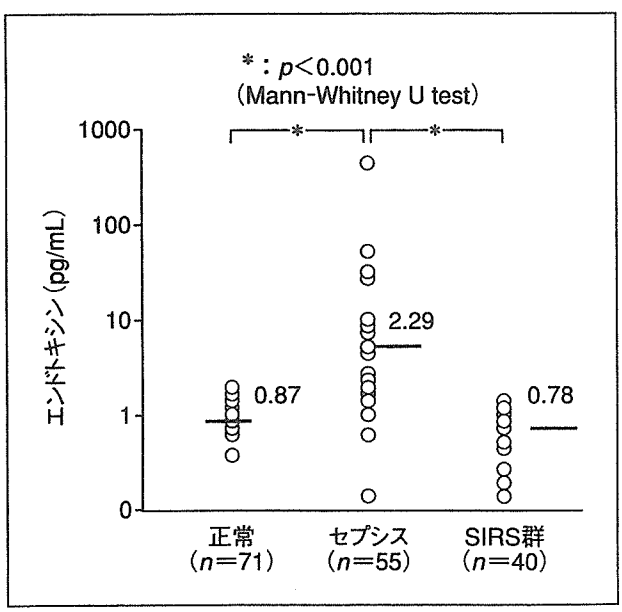

図1　高感度エンドトキシン測定による評価
セプシス患者，感染を合併しないSIRS患者および健常者のエンドトキシン値の比較

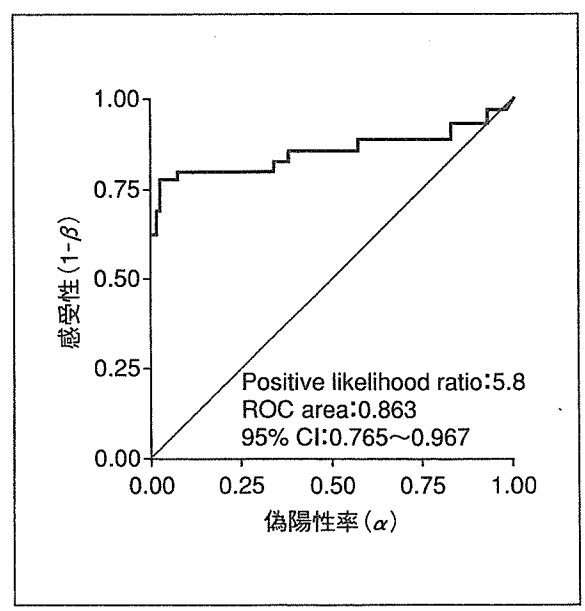

図2　エンドトキシン値によるセプシスの特異度と感度
セプシス患者におけるエンドトキシン値のROC曲線から最適カットオフ値は1.1 pg/mLとした

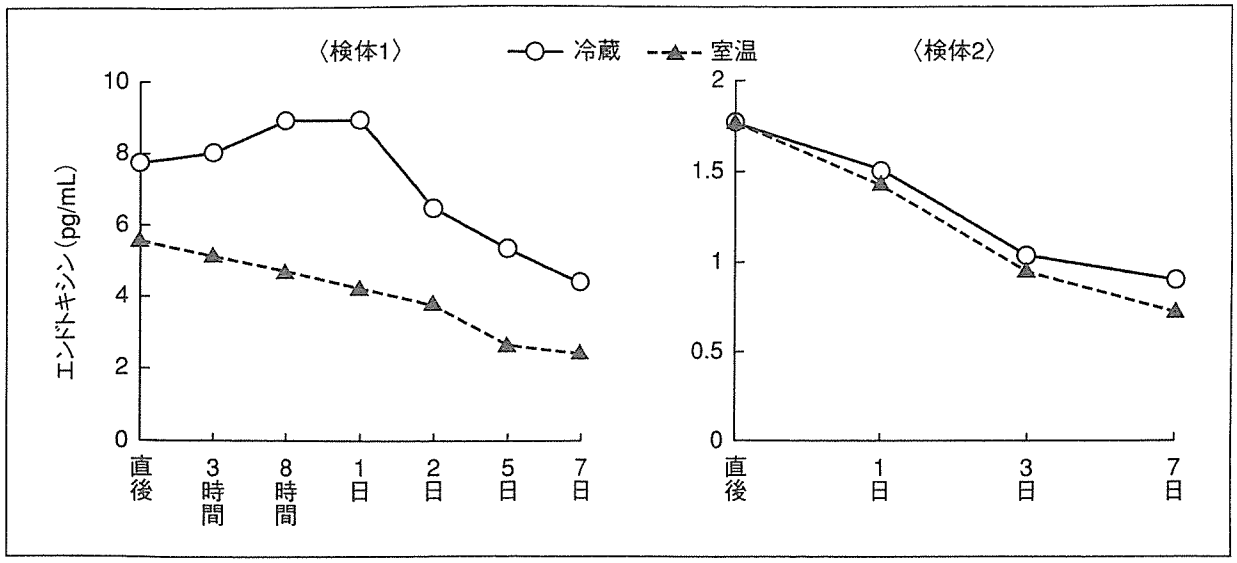

図 3　エンドトキシン測定までの保存と時間
検体の保存状態によりエンドトキシン値の乖離がみられる。

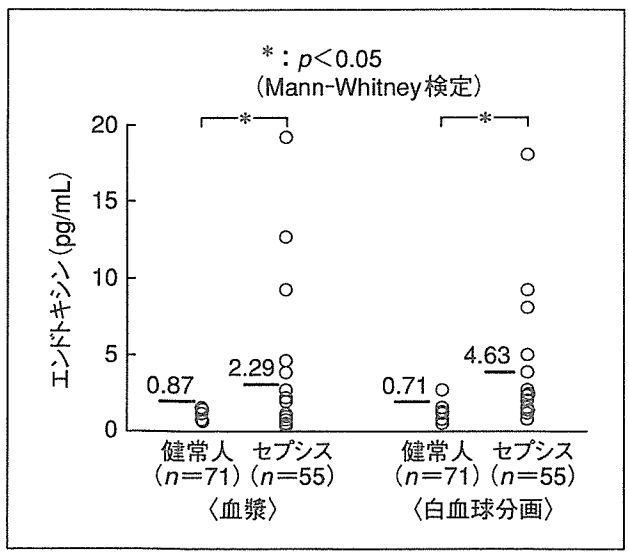

図 4　血漿と白血球分画のエンドトキシン値の比較
健常者，セプシス患者における血漿，白血球分画のエンドトキシン値。

分析法キット（和光純薬工業）がある。なお，このキットでは過剰量のグルカンを加えてG因子の活性化を抑制することでエンドトキシン特異的にしてある[4]。

● 高感度エンドトキシン測定法

比濁時間分析法はトキシノメーターを用いて，検体とカブトガニ血球から調整されたリムルス試薬を混和させた溶液のゲル化時間（リムルス反応）を測定する方法である。これには，リムルス反応を利用しているため特異度は非常に高い一方で，感度に関しては測定時間が短いと低下することや，試薬によりばらつきがでるという弱点があった。これまでのエンドトキシンのカットオフ値は，特異度と測定時間の短縮を重視し，3.5～5 pg/mL に設定されることが多く，感度が低いという問題があった。

MT-251 型のトキシノメーターは，生化学分析用に生産されているもので，カブトガニのライセートのゲル化時間を 999 分まで観察可能である。理論的には，0.01 pg/mL までのエンドトキシン濃度が測定できる。しかし，これでは臨床的に利用することに難点があり，われわれは，測定時間を 200 分とすることにより，0.1 pg/mL まで測定することができた。本法により，セプシス診断におけるエンドトキシン値の最適カットオフ値は 1.1 pg/mL とした（図 1）。これによるとセプシス診断における感度は 81.3%，特異度は 86.1%となり，従前のカットオフ値を 5.0 pg/mL に想定した場合に比べて，その感度は 3 倍以上になった[5]。これまでマスクされていた 1.1～5 pg/mL のエンドトキシン血症症例に対して，エンドトキシンをターゲットとして治療を行うことにより，著明な臨床効果が得られることは，エンドトキシン高感度測

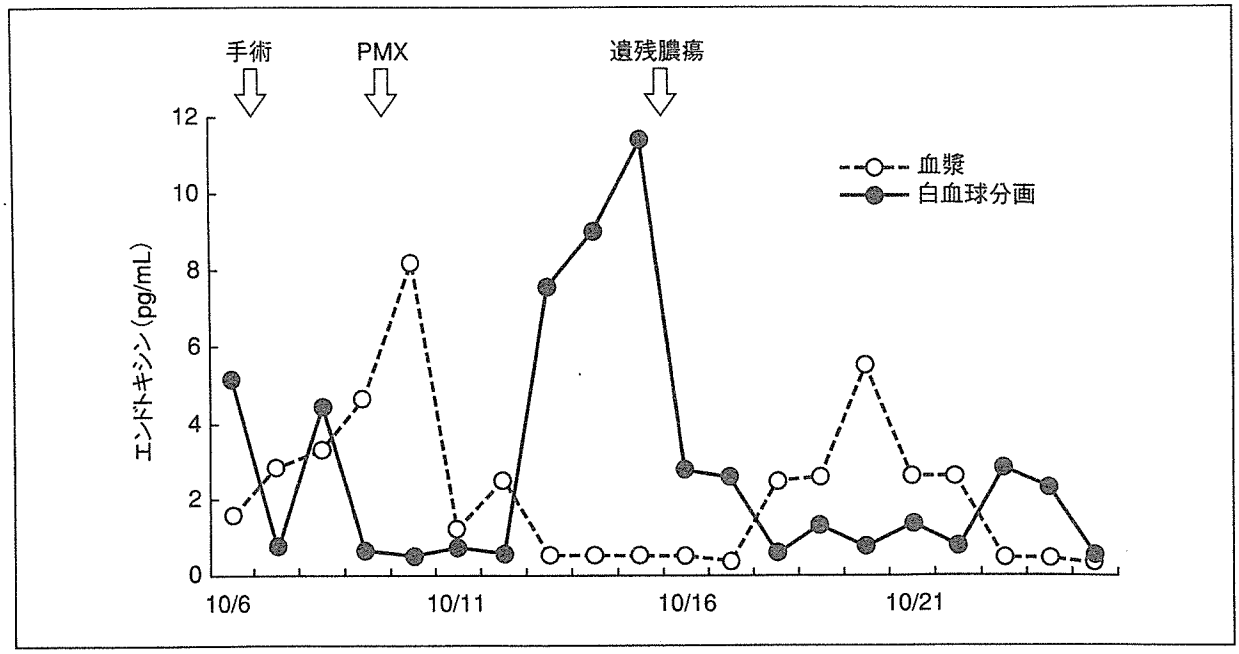

図 5　大腸穿孔
大腸穿孔患者の血漿，白血球分画のエンドトキシン値の推移。PMX：エンドトキシン吸着療法

定法の位置づけにおいて臨床上非常に重要であることを示唆するものである（**図 2**）[5]。

本法は，エンドトキシン測定法としては，現時点においてはゴールデンスタンダードであると思われ，すでに一部の臨床検査会社でも採用している。しかし実際の測定に際しては，採血後室温で長時間放置することなく，なるべく早期に血漿を分離し低温保存しておくことが重要である（**図 3**）[6]。

● 今後のエンドトキシン測定法

われわれは比濁時間分析による高感度化でセプシス診断での有用性の報告をしてきた。しかし，重症度の判定はこの方法を用いても困難であり，血漿解析だけではセプシス病態解明には限界がある。感染症防御は局所における白血球による細菌貪食が開始と考えられ，その際白血球分画に細菌の菌体成分が残っている可能性が高く，この部分のエンドトキシン測定は有用と考えられた。そこで白血球分画のエンドトキシン測定を考案した（図 4）。白血球分画は発症 1 日後から陽性となり，その経過は重症度と臨床経過によく相関していた（**図 5**）。前処理は簡便であり血漿との同時測定が可能であることから，白血球分画測定は実践的かつ有用と考えられる。血漿中および白血球分画のエンドトキシン血を同時に測定できるようになれば，エンドトキシン血症に対する今後新たな治療戦略も可能となるであろう。

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日集中医誌．2006;13:457～462.

血清サイトカイン濃度の推移を検討し得た治療抵抗性EBウイルス関連血球貪食症候群（EBV-AHS）の一例

斉藤　智誉*1　野間　祥子*1　河野　安宣*1　石原　晋*1
坂井　晃*1　兵頭英出夫*2　八重樫泰法*3　遠藤　重厚*3

要約：治療抵抗性であったEBウイルス関連血球貪食症候群（Epstein-Barr virus-associated hemophagocytic syndrome, EBV-AHS）症例における血清サイトカイン濃度について検討した。症例は30歳，男性。近医にて，重症感染症，disseminated intravascular coagulation（DIC）で加療中に急性腎不全を呈し，当院に紹介転院となった。ICU入室後，人工呼吸管理下に持続血液濾過透析（continuous hemodiafiltration, CHDF）を開始した。翌日，臨床症状，血液検査などから骨髄穿刺を施行し，血球貪食症候群（hemophagocytic syndrome, HPS）と診断した。重篤な肝機能障害のため免疫化学療法は施行せず，ステロイドパルス療法，血漿交換療法（plasma exchange, PE）を施行した。治療開始時の血清サイトカイン値は高値を示し，予後不良であったことが示唆された。また，サイトカインバランスに関しては抗炎症性サイトカインが優位な免疫不全状態であった。ステロイドパルス療法，PE施行後，サイトカインバランスは改善したが，臨床症状の改善には至らなかった。本症例のような重症例に対しては，早期の治療開始と，より有効なサイトカイン調節が必要であると考えられる。

Key words: ① hemophagocytic syndrome, ② Epstein-Barr virus, ③ cytokine

はじめに

血球貪食症候群（hemophagocytic syndrome, HPS）は，骨髄・脾臓等リンパ網内系組織での組織球の増殖と血球の貪食像を認め，高サイトカイン血症を特徴とする症候群で，成人例の多くは感染症，悪性腫瘍，膠原病等に併発するといわれている。今回我々は血漿交換療法（plasma exchange, PE），持続血液濾過透析（continuous hemodiafiltration, CHDF），ステロイドパルス療法などの治療に反応せず救命し得なかったEBウイルス関連血球貪食症候群（Epstein-Barr virus-associated hemophagocytic syndrome, EBV-AHS）症例を経験し，その血清サイトカイン値について検討した。

症　例

患者：30歳，男性。

既往歴：特記事項なし。

現病歴：38℃台の発熱，嘔気，息切れが出現したため夜間当番病院受診，黄疸を指摘され内科受診をすすめられた。その後も症状は改善せず，2日後に近医を受診し，高ビリルビン血症〔total bilirubin（T.Bil）11.4 mg·dl^{-1}〕がみられたため入院となった。発熱（40.4℃），皮膚および眼球結膜黄染，腹部CT検査にて肝脾腫，血液検査にて血球減少〔WBC 2,600 mm^{-3}, platelet（PLT）32,000 mm^{-3}〕，Dダイマーの上昇（16.9 μg·ml^{-1}）等から，重症感染症，disseminated intravascular coagulation（DIC）と診断され，抗生物質（アンピシリン 4 g·day^{-1}），メシル酸ガベキサート，低分子ヘパリンの投与が行われた。しかし，翌々日には無尿となり，精査加療目的にて当院救命救急センター紹介転院となった。

来院後経過：来院時現症では発熱，口渇，頻呼吸，全身倦怠感等を認め，安静を保つことが困難であった。血液検査では，血小板の低下，CRPの上昇，代謝性アシドーシス，凝固線溶系の異常，肝機能障害，腎機能障害を認めた。また，動脈血液ガス分析および胸部X線写真から，明らかな呼吸器系の異常を認め

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なかった（Table 1）。重症感染症によるDIC，臓器障害を疑い，抗生物質（メロペネム 0.5 g·day^{-1}），免疫グロブリン製剤の投与，人工呼吸管理下にCHDFを開始した。入院第2病日，①発熱，②2系統以上の血球減少（WBC 2,000 mm^{-3}，PLT 13,000 mm^{-3}），③高フェリチン血症（53,521 ng·ml^{-1}），④高LDH血症（5,950 U·l^{-1}），⑤肝脾腫等からHPSを疑い，骨髄穿刺を施行しHPSと診断した。血球減少，DICに対しては，補充療法（濃厚血小板，新鮮凍結血漿），抗凝固療法（メシル酸ガベキサート，アンチトロンビン製剤）を施行した。HPSに対する免疫化学療法に関しては，肝機能障害が著しいためこの時点でVP-16，シクロスポリン等を投与することは困難と判断し，まずステロイドパルス療法（メチルプレドニゾロン1 g·day^{-1}，iv，3日間），その後プレドニゾロン（60 mg·day^{-1}，iv）の投与を開始した。HPSの原因疾患として，単純ヘルペスウイルス（Herpes-simplex virus, HSV），水痘－帯状疱疹ウイルス（Varicella-Zoster virus, VZV）による感染の可能性を考慮し，アシクロビル（375 mg·day^{-1}, iv）の投与を行った。第3病日よりPE（血液流量120 ml·min^{-1}，3時間，新鮮凍結血漿40単位）を3日間施行した。施行後，GOT 246 IU·l^{-1}，GPT 100 IU·l^{-1}，LDH 2,720 IU·l^{-1}，T.Bil 9.3 mg·dl^{-1}と軽度低下したが（Fig. 1），十分な改善は得られなかったため，免疫化学療法の導入を断念した。第5病日にWBC 1,300 mm^{-3}まで低下したため（Fig. 1），フィルグラスチムの投与を開始した。経過中，喀痰·血液などの細菌培養を施行したが，いずれも陰性であった。第9病日より再度ステロイドパルス療法（メチルプレドニゾロン1 g·day^{-1}，3日間）を施行した。しかしながら，治療に反応せず多臓器不全が進行し，第20病日に永眠された。

HPSの発症原因について，経過中悪性リンパ腫，ウイルス感染，自己免疫疾患等の検索を行った。骨髄穿刺により表面マーカー解析，T細胞受容体解析による単クローン性増殖の有無を検索したが，悪性リンパ腫を示唆する所見は得られなかった。自己免疫疾患に関しては抗核抗体，抗ミトコンドリア抗体ともに陰性であった。ウイルス感染に関しては，HSV，VZV，A型肝炎ウイルス，B型肝炎ウイルス，C型肝炎ウイルス，サイトメガロウイルス等の検索を行ったが陰性であった。EBウイルス（EBV）に関しては，EBV-VCA IgG陽性，EBV-VCA IgM陰性，EBV-EBNA陰性であり，既感染のパターンを示していたが，後日，EBV-DNA（PCR）陽性および骨髄組織 *in situ* hybridizationによるEB virus-encoded RNA（EBER）陽性（濃紺部が陽性）（Fig. 2）が判明し，HPSの原因はEBV感染によるものと考えられた。

Table 1　Laboratory data on admission

Blood cell count		Blood biochemistry	
WBC	4,100 mm^{-3}	ALP	217 IU·l^{-1}
Band	42%	GOT	424 IU·l^{-1}
Segmented	11%	GPT	262 IU·l^{-1}
Lymphocyte	12%	LDH	4,595 IU·l^{-1}
Monocyte	2%	T.Bil	11.2 mg·dl^{-1}
Atypical lymphocyte	1.5%	BUN	70.3 mg·dl^{-1}
RBC	395 × 10^4 mm^{-3}	Cr	5.1 mg·dl^{-1}
Hb	12.4 g·dl^{-1}	TP	4.7 g·dl^{-1}
Ht	37.2 %	Alb	2.7 g·dl^{-1}
PLT	16 × 10^3 mm^{-3}	NH_3	41 μg·dl^{-1}
		CRP	24.2 mg·dl^{-1}
Coagulation test		**Blood gas analysis**	
PT	13.2 sec	pH	7.309
PT (%)	81%	$PaCO_2$	21.6 mmHg
APTT	44.4 sec	PaO_2	98.1 mmHg
FIB	227.2 mg·dl^{-1}	HCO_3^-	10.5 mmol·l^{-1}
FDP	38.3 μg·ml^{-1}	BE	−14.6 mmol·l^{-1}
D-dimer	31.0 μg·ml^{-1}		
AT-Ⅲ	52%		

PLT, platelet; PT, prothrombin time; APTT, activated partial thromboplastin time; FIB, fibrinogen; AT-Ⅲ, antithrombin-Ⅲ; ALP, alkaline phosphatase; T.Bil, total bilirubin; BUN, blood urea nitrogen; Cr, creatinine; TP, total protein; Alb, albumin; NH_3, ammonia.

血清サイトカインの推移：血清interleukin-6（IL-6），IL-8，IL-10，interferon-γ（IFN-γ），tumor necrosis factor-α（TNF-α），soluble IL-2 receptor（sIL-2R）を後日，酵素免疫測定法（enzyme-linked immunosorbent assay, ELISA）で測定した。PE施行時にはその前後で血液採取を行った。PE施行前の血清IL-6，IL-8は，それぞれ520 pg·ml^{-1}，468 pg·ml^{-1}と高値を示した。これらはPE，ステロイドパルス療法終了後一時低下したが，2度目のステロイドパルス療法終了後，再び上昇に転じた。IL-10はIL-6，IL-8に比べて著しく高値を示し，PE，ステロイドパルス療法終了後から低下傾向となった。IL-6/IL-10比は1以下であったが，ステロイドパルス療法，PE終了後徐々に改善し，1以上となった。また，血清sIL-2Rも同様に6,614 pg·ml^{-1}と高値を示し，血清IFN-γ，TNF-αも504 pg·ml^{-1}，289 pg·ml^{-1}と高値を示した。血清IFN-γはPE，ステロイドパルス療法終了後，徐々に低下傾向を示した。血清sIL-2R，TNF-αも低下傾向を示したが，2度目のステロイドパルス療法終了後は高値のまま平衡状態となった

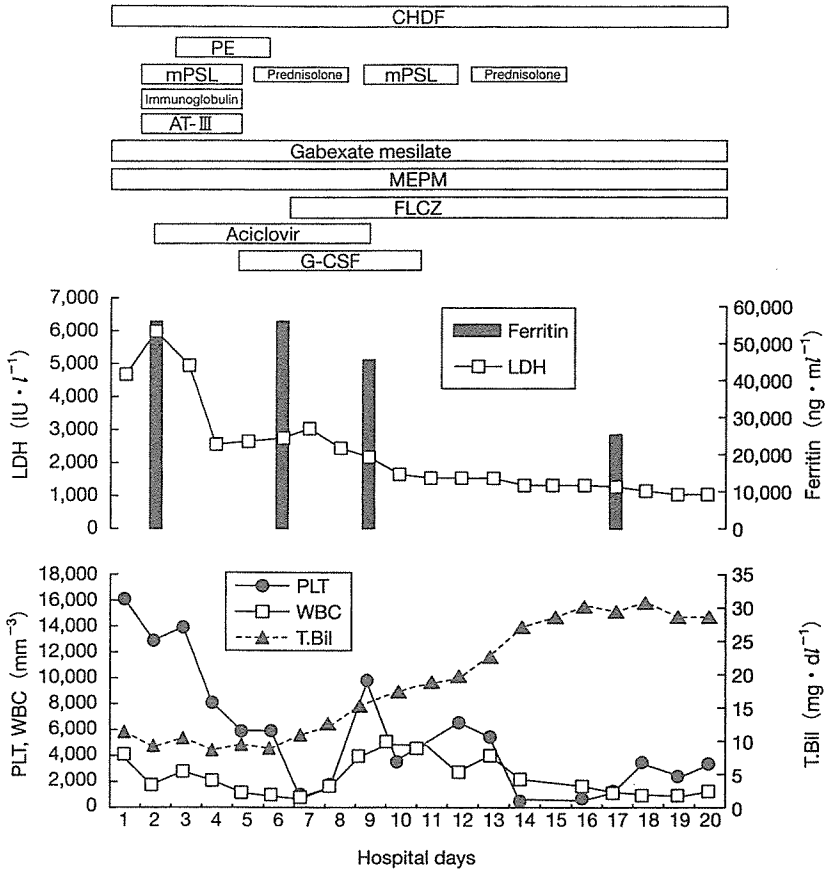

Fig. 1 Changes in blood biochemistry data during the clinical course
CHDF, continuous hemodiafiltration; PE, plasma exchange; mPSL, methylprednisolone; AT-Ⅲ, antithrombin-Ⅲ; MEPM, meropenem; FLCZ, fluconazole; G-CSF, granulocyte colony stimulating factor; PLT, platelet; T.Bil; total bilirubin.

(Fig. 3)。また，これらサイトカインのPE前後の推移について，Table 2に示した。血清IL-6はその前後で低下し，血清IL-8はやや低下傾向を示した。その他のサイトカインについては，明らかな変化を認めなかった。

考　察

HPSの治療は，原因や基礎疾患に対する治療とHPSに対する治療に分類できる。HPSに対する治療は3段階に分けられ，軽症型ではプレドニゾロン，VP-16，シクロスポリンなどによる単剤あるいは2剤併用療法，中等症型ではCHOP，HDCAなどの多剤併用療法を行い，これらの治療に抵抗性で再燃を繰り返す重症型では造血幹細胞移植を施行するとされている[1]。また，これら以外にもPE，CHDFなどがHPSの有効な治療法として報告されている[2)~4)]。本症例では，著しい肝機能障害のため免疫化学療法自体が肝機能を悪化させ，肝不全に至る可能性も十分に考えられた。このため，まずCHDF，PE，ステロイドパルス療法を行った上で，次の治療法を考慮することにした。そ

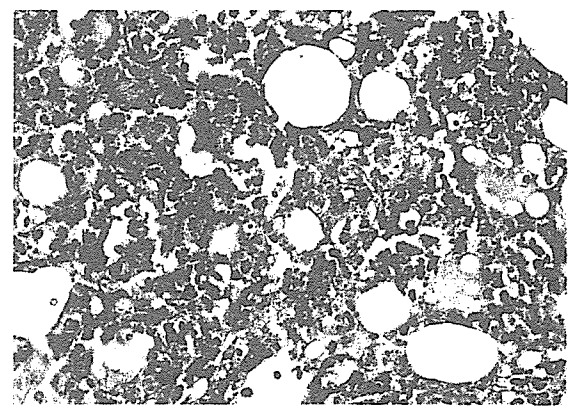

Fig. 2 Positive signal in bone marrow tissue after EBV-encoded RNA (EBER) *in situ* hybridization

の結果，これらの治療によって肝機能障害の十分な改善は得られず，免疫化学療法に踏み切ることができなかった。

通常EBVはB細胞に感染し，それを細胞障害性Tリンパ球やNK細胞が排除するが，本症例のようなEBV-AHSでは，T細胞あるいはNK細胞へのEBVの感染が本質的な問題であると想定されている。感染し

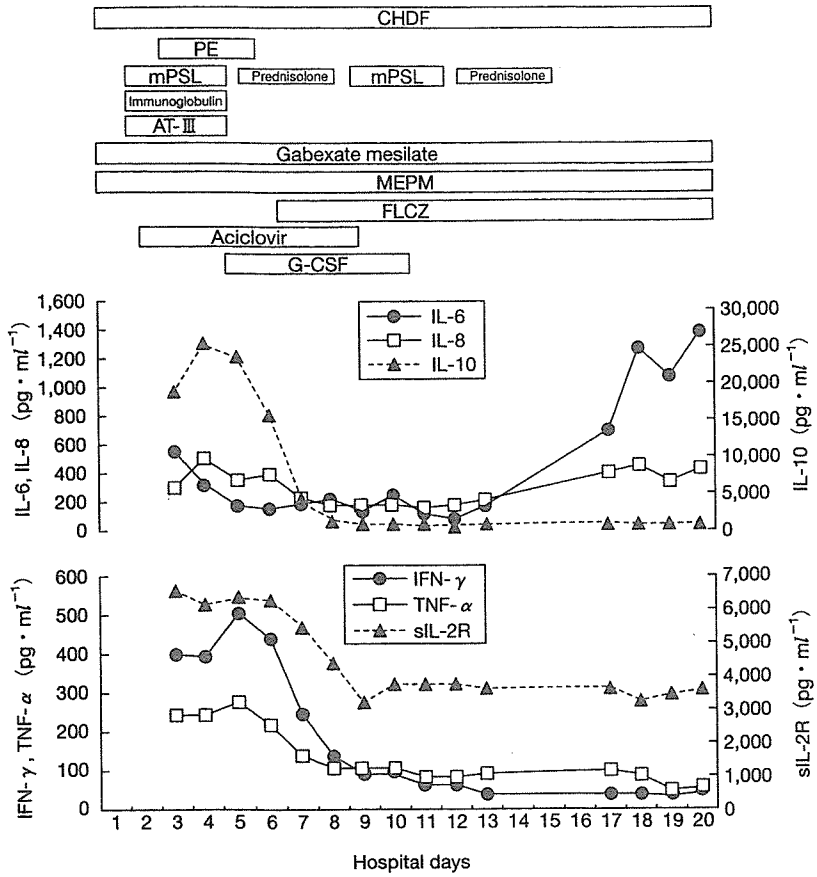

Fig. 3 Changes in levels of serum cytokines during the clinical course
CHDF, continuous hemodiafiltration; PE, plasma exchange; mPSL, methylprednisolone; AT-Ⅲ, antithrombin-Ⅲ; MEPM, meropenem; FLCZ, fluconazole; G-CSF, granulocyte colony stimulating factor; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin 10; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alfa; sIL-2R, soluble IL-2 receptor.

Table 2 Changes in levels of serum cytokines during plasma exchange

Plasma exchange		IL-6 (pg·ml^{-1})	IL-8 (pg·ml^{-1})	IL-10 (pg·ml^{-1})	IFN-γ (pg·ml^{-1})	TNF-α (pg·ml^{-1})	sIL-2R (pg·ml^{-1})
1st	Before	520	246	17,900	400	224	6,615
	After	284	468	24,600	390	231	6,182
2nd	Before	350	319	27,700	447	289	6,432
	After	170	260	21,800	385	220	5,973
3rd	Before	123	310	22,900	504	270	6,396
	After	104	286	25,600	448	274	6,625

IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin 10; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alfa; sIL-2R, soluble IL-2 receptor.

た細胞はIFN-γ，TNF-α，IL-6などの様々なサイトカインを分泌し，組織障害を引き起こすと共に，コントロール不能なマクロファージ活性化を誘導し，これがさらにサイトカインの上昇を促進するといわれている[5]。このサイトカイン値はHPSの重症度や予後を示す因子としてその有用性が報告され，特にIFN-γ，TNF，sIL-2Rの高値例は予後不良とされている[6)~9)]。本症例においても，治療開始時におけるこれらの血清サイトカイン値は著明に高値であり，予後不良であったものと考えられる。

これまでもHPS症例において，抗炎症性サイトカインの代表といわれるIL-10の高値例が報告されているが[10]，本症例のような異常高値例は報告されていない。この状態はIL-6/IL-10比が1以下であり，compensatory anti-inflammatory response syndrome（CARS）といわれる免疫不全状態であったと考えられる[11]。我々は

高度の肝機能障害を理由に免疫抑制薬や抗腫瘍薬を結果的に投与しなかったが，もし投与していたとしても免疫状態をさらに抑制し，病態を悪化させていた可能性が高いと考えられる。須佐らは[12]，このようなCARSの状態下でのPEは，サイトカインバランスや免疫状態のベクトルを変えるimmunomodulationになりうる有用な治療法であると述べている。本症例もPEによってIL-6，IL-8は低下し，IL-10に関してもPE施行直後には低下していないもののPE終了後からは著明に低下し，IL-6/IL-10比も1以上に改善したため，一定のimmunomodulationを行うことができたものと考えられる。このように炎症性サイトカインと抗炎症性サイトカインを測定することによって，免疫状態を把握することが可能になり，病態に合った治療を行うことができるものと考えられる，しかしながら，本症例においては結果として，サイトカイン値の改善による病態の改善には至らなかった。これは既に臓器障害が重度であったためか，あるいはsIL-2RやTNF-αにみられたように，血清サイトカイン値の改善の程度が病態を改善させるには不十分であったためと思われる。

本症例は，血清サイトカイン値からみると，PE，CHDF，ステロイドパルス療法などの治療によって一定の改善を認め，その有用性が確認された。特にPEによるサイトカインの除去効果は明らかであった。また，血清サイトカイン値は，重症度や予後を推定する指標として有用である上に，サイトカインバランスを把握することが適切な治療法選択に有用であると考えられた。しかしながら，本症例のような高度の臓器障害に進展した症例の治療は非常に困難であり，救命のためにはできる限り早期に適切な治療を開始し，有効なサイトカイン調節が必要と考えられる。

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A study of hematopoietic factors in the presence of disseminated intravascular coagulation associated with diffuse peritonitis

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Abstract : Hematopoietic factors in disseminated intravascular coagulation (DIC) associated with diffuse peritonitis were investigated. Thrombopoietin and interleukin 11 levels were significantly elevated in the group having DIC associated with diffuse peritonitis. In the group of deceased patients, thrombopoietin and stem cell factor were significantly elevated. The results suggest that these hematopoietic factors are possibly involved in the formation of pathological conditions underlying DIC associated with diffuse peritonitis.

Key words : DIC, thrombopoietin, IL-11, SCF

Introduction

Some reports have shown that cytokines play important roles in manifestations of pathological conditions of disseminated intravascular coagulation (DIC) and of visceral injuries associated with DIC[1, 2]. Monocytes, macrophages and hemangioendothelial cells, which are activated by cytokines, enhance generation of tissue factors and so on[3]. Diffuse peritonitis is often accompanied by septic DIC. Thrombin, fibrin, fibrin degradation products and plasmin, the activation of complements, which accompanies their generation, and neutrophil activation have all been considered to exert synergistic effects whereby visceral injuries progress in septic DIC[4].

The platelet count is decreased in the presence of DIC. Generation of platelets results from proliferation of colony-forming unit-megakaryocytes (CFU-Meg), proliferation and maturation of megakaryocytes, and separation of platelets from the megakaryocytic plasma membrane.

Cytokines which act to increase the platelet count include interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 11 (IL-11), stem cell factor (SCF), leukemia inhibitory factor (LIF), thrombopoietin (TPO) and so on[5~9].

We have also studied and reported on hematopoietic factors in the presence of DIC[11, 12]. In

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the present study, hematopoietic factors in patients with septic DIC, associated with diffuse peritonitis, were investigated.

Materials and Methods

Prior to initiating the present study, we obtained informed consent from patients, or members of their families, and approval from the Ethics Committee of Iwate Medical University.

There were 32 patients (21 males and 11 females) with a mean age of 56.5 ± 18.36 years (range; 18 to 85 years).

In diagnosing DIC, we adhered to the diagnostic criteria reported by *Aoki* et al.[13]. Similarly, for sepsis the criteria established by the ACCP/SCMCC Consensus Conference Committee were applied[13].

IL-3 levels were measured by ELISA (R & D System Inc., Minneapolis, MN, USA). IL-11 levels were also measured by ELISA(R & D System Inc., Minneapolis, MN, USA). The measurement limit for each factor was 4 pg/mL. Levels of SCF, LIF and TPO were also measured by ELISA (Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan). The measurement limits for these factors were 4, 4 and 50 pg/mL, respectively. The comparison of data for each factor used the maximum level at the time the diagnosis of DIC was made in the group with associated DIC and the maximum level during the course (of diffuse peritonitis) in the group without associated DIC. All data were expressed as the mean ± standard deviation (SD). The significance of differences was analyzed using the non-matched Wilcoxon's test, and the significance of correlations was analyzed by Pearson's test. Differences and correlations at $p < 0.05$ were considered significant.

Results

In all of the present patients, sepsis was associated with diffuse peritonitis.

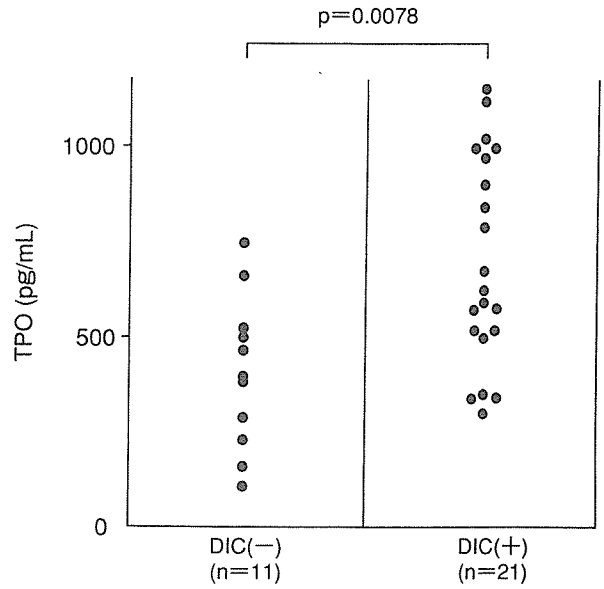

Fig. 1 TPO levels in patients with and without DIC

DIC was associated with these conditions in 21, but not in the other 11 patients. The mean age of the patients in the group with DIC was 62.4 ± 15.2 years, and in the other group was 53.2 ± 17.4 years. Thus, age was significantly ($p = 0.0492$) higher in the group with DIC.

Twenty-two of the 32 patients survived (survival group), and 10 patients died (deceased group). The mean ages were 58.2 ± 14.1 and 61.5 ± 16.6 years, respectively. Thus, there was no significant ($p = 0.3387$) difference in age between these groups.

The mean TPO level was 682 ± 272 pg/mL in the group with DIC, while the corresponding level was 403 ± 201 pg/mL in the group without DIC. Thus, the TPO levels were significantly higher in the group with DIC than in that without this association **(Fig. 1)**.

The mean IL-11 level was 23.7 ± 27.8 pg/mL in the group with DIC, while the corresponding level was 9.4 ± 10.9 pg/mL in that without this association. Thus, the IL-11 levels were significantly higher in the group with than in that without DIC **(Fig. 2)**.

The mean SCF level was 4,360 ± 2,362 pg/mL in the group with DIC, while the corresponding

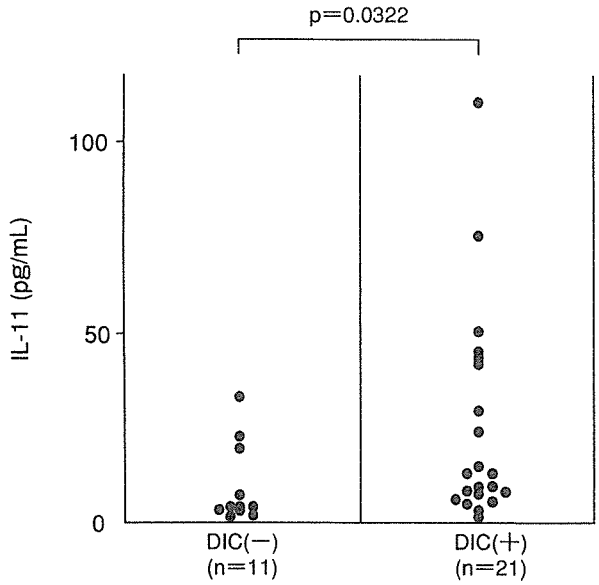

Fig. 2 IL-11 levels on patients with and without DIC

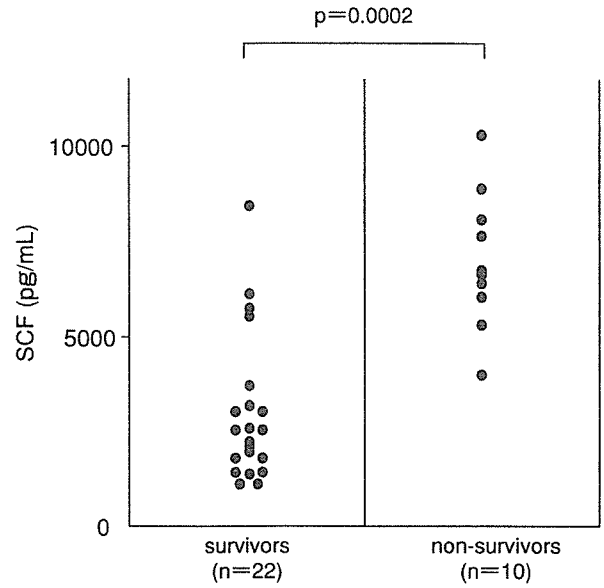

Fig. 4 SCF levels in survivors and non-survivors

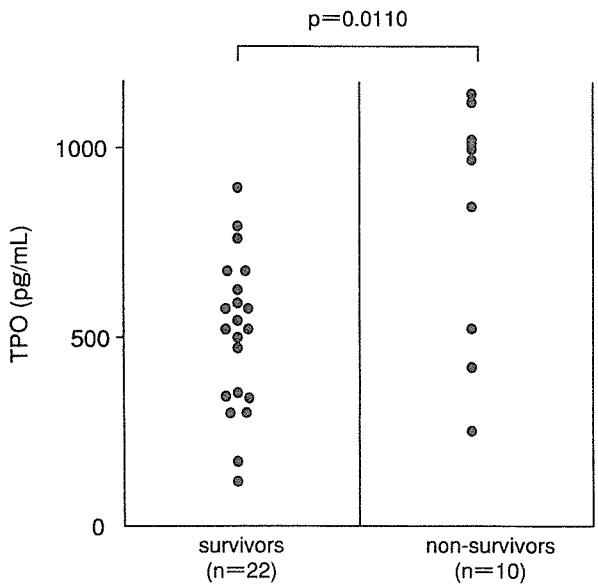

Fig. 3 TPO levels in survivors and non-survivors

level was 3,751 ± 2,993 pg/mL in the group without DIC. Thus, there was no significant (p = 0.3827) difference between the groups.

Both IL-3 and LIF levels exceeded measurement sensitivity in 2 of the 21 patients in the group with DIC.

The mean TPO level was 485 ± 198 pg/mL in the survival group and 808 ± 316 pg/mL in the deceased group. Thus, the levels were significantly higher in the deceased group **(Fig. 3)**.

The mean IL-11 level was 13.0 ± 14.3 pg/mL in the survival group and 31.4 ± 35.7 pg/mL in the deceased group. Thus, there was no significant (p = 0.1378) difference between the groups.

The mean SCF level was 2,974 ± 1,919 pg/mL in the survival group and 6,741 ± 1,795 pg/mL in the deceased group. Thus, the levels were significantly higher in the deceased group **(Fig. 4)**.

Discussion

While the actions of thrombomodulin in endothelial cells are decreased, thrombin receptors are increased in the presence of hypercytokinemia[14]. Cytokine-induced augmentation of the hemostatic system on hemangioendothelial cells, monocytes and macrophages causes thrombosis and DIC.

IL-11 alone has no influence on megakaryocyte colony formation, whereas IL-11 with IL-3 increases the number of megakaryocyte colonies[15]. IL-11 is involved in reactions in the acute stage as well and induces acute-stage proteins[16].

SCF exerts no hematopoietic effect when acting alone. However, when acting in conjunc-

tion with each of the other cytokines, such as IL-1, CSF and IL-6, it exerts actions promoting differentiation and proliferation of hematopoietic stem cells via synergic stimulation[17].

The present study revealed that cytokines including TPO, IL-11 and SCF, which act to raise the platelet count, are increased in pathological conditions such as DIC, in which the platelet count is decreased. In pathological states, TPO and IL-11 levels in particular were markedly elevated. There was no difference in SCF level between the groups with and without DIC. Levels of tumor necrosis factor α (TNF-α), IL-6 and IL-8, which are inflammatory cytokines, were significantly higher in the presence of septic DIC, as compared to those in the presence of DIC without infection. This raises the possibility of these inflammatory cytokines playing stimulatory roles in the generation of hematopoietic factors.

With regard to the involvement of these factors in the outcomes of patients, the outcomes were poor for those who showed high levels of TPO and SCF.

Whether the increased levels of these cytokines, which act to raise platelet counts in the presence of DIC, is a bio-reaction relevant to the increased platelet count or merely a reflection of an inflammatory reaction, remains unclear. These cytokines, in the presence of DIC associated with various underlying diseases, merit further studies.

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Nuclear matrix protein and tumor necrosis factor a levels in patients with septic acute lung injury/acute respiratory distress syndrome

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Abstract : Blood levels of nuclear matrix protein (NMP), as an indicator of apoptosis, were measured in patients with septic acute lung injury (ALI)/acute respiratory distress syndrome (ARDS). The subjects were 46 sepsis patients, 28 of whom had ALI/ARDS associated with sepsis. The blood NMP level was 822±881 U/mL in the patients with ALI/ARDS, which was significantly higher than the 198±171 U/mL in those without ALI/ARDS. The blood level of tumor necrosis factor a (TNF-α) was 180±294 pg/mL in the patients with ALI/ARDS, which was significantly higher than the 40±27 pg/mL in those without ALI/ARDS. There was also a significant ($p = 0.0001$) correlation between NMP and TNF-α levels ($r = 0.5349$). These results suggest apoptosis to be associated with septic ALI/ARDS and that TNF-α is involved in triggering apoptosis.

Key words : ALI, ARDS, NMP, TNF-α

Introduction

We have reported that several mediators are produced via cytokines in the presence of multiple organ dysfunction syndrome (MODS) and that these substances directly or indirectly induce hemangioendothelial disturbances[1~7].

Shock, particularly septic shock, is well known to be closely associated with the etiology of MODS[8]. The microcirculatory disturbance following septic shock has been believed to induce cell and tissue damage, ultimately leading to visceral injuries. It is also well known that cytokines and nitric oxide (NO) are intimately involved with manifestation of septic shock[2, 4, 9~15].

Respiratory disturbances are mostly associated with MODS. The entity of acute lung injury (ALI) involves damage to pulmonary micro-hemangioendothelial cells.

The importance of apoptosis in the process of lung injury has also been drawing attention. TNF-α reportedly induces apoptosis[16]. It has also been reported that NO is involved in production of the Fas antigen, which in turn induces apoptosis[17].

We have shown that blood levels of nitrite/

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