透析療法のリスクマネジメント ©

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しかし，その活動により，感染事故の撲滅が得られれば，①透析施設の診療内容に対する評価の向上—患者の満足，②医療者の専門職としての充実感，③経済効果など，その努力に見合う効果の得られる領域である．現場の医療従事者から院長など経営責任を果たすべき医師まで，その重要性を認識して，診療の一部として組み込んでいただきたい．

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（秋葉　隆）

2．検査と診断

透析施設において，感染症を早期に正しく診断することは，早期治療や隔離，感染経路遮断など院内感染対策上，非常に重要である．そのためには，日常からの定期検査や症状発現時の検査を効率よく施行して，診断の助けとする必要がある．検査・診断の要点を表7-1にまとめた．また，検査結果や診断結果はプライバシー保護に努めながら，スタッフに周知徹底し，患者本人にも告知することが必要である．

A ウイルス肝炎

臨床像は非透析患者と同様であるが，肝酵素〔AST（GOT），ALT（GPT）〕の基礎値は透析患者では低値であり，肝炎の際の上昇も軽微である．日本透析医学会の統計では，GPTの正常平均値は14 IU/*l*であり，NakayamaらもHCV抗体陰性の透析患者のGOTは平均12.6 IU/*l*，GPTは平均15.3 IU/*l*であるのに対し，HCV抗体陽性患者では各々22.9 IU/*l*，22.7 IU/*l*と正常範囲内にとどまっていると述べている．ウイルス肝炎の診断はおもに後述する肝炎ウイルスマーカーによってなされる．

1. B型肝炎

HBs抗原はHBV感染状態であることを示し，急性B型肝炎では肝機能異常に2～4週間先立っ

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表 7-1　透析患者感染症の検査・診断の要点

B 型肝炎
- 肝酵素（GOT, GPT）の上昇：軽微ないし正常なこともある．
- HBs 抗原陽性：現在の感染状態
- HBc 抗体高力価：キャリア
- IgM-HBc 抗体：急性感染
- HBe 抗原：ウイルスの増殖状態で感染性を示す．
- HBe 抗体：ウイルスの非増殖状態で非感染性を示す（Hbe 抗原非産生変異株の場合は感染性）．
- HBV DNA：ウイルスの直接証明，治療効果の推測
- HBV 関連ポリメラーゼ：ウイルス量と相関

C 型肝炎
- 肝酵素（GOT, GPT）の上昇：軽微ないし正常なこともある．
- HCV 抗体：高値なら現在の感染，低値なら過去の感染
- HCV RNA：ウイルスの直接証明，治療効果を予測
- HCV サブタイプ：治療効果を予測，感染経路の検索

結核症
- 感染の既往の有無
- 胸部 X 線写真，リンパ節腫脹，CT
- ツベルクリン反応：透析患者では反応低下
- 塗抹・培養・PCR 検査：陰性でも否定できない．
- 胸水，腹水：滲出液，ADA 高値

ブラッドアクセス関連菌血症
- 透析用カテーテルやグラフトの存在，局所の所見
- 血液培養：カテーテルの培養結果と一致するか
- 心エコー（経胸および経食道エコー）：感染性心内膜炎のチェック

MRSA 感染症
- 血液，髄液，胸水：コンタミネーションが否定されれば診断価値高い．
- 喀痰，皮膚，尿，便，鼻腔，咽頭，開放性分泌物：感染か定着か鑑別を要する．

て認められる．HBc 抗体は HBV 感染後早期よりみられ，急速に上昇して生涯持続する．HBc 抗体の抗体価が高値であれば HBV の持続感染が，低値であれば一過性の過去の感染を示す．急性または最近の感染は感染後 6 カ月間に限って認められる IgM-HBc 抗体により診断する．免疫能の正常な成人では，多くの場合，2〜3 カ月で HBs 抗原が排除され，回復期に HBs 抗体が上昇するが，免疫能の低下した透析患者では慢性感染に移行することがまれでない．6 カ月以上の間隔をおいて HBs 抗原が検出されれば，持続感染と考えられる．HBe 抗原は，HBV のコア抗原蛋白 C 末端の一部が切断され，血中に放出されたもので，血中 HBV 量と相関し，感染性の強さを表わす．HBe 抗体はウイルス量の減少に関連し，これが陽性の患者は感染性が弱く，大部分が無症候性キャリアである．しかし，HBV の pre-C 領域に変異を来した HBe 抗原を産生しないウイルス株もあり，劇症肝炎の原因となるので，HBe 抗原・抗体の解釈には注意を要する．HBV DNA および HBV 関連 DNA ポリメラーゼはこの変異ウイルスでもウイルス量を評価でき，肝炎の活動

性や抗ウイルス薬の効果判定に有用である.

2. C型肝炎

HCV抗体はコア領域, NS3, NS4, NS5（第3世代のみ）に対応する第2・3世代の検査になって, 特異性, 感度ともに良好になった. HCVの感染が成立してから, HCV抗体が陽性を示すまでの期間（window period）は第2世代検査で約10週間, 第3世代検査で約2〜3週間まで短縮された. HCV抗体検査は経費を比較的要しない点や, 検査方法の簡便さ, 自動化のしやすさなどスクリーニング検査には適している. 偽陰性が少ない点やwindow periodが短い点などから, 第3世代のHCV抗体検査の方が第2世代のものよりも優れている.

HCV RNA検査はウイルス自体を検出し, HCV感染を診断するにはHCV抗体よりも優れている. ウイルス量が多いほど治療抵抗性が高く, 病態とも相関するので, HCV RNA量の測定は治療効果の予測および治療後の経過観察に有用である. また, 急性C型肝炎診断の際には, HCV抗体が陽性を示す例は約50%であり, HCV RNAの方がより早く陽性化するので, 迅速な診断にはHCV RNAが有用である. HCVのジェノタイプの検査はHCV感染の診断自体には不要だが, 感染源の同定や感染経路の推定に役立ち, インターフェロンによる治療効果の予測にも有用である. HCV抗体陽性でHCV RNAが陰性を示す場合は, ①C型肝炎の既感染で現在はウイルスがない, ②血液以外の肝臓, 単核球にウイルスが存在する, ③ウイルスが間欠的に血中に認められる, ④ウイルス量が少なく, HCV RNA検査の測定感度以下である, ⑤輸血でHCV抗体が一時的に移入された, などが考えられる. 逆に, HCV抗体陰性でHCV RNAが陽性を示す場合は, ①HCV抗体価が低くて検出できない, ②HCV抗体検査がある種のジェノタイプには反応しない抗原を用いている, ③免疫能の低下によりHCV抗体反応がみられない, などが考えられる.

3. 院内感染対策としての肝炎ウイルス検査

B型肝炎ウイルス（HBs抗原), C型肝炎ウイルス（HCV抗体）については, 年2回の定期検査を行う. HBs抗原陽性者に対しては, HBe抗原, HBe抗体, HBc抗体についても施行し, HCV抗体陽性患者に対してはHCV RNA検査も施行する.

もともと肝炎ウイルスが陰性であった患者において, 肝機能検査（月1, 2回）で通常の値よりも高値を示す際には, 定期外に肝炎ウイルスマーカーを検査する必要がある. 早期診断をするには, B型肝炎ではIgM-HBc抗体, C型肝炎ではHCV RNAが適当である.

B 結核症

免疫能の低下した透析患者では特に重要な感染症であり, 早期発見が求められる. 透析患者では, 肺外結核が結核の約半数を占めるほど多く, 粟粒結核も比較的多いのが特徴である. 肺外結核の部位としては, リンパ節がもっとも多く, 腎・尿路, 腹膜, 肝臓, 骨関節が続く. 発病時期は, 透析導入6カ月前後がもっとも多い. 発見の動機となった症状としては, 一般抗菌薬無効の

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発熱，リンパ節腫脹，倦怠感，膿尿などが多い．肺結核に関しては，原因不明の発熱や咳が2，3週間以上持続する際には，胸部X線検査，喀痰検査などをする必要がある．透析患者では，病巣の大きさの割に，発熱や炎症反応が強く，急性炎症の様相を呈することも少なくない．確定診断には結核菌を証明することが必要であるが，結核菌が検出されないことも少なくない．喀痰塗抹検査は3日連続で行う必要がある．培養検査が最も確実な検査法であるが，中間培養で4週間，最終培養には8週間要するのが難点である．PCR法は早期診断や非定型抗酸菌の診断に有用だが，コンタミネーションや死菌による陽性が問題である．ツベルクリン反応は免疫能の低下した透析患者では陽性になることが少ないので，陽性を示したら結核感染を疑う．臨床的に結核が疑われるが，結核菌が検出されない場合，INHを200 mg/日投与し，熱型や炎症反応などに改善が認められるかをみる診断的治療が必要になる．胸水，腹水ではadenosine deaminase（ADA）の高値も診断的価値が高い．

C ブラッドアクセス関連菌血症

ブラッドアクセスの感染（275頁参照）は透析患者菌血症の原因の48.73%を占める最も多い原因で，起炎菌は黄色ブドウ球菌，表皮ブドウ球菌がほとんどである．ブラッドアクセス用カテーテルを留置し，突然の悪寒・発熱を認め，他に明らかな感染源がなければ，カテーテル関連菌血症の診断は容易である．迅速な血液培養が必須の検査である．人工血管は内シャントよりもはるかに感染のリスクが高く，感染徴候（発赤，腫脹，疼痛，排膿など）と発熱が認められれば，グラフト感染と診断できるが，なかには，局所の感染徴候が乏しく，発熱も微熱程度のこともあるので注意が必要である．このような感染徴候の明らかでないブラッドアクセス感染を診断するには，インジウム-111標識白血球によるシンチが有用である．

菌血症の合併症としては，細菌性心内膜炎が重要であり，心エコー検査（経胸的のみならず，必要に応じて経食道エコーも有用である）を施行する．

D MRSA感染症

MRSAによる感染症では，敗血症，肺炎，腸炎などが臨床的に重要である．MRSA感染症の検査・診断の際には，感染か定着かが，その後の治療方法などの点で異なっており，鑑別することが重要である．本来無菌の部位（血液，髄液，胸水など）から検出された場合，コンタミネーションさえ否定できれば感染である可能性が高い．一方，本来非無菌の部位（喀痰，皮膚，尿，便，鼻腔，咽頭，開放性分泌物）から検出された場合，菌数が多い（喀痰で10^7/ml以上，尿で10^5/ml以上），グラム染色で白血球貪食像がみられる，膿性所見である，発熱や局所の感染症状がある，白血球増加，CRP上昇などを伴えば感染と判断するが，そうでなければ定着と判断する．

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(安藤亮一)

3. 消毒と滅菌

透析患者は，細胞性免疫の低下した状態にあり，血液透析を行うことで日常的に血液が体外に導き出されること，また透析室は集団治療の場所であることなどから，感染症を発症，伝播しやすい環境下におかれている．

患者が安全に透析治療を受けられる環境を維持していくには，基本に忠実なケアを実践することと，透析室内を常に清潔な環境に整えるよう，手洗いの励行や使用する物品・器具を適切に取り扱うことが重要である．

A 滅菌と消毒

1. 滅菌と消毒

滅菌とは病原菌および非病原菌を問わず，すべての微生物を殺菌除去することをいい，その方法として，高圧蒸気滅菌，エチレンオキサイドガス（EOG）滅菌，乾熱滅菌，濾過滅菌法などがある．消毒とは，人体に有害な細菌を感染性がなくなるまで希釈するか，増殖できない状態にすることをいい，煮沸消毒，紫外線殺菌，薬液消毒などがある．

滅菌と消毒の方法は，目的や材質に応じて最も適切なものを選択する（表 7-2）．さらに，滅菌や消毒方法が的確に行われているかを日々監視していくことが重要である．

2. 消毒薬の使用方法

多種類の消毒薬が市販されているが，すべての微生物に有効な消毒薬はなく，消毒薬それぞれの効果特性を知り，その用途にあったものを選択し，適切に使用する．

a）消毒薬の濃度と作用時間

消毒薬の濃度は消毒効果に大きく影響するため，用途に応じた適切な濃度に調節して使用することが重要である．調整時は，消毒薬や希釈水を目分量で測定するのではなく，シリンダーなどを用いて正しく計測する．また，消毒薬の中には経時変化を起こすものもあるので，使用時に調整することが望ましい．使用中の消毒薬に新しい消毒薬を継ぎたすことは避けなければならない．

消毒時間は，消毒薬の種類・濃度，対象物品によって異なり，それらに応じた適切な時間の消

【雑　誌】

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Prevalence of Hepatitis E Virus Infection in Regular Hemodialysis Patients

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Abstract: The percentage of patients infected with blood-borne diseases, including hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, is high in patients undergoing hemodialysis regularly. Hepatitis E virus (HEV) is transmitted via the fecal-oral route, and blood-borne HEV infection has also been reported recently. On the basis of these findings, we investigated the actual status of HEV infection in regular hemodialysis patients. Out of 1077 patients undergoing hemodialysis at two key hospitals and three outpatient hemodialysis clinics, 300 were randomly selected as the subjects. Among these 300 hemodialysis patients, 19.0% were IgG-type anti-HEV antibody-positive. The percentage of HEV-infected patients increased with patient age and it was particularly high in patients 40 years of age or older. The percent IgG-type anti-HEV antibody positivity was not significantly different ($P = 0.14$) between anti-HCV antibody-positive patients (27.8%) and anti-HCV antibody-negative patients (17.8%). The percentage of HEV-infected patients among the hemodialysis patients was higher than that previously reported among patients with healthy kidneys. No correlation was observed between the percentage of HEV-infected patients and HCV infection incidence or a history of blood transfusion. The percent IgG-type anti-HEV antibody positivities were significantly different among the facilities. It was impossible to specify the route of infection, and the correlation between the incidence of infection and hemodialysis therapy was not clear. Because more routes of infection are possible for patients undergoing dialysis than for persons with normal kidney function, it seems necessary to analyze the significance of infection incidence, the route of infection and infection prevention measures.
Key Words: Antibody, Hemodialysis patient, Hepatitis E virus.

Hepatitis E virus (HEV) is an unclassified nonenveloped RNA virus. Mass outbreaks of HEV infection caused by contaminated drinking water have frequently been reported in many developing countries of Asia and Africa, where sanitation is suboptimal. Thus, HEV infection has been referred to as water-borne hepatitis (1–3). In Japan, where excellent water supply and sewage systems are available, all cases of HEV infection had previously been considered to be imported. However, the HEV infection of patients who have not traveled abroad has been reported recently (4,5). HEV infection via blood product transfusion has been reported (6) and evidence that HEV, mainly transmitted via the fecal-oral route (7), can also be transmitted via the blood-borne route has also been reported. Mitsui recently reported that hemodialysis patients in Japan were infected with a genotype 3 HEV by blood transfusion (8). The present study was designed to determine the actual status and significance of HEV infection in hemodialysis patients by calculating the percentage of HEV-infected patients for whom the percentage of blood-borne infections, including hepatitis B virus (HBV) and hepatitis C virus (HCV), was high and to determine the correlation between the findings and patient demographics.

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MATERIALS AND METHODS

Patients

Out of 1077 regular hemodialysis patients treated at the hemodialysis departments of two key hospitals and at three outpatient hemodialysis clinics, 300 were randomly selected to participate in the present study. The titers of the antibodies to HCV (Lumipulse), IgG-type HEV and IgM-type HEV were determined in these patients. The 1077 patients at the facilities

consisted of 690 male patients and 387 female patients with a mean age of 59.8 ± 12.8 years and a hemodialysis duration of 9.1 ± 6.0 years. The 300 selected subjects consisted of 188 male patients and 112 female patients, with a mean age of 60.1 ± 13.1 years and a hemodialysis duration of 9.7 ± 7.3 years. The demographics of the patients attending these facilities and the 300 selected subjects were not significantly different from those of patients in the nationwide survey conducted by the Japanese Society for Dialysis Therapy (9). The following patient demographics were determined and analyzed: patient age, sex, hemodialysis duration, history of blood transfusion, status of HCV infection, platelet (Plt) count, serum albumin (Alb) level and serum alanine aminotransferase (ALT) level. The obtained values of various parameters are shown as the mean ± standard deviation. The χ^2 test was used in the statistical analysis of data.

Measurements

An enzyme-linked immunosorbent assay (ELISA) for detecting anti-HEV antibodies was carried out using a recombinant ORF2 antigen generated on the basis of the gene sequence of genotype IV HEV for an IgG-type anti-HEV antibody (VIRAGENT anti-HEV antibody (human IgG), Cosmic Corporation, Tokyo, Japan) and an IgM-type anti-HEV antibody (VIRAGENT anti-HEV antibody (human IgM), Cosmic Corporation, Tokyo, Japan). A cut-off index of positivity for the IgG-type anti-HEV antibody was set at 13 according to the manufacturer's instructions, and patients with indices of 13 or higher were considered positive for the IgG-type anti-HEV antibody. A cut-off index of positivity for the IgM-type anti-HEV antibody was set at 30 according to the manufacturer's instructions, and patients with indices of 30 or higher were considered positive for the IgM-type anti-HEV antibody.

Detection of hepatitis E virus RNA

Total RNAs were extracted from the serum sample with the TRIZOL LS reagent (Invitrogen, Tokyo, Japan). The RNA was reverse transcribed with SuperScript II RNase H reverse transcriptase (Gibco-BRL, Tokyo, Japan) and an antisense primer (primer HE040; 5′-CCC TTR TCC TGC TGA GCR TTC TC-3′ (R = A or G)) specific for the HEV ORF2 sequence and was then subjected to nested PCR in the presence of TaKaRa Ex Taq (TaKaRa Shuzo, Shiga, Japan). A part of the ORF2 sequence was amplified with the primer pair HE044 (sense primer; 5′-CAA GGH TGG CGY TCK GTT GAG AC-3′ [H = A, T, or C; Y = T or C; and K = G or T)) and HE040 in the first round and HE110-2 (sense primer; mixture of three sequences, 5′-GYT CKG TTG AGA CCT CYG GGG T-3′, 5′-GYT CKG TTG AGA CCA CGG GYG T-3′, and 5′-GYT CKG TTG AGA CCT CTG GTG T-3′ (common nucleotides are underlined)) and HE041 (antisense primer; 5′-TTM ACW GTC RGC TCG CCA TTG GC-3′(M _ = A or C, W = A or T)) in the second round (ORF2 PCR). The PCR amplification was carried out for 35 cycles in the first round (94°C for 30 s (an additional 2 min was used in the first cycle), 55°C for 30 s, 72°C for 75 s (an additional 7 min was used in the last cycle)) and for 25 cycles in the second round under the same conditions used for the first round except that extension was carried out for 60 s. The size of the amplification product of the first-round PCR was 506 bp, and that of the amplification product of the second-round PCR was 458 bp. The amplification products were electrophoresed on a 1.5% (wt/vol) agarose gel, stained with ethidium bromide, and photographed under UV light. The RT-PCR assay was carried out in duplicate, and reproducibility was confirmed.

RESULTS

Patient demographics and laboratory test results

IgG-type anti-HEV antibody-positive patients consisted of 41 male patients and 16 female patients with a mean age of 62.4 ± 11.6 years and a mean hemodialysis duration of 9.1 ± 7.7 years (Table 1). IgG-type anti-HEV antibody-negative patients consisted of 147 male patients and 96 female patients

TABLE 1. *Patient profiles*

	Total	Anti-HEV pos.	Anti-HEV neg.	*P*-value
Age (year)	60.1 ± 13.1	62.4 ± 11.6	59.6 ± 13.4	0.17
Duration of dialysis (year)	9.7 ± 7.3	9.1 ± 7.7	9.8 ± 7.2	0.60
Gender (M/F)	188/112	41/16	147/96	0.10
Platelet (10^4/μL)	20.1 ± 7.3	21.5 ± 11.0	19.7 ± 6.2	0.10
Serum Albumin (g/dL)	3.94 ± 0.39	3.94 ± 0.35	3.94 ± 0.15	0.99
ALT (IU/L)	12.5 ± 8.0	11.9 ± 6.6	12.7 ± 8.3	0.54
HCV (%)	13.3	19.3	11.9	0.14
Blood Transfusion (%)	55.3	45.0	57.3	0.30

with a mean age of 59.6 ± 13.4 years and a mean hemodialysis duration of 9.8 ± 7.2 years. Laboratory tests showed a Plt count of 21.5 ± 11.0 × 10^4/mm^3, a serum Alb level of 3.94 ± 0.35 g/dL and a serum ALT level of 11.9 ± 6.6 IU/L in the IgG-type anti-HEV antibody-positive patients. The tests also showed a Plt count of 19.7 ± 6.2 × 10^4/mm^3, a serum Alb level of 3.94 ± 0.15 g/dL, and a serum ALT level of 12.7 ± 8.3 IU/L in the IgG-type HEV antibody-negative patients. No significant differences were observed in the laboratory test results between the two patient groups. There were no significant differences of mean age and gender among facilities.

Percentage of patients positive for anti-hepatitis C virus antibody, IgG-type anti-hepatitis E virus antibody and IgM-type anti-hepatitis E virus antibody

Forty patients (13.3%) were positive for the anti-HCV antibody, 57 patients (19.0%) for the IgG-type anti-HEV antibody, and one patient (0.3%) for the IgM-type anti-HEV antibody. IgG-type anti-HEV antibody was not positive in the IgM-type anti-HEV antibody-positive patients, and RT-PCR assay for HEV RNA was not detected by RT-PCR assay in this patient. The percent IgG-type anti-HEV antibody positivity was higher in the male patients (21.6%) than in the female patients (14.3%).

Correlations of patient age and hemodialysis duration with the percent anti-hepatitis E virus antibody positivity and percent IgG-type anti-hepatitis E virus antibody positivity

The percent anti-HCV antibody positivity increased with the duration of dialysis; patients with a history of 20 years of hemodialysis showed a markedly high HCV infection prevalence of 42.9% (Figs 1,2). However, no similar correlation was observed between the percent IgG-type anti-HEV antibody positivity and the length of hemodialysis duration. The percent IgG-type HEV antibody positivity increased with age whereas no similar correlation was observed between percent anti-HCV antibody positivity and patient age.

Correlation of hepatitis C virus infection with history of blood transfusion and IgG-type anti-hepatitis C virus antibody positivity

The percent IgG-type anti-HEV antibody positivity in the anti-HCV antibody-positive patients (27.8%) was higher than that in anti-HCV antibody-negative patients (17.8%) but the difference did not reach statistical significance ($P = 0.14$). Patients with a history of blood transfusion (+) had an IgG-type

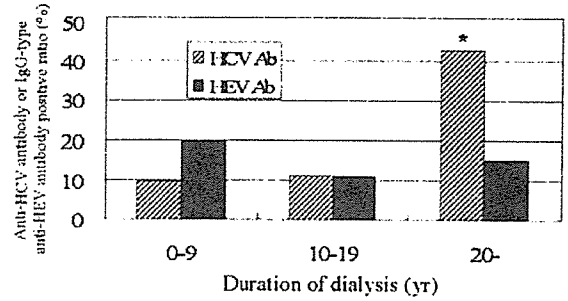

FIG. 1. The relation between duration of hemodialysis and anti-hepatitis C virus antibody or IgG-type anti-hepatitis E virus antibody positive ratio. The percent anti-hepatitis C virus antibody positivity increased with the length of hemodialysis duration. However no similar correlation was observed between the percent IgG-type anti-hepatitis E virus antibody positivity and length of hemodialysis duration. *$P < 0.001$ compared with duration of 0–9 years.

anti-HEV antibody positivity of 45.0% (11/24) and patients without a history of blood transfusion (–) had that of 57.3% (67/117), which were not significantly different ($P = 0.30$).

Percent IgG-type anti-hepatitis E virus antibody positivities for the hemodialysis facilities

The percent IgG-type anti-HEV antibody positivities were 20.2% for hemodialysis clinic A located in one of the 23 wards of Tokyo, 24.2% for hemodialysis clinic B located in one of the 23 wards of Tokyo, 13.6% for hemodialysis clinic C located in one of the 23 wards of Tokyo, 6.45% for a hemodialysis hospital in Chiba and 23.5% for a hemodialysis hospital in Gunma Prefecture, north of Tokyo (Fig. 3). These

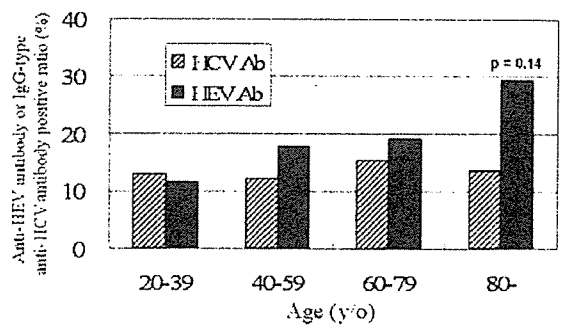

FIG. 2. The relation between age of hemodialysis patients and anti-hepatitis C virus antibody or IgG-type anti-hepatitis E virus antibody positive ratio. The percent IgG-type hepatitis E virus antibody positivity increased with patient age, whereas no similar correlation was observed between percent anti-hepatitis C virus antibody positivity and patient age. *P* compared with age value of 20–39 years old.

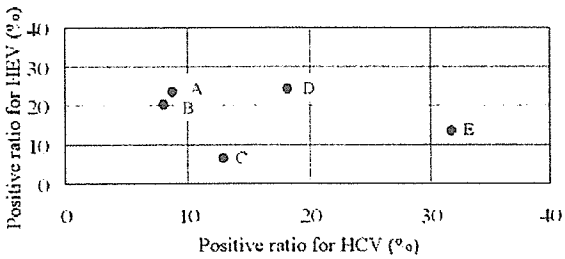

FIG. 3. The anti-hepatitis C virus antibody and IgG-type anti-hepatitis E virus antibody positive ratio for the hemodialysis facilities. These percent IgG-type anti-HEV antibody positivities were significantly different among the hemodialysis facilities. (A) hemodialysis hospital in Gunma, north of Tokyo. (B) hemodialysis clinic A located in one of the 23 wards of Tokyo, center of Tokyo. (C) hemodialysis hospital in Chiba, east of Tokyo. (D) hemodialysis clinic B located in one of the 23 wards of Tokyo, center of Tokyo. (E) hemodialysis clinic C located in one of the 23 wards of Tokyo, north part of Tokyo.

percent IgG-type anti-HEV antibody positivities were significantly different among the hemodialysis facilities.

Distribution of the IgG-type anti-HEV antibody titers

The histogram of the Figure 4 shows distribution of IgG-type HEV antibody titers. The cut-off index of 13 for the positive was used according to manufacturer's instructions. There are several patients close to the cut-off index, suggesting the existence of pseudo-positive or pseudo-negative patients.

DISCUSSION

HEV infection in Japan was previously considered to be imported. However, the high percent IgG-type anti-HEV antibody positivity (19.0%) among hemodialysis patients in the present study and the observation that many of the HEV-infected patients had never been abroad suggest the existence of other routes of infection. The percent IgG-type anti-HEV antibody positivity in our subjects was higher than the percent IgG-type anti-HEV antibody positivity reported in hepatic disease patients, hemodialysis patients, medical staff members, and subjects with healthy kidneys in Tokyo (9.8%; 101/1033), and in the general population of Japan (5.4%,49/900) (10,11). When IgG-type anti-HEV antibody-positive patients were classified by sex, the percent positivities were 21.6% for the male patients and 14.3% for the female patients, which are similar to these for subjects with healthy kidneys. The distribution of IgG-type anti-HEV antibody titers suggests the existence of pseudo-antibody-positive or pseudo-antibody- negative patients. However, the percent IgG-type anti-HEV antibody positivity is still considered to be high, even when these findings and their implications are taken into account. In contrast, there was only one IgM-type anti-HEV antibody-positive patient. This patient was suspected to be pseudo-antibody -positive on the basis of an IgM antibody titer of 44, an IgG antibody titer of less than 5, and a negative PCR result.

With regards to the laboratory test results, no significant differences were observed in serum ALT level, Alb level or Plt count between the IgG-type anti-HEV antibody-positive and -negative patients. Considering that the IgG-type anti-HEV antibody-positive patients had already been infected, the infection might have an influence on hepatic impairment.

The percent IgG-type anti-HEV antibody positivity varied significantly among hemodialysis facilities from a minimum of 6.5% to a maximum of 24.2%. The percent IgG-type anti-HEV antibody positivities in the general population with healthy kidneys were reported to be 1.9% (6/316) in southern Japan, 3.3% (12/364) in central Japan and 14.1% (31/220) in northern Japan. The higher percent IgG-type anti-HEV antibody positivity in northern Japan was reported (11). However, local differences in the Kanto area were also observed. The cause of these differences was not clear (Fig. 5). In comparison with hemodialysis patients in other countries (10,12–16), hemodialysis patients in Japan showed a higher IgG-type anti-HEV antibody positivity, but it was unclear whether some patient demographics or details of hemodialysis treatment account for this higher value. It was reported that percent IgG-type anti-HEV antibody positivity increases with age in Japanese subjects with healthy kidneys aged 30 years or older

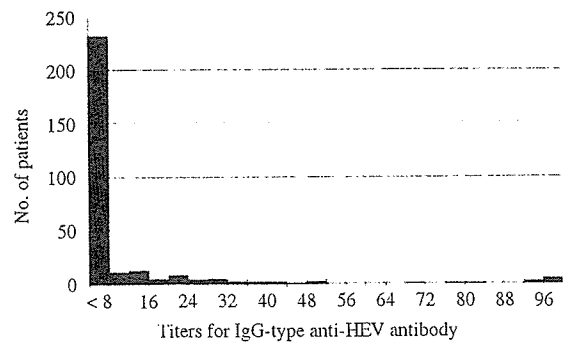

FIG. 4. Distribution of IgG-type anti-hepatitis E virus Ab titers. Some patients had titers close to the cut-off index of 13, suggesting the existence of pseudo-antibody-positive or pseudo-antibody-negative patients.

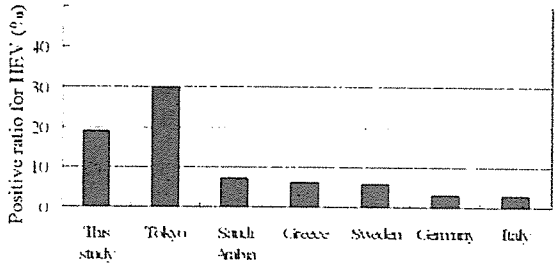

FIG. 5. The IgG-type anti-hepatitis E virus antibody positive ratio for the countries.Hemodialysis patients in Japan showed a higher ratio in comparison with other countries.

(10). Another report indicated that the percentage is higher in subjects aged 40 years or older (6). The percent IgG-type anti-HEV antibody positivities in Sweden were reported to be 2.5% in hemodialysis patients under 40 years of age and 7.4% in hemodialysis patients aged 40 years or older (14). Hemodialysis patients in Japan were similar to subjects with healthy kidneys and hemodialysis patients in Sweden. In Japan, a high incidence of *Helicobacter pylori* infection in subjects aged 40 years of age or older was reported and the infection was assumed to spread via sanitation equipment, water supply and sewage systems (17). This suggests that water-borne infection might underlie the findings of the present study, although *H. pylori* infection was not examined in the present study.

HCV antibody-positive was high ratio in the HEV positive patient, although it was not statistically significant, and blood transfusion was not risk factor. Thus. blood-borne infection could be one of infection routes in the dialysis patients.

Various routes of HEV infection in developed countries were reported or assumed, including water-borne, food-borne (18), and blood-borne infections, and cross infections, unlike the conventional water-borne HEV infection among developing countries. HEV infection in hemodialysis patients is also considered to occur via different routes, including blood-borne infections.

In conclusion, it is likely that the high prevalence of HEV infection in hemodialysis patients is caused mainly by water-borne infection, while blood-borne infection or false positive also can be attributed to that. It is necessary to further assess the significance of the high percent IgG-type anti-HEV antibody positivity among hemodialysis patients in terms of the route of infection, the effect of HEV infection on the prognosis of patients undergoing hemodialysis, and HEV infection preventive measures.

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Reduced Capacity of Mononuclear Cells to Synthesize Cytokines against an Inflammatory Stimulus in Uremic Patients

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Key Words
Intracellular cytokine staining · Phagocytosis · Monocyte · Helper T cell · Polymorphonuclear leukocytes

Abstract
Background: The local production of cytokines and phagocytosis is pivotal in innate immunity. Uremic patients have a high infectious morbidity, but it remains unclear if this arises from incompetence of these local cellular functions. ***Methods:*** In 30 predialysis uremic patients and 20 controls, we studied the intracellular cytokine synthesis by mononuclear cells in response to stimulation. Moreover, phagocytic activity by leukocytes was tested. Lipopolysaccharide- or mitogen-stimulated peripheral blood cells were labeled with anti-CD14 and -CD4 antibodies, respectively and subjected to intracellular cytokine staining and flow cytometry. Tumor necrosis factor (TNF)-α, IL-1β, IL-6, and IL-8 synthesis was examined in $CD14^+$ monocytes. IFN-γ and IL-4 synthesis was examined in $CD4^+$ helper T cells to determine their Th1 or Th2 phenotype. The flow cytometric analysis of phagocytosis of opsonized bacteria was performed in whole blood. ***Results:*** Uremic patients exhibited a significantly reduced monokine response and inhibited development of helper T cells into Th1 or Th2 phenotypes compared with control subjects. Their phagocytic activity was comparable to control subjects. No clinical parameters were linked to in vitro cytokine production and phagocytic activity. ***Conclusions:*** Mononuclear cells in uremic patients are hyporeactive to inflammatory challenge and this may be one reason why uremic patients are vulnerable to infections.

Introduction

In general, patients with chronic kidney disease (CKD) as well as dialysis patients have an increased incidence of infections and are susceptible to infectious diseases [1]. While previous data suggest impairment of immune cellular functions in uremic patients including pre- and postdialysis patients [2], it remains unclear whether this is associated with their innate host defense. Peripheral mononuclear cells and polymorphonuclear leukocytes (PMNLs) play a key role in innate host defense. In particular, mononuclear cells initiate and orchestrate the innate immune response primarily by producing cytokines, which are also responsible for activating the adaptive immune system that gradually takes over from the innate immunity. In these innate host defense systems, pro-inflammatory cytokines are vital in overcoming infections and may be crucial for host defense [3, 4]. Cytokines exert their major physiologic and pathophysiologic effects as autocrine or paracrine factors. Therefore, altered local

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processing and release of pro-inflammatory cytokines are relevant to the pathogenesis of defective innate host defense. Although this line of study has been conducted mainly in hemodialysis (HD) patients [5], it is well known that HD has a major impact on immune cells. Thus, uremic patients should be studied before they require dialysis in order to examine the influence of uremia per se on local cytokine responses by immune cells to invading pathogens.

The study of circulating cytokine levels has limited value and gives little information as a result of their short half-lives and local effects. In contrast, measuring the capacity of peripheral blood mononuclear cells to synthesize pro-inflammatory cytokines in response to pathogenic stimuli may provide a more precise picture in clinical practice. We therefore decided to investigate the intracellular synthesis of cytokines by mononuclear cells in response to ex vivo stimuli in pre-dialysis CKD patients. In addition, the phagocytosis of opsonized bacteria by PMNLs and monocytes was studied in whole blood.

Materials and Methods

Subjects

The study included 30 predialysis CKD patients (male/female, 16/14; mean age, 54.8 ± 4.19 years) and 20 healthy subjects without any infectious or inflammatory diseases (male/female, 10/10; mean age, 52.3 ± 5.14 years). There was no significant difference in age between groups. The chronic renal failure of the CKD patients was due to the following diseases: nephrosclerosis (n = 16), chronic glomerulonephritis (n = 12) and polycystic kidney diseases (n = 2). Patients with renal failure due to collagen diseases, diabetes mellitus or hepatitis C were strictly excluded from the study since the immune cells of such patients are considered to be functionally abnormal. Several drugs that can influence cytokine expression such as steroids, statins and vitamin D were discontinued in the patients at least 1 month before the study. The study was approved by the institutional review board of the hospital and was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all subjects.

Reagents and Antibodies

Brefeldin-A, lipopolysaccharide (LPS), phorbol 12-myristate-13-acetate (PMA) and ionomycin were purchased from Sigma Chemicals (Tokyo, Japan). The following reagents were purchased from Becton Dickinson (Calif., USA): FACS Lysing Solution®, Permeabilizing Solution®, FITC-conjugated anti-cytokine mAbs to IFN-γ anti-human IFN-γ FITC , TNF-α anti-human TNF-α FITC) and IL-6 (anti-human IL-6 FITC), PE-conjugated anti-cytokine mAbs to IL-1β anti-human IL1β PE, IL-4 (anti-human IL4 PE) and IL-8 (anti-human IL-8 PE), and isotype-matched Abs of irrelevant specificities (FITC- or PE-labeled mouse IgG). PC5-conjugated monoclonal antibodies (mAbs) to $CD4^+$ or $CD14^+$ were purchased from Immunotech (Tokyo, Japan). RPMI 1640 medium, phosphate-buffered saline (PBS), paraformaldehyde, and bovine serum albumin (BSA) were purchased from GIBCO (Tokyo, Japan). Phagotest® (ORPEGEN Pharma: Heidelberg, Germany) was purchased from BIOCARTA (Calif., USA).

Blood Collection, Measurements and Mononuclear Cell Labeling

Blood samples were collected from the pre-dialysis CKD patients and the control subjects before having breakfast in the morning. We used the four-variable (abbreviated) Modification of Diet in Renal Disease (MDRD) equation to estimate the glomerular filtration rate (GFR). To measure the stimulation-induced cytokine production of these patients, we used intracellular cytokine staining using the FASTIMMUNE Assay System® kit (Becton Dickinson) that is based upon the method described by Jung et al. [6] and Picker et al. [7]. The dose of stimulants and optimal stimulation time for cytokine synthesis had been determined by a previous kinetic study as described in reference [8]. In brief, 1 ml of peripheral blood was diluted with 1 ml of RPMI 1640 medium and then incubated with 15 μg/ml of brefeldin-A, which disrupts intracellular Golgi-mediated transport and allows cytokines to accumulate. This yields an enhanced cytokine signal. Thereafter, 0.1 μg/ml of LPS was added to activate monocytes or 40 ng/ml of PMA plus 4 μg/ml of ionomycin was added to activate lymphocytes. The blood was then incubated for 4 h at 37°C in a CO_2 incubator (5% CO_2). The viability of mononuclear cells (>95%) in whole blood was evaluated using trypan blue exclusion. Thereafter, 500 μl of the activated blood was labeled with anti-CD4 or CD14 Abs for 15 min at room temperature.

Permeabilization and Intracellular Staining

FACS lysing solution® (4 ml) was added to the activated, labeled blood and incubated for 10 min. The sample was then washed twice with PBS/0.1% BSA by centrifugation and the supernatant was removed. Permeabilizing solution® (500 μl) was added and the specimen was left for 10 min in the dark. After washing the cells twice, the cell pellets in 50 μl of RPMI medium were incubated with the fluorescent anti-cytokine mAbs in the tubes for 30 min in the dark. After washing the cells by centrifugation, the supernatants were removed and 500 μl of PBS/1% paraformaldehyde was added. The samples were promptly analyzed with a flow cytometer. Accumulated intracellular cytokines including TNF-α, IL-1β, IL-6, IL-8, IFN-γ and IL-4, were identified by specific mAbs. Helper T cell type 1 (Th1) cells were defined as IFN-γ (+), IL-4 (–), $CD4^+$ cells, while type 2 (Th2) cells were defined as IFN-γ (–), IL-4 (+), $CD4^+$ cells. Isotype control mAbs of irrelevant specificities were added at matching concentrations in control samples to detect the nonspecific binding of the mAbs to the cells.

Intracellular Cytokine Measurement by Flow Cytometry

The samples were analyzed by using a flow cytometer (EPICS XL/MCL System II; Beckman Coulter, Calif., USA) and the data were assessed with EXPO 32 (Beckman Coulter). The light scatter and fluorescence channels were set at a logarithmic gain. Only cells that were positive for CD14 (monocytes) or CD4 (helper T cells) were gated (R1) on the SSC-FL3 plot. Each analysis involved 10,000 monocytes or helper T cells within the R1 gate. The cells

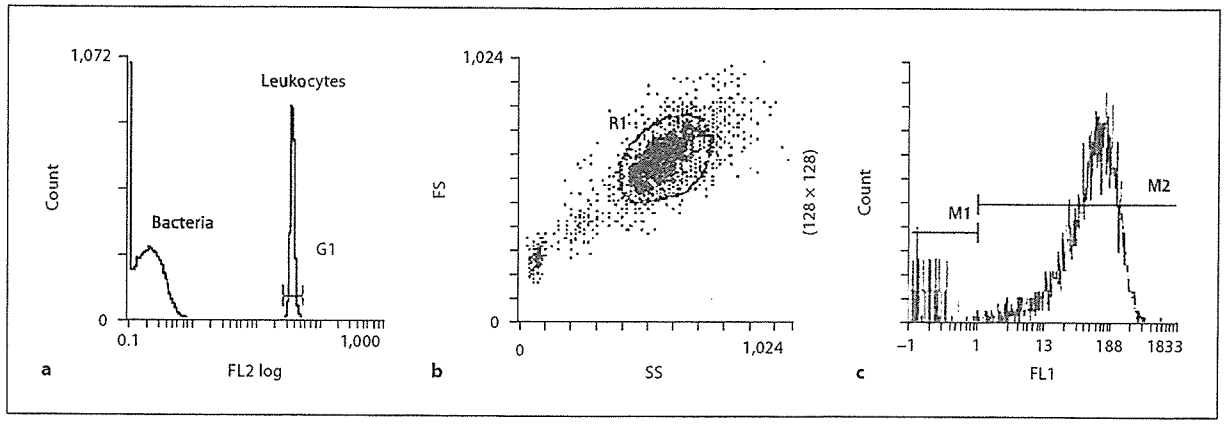

Fig. 1. Flow cytometric analysis of phagocytosis in polymorphonuclear leukocytes (PMNLs). During data acquisition, a 'live' cell gate (G1) is set in the FL2 fluorescence histogram on those events which have at least the same DNA content as a human diploid cell in order to exclude bacterial aggregates having the same scatter light properties as leukocytes (**a**). Typical dot plots FS/SS of living PMNLs gated by the G1 in a control subject (**b**) and the FL1 histogram showing the phagocytosis (as an M2 bar in **c**). The histogram for the control sample maintained at 0°C is presented on the left (as an M1 bar in **c**).

labeled with specific anti-cytokine mAbs were profiled on the FL1-FL2 plot and compared to the samples that had been treated with the isotype control mAbs (negative controls). Cutoff markers were set individually for each measurement on the basis of the negative control. The percentage of cells that were positive for each cytokine antibody (% positive cells) was calculated on the quadrant diagram for the assessment, as described in our previous article [5]. The mean fluorescence intensity (MFI) of positive cells was measured by a logarithmic scale to determine cytokine synthesis. All experiments were performed in duplicate and their mean values were used for analysis. The intra-assay precision was determined on triplicate whole blood samples from CKD patients. The coefficient of variability for the assay was 7.4 ± 4.1%.

Measurement of Phagocytic Activity

Phagocytic activity in peripheral PMNLs and monocytes was investigated with Phagotest®. This test kit allows the quantitative determination of leukocyte phagocytosis (ingestion of bacteria) using whole blood to which opsonized bacteria are added. It measures the percentage of phagocytes which have ingested bacteria as well as their phagocytic activity (number of bacteria per cell is determined by MFI) using a flow cytometry. The procedure was performed following the manufacturer's protocol. In brief, 100 µl of heparinized whole blood was incubated with 20 µl of the FITC-labelled *Escherichia coli* bacteria (~5 × 10^7) for 10 min at 37°C with the negative control sample remaining on ice. Phagocytosis is stopped by placing the samples on ice and adding quenching solution. This solution allows the discrimination between attachment and internalization of bacteria by quenching the FITC-fluorescence of surface bound bacteria leaving the fluorescence of internalized particles unaltered. After two washing steps, erythrocytes are then removed by addition of lysing solution. The DNA staining solution, which is added just prior to flow cytometric analysis, excludes aggregation artifacts of bacteria or platelets. Cells that phagocytosed bacteria are analyzed by flow cytometry. During data acquisition a 'live' gate is set in the fluorescence histogram on those events which have at least the same DNA content as a human diploid cell to exclude aggregation of bacteria or platelets. The percentage of cells having performed phagocytosis is analyzed as well as their MFI (total number of ingested bacteria). All experiments were performed in duplicate and their mean values were used for analysis. The intra-assay precision was determined on triplicate whole blood samples from uremic patients. The coefficient of variability for the assay was 8.4 ± 2.9%. The representative figures showing the analysis of PMNLs from a CKD patient are shown in figure 1.

Statistics

The data are expressed as mean ± SD unless otherwise stated. Statistical analysis was performed using the Mann-Whitney U test to compare data between the two study groups. $p < 0.05$ was considered to be statistically significant. Bivariable analyses exploring the relationship of in vitro cytokine synthesis to clinical variables were performed using the nonparametric Spearman rank correlation test.

Results

Laboratory Data of the Subjects

The laboratory data of the subjects are shown in table 1. The CKD and control groups did not statistically differ with respect to total white blood cell numbers or

Table 1. Laboratory data

	CKD (n = 30)	Control (n = 20)	p values
WBC counts, /μl	5,011 ± 1,365	5,218 ± 1,267	n.s.
Monocytes, /μl	423 ± 202	437 ± 198	n.s.
Lymphocytes, /μl	1,161 ± 575	1,232 ± 688	n.s.
$CD4^+$ helper T cells, /μl	468 ± 254	481 ± 291	n.s.
Hct, %	30.1 ± 5.1	43.5 ± 3.4	<0.05
Serum data			
TP, g/dl	6.9 ± 1.6	7.3 ± 1.3	n.s.
Alb, g/dl	3.9 ± 1.2	4.3 ± 0.3	n.s.
UN, mg/dl	68.1 ± 7.3	12.8 ± 3.4	<0.01
Cr, mg/dl	6.9 ± 4.3	0.88 ± 0.3	<0.01
β_2MG, mg/dl	13.5 ± 5.21	0.93 ± 0.14	<0.01
TC, mg/dl	205 ± 23.2	233 ± 65.9	<0.05
TG, mg/dl	151 ± 66.6	104 ± 21.2	<0.05
ESR, mm/h	20.1 ± 8.11	8.50 ± 1.22	<0.05
CRP, mg/dl	0.1 ± 0.1	0.1 ± 0.2	n.s.
Estimated GFR, ml/min/1.73 m^2	9.4 ± 1.71	89.2 ± 7.33	<0.01

Data are expressed as mean ± SD.

WBC = White blood cell; Hct = hematocrit; TP = total protein; Alb = albumin; UN = urea nitrogen; Cr = creatinine; β_2MG = β_2-microglobulin; TC = total cholesterol; TG = triglycerides; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; GFR = glomerular filtration rate (estimated by Modification of Diet in Renal Disease equation).

monocyte and lymphocyte counts. Hematocrit was significantly lower in the CKD patients. The serum TNF-α, IL-1β, IL-6 and IL-8 levels were not different between the CKD and control groups. Significant differences were observed between the CKD and control groups with regard to serum parameters that were related to uremia (estimated GFR, urea nitrogen, creatinine, β_2-microglobulin) and serum lipids levels, but were not observed with regard to nutrition such as total protein and albumin levels. Erythrocyte sedimentation rate was significantly higher in the CKD patients, but C-reactive protein levels were comparable.

Intracellular Cytokine Induction by Monocytes in Response to LPS

There were no differences in the intracellular monocyte cytokine levels between the two groups at the baseline in the absence of LPS stimulation (data not shown) as published recently [5]. However, significant differences were detected following LPS stimulation. Figure 2 shows the percentage of cells that were positive for TNF-α, IL-1β, IL-6 and IL-8 (frequency) and the level of cytokine synthesis expressed by MFI (intensity) in the CKD patients and controls. The CKD patients exhibited a significantly reduced frequency and intensity in all monokines analyzed compared with controls.

Intracellular Cytokine Induction and Polarization of Helper T Cells in Response to Mitogen

The data for intracellular cytokine induction and polarization of helper T cells are shown in figure 3. The polarization of helper T cells in response to PMA-ionomycin stimulation was blunted in the CKD patients, resulting in a significant decrease of both IFN-γ-producing T cells (Th1 cells) and IL-4-producing cells (Th2 cells) compared with controls. The Th1/Th2 ratio of the CKD patients was preserved and comparable with that seen in the control subjects.

Phagocytic Activity of PMNLs and Monocytes

The data for phagocytic activity of PMNLs and monocytes are shown in table 2. The percentage of phagocytes which have ingested bacteria and their activity (MFI) did not significantly differ between the CKD patients and controls.

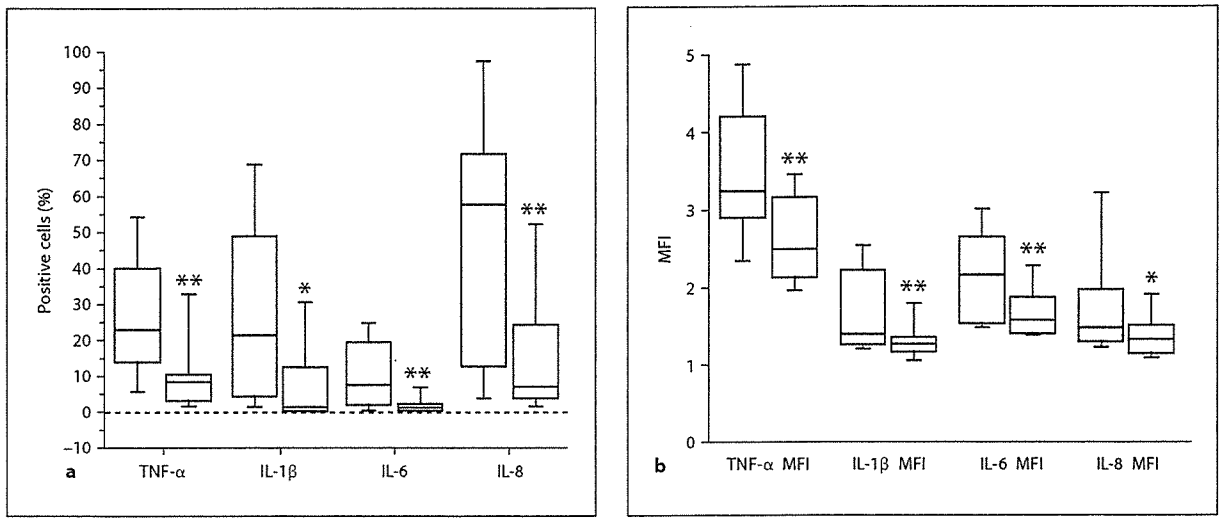

Fig. 2. Intracellular cytokine synthesis by monocytes from patients with chronic kidney failure (CKD) and control subjects (CONT) in response to ex vivo LPS stimulation. The intracellular cytokine synthesis is shown by the percentage of cells that are positive for each cytokine antibody (**a**). Their mean fluorescence intensity (MFI) was also measured to determine the cytokine synthesis (**b**). The data are shown as box and whisker plots; the box represents the 25–75th percentile with a horizontal line at the median; the whiskers extend to the highest and lowest values. p values represent the comparisons between CKD (open column) and CONT (hatched column). * $p < 0.05$, CKD vs. CONT and ** $p < 0.01$, CKD vs. CONT.

Table 2. Phagocytic activity in PMNLs and monocytes

	CKD (n = 30)	Control (n = 20)	p values
PMNLs, %	81.8 ± 12.9	84.3 ± 7.88	n.s.
PMNLs, MFI	122.5 ± 21.3	123.9 ± 25.6	n.s.
Monocytes, %	51.1 ± 14.6	48.2 ± 8.78	n.s.
Monocytes, MFI	59.0 ± 20.7	58.6 ± 18.6	n.s.

Data are expressed as mean ± SD.
% = % phagocytosing cells; MFI = mean fluorescence intensity.

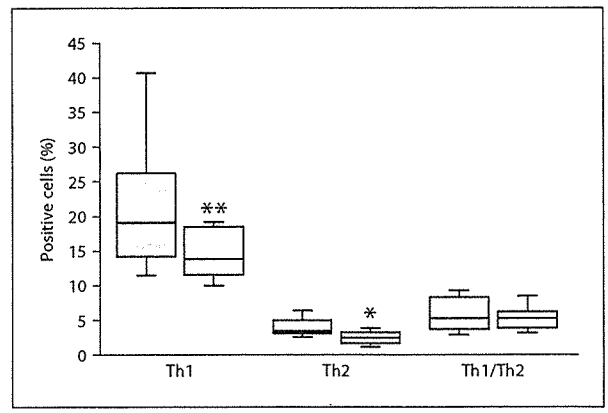

Fig. 3. Polarization of the helper T cells in patients with chronic kidney failure (CKD) and control subjects (CONT) in response to ex vivo PMA-ionomycin stimulation. Intracellular synthesis of IFN-γ and IL-4 was assessed in order to differentiate helper T type 1 (Th1) and helper T type 2 (Th2) cell subsets. These cells are shown by the percentage of cells that are positive for each cytokine antibody. The data are shown as box and whisker plots; the box represents the 25–75th percentile with a horizontal line at the median; the whiskers extend to the highest and lowest values. p values represent the comparisons between CKD (open column) and CONT (hatched column). * $p < 0.05$, CKD vs. CONT and ** $p < 0.01$, CKD vs. CONT.

Correlations of Intracellular Cytokine Synthesis with Clinical Variables and Phagocytic Activity

The intensity and frequency of intracellular cytokine synthesis were not significantly correlated with any clinical variable associated with renal function such as serum creatinine, urea nitrogen, β_2-microglobulin, and estimated GFR. Phagocytic activity in PMNLs and monocytes was not significantly correlated with intracellular production of each cytokine and other clinical variables on bivariable analyses (data not shown).

Discussion

The intracellular cytokine synthesis by mononuclear cells was studied after stimulation with LPS or PMA and ionomycin. Monocytes derived from CKD patients were hyporeactive to ex vivo LPS stimulation compared with those from healthy control subjects. Moreover, the development of helper T cells into both Th1 and Th2 subsets was significantly reduced in those patients. In contrast, PMNLs and monocytes from CKD patients exhibited normal phagocytic activity. No renal parameters exhibited a significant correlation to this hyporesponsiveness in cytokine synthesis. These findings suggest that the local pro-inflammatory cytokine response of immune cells against invasive pathogens is impaired in chronically uremic patients before the initiation of dialysis.

The reduced capacity of monocytes to synthesize pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-8, in response to an inflammatory stimulus may result in vulnerable host defense in uremia patients. Cytokines act as autocrine or paracrine mediators in exerting their major physiologic and pathophysiologic effects. Insufficient or delayed cytokine release into the local environment may decrease the immune response and increase the risk of infection [4, 9, 10]. In fact, several studies have shown that a reduction of pro-inflammatory monokine synthesis in response to stimuli, even though the plasma level is quite high, is correlated with a higher mortality in septic patients [11, 12]. In addition to the relevance to host defense, this lower cytokine response in local lesions could be a possible cause of the ineffective low-grade inflammation which is frequently observed in uremic patients [13]. There is scant data regarding the inhibitory mechanisms regulating peripheral mononuclear cell production of cytokines in response to stimuli under acute and chronic uremia [14]. Although we postulated that residual GFR or degree of uremia may affect the cytokine response, our results did not demonstrate any significant correlation between the in vitro cytokine responses and clinical parameters for kidney function on bivariable analyses (data not shown). Our study did not address the profile of anti-inflammatory cytokines or the balance between pro- and anti-inflammatory cytokine responses such as the ratio of IL-10 to TNF-α which also need to be considered in host defense. We believe, however, that the reduction of pro-inflammatory monokines is directly relevant to the susceptibility to bacterial infections [9–12].

Our study showed that the PMA/mitomycin-induced polarization of helper T cells into both subsets was reduced in the CKD patients. Our results are not consistent with a report by Sester et al. [17] showing that helper T polarization in HD patients is skewed towards a Th1 direction. HD induces significant systemic inflammatory changes in immune cells, which may be responsible for the difference between the two studies. Moreover, when both data are compared, it should be considered that PMA/mitomycin can stimulate the differentiated T cells as well as naïve T cells and thus the amount of Th1 and Th2 cells evaluated by this method might in part reflect the number of Th1 and Th2 cells which were already present. Among Th1 cytokines, IFN-γ is placed in a pivotal position in the immune networks induced by microbacterial invasion. This cytokine amplifies the innate immune defense by activating macrophages and neutrophils. It also orchestrates a number of biological events that are related to the progression of innate to adaptive host responses. Thus, the decreased polarization into Th1 cells and number of Th1 cells in CKD patients is not conducive to acquired immunity. The antibody response to hepatitis B vaccination and skin testing for tuberculosis with tuberculin-purified protein derivative (PPD) have been used as sensitive markers for the extent of individual immune incompetence. Hyporesponsiveness to these tests is confirmed in uremic patients suggesting impairment of both cellular and humoral immunity [18, 19]. However, the mechanisms underlying these clinical phenomena are unclear. It is conceivable that reduced differentiation of helper T cell into both subsets is in part responsible for hyporesponsiveness of CKD patients in these clinical tests.

Phagocytic activity as determined with Phagotest was comparable to control subjects. Phagocytosis by PMNLs and monocytes constitutes an essential arm of host innate defense against bacterial and fungal infections. The phagocytic process can be separated into four major stages: chemotaxis (migration of phagocytes to inflammatory sites), attachment of particles to the cell surface of phagocytes, ingestion (phagocytosis) and intracellular killing by oxygen-dependent and oxygen-independent mechanisms. Phagotest can investigate both the attachment process and the ingestion capability under simulated in vivo conditions. Our results mean that at least two stages in the phagocytic process may be preserved in CKD patients. In contrast, there are several papers indicating impaired phagocytotic activity by PMNLs in HD patients [3, 20, 21]. Nevertheless, data derived from HD patients do not always hold true for pre-dialysis CKD patients since dialysis sessions inevitably change cellular functions through the contact of immune cells with dia-

lyzer membranes [2, 3, 17, 20–22]. To date, there have been no thorough studies testing phagocytic activity in pre-dialysis CKD patients. The analysis of other phagocytic stages such as chemotaxis and intracellular bactericidal activity was not examined in this study and it will be necessary to make a total assessment of phagocytic activity in CKD patients.

In conclusion, ex vivo stimulated whole blood was employed in this study to understand the capacity of peripheral blood mononuclear cells to synthesize proinflammatory cytokines under natural infectious conditions [4, 12]. Our results indicate that pre-dialysis CKD patients exhibit inadequate innate immunity with respect to the ability to generate appropriate cytokine synthesis against invading pathogens. This compromised cytokine response may represent a reason why uremia is involved in infectious diseases.

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原　著

慢性血液透析患者におけるC型肝炎ウイルス感染のサーベイランス

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（受理　平成17年12月14日）

Surveillance of Hepatitis C Virus Infection in Hemodialysis Patient

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In Japan, the rate of hepatitis C Virus (HCV) infection among hemodialysis patients is high (12.1%); yet, early detection of HCV infection among these patients is limited because their AST/ALT increase is small, an index or a standardized surveillance method is unavailable, the measurement of HCV antibody varies among facilities, and the conventional HCV-RNA test is costly. We applied the public HCV surveillance method to hemodialysis patients and examined its effectiveness. A total of 1,077 hemodialysis patient (690 males, 387 female ; mean age 59.8 ± 12.8 years old; dialysis history 9.1 ± 6.0 years) were included. The public HCV surveillance method included a combination of HCV antibody, HCV core antigen, and HCV-RNA tests. Using this method, we detected 151 (14%) HCV antibody-positive patients, of whom the antibody was high in 89, medium in 50, and low in 12 patients. The conventional method would require all HCV antibody positive patients (n = 151) to undergo HCV-RNA test; however, we only required 27 patients to undergo HCV-RNA test. The 82.1% reduction in the requirement for further testing shows that our method is cost effective and efficient. The surveillance method should be introduced to help reduce infection in hemodialysis patients.

Key words: hemodialysis, hepatitis C virus, surveillance, HCV core antigen, alpha glutathione S-transferase

緒　言

わが国の慢性透析患者は年々増加しており，2003年末で237,710人にのぼり，この透析患者のHCV抗体陽性率は12.1%と高率である[1]．1993年に行われた日本透析医学会の調査では，透析患者のHCV抗体陽性率は，23.9%とかなり高率であった．輸血製剤のスクリーニング，エリスロポエチン製剤の保険適用，ディスポーザブル製品の普及により，HCV抗体の陽性率は年々減少している[2][3]．しかし，年あたりのC型肝炎ウイルス（HCV）新規感染率は2001年で2.2%と報告されており，いまだに驚くべき高率である[4][5]．

透析患者は観血的な透析操作，頻回の通院などHCVに曝露される機会も多く，透析室内での水平感染の存在も指摘されている[6]．透析患者はHCV感染のハイリスクグループであり，長期維持透析患者・高齢透析患者が増加している現状では，肝硬変・肝細胞癌に進行する症例の増加が危惧される[7]．

厚生労働科学研究により「透析医療における標準的な透析操作と院内感染予防に関するマニュアル」（感染マニュアル）が作成され，透析施設での感染対策に注意喚起が促された[8]．しかし，依然として各透析施設でのHCV対策には施設間差があり，HCVのサーベイランスも充分に行われていない．また，HCV抗体が出現する前にHCV新規感染の兆候をつかまえるとしても，asparatate aminotransferase (AST)，alanine aminotransferase (ALT) の上昇はごくわずかでHCV感染の指標がないことから[7]，HCV新規感染者の早期発見ができ難い状況にある．

厚生労働科学研究により確立したHCV検診法[9][10]（図1）は，2002年4月より40歳以上の一般住民に