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8. 昆　吏規 大高徹也 小林光樹	血液透析患者の心機能に対するC型肝炎ウイルス感染の影響について	東北大学医学部保健学科紀要	17	23-27	2008

Ⅳ. 研究成果の刊行物・別刷

【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 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 ($P = 0.30$).

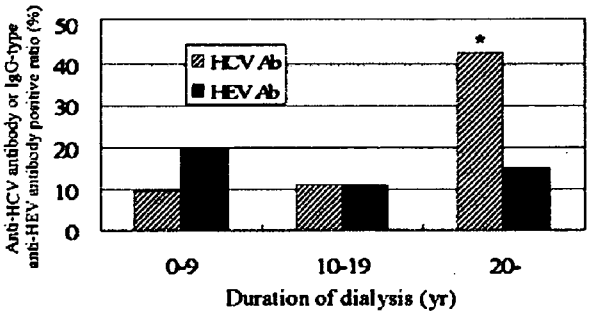

FIG. 1. The relation between duration of hemodialysis and anti-hepatitis C virus antibody or IgG-type anti-hepatitis E virus antibody positive ratio. The percent anti-hepatitis C virus antibody positivity increased with the length of hemodialysis duration. However no similar correlation was observed 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.

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

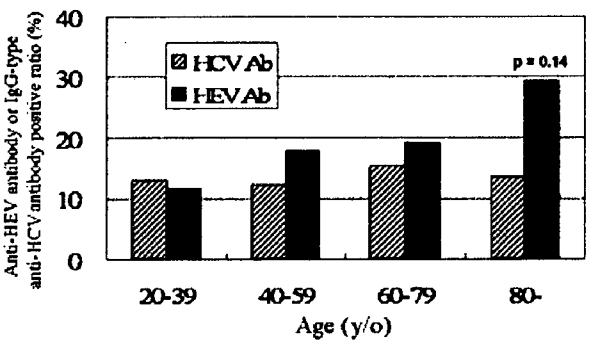

FIG. 2. The relation between age of hemodialysis patients and anti-hepatitis C virus antibody or IgG-type anti-hepatitis E virus antibody positive ratio. The percent IgG-type hepatitis E virus antibody positivity increased with patient age, whereas no similar correlation was observed between percent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

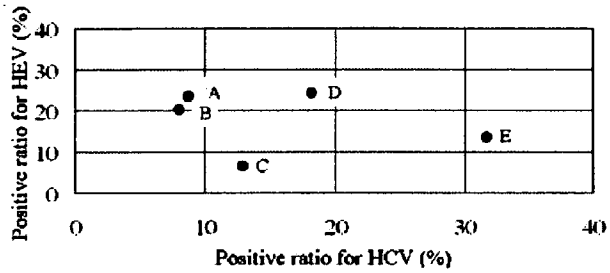

FIG. 3. The anti-hepatitis C virus antibody and IgG-type anti-hepatitis E virus antibody positive ratio for the hemodialysis facilities. These percent IgG-type anti-HEV antibody positivities were significantly different among the hemodialysis facilities. (A) hemodialysis hospital in Gunma, north of Tokyo. (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

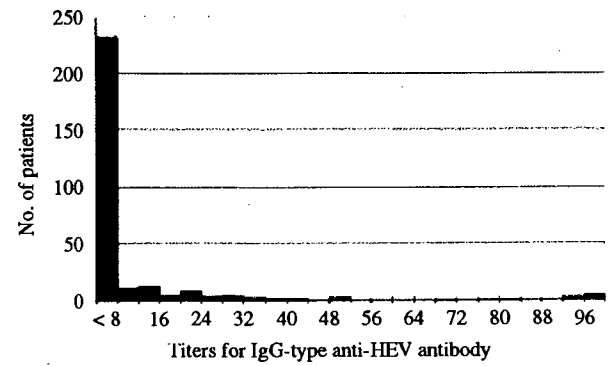

FIG. 4. Distribution of IgG-type anti-hepatitis E virus Ab titers.Some patients had titers close to the cut-off index of 13, suggesting the existence of pseudo-antibody-positive or pseudo-antibody-negative patients.

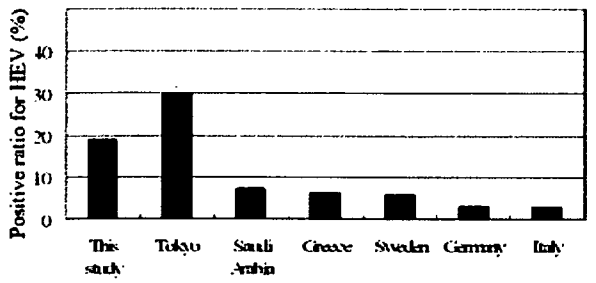

FIG. 5. The IgG-type anti-hepatitis E virus antibody positive ratio for the countries.Hemodialysis patients in Japan showed a higher ratio in comparison with other countries.

(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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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

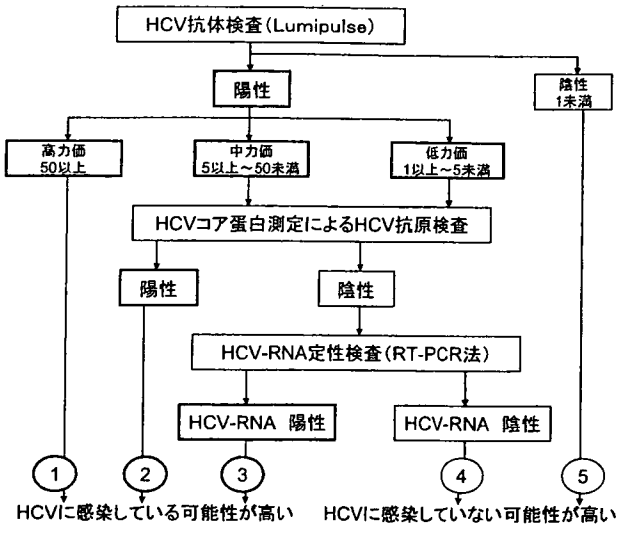

図1 HCV サーベイランス法

対する節目検診に用いられている. HCV 抗体検査で陽性と判定された人は, HCV キャリアと感染既往者に大別されることはよく知られている. 一般に HCV キャリアは HCV 抗体価が高い値（HCV 抗体「高力価」陽性）を示すのに対して, 感染既往者では低値（HCV 抗体「低力価」陽性）を示す. HCV 抗体価が中等度の値（HCV 抗体「中力価」陽性）を示す集団の中には, HCV キャリアであるにも関わらず, HCV 抗体が十分に作られない人と, HCV が身体から排除されて十分に時間が経っていないために HCV 抗体が「低力価」陽性になっていない人が混在することが知られている. このことから, HCV キャリアを見出すための検査手順とし, HCV 抗体の測定（HCV 抗体陽性の場合には, HCV 抗体「高力価」,「中力価」,「低力価」の 3 群に分別）と HCV の存在診断のため HCV コア抗原の測定, HCV-RNA の測定の三者を組み合わせた方法が採用されている.

しかしこの HCV 検診法が, 免疫能の異常があり感染リスクの高い血液透析患者の HCV 感染のスクリーニングとして使用可能かどうかの報告はない. そこで慢性血液透析患者の HCV サーベイランスとし, 一般住民に対する HCV 検診法が有用な方法であるか検討した. また, 肝逸脱酵素の 1 つである alpha glutathione S-transferase[11]（α-GST）が透析患者の肝障害マーカーとし使用可能かどうか検討した.

対象および方法

1. 対象

関東地域の基幹病院透析室 2 施設, 無床外来透析施設 3 施設で 3 ヵ月以上血液透析を継続している患者で, 検査の同意が得られた症例を対象とした.

2. 方法

この症例で, 年齢, 性別, 透析歴, 原疾患および輸血歴を調査した. 血液検査は, 週初め血液透析前に動脈側より採血し, AST, ALT, α-GST, γ-glutamyltranspeptidase (γ-GTP), 総ビリルビン (T-Bil), フェリチン, 血算を測定した. 全症例に HCV 抗体（ルミパルス®）検査と HCV コア抗原検査を実施した. HCV 抗体が 1 以上かつ HCV コア抗原が 20 fmol/l 以上の症例では HCV-RNA 定量検査を実施した. HCV 抗体が 1 以上かつ HCV コア抗原が 20 fmol/l 未満では, HCV-RNA 定性を実施した. HCV-RNA 定性が陽性の症例に対し HCV-RNA 定量を実施した. HCV 抗体が 1 未満かつ HCV コア抗原が 20 fmol/l 未満の症例は検査終了とした. この結果を HCV 検診法に適用した.

3. 測定

HCV 抗体は化学発光酵素免疫測定法試薬, ルミパルス®II オーソ®HCV（オーソ・クリニカル・ダイアグノスティックス, 東京）を使用した. HCV 抗体価 1 以上を陽性とし, 低力価 1 以上 5 未満, 中力価 5 以上 50 未満, 高力価 50 以上 100 以下とした[10)12)]. HCV コア抗原は RIA 固相法を測定原理とする, オーソ HCV 抗原 IRMA テスト（オーソ・クリニカル・ダイアグノスティックス, 東京）を使用した. オーソ・クリニカル・ダイアグノスティックスのマニュアルに従い, HCV 抗原 20fmol/l 以上を陽性と判定した[13)].

HCV-RNA 定性・定量検査には, reverse transcription-polymerase chain reaction（RT-PCR）を測定原理とするアンプリコア HCV V2.0（ロシュ・ダイアグノスティックス, 東京）を使用した[14)]. 定量検査は 5KIU/mL 以上を陽性とした. α-GST は, EIA を測定原理とする BIotrin HEPKIT-Alpha Human GST-Alpha (Biotrin International, Ireland) を使用した[11)].

4. 統計

データのうち計量値については, 平均値±標準偏差で表示した. 統計学的処理には χ^2 検定, t 検定, ピアソンの相関係数および単回帰分析を用いた. $p<0.05$ を有意と判定した

結　果

1. 患者背景と臨床検査成績（表 1, 2）

患者は 1,077 例で, 性別は男性 690 人, 女性 387 人, 年齢 59.8±12.8 歳, 透析歴 9.10±5.95 年であっ

表1　HCV 抗体陽性・陰性別の原疾患

原疾患	HCV 抗体陽性	HCV 抗体陰性	計（人）
慢性糸球体腎炎	48	297	345
糖尿病	14	172	186
腎硬化症	2	20	22
多発性のう胞腎	3	16	19
ネフローゼ症候群	1	5	6
その他	18	12	30
不明	65	404	469
計（人）	151	926	1,077

表2　臨床検査結果

	HCV 抗体陽性	HCV 抗体陰性	p 値
血小板（$\times 10^4/\mu L$）	16.5 ± 5.7	20.0 ± 6.9	0.009
アルブミン（g/dL）	3.79 ± 0.36	3.93 ± 0.39	0.16
T-Bil（mg/dL）	0.21 ± 0.12	0.21 ± 0.18	< 0.001
AST（U/L）	20.4 ± 8.2	15.0 ± 7.46	< 0.001
ALT（U/L）	18.0 ± 11.4	11.8 ± 7.1	< 0.001
γ-GTP（U/L）	41.0 ± 48.5	31.9 ± 67.8	< 0.001
フェリチン（ng/mL）	120.5 ± 165.7	97.3 ± 125.9	< 0.001
α-GST（μg/L）	3.94 ± 2.46	1.90 ± 1.64	0.002

表3　HCV 抗体と輸血歴

	HCV 抗体陽性	HCV 抗体陰性	合計（人）
輸血あり	23	117	140
輸血なし	6	121	127
合計（人）	29	238	267

χ^2 検定　p = 0.002

た．HCV 抗体陽性・陰性別の原疾患の人数は表1のごとくであった．HCV 抗体陽性患者は 151 人で HCV 抗体陽性率は 14.0% であった．臨床検査成績（表2）は総ビリルビンとアルブミン以外のすべての項目に有意差を認めた．肝障害のマーカーである AST・ALT の平均値は，HCV 抗体陽性群・陰性群ともに正常値内であったが，HCV 抗体陽性群で有意に上昇していた．肝線維化のマーカーである血小板数は，HCV 抗体陽性群で有意に低下していた．また，肝障害マーカーとして測定した α-GST は HCV 抗体陽性群で有意に上昇していた．

2. HCV 抗体と輸血歴（表3）

輸血歴あり群の HCV 抗体陽性率は 16.4%（23/140），輸血歴なし群の HCV 抗体陽性率は 4.7%（6/127）であった．輸血歴あり群と輸血歴なし群の HCV 抗体陽性率は，χ^2 検定で有意差（p = 0.002）を認めた．

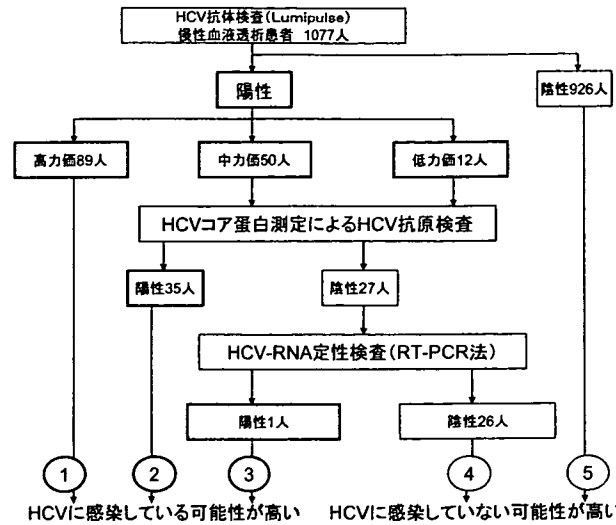

図2　HCV サーベイランスの結果

3. HCV サーベイランスの結果（図2）

HCV 抗体陰性患者は 926 人，HCV 抗体陽性患者は 151 人であった．HCV 抗体陰性患者は全症例が HCV コア抗原陰性であった．HCV 抗体高力価群は 89 人で，HCV-RNA 定性または定量で 88 人が陽性，1 例は HCV-RNA 定性が陰性であった．この陰性症例は 12 ヵ月後の検査で HCV 抗体価が 1.6 と低下を確認した．中力価群は 50 人で，HCV コア抗原陰性患者は 15 人で 1 例は HCV-RNA 定性が陽性であった．HCV 抗原陽性患者は 35 人で，1 例は HCV-RNA 定性が陰性であった．この症例は 12 ヵ月後の検査で HCV 抗体が 1.1 と低下を確認した．低力価群は 12 人で，全症例が HCV 抗原・HCV-RNA 定性とも陰性であった．

4. HCV 抗体と透析歴，年齢の関係（図3，4）

HCV 抗体の陽性率は透析歴とともに上昇し，透析歴と HCV 抗体陽性率は $r = 0.78$，$p < 0.001$ と強い正相関を認めた．10 年ごとの透析期間と HCV 抗体陽性率は，10 年未満で 8.3%，10 年以上 19 年未満で 11.0%，20 年以上 29 年未満で 42.5%，30 年以上で 60.0% であった．

HCV 抗体の陽性率は年齢とともに低下し，年齢と HCV 抗体陽性率は $r = -0.44$，$p < 0.001$ と負の相関を認めた．10 歳ごとの年齢と HCV 抗体陽性率は，年齢 20 歳以上 29 歳未満で 22.2%，30 歳以上 39 歳未満で 13.6%，40 歳以上 49 歳未満で 18.3%，50 歳以上 59 歳未満で 14.0%，60 歳以上 69 歳未満で 13.1%，70 歳以上 79 歳未満で 14.5%，80 歳以上で 8.2% であった．

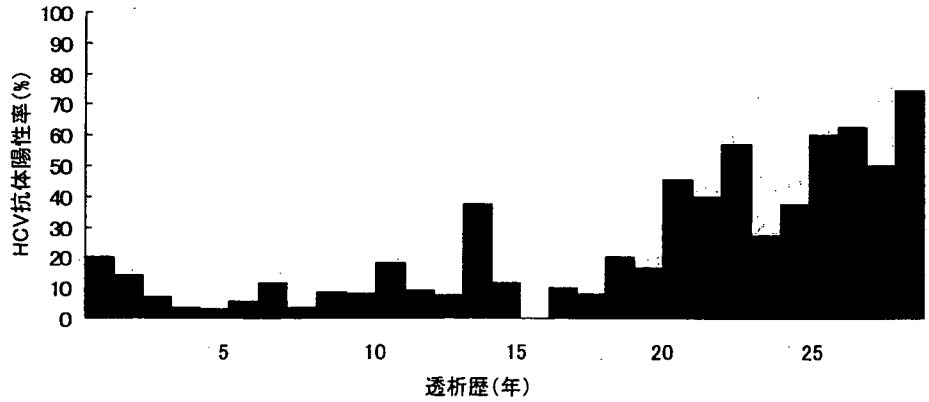

図3　HCV 抗体と透析歴

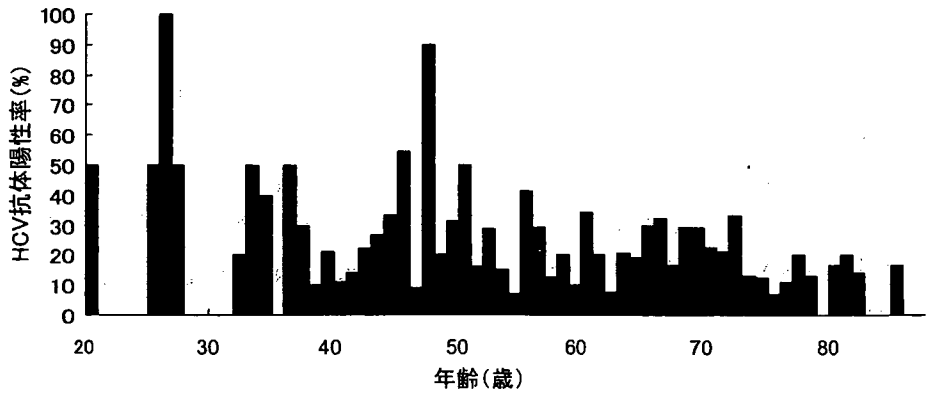

図4　HCV 抗体と年齢

5. HCV マーカー

HCV 抗体と HCV コア抗原は r＝0.50，p＜0.001 と正相関を認め，HCV 抗原と HCV-RNA 定量は r＝0.85，p＜0.001 と強い正相関を認めた．

6. 肝障害マーカーと HCV 抗体陽性率・HCV コア抗原陽性率

HCV 抗体陽性率・HCV コア抗原陽性率は，ALT の上昇とともに上昇した．ALT と HCV 抗体陽性率・HCV コア抗原陽性率は，ALT 値 5U/L 以上 10 U/L 未満で 6.8%・3.9%，10U/L 以上 15U/L 未満で 12.5%・9.6%，15U/L 以上 20U/L 未満で 18.7%・17.9%，20U/L 以上 25U/L 未満で 35.9%・33.3%，25 U/L 以上 30U/L 未満で 36.4%・27.3%，30U/L 以上で 43.8%・37.5% であった．特に ALT 20U/L 以上で，HCV 抗体陽性率・HCV コア抗原陽性率は有意（p＜0.05）に上昇した．

また，HCV 抗体陽性率・HCV コア抗原陽性率は，α-GST の上昇とともに上昇した．α-GST と HCV 抗体陽性率・HCV コア抗原陽性率は，α-GST 値 2.0μg/L 未満で 7.6%・3.8%，2.0μg/L 以上 4.0U/L 未満で 27.8%・23.7%，4.0μg/L 以上 6.0U/L 未満で 35.5%・35.5%，6.0μg/L 以上 8.0U/L 未満で 50.0%・50.0%，8.0μg/L 以上 10.0U/L 未満で 55.6%・55.6% であった．特に α-GST 2.0μg/L 以上で，HCV 抗体陽性率・HCV コア抗原陽性率は有意（p＜0.001）に上昇した．

考　察

2003 年末現在の日本における慢性透析患者での HCV 抗体陽性患者数は 20,864 人で，HCV-RNA 定性検査が施行された患者数は 6,526 人（31.3%）と低率となっている[1]．感染症マニュアルでは，透析施設での水平感染の予防目的で HCV 抗体を年に 2 回測定し，感染性有無の判断のため，抗体陽性患者全員に HCV-RNA 定性検査の施行を推奨している．

本研究の患者 1,077 例の HCV 抗体陽性者は 151 例（14.0%）であり，感染マニュアルに従うと HCV 抗体陽性患者の感染性を評価するために，HCV 抗体陽性者 151 例全員について HCV-RNA 検査を必要とした．しかし，本サーベイランス法では，HCV-RNA 検査が必要とされるのは 27 例と，HCV-RNA 検査を 82.1% も減少することができた．結果報告までの時間や検査費用を考慮すると，本サーベイラン

ス法は有用であり，普及させるべきと考えられた．

本サーベイランス法では，1,077例中2例の不一致例を認めた．高力価の患者1例，中力価・HCV抗原陽性の患者1例がHCV-RNA定性陰性であった．その原因として，C型肝炎の自然経過でHCVが陰性化し，HCV抗体価が低下してくる過程であると考えられた．本サーベイランスを採用すれば，安価な検査費でHCV感染の有無とその感染性を高い確率で診断が可能となり，透析施設における院内感染予防上，有用な方法である．

非腎不全患者のHCV抗体陽性率は年齢とともに上昇することが明らかとなっているが，血液透析患者は年齢と逆相関し透析歴と正相関することが明らかとなった．血液透析患者の透析導入平均年齢は年々上昇しており65.76歳と高齢である[15]．透析歴が短期の群では，長期の群と比較しHCV抗体の陽性率は低率であり，この低率の群が高年齢の群と重なることが逆相関する要因の1つと考えられた．

1989年より輸血製剤のHCV抗体のスクリーニングが開始され，輸血によるHCV感染の機会が減少し，1990年よりエリスロポエチン製剤が保険適用となり，輸血の機会も減少した．HCV抗体のスクリーニング前・エリスロポエチン製剤が保険適用前の長期透析患者はHCV抗体陽性率が高率であり，輸血歴あり群と輸血歴なし群のHCV抗体陽性率は有意差を認めたことから，長期血液透析患者の高いHCV抗体陽性率は輸血が原因と考えられた．しかし，透析歴が短期の群においても，非慢性腎不全患者よりHCV抗体陽性率は高率であり，透析歴とともに抗体陽性率が上昇することから輸血以外の感染の可能性が示唆された．

以前より透析患者のALT値は低値であることが知られている．原因として，透析患者の免疫能の低下，透析患者のビタミンB6の低下，透析による肝細胞増殖因子の上昇，透析によるウイルス量の減少などが考えられている[16)~22)]．本研究においても，慢性血液透析患者のALT値はHCV抗体陽性・陰性に関わらず，非腎不全患者の正常値内の値であった．しかし，陽性患者群のALT値は有意に上昇していた．非腎不全患者ではALT値が高値を示す集団では，HCVに感染している頻度が高いことが知られている[10]．本研究でのALT値とHCV抗体陽性率・HCV抗原陽性率の関係では，ALT 20U/L以上で陽性率が有意に上昇することから，正常値内の上昇であっても，血液透析患者はALT 20U/L以上で肝炎の可能性を疑う必要性がある．

結　語

HCV感染は慢性血液透析患者の予後を決定する重要な因子の一つであることが明らかとなっている[23)]．効率的なサーベイランス法である本法の普及が，キャリアの把握，新規感染者の発見に寄与するものと考えられる．感染対策によるHCVの拡大防止，キャリアの把握でC型肝炎の早期治療が可能となり，透析施設，患者の双方に貢献できると考えられた．

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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

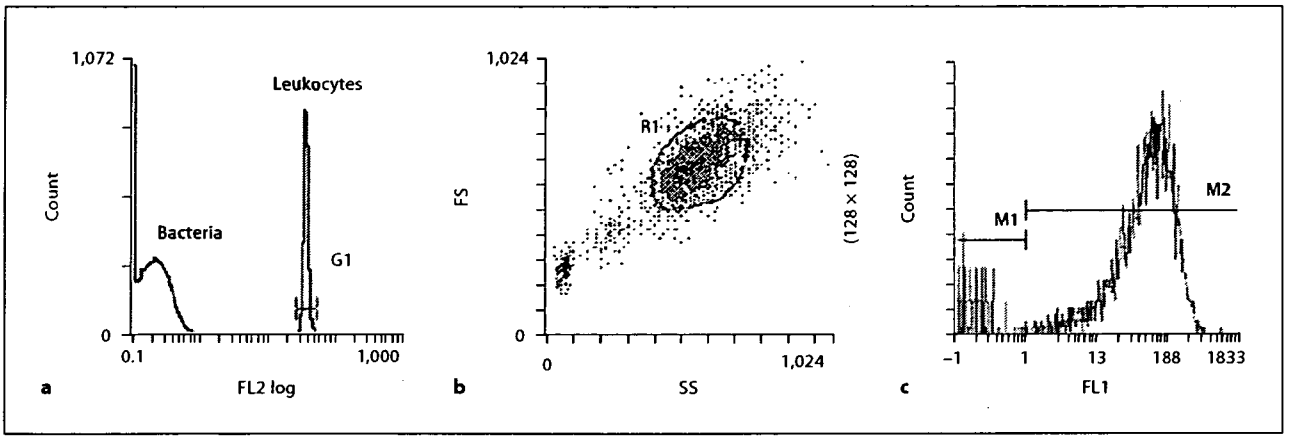

Fig. 1. Flow cytometric analysis of phagocytosis in polymorphonuclear leukocytes (PMNLs). During data acquisition, a 'live' cell gate (G1) is set in the FL2 fluorescence histogram on those events which have at least the same DNA content as a human diploid cell in order to exclude bacterial aggregates 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.

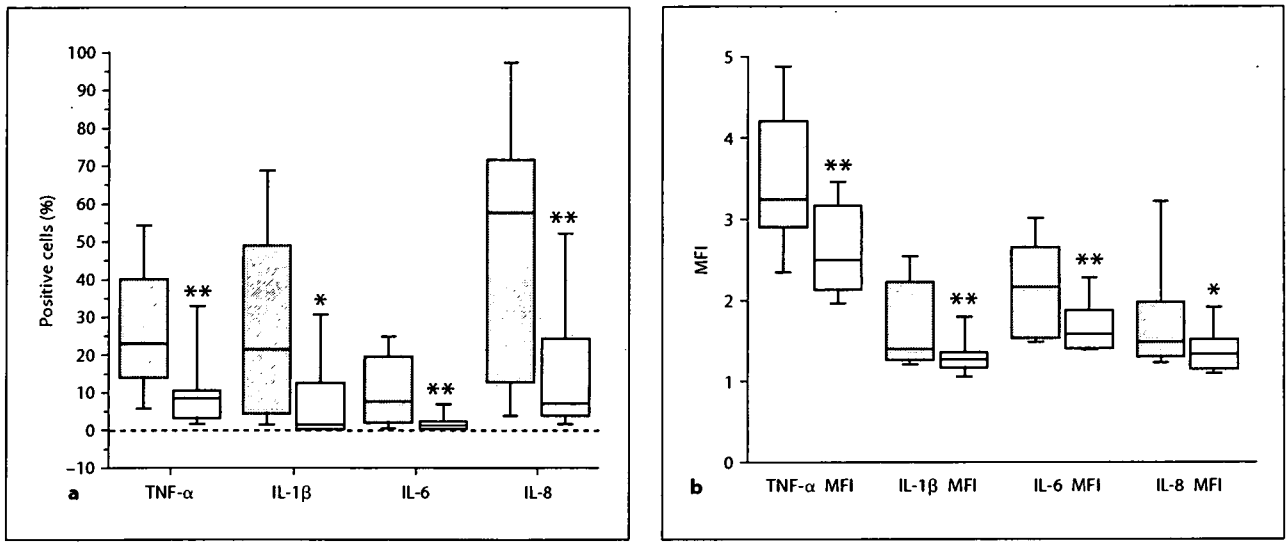

Fig. 2. Intracellular cytokine synthesis by monocytes from patients with chronic kidney failure (CKD) and control subjects (CONT) in response to ex vivo LPS stimulation. The intracellular cytokine synthesis is shown by the percentage of cells that are positive for each cytokine antibody (**a**). Their 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.

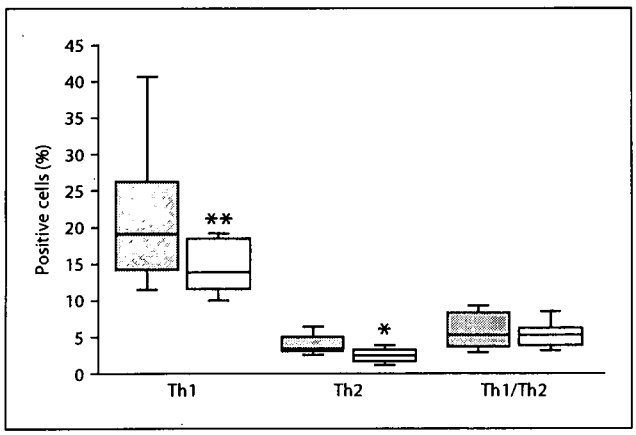

Fig. 3. Polarization of the helper T cells in patients with chronic kidney failure (CKD) and control subjects (CONT) in response to ex vivo PMA-ionomycin stimulation. Intracellular synthesis of IFN-γ and IL-4 was assessed in order to differentiate helper T type 1 (Th1) and helper T type 2 (Th2) cell 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).