Journal of Clinical Laboratory Analysis 16:40–46 (2002)

Cytokine Serum Levels in Patients With Chronic HCV Infection

Nick E. Spanakis,[1]* George A. Garinis,[1] Evangelos C. Alexopoulos,[2] George P. Patrinos,[3] Panayotis G. Menounos,[3] Alexandra Sklavounou,[4] Evangelos N. Manolis,[5] Vassilis G. Gorgoulis,[6] and Dimitrios Valis[1]

[1]Medicanalysis Institute of Molecular Biology Applications, Athens, Greece
[2]Department of Hygiene and Epidemiology, School of Medicine, University of Athens, Athens, Greece
[3]Nursing Military Academy, Laboratory of Research, Athens, Greece
[4]Department of Oral Pathology, Faculty of Dentistry, University of Athens, Athens, Greece
[5]Department of Histology, School of Nursing, University of Athens, Athens, Greece
[6]Department of Histology and Embryology, School of Medicine, University of Athens, Athens, Greece

The pathogenic role of immune-mediated mechanisms in chronic hepatitis C virus (HCV) infection has not yet been elucidated. In this study, we report different cytokine expression profiles from hemodialysis (HD) and non-HD HCV (+) patients. IL-1β, IL-2, IL-4, IL-6, TNF-α, and TGF-β1 serum levels, and liver biochemical parameters were determined in 85 individuals (41 HD patients and 44 non-HD patients). Screening for HCV RNA and anti-HCV antibodies was performed using qualitative and quantitative reverse transcription polymerase chain reaction (RT-PCR), and standardized enzyme-linked immunosorbent assay (ELISA) and recombinant immunoblot assay (RIBA) methods, respectively. IL-4 and IL-1β demonstrated decreased serum levels in non-HD HCV carriers compared with healthy controls. Both T helper (Th) 1 and Th2 lymphocytes were highly associated with chronic HCV infection, as indicated by the increased IL-2, IL-4, and IL-6 cytokine circulating levels in all chronic active hepatitis (CAH) patients examined. An enhanced Th2 response (IL-4 and IL-6) coupled with increased TNF-α and IL-1β serum levels was reported in HD HCV (–) patients. In conclusion, our data show that a virus-induced Th2 and IL-1β immunosuppression is an early event in HCV-related chronicity. Long-term HD specifically exerts a chronic effect on IL-6, IL-1β, and TNF-α serum circulating levels. Irrespective of the HD status, HCV viremia, and liver biochemistry parameters, both Th1 and Th2 responses are highly associated with chronic HCV infection. J. Clin. Lab. Anal. 16:40–46, 2002.

Key words: hepatitis C virus; interleukin; serum; transforming growth factor-β1; tumor necrosis factor-α; viremia

INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded, positive-sense RNA species that is now recognized as the major etiologic agent of non-A, non-B hepatitis (1). HCV infection is found to induce chronic active hepatitis (CAH) in approximately 50–70% of patients, while a significant proportion of them progress to cirrhosis and hepatocellular carcinoma (2). Hemodialysis (HD) patients may be at a higher risk of acquiring HCV infection, since the prevalence of anti-HCV antibody in these patients is consistently higher than in healthy individuals (3).

Although the exact mechanism responsible for hepatocellular damage is not known, cellular immunity-mediated mechanisms are believed to play an important role in chronic hepatitis C (4). Two subpopulations of murine CD4+ T-helper lymphocytes, termed T-helper type (Th) 1 and Th2, have been previously identified according to the different cytokine expression profiles that were originally described in mouse T-cell clones (5). Th1 cells generate cell-mediated and cytotoxic T lymphocyte responses promoted predominantly by interleukin (IL)-2, interferon gamma (IFN-γ), and tumor necrosis factor (TNF)-α. Th2 cells produce cytokines, such as

Grant sponsor: Medicanalysis Institute of Molecular Biology Applications.

George P. Patrinos is now at the Department of Cell Biology and Genetics, Faculty of Medicine, Erasmus University Rotterdam, Rotterdam, The Netherlands.

*Correspondence to: Nick E. Spanakis, Medicanalysis Institute of Molecular Biology Applications, Fleming 18, GR-151 26 Nea Filothei, Athens, Greece. E-mail: Valisdial@hol.gr

Received 14 May 2001; Accepted 12 October 2001

DOI 10.1002/jcla.2060

IL-4 and IL-6, which mediate the humoral response. Th1 cellular immune responses, networked by their elaborated cytokines, are associated with resistance to infection, while Th2-related cytokines exhibit negative immunoregulatory functions (6,7). In addition, some of the structurally unrelated cytokines examined in this study may often display common properties (8), while others seem to participate (in a synergistic or antagonistic manner) in overlapping pathways (9), thereby justifying their overall study.

From different lines of evidence, it seems plausible that a shift in the balance between Th2 and Th1 immune responses may play a role in the development of HCV chronicity (4,7). However, few studies have described the effect of long-term HD on the circulating levels of these cytokines, or on the levels of IL-2, IL-4, and transforming growth factor (TGF)-β1 in HD patients infected with HCV. In view of the above observations, we investigated the role of IL-1β, IL-2, IL-4, IL-6, TNF-α, and TGF-β-l in the pathogenesis of type C chronic liver disease by examining their serum levels in HD and non-HD patients with HCV-related CAH of varying activity.

MATERIALS AND METHODS

Patients and Controls

Twenty-one HCV (+) HD and 24 HCV (+) non-HD patients (22 males and 23 females; mean age: 55, range: 43–69) were included in this study. They were selected under the following criteria: all were positive for HCV RNA and were diagnosed histologically during approximately the same period for chronic HCV infection. The Scheuer liver histology score was used for histopathological examination to discriminate active from non-active liver involvement (10). The various groups of HCV (+) patients (with or without CAH, on HD or not) were categorized histologically as follows: HD HCV (+) and non-HD patients with CAH were characterized for portal/periportal activity with a mild (n = 9 (4 HD and 5 non-HD)), moderate (n = 12 (5 HD and 7 non-HD)), or piecemeal necrosis (n = 1 HD); and for lobular activity with focal necrosis or acidophilic bodies (n = 21 (10 HD and 11 non-HD)), severe focal cell damage (n = 1 (non-HD)), and damage with bridging necrosis (n = 0). HD HCV (+) and non-HD carriers included either asymptomatic patients (APs) (n = 4) who exhibited normal serum ALT levels (<45 U/L for >1 year) and/or patients with minimal symptoms (elevated serum ALT levels for >6 months) of chronic persistent hepatitis. These patients were characterized with no or minimal portal/periportal and/or lobular inflammation. No biopsy data are available for AP-HCV carriers (in HD or not). Patients with active infection (positive for hepatitis B surface antigen, HIV, and systemic vasculitis) were excluded. No alcohol-, allergy-, or drug-induced (immunostimulating and/or immunosupppressive-treated) hepatitis, autoimmune hepatitis, or previous transplantation cases were included in this study. Selected patients who met the inclusion criteria were divided into the following categories: 1) HD CAH patients (n = 10); 2) HD HCV (+) carriers (n = 11); 3) non-HD CAH patients (n = 12); and 4) non-HD HCV (+) carriers (n = 12).

Determination of Serum Levels for IL-1β, IL-2, IL-4, IL-6, TNF-α, and TGF-β1

For the cytokine quantitative assays, 20 HCV (–) HD patients (11 females and 9 males; age: 35 ± 5) and 20 healthy individuals (10 females and 10 males; age: 37 ± 6) without a clinical history of hepatitis and without symptoms or signs of liver disease were selected as negative controls for HD HCV (+) and non-HD HCV (+) groups, respectively. All blood samples were centrifuged and collected sera were stored at –20°C for up to 1 week. All HD patients had an internal vascular a-v fistula as vascular access. Each HD session had an average duration of 4 hr 30 min. New dialyzers were employed in each case. No critical adverse reactions to the HD procedure were observed. All HD patients had been treated by means of the same type of membrane (Hemophan, Bellco, Italy). To study the long-term effect of HD on the cytokine circulating levels, sera from HD patients were collected before the first HD session of the week. Determination of serum levels (pg/ml) of IL-1β, IL-2, IL-4, IL-6, TNF-α, and TGF-β1 was performed, according to the assay's protocol (PREDICTA®; Genzyme Diagnostics, Cambridge, MA). Mean yearly values of hematocrit (Hct), were obtained for each HD patient. In the case of HD patients, a relatively large interindividual Hct variability (–3.6% to 0.5%) has been observed, and the mean Hct of HD HCV (+) patients did not differ significantly from that of HD HCV (–) patients ($P > 0.05$). There are no data available for non-HD patients of either group.

Detection of Anti-HCV Antibodies, HCV RNA Qualitative and Quantitative Determination, and Liver Biochemistry Parameters

Serum samples were routinely tested three consecutive times during the study for the presence of anti-HCV antibodies by MONOLISA HCV New-Ag (MONOLISA® HCV PLUS; Sanofi Diagnostics Pasteur, Inc., Chaska, MN), and weakly positive or indeterminate results were confirmed by RIBA 3.0. The procedure was described in detail in our previous work (11). HCV reverse transcription polymerase chain reaction (RT-PCR) qualitative (Amplicor® HCV Test, Roche Diagnostic Systems, Basel, Switzerland) and quantitative (HCV-Monitor® Test, Roche Diagnostic Systems) determinations were performed with previously described (11,12) standardized protocols. Results were expressed as viral copies/ml of human serum (lower detection limit: ~10^2 viral copies/ml of human serum). The duration of infection for non-HD patients should be considered as indicative since

the actual duration of infection could be longer than what was estimated. Measurement of ALT, AST, and γ-GT was performed immediately after collection in nonhemolyzed fresh serum, collected from all patients by means of a screening photometer. Results were reported in U/L.

Statistical Analysis

Comparisons of mean values of cytokines in various subgroups were examined by nonparametric Mann-Whitney U-test statistics. We used a 0.05 alpha level, but we multiplied all the single-association *P*-values by the number of associa-

TABLE 1. HCV (+) patients' viral profile and biochemistry data

No.	Sex/ age	VL (no. copies/ml)	γ-GT (U/L) (11–50)	ALT (U/L) (0–42)	AST (U/L) (0–42)	DI[a] (yrs)	DD (yrs)
HCV (+) HD CAH patients							
1	M/45	700,000	33	122	135	4	10
2	M/65	<2,000	62	100	119	5	7
3	M/54	500,000	68	78	121	6	5
4	M/57	125,000	27	50	72	4	8
5	M/51	200,000	25	60	123	5	7
6	F/57	130,000	30	36	89	3	9
7	F/45	<2,000	51	131	34	6	12
8	F/47	100,000	52	78	120	5	9
9	F/43	200,000	43	120	121	4	8
10	F/54	150,000	30	90	70	7	9
HCV (+) HD carriers							
1	F/66	500,000	33	41	15	8	10
2	F/45	190,000	34	30	34	3	8
3	M/65	250,000	41	56	13	4	7
4	F/55	80,000	13	706	32	2	9
5	F/50	250,000	43	89	24	5	7
6	F/69	100,000	12	78	15	6	7
7	F/45	<2,000	11	54	11	4	9
8	M/64	150,000	26	37	25	5	9
9	M/51	40,000	11	45	26	6	10
10	M/55	50,000	27	56	35	4	9
11	M/53	150,000	26	100	22	5	9
Non-HD CAH patients							
1	F/55	200,000	53	124	120	6	–
2	M/54	100,000	26	36	24	4	–
3	M/57	<2,000	22	128	122	3	–
4	F/64	120,000	19	130	113	6	–
5	F/65	500,000	11	146	21	5	–
6	F/45	100,000	25	29	110	7	–
7	M/56	150,000	14	145	90	4	–
8	M/66	<2,000	19	25	124	9	–
9	M/55	100,000	21	134	58	3	–
10	F/45	150,000	34	79	115	2	–
11	F/55	200,000	32	45	92	2	–
12	F/51	100,000	20	80	111	10	–
HCV (+) non-HD carriers							
1	F/56	100,000	18	67	46	4	–
2	M/54	50,000	11	56	45	3	–
3	F/45	50,000	23	43	26	2	–
4	M/44	20,000	13	66	49	5	–
5	M/55	80,000	14	52	25	6	–
6	F/67	10,000	16	45	32	5	–
7	F/66	12,000	11	71	51	3	–
8	F/45	500,000	18	52	55	8	–
9	M/65	100,000	15	33	46	7	–
10	M/55	150,000	12	68	55	6	–
11	M/65	100,000	21	87	53	5	–
12	M/45	500,000	22	90	45	4	–

VL, viral load; DI, duration of infection; DD, duration of dialysis.

[a]In case of HD patients duration of infection was estimated from the initial detection of anti-HCV antibodies.

TABLE 2. Mean values of the serum levels (in pg/ml) of IL-2, IL-4, IL-6, IL-1β, TGF-β1 and TNF-α in subgroups of patients (HD and/or non-HD) with HCV-related hepatitis of varying activity

Subgroups	IL-2	IL-4	IL-6	IL-1β	TGF-β1	TNF-α
A Healthy individuals	45.8	65.0	49.5	22.0	73.0	61.2
B Non-HD HCV	36.3	40.8	45.4	14.3	92.7	51.7
C HD HCV (–)	53.8	77.4	92.0	51.5	98.6	131.2
D HD HCV (+)	69.5	76.9	82.8	35.0	145.2	104.8
E Non-HD CAH	95.0	98.1	76.0	14.9	162.7	102.9
F HD CAH	100.1	124.2	147.8	57.0	250.2	193.4

tions (36, given the six groups and six cytokines) before comparing them to the alpha level. This procedure is equivalent to the Bonferroni method of adjusting for multiple comparisons. Linear regression analysis was used to investigate the relationship between covariates (HCV viremia, liver histology, and liver biochemical parameters) and cytokine serum levels. The statistical analyses were performed with the SPSS for Windows 95 statistical package.

RESULTS

Prevalence of HCV RNA (+) and Anti-HCV (+) Patients

A total of 45 patients (21 HD and 24 non-HD) were screened three consecutive times and were reported as anti-HCV (+) by means of ELISA II and subsequent confirmation by RIBA 3.0. These patients were also tested for HCV RNA and found to be HCV (+). The HCV viral load (number of HCV copies per ml of human serum) was measured twice and remained practically constant during an 18-month period (Table 1).

Serum Levels of Cytokines IL-1β,IL-2, IL-4, IL-6, TGF-β1, and TNF-α

Non-HD HCV (+) carriers and non-HD CAH patients

Non-HD HCV (+) carriers exhibited decreased IL-4 and IL-1β serum levels compared with healthy individuals. Non-HD CAH cytokine serum levels demonstrated (with the exception of IL-1β), significantly higher levels compared with non-HD HCV (+) carriers. However, these findings were different (in the case of IL-2, TGF β1, and IL-1β) when we compared non-HD CAH with HD CAH patients (Table 2). The remaining comparisons between these groups and others are listed in Table 3.

TABLE 3. *P* values, obtained by Mann-Whitney-U test (two-tailed significance) and corrected with Bonferroni test

C	IL-2	IL-4	IL-6	IL-1β	TGF-β1	TNF-α
C vs. F	0.000	0.000	0.000	**0.172**	0.000	0.000
C vs. D	**0.016**	**0.869**	**0.072**	**0.005**	0.000	0.002
A vs. E	0.000	0.000	0.000	0.000	0.000	0.000
A vs. B	**0.007**	0.000	**0.161**	0.000	**0.035**	**0.041**
E vs. F	**0.337**	0.000	0.000	0.000	**0.002**	0.000
B vs. D	0.001	0.000	0.000	0.000	0.000	0.000
D vs. F	0.000	0.000	0.000	0.001	0.000	0.000
B vs. E	0.000	0.000	0.000	**0.908**	0.000	0.000

Not significant *P* values are in bold.

HD HCV (+) carriers and HD CAH patients

Increased TGF β1 serum levels were reported in HD HCV (+) compared with HD HCV (–) patients. However, when HD HCV (+) cytokine data were judged against those from non-HD HCV (+) patients, all cytokine serum levels showed significantly increased levels (see Table 3). HD CAH patients' cytokine serum levels exhibited (with the exception of IL-1β), significantly higher levels compared with HD HCV (+) carriers. These findings displayed a different profile when HD CAH cytokine serum levels compared with HD HCV (–) and non-HD CAH patients. Differences between these two groups of patients were observed in IL-2 and TGF-β1, and in IL-1β, respectively.

Patient demographics data

With respect to age, gender, known duration of liver disease, and levels of serum ALT, AST, γ-GT, and HCV RNA, there was no significant correlation to the amounts of cytokine levels.

DISCUSSION

The aim of this study was to assess whether: 1) a cytokine imbalance, oriented toward a Th1- or Th2-type response (including cross species), plays a role in chronic hepatitis C; and 2) the reported cytokine profile was actually differentiated in case of those HCV (+) patients enrolled in long-term HD. Within this context, we investigated the role of IL-1β, IL-2, IL-4, IL-6, TNF-α, and TGF-β1 by measuring their peripheral circulating levels in the sera of HD and non-HD patients with CAH C of varying activity.

Our results demonstrated decreased IL-4 serum levels coupled with a decreased IL-1β response in non-HD HCV carriers. These findings suggest that the aforementioned responses comprise an early event in HCV-related chronicity and support the hypothesis for a selective virus-induced immunosuppression (8). Thus, IL-4- and IL-1β-impaired production may actually contribute to the progression of HCV persistence, notwithstanding the presence of immune defense mechanisms such as cytotoxic killer cells and antibody directed against HCV. Alternatively, the lack of a uniform Th2 response among the various Th2 type cytokines reported in non-HD HCV carriers indicates that in vivo responses may differ among the various HCV antigens, although the decreased levels of IL-4 and IL-1β could actually represent a systemic response rather than local production within the liver.

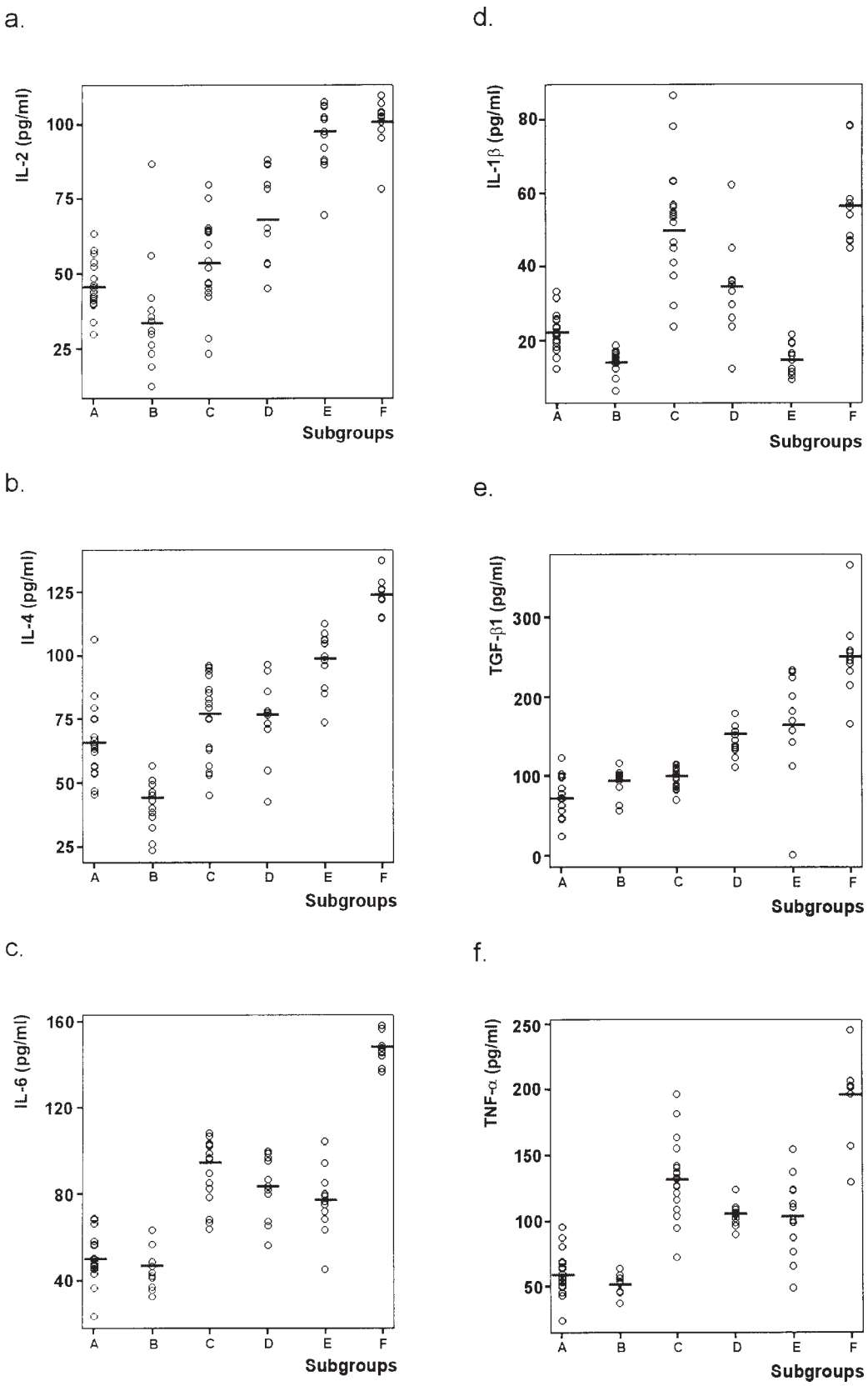

Fig. 1. IL-2, IL-4, IL-6, IL-1β, TGF-β1, and TNF-α in subgroups of patients (HD and/or non-HD) with HCV-related hepatitis of varying activity. Subgroups: (**A**) healthy individuals, (**B**) non-HD HCV, (**C**) HD HCV (–), (**D**) HD HCV, (**E**) non-HD CAH, and (**F**) HD CAH.

In HCV carriers enrolled in long-term HD, the predominance of TGF-β1 response was more profound (Table 3). It is possible that the difference in patient etiology and the effect of long-term HD through endotoxin contamination (13), quality of dialysate (14), and membrane characteristics (15) may mask or alter the initial cytokine profile.

Interestingly, when we attempted to compare TNF-α and IL-1β serum levels in HD CAH patients vs. HD HCV carriers, these cytokines, which were previously shown to share common biological activities (16), demonstrated similar immunoserological profiles (Table 2 and Fig. 1). This was also the case when HD HCV carriers were compared with HD HCV (–) patients. However, when we compared non-HD CAH patients and non-HD HCV carriers, TNF-α and IL-1β did not parallel each other. This was also confirmed when TNF-α and IL-1β serum levels from non-HD HCV carriers were judged against healthy controls. Our findings are in accordance with those reported by Mege et al. (17). Furthermore, these authors showed that the effect of TNF-α and IL-1β is directly related to the type of membrane (Hemophan). Since the membrane type used in our study was also of the Hemophan type, our results are in line with the hypothesis that the parallel TNF-α and IL-1β response probably reflects an HD-dependent, membrane-specific behavior. However, it should be noted that IL-1β often presents very large variations, even in normal persons, and the possibility of the true mean value of IL-1β being measured inaccurately cannot be neglected (17). Otherwise, for these cytokines, several effects exerted by the long-term HD procedure as described above (13–15) may account, to a certain extent, for their relevant circulating levels.

The role of the Th1 and/or Th2 type of cytokine secretion in the evolution from acute to chronic HCV infection has not yet been elucidated. A study by Cribier et al. (18) indicated that Th1 type lymphocytes predominate in chronic HCV infection. In our study, we did not observe a particular Th1 or Th2 response, and both types of $CD4^+$ T-helper lymphocytes were significantly associated with chronic HCV infection, as indicated by the increased IL-2, IL-4, and IL-6 production in all CAH patients examined (on HD or not). IL-4 usually behaves as an antiinflammatory and inhibitory cytokine to the lymphocyte-monocyte network (9). The fact that in our study IL-4 paralleled the other proinflammatory cytokine levels may reflect a secondary adaptive response to the action of proinflammatory cytokines in HCV (+) patients.

Although several investigations have shown a positive correlation of ALT levels with liver injury progression, a normal ALT level does not exclude viral replication and/or liver damage, and does not correlate with postviral clearance (19). In our study, normal ALT/AST/γ-GT levels did not exclude chronic HCV infection and/or elevated cytokine levels, supporting the observation that biochemical parameters alone may not adequately inform about disease severity (20). In fact, there is a need for additional biomarkers that could more precisely describe host–virus interactions. Furthermore, neither the cytokines nor the biochemical markers examined in this study correlated with the hepatitis C viral load.

In conclusion, our data provide evidence that a virus-induced Th2 and IL-1β immunosuppression is an early event in HCV-related chronicity. In addition, long-term HD specifically exerts a chronic effect on IL-1β and TNF-α serum circulating levels, while irrespective of the HD status, HCV, viremia, and liver biochemistry parameters, both Th1 and Th2 are highly associated with progressive chronic HCV infection.

ACKNOWLEDGMENTS

The authors are indebted to V. Theodorou and T. Ioannides for outstanding technical assistance.

REFERENCES

1. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244:359–362.
2. Di Bisceglie AM. Hepatitis C and hepatocellular carcinoma. Hepatology 1997;26(3 Suppl 1):34S–38S.
3. Pereira BJ. Hepatitis C virus infection in dialysis: a continuing problem. Artif Organs 1999;23:51–60.
4. Ferrari C, Penna A, Bertoletti A, et al. Antiviral cell-mediated immune responses during hepatitis B and hepatitis C virus infections. Recent Results Cancer Res 1998;154:330–336.
5. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136:2348–2357.
6. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 1996;17:138–146.
7. Lucey DR, Clerici M, Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev 1996;9:532–562.
8. Eckels DD, Tabatabail N, Bian TH, et al. In vitro human Th-cell responses to a recombinant hepatitis C virus antigen: failure in IL-2 production despite proliferation. Hum Immunol 1999;60:187–199.
9. Varano B, Fantuzzi L, Puddu P, Borghi P, Belardelli F, Gessani S. Inhibition of the constitutive and induced IFN-beta production by IL-4 and IL-10 in murine peritoneal macrophages. Virology 2000;277:270–277.
10. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–1519.
11. Garinis G, Spanakis N, Theodorou V, et al. Comparison of the enzyme-linked immunosorbant assay III, recombinant immunoblot third generation assay, and polymerase chain reaction method in the detection of hepatitis C virus infection in haemodialysis patients. J Clin Lab Anal 1999;13:122–125.
12. Garinis G, Patrinos GP, Menounos P, et al. Evaluation of a minipool reverse transcription-PCR screening method for the detection of hepatitis C virus infection in hemodialysis patients. Clin Chem 2000;46: 583–584.
13. Pearson FC, Dubczak J, Weary M, Anderson J. Determination of endotoxin levels and their impact on interleukin-1 generation in continuous ambulatory peritoneal dialysis and hemodialysis. Blood Purif 1988;6:207–212.
14. Port FK, Vandekerkhove KM, Kunkel SL, Kluger MJ. The role of dialysate in the stimulation of interleukin-1 production during clinical hemodialysis. Am J Kidney Dis 1987;2:118–122.

15. Haeffner-Cavaillon N, Cavaillon JM, Ciancioni C, Bacle F, Delons S, Kazatchkine MD. In vivo induction of interleukin-1 during hemodialysis. Kidney Int 1989;35:1212–1218.
16. Dinarello CA. Interleukin-1. Cytokine Growth Factor Rev 1997;8: 253–265.
17. Mege JL, Olmer M, Purgus R, et al. Haemodialysis membranes modulate chronically the production of TNF alpha, IL1 beta and IL6. Nephrol Dial Transplant 1991;6:868–875.
18. Cribier B, Schmitt C, Rey D, Lang JM, Kirn A, Stoll-Keller F. Production of cytokines in patients infected by hepatitis C virus. J Med Virol 1998;55:89–91.
19. Alter HJ, Conry-Cantilena C, Melpolder J, et al. Hepatitis C in asymptomatic blood donors. Hepatology 1997;26(3 Suppl 1):29S–33S.
20. Puoti C, Stati T, Magrini A. Serum HCV RNA titer does not predict the severity of liver damage in HCV carriers with normal aminotransferase levels. Liver 1999;19:104–109.