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The effect of cytokine profiles on the viral response to re-treatment in antiviral-experienced patients with chronic hepatitis C virus infection

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ABSTRACT

Background: There have been few studies on the potential immunological factors associated with viral controls in antiviral-experienced patients on a second round of combination therapy. In this study, we evaluated the level of systemic cytokines and potential impact on combination therapy in both antiviral-naïve and -experienced patients chronically infected with hepatitis C virus.

Methods: Longitudinal analysis of 27 cytokines and chemokines was performed using the multiplex Biorad 27 plex assay in 37 antiviral-naïve and 24 experienced chronically HCV-1b-infected patients during combination therapy with peginterferon-alfa and ribavirin. A group of healthy donors was included as the control ($n = 11$).

Results: Fifty percent of antiviral-experienced chronically HCV-patients could achieve a delayed and slow virologic response after 48 weeks combination therapy, comparing with an early and fast virologic response in antiviral-naïve patients. A distinction of immune mediators profiling before and during antiviral therapy between antiviral-naïve and -experienced patients was identified, IL-4, IFN-γ and CCL-3 (MIP-1a) were significantly higher in naïve patients than those in experienced patients ($P = 0.005$, 0.047 and 0.017, respectively) while G-CSF in naïve was lower than in experienced patients ($P < 0.05$). Notably, higher Th1 type cytokine IFN-γ and lower Th2 type cytokine IL-4 at baseline and week 4 were associated with HCV clearance in naïve patients, and a similar trend appeared at week 12 in experienced patients.

Conclusions: We found a successful second round therapy in antiviral-experienced patients appears to be associated with the host immune response. Dominant Th1-polar cytokines, especially IFN-γ, is a potential predictor of viral responsiveness.

1. Introduction

Hepatitis C virus (HCV) infection is a worldwide epidemic with approximately 170 million infected individuals (Lauer and Walker, 2001; Poynard et al., 2003). Seventy to eighty-five percent of individuals do not spontaneously resolve acute HCV infection, resulting in chronic infection with the potential to develop cirrhosis, end-stage liver disease and hepatocellular carcinoma (Inchauspé et al., 2008). Combination therapy with peginterferon-alfa and ribavirin is currently the standard antiviral therapy for chronic HCV leading to long-term resolution of infection in only 45–80% of individuals depending on the viral genotype (Fried et al., 2002; Hadziyannis et al., 2004; Manns et al., 2001). Despite significant advances in treatment efficacy, non-virological responders remain a large part of the population, and are becoming the main population of chronically HCV-infected patients, especially in China and India, where the majority of patients are infected with HCV genotype 1 and 4 (Chen et al., 2002; Raghuraman et al., 2004). Consequently, understanding of the efficacy and factors affecting antiviral therapy in antiviral-experienced patients is necessary to evaluate and optimize current treatment strategies.

The factors that determine the outcome of therapy are poorly understood, but host genetics, viral subtypes and high viral loads have significant effects on their virological response (Ge et al., 2009; Ghany et al., 2009; Thomas et al., 2009). Some studies suggest that HCV clearance may be mediated by a combination of

Abbreviations: HCV, hepatitis C virus; RVR, rapid virologic response; EVR, early virologic response; ETVR, end-of-treatment virologic response.

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direct antiviral effects and stimulation of immune function during therapy (Neumann et al., 1998). Furthermore, the levels of cytokines/chemokines in peripheral blood may be a predictor of responsiveness to antiviral therapy with peginterferon-alfa and ribavirin. For example, cytokines induced by IFN-γ (MIG) (Shields et al., 1999), IFN-γ-inducible protein 10 (IP-10) (Narumi et al., 1997) and IFN-inducible T-cell α chemoattractant (I-TAC) (Helbig et al., 2004) may predict the efficacy of treatment (Butera et al., 2005). While most of studies were focused on antiviral-naïve patients, there were little data on antiviral-experienced patients, who were re-treated with combination therapy with peginterferon-alfa and ribavirin.

Understanding the difference of cytokines/chemokines between antiviral-naïve and antiviral-experienced patients before, during and after antiviral therapy is of great importance in the analysis of the effectiveness and optimization of current treatment strategies. In order to address this issue, we performed a prospective longitudinal study to identify the distinction of cytokines/chemokines in peripheral blood between antiviral-naïve and antiviral-experienced patients infected with hepatitis C genotype 1b before and during therapy for 4 and 12 weeks with peginterferon-alfa and ribavirin, using the multiplex Biorad 27 plex assay to evaluate 27 cytokines.

2. Materials and methods

2.1. Patients

Sixty-one HCV genotype 1b-infected patients (37 antiviral-naïve and 24 antiviral-experienced), who were participating in a clinical study designed to assess the efficacy of the treatment regime described in detail below, were enrolled for the study. Ethical approval was obtained from Beijing You'an Hospital. All patients were negative for HIV and hepatitis B virus antibodies and underwent combination therapy with peginterferon-alfa 2a (180 μg) and ribavirin (10.6–15 mg/kg/day) for a total of 48 weeks (National Institutes of Health Consensus Development Conference Statement, 2002). Control subjects included 11 self-reported healthy volunteers, who were HCV, HBV, and HIV antibody negative. Clinical and demographic characteristics of these patients have been detailed in Table 1. Serial blood samples were collected before treatment (week 0), at week 4 and 12 after the start of treatment. Serum was frozen at -20 °C.

2.2. Viral load

HCV RNA was measured in serum using the qualitative Roche COBAS Amplicor assay (version 2.0; Roche Molecular Systems. Lower limit of detection: 50 IU/ml).

2.3. Clinical definitions

Clinical definitions are based on viral clearance. Rapid virologic response (RVR) was defined as an undetectable serum HCV RNA level (<50 IU/ml) at week 4 of therapy. Early virologic response (EVR) was defined as at least a 2-log reduction in serum HCV RNA level from baseline to week 12 of antiviral therapy. End-of-treatment virologic response (ETVR) was defined as an undetectable serum HCV RNA level at the end-of-treatment.

2.4. Measurements of cytokines and chemokines

Serum cytokine and chemokine levels were measured using Human Cytokine 27-plex assay kit (Bio-Rad, Hercules, CA) with Bio-Plex Manager software version 6.0 in Bio-Plex™ 200 system (Bio-Rad). This system allows quantitative measurement of 27 different chemokines, cytokines, growth factors and immune mediators, including IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17, Eotaxin, FGF basic, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1(MCAF), MIP-1a, PDGF-bb, MIP-1b, RANTES, TNF-α and VEGF, while consuming 12.5 μl-vol samples. Cytokines were evaluated according to the Manufacturer's instructions.

2.5. Statistical analysis

Cytokines/chemokines at each time point were compared with baseline (pretreatment) values using the paired-samples *T* test. Comparisons between patients with ETVR and without ETVR before and during treatment were made using the independent-samples *T* test. Bonferroni correction was performed in the multiple *T* test. Log-rank (Mantel–Cox) test was employed to illustrate the dynamic of HCV RNA and normalization of ALT. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Efficacy of combination therapy and different dynamics of HCV viral load between antiviral-naïve and -experienced patients

Overall, 12 (50%) out of the 24 antiviral-experienced chronic HCV patients could achieve a viral response after 48 weeks combination therapy, compared with 27 (72.97%) of the 37 antiviral-naïve patients who achieved a viral response at the end of 48-week therapy ($P = 0.068$). Furthermore, the difference of RVR (43.24% vs. 12.5%; $P = 0.011$) and EVR (70.27% vs. 41.67%; $P = 0.026$) between antiviral-naïve and -experienced patients indicated that antiviral experienced patients expressed a delayed and slow virologic response, while antiviral-naïve patients demonstrated an early and fast virologic response (Figs. 1 and 2a).

3.2. Different dynamics of ALT between antiviral-naïve and -experienced patients

The dynamics of ALT during antiviral therapy were compared between antiviral-naïve and -experienced patients, which demonstrated that the level of ALT in antiviral-naïve patients decreased from baseline (92.56 ± 113.9 IU/L) to the 32.05 ± 23.18 IU/L in 4 weeks, while the value of ALT in antiviral-experienced patients fluctuated at baseline level (56.32 ± 46.90 IU/L) until week 12, and then decreased to 34.03 ± 24.06 IU/L at week 24. Furthermore, Log-rank (Mantel–Cox) test illustrated a rapid biochemical response, notably normalization of ALT, in antiviral-naïve, compared with a slow and delayed biochemical response in experienced patients ($P = 0.0323$) (Figs. 2b and 3).

3.3. Different profiling of Immune mediators before therapy between antiviral-naïve and -experienced patients

HCV in chronic HCV patients, including antiviral-naïve and -experienced patients, induced a systemic elevation of 12 cytokines/chemokines, shown to be expressed before antiviral therapy; IL-1b, IL-4, IL-6, IL-7, IL-9, IL-13, FGF, GM-CSF, IFN-γ, CCL-2 (MCP-1), CCL-3 (MIP-1a), and CCL-4 (MIP-1β). Significant differences in the levels of these mediators were found between chronic HCV patients and healthy donors ($P < 0.05$, respectively), while G-CSF level in chronic HCV patients was lower than that in healthy controls ($P < 0.05$) (Table 2). Among these mediators, the distinction between antiviral-naïve and -experienced patients is highlighted in Fig. 4, in which IL-4, IFN-γ and CCL-3 (MIP-1a) were significantly higher in naïve patients than in experienced patients

Table 1
Variable virological responses of chronically HCV-infected patients during combination therapy between antiviral-naïve and -experienced patients.

Group	ETVR	Age	Gender	HCV RNA log(IU/ml)					
				0 week	2 weeks	4 weeks	12 weeks	24 weeks	48 weeks
Antiviral-naïve patients	With ETVR	39	M	6.29	ND	1.95	<LDL	<LDL	<LDL
		33	F	3.54	<LDL	<LDL	<LDL	<LDL	<LDL
		17	M	4.06	<LDL	<LDL	<LDL	<LDL	<LDL
		61	M	7.07	5.38	4.51	<LDL	<LDL	<LDL
		41	M	7.74	4.47	4.26	<LDL	<LDL	<LDL
		37	F	6.00	2.16	<LDL	<LDL	<LDL	<LDL
		53	F	6.39	3.40	<LDL	<LDL	<LDL	<LDL
		54	F	6.30	3.03	<LDL	1.34	<LDL	<LDL
		20	M	6.01	4.68	1.96	<LDL	<LDL	<LDL
		19	F	6.40	3.31	<LDL	<LDL	<LDL	<LDL
		20	M	6.22	5.21	<LDL	<LDL	<LDL	<LDL
		40	M	6.03	2.11	<LDL	<LDL	<LDL	<LDL
		17	M	5.31	<LDL	<LDL	<LDL	<LDL	<LDL
		18	F	7.30	4.83	3.08	<LDL	<LDL	<LDL
		57	F	6.32	3.98	<LDL	<LDL	<LDL	<LDL
		51	F	5.58	3.52	3.04	<LDL	<LDL	<LDL
		16	M	4.63	<LDL	<LDL	<LDL	<LDL	<LDL
		69	F	5.71	<LDL	<LDL	<LDL	<LDL	<LDL
		41	M	3.90	<LDL	<LDL	<LDL	<LDL	<LDL
		36	M	6.61	2.09	<LDL	<LDL	<LDL	<LDL
		51	F	5.95	5.65	4.68	<LDL	<LDL	<LDL
		56	F	3.37	3.01	2.25	<LDL	<LDL	<LDL
		39	M	6.78	4.69	1.72	<LDL	<LDL	<LDL
		20	M	7.40	4.68	3.50	<LDL	<LDL	<LDL
		54	F	7.16	2.40	<LDL	<LDL	<LDL	<LDL
		34	M	7.05	<LDL	<LDL	<LDL	<LDL	<LDL
		20	F	7.31	4.12	4.50	2.13	<LDL	<LDL
	Without ETVR	57	F	6.38	5.60	5.67	5.45	6.06	6.37
		57	F	6.17	6.37	5.84	5.66	5.05	5.34
		52	F	6.70	5.74	4.97	4.80	4.38	5.41
		41	M	7.03	4.68	4.54	5.00	4.93	4.81
		57	M	6.33	5.29	5.42	5.66	5.76	5.84
		17	M	7.07	ND	6.52	6.44	6.50	6.63
		53	M	5.60	6.47	6.15	6.53	6.18	5.81
		40	F	6.98	5.87	5.14	5.45	5.94	5.94
		16	M	7.00	6.99	6.57	6.47	6.51	6.50
		52	F	7.16	6.61	6.61	6.59	5.87	5.75
Antiviral-experienced patients	With ETVR	21	M	5.23	4.75	<LDL	<LDL	<LDL	<LDL
		37	F	5.67	4.23	3.00	<LDL	<LDL	<LDL
		50	M	7.06	4.24	3.83	<LDL	<LDL	<LDL
		54	F	7.22	5.37	4.65	<LDL	<LDL	<LDL
		50	M	6.83	5.10	3.87	<LDL	<LDL	<LDL
		46	F	6.51	6.25	5.42	<LDL	<LDL	<LDL
		55	M	6.03	6.47	5.54	<LDL	<LDL	<LDL
		32	M	5.31	4.94	4.08	<LDL	<LDL	<LDL
		59	M	8.10	5.30	4.37	<LDL	<LDL	<LDL
		31	M	4.33	4.05	<LDL	<LDL	<LDL	<LDL
		43	F	6.76	6.06	5.99	4.78	3.87	<LDL
		34	F	6.81	6.70	5.03	3.82	<LDL	<LDL
	Without ETVR	54	M	5.66	5.25	5.24	4.62	4.82	4.72
		62	M	6.78	6.03	6.55	6.22	6.37	6.32
		19	F	6.27	6.74	ND	6.80	6.84	6.48
		22	M	5.96	5.58	5.71	6.02	5.15	5.18
		18	M	7.12	6.58	6.87	5.58	5.92	5.73
		19	F	7.34	7.12	7.13	5.17	5.41	5.64
		46	F	6.50	6.11	6.20	6.38	6.35	6.23
		20	F	5.87	5.67	5.93	5.75	5.87	5.66
		52	F	7.00	6.99	6.57	6.47	6.55	6.48
		21	M	7.16	6.61	6.61	6.59	6.87	6.54
		32	M	6.69	6.52	5.36	5.56	5.28	5.33
		37	F	6.58	6.13	6.24	6.73	6.60	6.09

ND: not detected.
<LDL: lower than the detection level.

($P = 0.005$, 0.047 and 0.017, respectively,) while G-CSF in naïve was lower than in experienced patients ($P < 0.05$).

3.4. IFN-γ is a potential predictor of viral responses and of key value to evaluate the status of immune tolerance

The difference of IFN-γ between patients with and without ETVR is significant, not only at baseline (649.73 ± 424.74 vs. 370.75 ± 219.02 pg/ml, $P = 0.001$) but also at week 4 (579.68 ± 510.87 vs. 299.5 ± 182.17 pg/ml, $P = 0.016$) and week 12 (551.73 ± 428.58 vs. 315.69 ± 195.02 pg/ml, $P = 0.005$). A further analysis to evaluate the predictive value of IFN-γ according to the level above or below 500 pg/ml before therapy was performed and found that 85.19% (23/27) of patients with an IFN-γ level above 500 pg/ml achieved ETVR while 53% (18/34) of patients with IFN-γ below 500 pg/ml could not achieve ETVR, $P = 0.002$. In

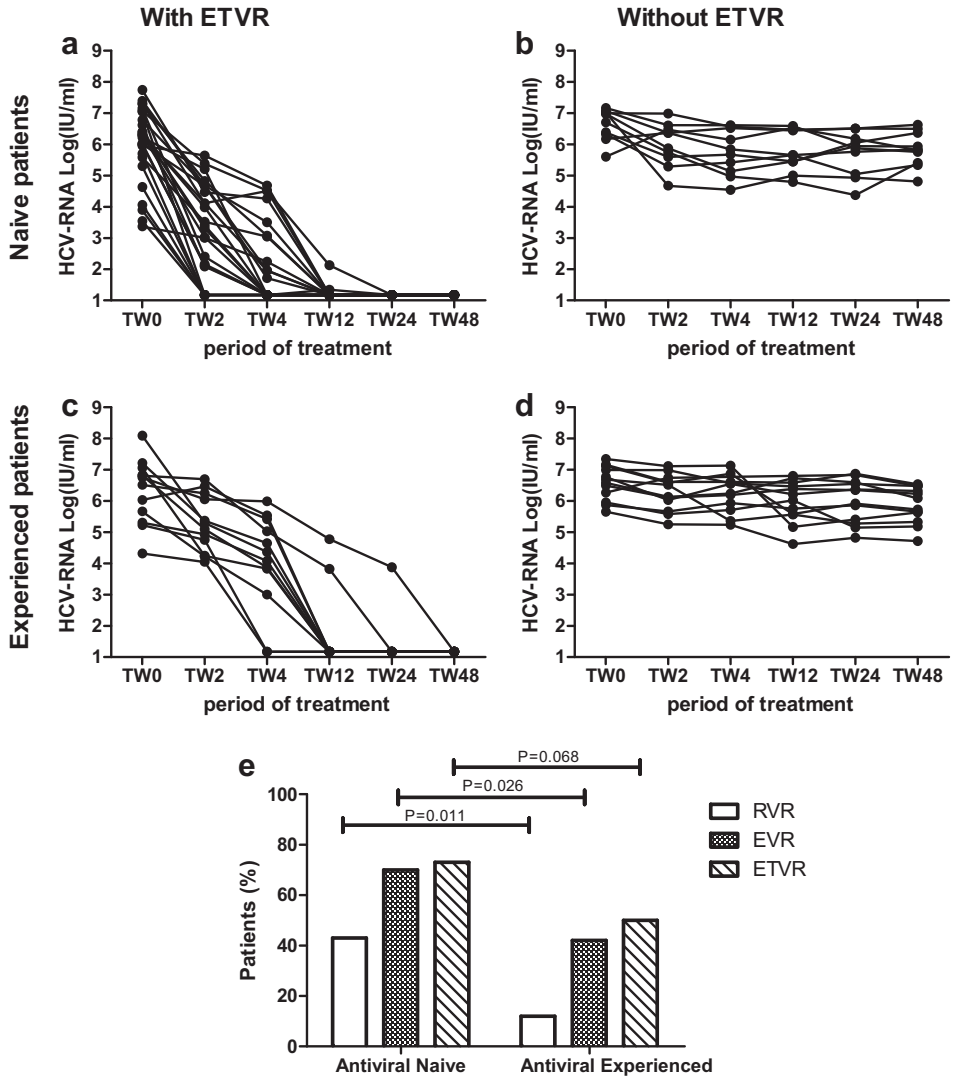


Fig. 1. The dynamics of HCV viral load between naïve and experienced patients. HCV viral load was detected in 61 chronic HCV patients, the kinetic of HCV during combination therapy were presented amongst naïve patients with ETVR (1a), naïve patients without ETVR (1b), experienced patients with ETVR (1c) and experienced patients without ETVR (1d). The comparison of RVR, EVR and ETVR between antiviral-naïve and -experienced patients indicated a delayed and slow virologic response in antiviral-experienced patients comparing with an early and fast virologic response in antiviral-naïve patients (1e). RVR, rapid virologic response; EVR, early virologic response; ETVR, end-of-treatment virologic response.

addition, the predictive values of IFN-γ in antiviral-naïve and -experienced patients were evaluated. The positive predictive value was 89.47% in naïve patients and 75% in experienced patients; negative predictive value was 44.44% and 62.5%, respectively.

3.5. Dominant Th1-polar cytokines were associated with a favorable viral response

A comparison of Th1/Th2 cytokines between patients with and without ETVR demonstrated a significant trend, in which a preferential shift towards a Th1-polar cytokines was associated with a favorable outcome of antiviral therapy. The level of IL-4 was significantly lower in patients with ETVR than those without ETVR, while patients with ETVR showed higher levels of IFN-γ and TNF-α than those without ETVR. Interestingly, this trend was associated with the dynamics of HCV viral load, which appeared in the early of antiviral therapy (week 0 and 4) in naïve patients, and at week 12 of therapy in experienced patients (Fig. 5).

4. Discussion

Although host genetics, viral subtypes and high viral loads may have a significant role in predicting the outcome of antiviral therapy in antiviral-naïve patients (Ge et al., 2009; Ghany et al., 2009; Thomas et al., 2009), these factors only have a small role in the prediction of virological response in antiviral-experienced patients who received the second round antiviral therapy (Yoshida et al., 2009). Host immune responses might play a key role in the HCV clearance during therapy (Neumann et al., 1998) although the immune mediators associated with favorable viral response in antiviral-experienced patients have not been well characterized.

The main findings of our analysis clearly demonstrate that the difference of immune mediator profiling before and during antiviral therapy between antiviral-naïve and -experienced patients, which is obviously associated with virologic responses in antiviral-naïve and -experienced chronic HCV patients. Notably, IFN-γ is a potential predictor of viral responses and key to evaluate the status of immune tolerance, which is very valuable in optimizing

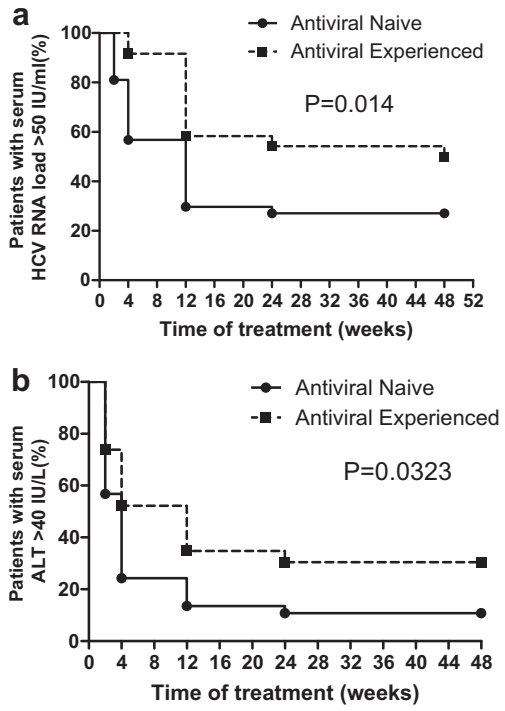


Fig. 2. Kaplan–Meier curve of cumulative HCV RNA negative (defined as serum viral load ⩽50 IU/ml) and normalization of ALT (defined as serum ALT ⩽40 IU/L) during 48 weeks of treatment. Patients are grouped as antiviral-naïve and -experienced patients at baseline, before the onset of combination therapy with peginterferon-alfa and ribavirin. Significant differences in HCV clearance (2a) and normalization of ALT (2b) between naïve and experienced patients were found ($P = 0.014$ and 0.0323, respectively). P values were obtained using the Log-rank (Mantel–Cox) test.

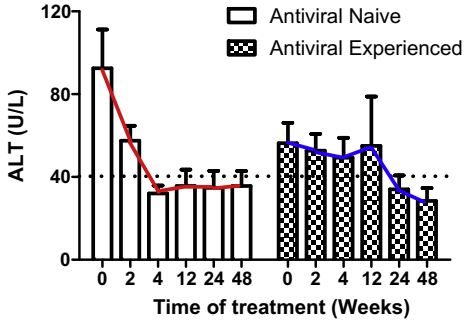


Fig. 3. The dynamics of ALT during antiviral therapy between antiviral-naïve and -experienced patients. ALT was detected at baseline (week 0), week 2, 4, 12, 24 and 48. Columns represent the means with SD at each time point, and connecting line to express the dynamics of ALT between antiviral-naïve (red) and antiviral-experienced (blue) patients. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

current treatment strategies or guiding personalized therapy, especially in antiviral-experienced patients.

One of the most intriguing observations in our present study is that the success of antiviral-experienced patients does not appear to depend of host genetics or viral subtypes, but host immune response. Firstly, we determined an obvious trend that antiviral-experienced patients express a significant weakness of immune mediators before the second round therapy as compared to antiviral-naïve patients. IFN-γ is the first line of immune mediator, which was secreted by infected hepatocytes in response to HCV infection (Feld and Hoofnagle, 2005). IL-4 is the major stimulus for the development of Th2 cells from naïve CD4+ helper T cells and serves as the signature cytokine of the Th2 subset (Paul and Zhu, 2010). MIP-1a is secreted by macrophages and epithelial cells and is pro-inflammatory in murine T cell-mediated hepatitis (Ajuebor et al., 2004). These three cytokines not only mediate antiviral functions, but also pro-inflammatory responses. Increases in IL-4, IFN-γ and CCL-3 (MIP-1a) in chronic HCV patients indicate the role of the host immune system in response to HCV infection. However significantly lower levels of these mediators in antiviral-experienced patients compared to naïve patients could reflect a status of immune tolerance in this particular subset of patients, which might to be one of the explanations why these patients failed in first course of antiviral therapy.

The differential kinetic profiles of HCV load and ALT level during combination therapy between naïve and experienced patients could indirectly support the notion that host immune response play a significant role to viral clearance and biochemical response in antiviral-experienced patients. The dynamics of HCV and ALT in antiviral-naïve patients in our study presents an early and fast virologic/biochemical response, followed by a second, slower phase of decline, which coincides with the classical pattern of decrease in HCV load and normalization of ALT during interferon therapy (Neumann et al., 1998). The delayed and slow virologic clearance and biochemical response in experienced patients might be due to the clearance of virus-infected cells by cell death or by eradication of viral replication in the cell (Feld and Hoofnagle, 2005).

The coincidence between dominant Th1-polar cytokines and favorable viral response further confirms the critical responsibility of host immune response in the outcome of antiviral-experienced patients. In the current study, HCV clearance in most naïve patients appears at week 2 and 4 after combination therapy, when Th1-polar cytokines, the higher level of Th-1 cytokines and the lower level of Th-2 mediators, demonstrate dominant immune mediators. The consistency between the profile of immune mediators and viral clearance is also found in experienced patients, who displayed a delayed and slow viral response. Based on those observations, we postulate that Th-1 polar cytokine profiles herald a favorable combination therapy outcome. While association between high level of circulating Th1 cytokines and viral clearance is well known from previous reports on antiviral-naïve patients (Amati et al., 2002), this is the first report evidencing dominant Th-1 polar cytokines as a signature of favorable viral response in antiviral experienced patients at the second round therapy. The results obtained here support the development of further studies to clarify the role of host immune response in the success of re-treatment in antiviral-experienced patients.

Recently, IL28B was identified as a primary factor in natural clearance of HCV (Thomas et al., 2009) and a main human genetic contributor to anti-HCV treatment (Ge et al., 2009), while our results (data not shown) did not demonstrate this trend, which might be due to the difference of race and the scale of cohort. The IP-10 level is also a predictive of the response to HCV treatment. Low circulating IP-10 levels at baseline were associated with a favorable initial reduction in serum viral loads, as well as with a sustained elimination of the virus after the completion of treatment (Romero et al., 2006). The correlation between IP-10 and virologic response was not found in antiviral-experienced patients in our study. This might be explained by the status of immune tolerance, which may affect the secretion and expression of IP-10 in antiviral-experienced patients.

We conclude that the host immune response plays an important role in virus control in combination therapy, We also recognize that our analysis is merely focused on circulating immune mediators; further study on the association between immune response in the liver (Thomson et al., 2003) and virus control during combination therapy in antiviral-experienced patients will help to delineate new immunotherapeutic strategies and improve current treatment regimens.

Table 2
Summary of 27 cytokines in antiviral-naïve, -experienced patients and healthy controls.

Cytokines chemokines	Antiviral naïve Mean ± SD (pg/ml)	Antiviral experienced Mean ± SD (pg/ml)	Healthy control Mean ± SD (pg/ml)
IL1b	4.27 ± 2.35[*,¶]	3.64 ± 1.5[*,#]	1.6 ± 0.55
IL1ra	356.95 ± 301.96	331.1 ± 208.82	253.22 ± 124
IL2	19.43 ± 12.18[*,#]	17.84 ± 27.78	8.47 ± 5.75
IL4	39.79 ± 36.37[*,#,¶,**]	13.36 ± 14.35[*]	4.3 ± 1.37
IL5	7.8 ± 6.05	6.06 ± 3.23	4.33 ± 2.68
IL6	22.62 ± 10.44[*]	19.75 ± 9.88[*]	12.24 ± 5.92
IL7	15.19 ± 7.9[*,#]	13.74 ± 6.53[*,#]	7.13 ± 2.38
IL8	20.07 ± 7.15[¶]	16.22 ± 5.97	16.28 ± 10.78
IL9	105.13 ± 100.58[*]	82.74 ± 85.45[*]	28.96 ± 12.86
IL10	8.42 ± 3.72[*,¶]	6.54 ± 3.35	4.57 ± 2.94
IL12	27.12 ± 15.17	29.36 ± 15.03[*]	18.47 ± 9.16
IL13	14.72 ± 8.3[*,#]	14.82 ± 6.59[*,#]	1.58 ± 2.62
IL15	11.96 ± 12.87	14.12 ± 18.41	10.11 ± 9.61
IL17	56.65 ± 30.02[*]	43.45 ± 32.39	29.6 ± 21.85
Eotaxin	206.02 ± 68.6	238.26 ± 340.13	175.81 ± 120.31
FGFbasic	96.23 ± 65.82[*,#,¶]	61.92 ± 46.04[*]	26.87 ± 20.12
GCSF	86.42 ± 44.37[*,#,¶]	132.68 ± 77.3[*,#]	239.9 ± 65.03
GMCSF	143.89 ± 122.32[*,#]	113.27 ± 126.81[*,#]	11.97 ± 32.1
IFNg	623.22 ± 418.44[*,#]	434.86 ± 304.87[*]	207.08 ± 109.22
IP10	4367.94 ± 3271.17[*,#]	4950.66 ± 7659.46	1262.02 ± 414.15
MCP1	40.73 ± 18.53[*,#]	35.11 ± 17.11[*,#]	12.17 ± 6.62
MIP1a	18.66 ± 9.36[*,#,¶]	13.1 ± 7.41[*,#]	3.84 ± 2.96
PDGFbb	5489.95 ± 3869.53[¶]	2946.23 ± 2093.28	3225.83 ± 1636.53
MIP1b	157.91 ± 60.55[*,#]	142.12 ± 68.64[*]	87.46 ± 52.74
RANTES	12782.55 ± 24761.25[¶]	3517.33 ± 1507.56[*]	15327.9 ± 9531.7
TNFa	66.32 ± 46.33[*,#,¶]	232.64 ± 252.06	270.08 ± 113.22
VEGF	79.75 ± 56.81	68.68 ± 60.18	58.39 ± 88.01

[#] Compare with healthy control, Bonferroni correction $p < 0.00185$.
[¶] Compare between naïve and experienced, $p < 0.05$.
[*] Compare with healthy control, $p < 0.05$.
[**] Compare between naïve and experienced, Bonferroni correction $p < 0.00185$.

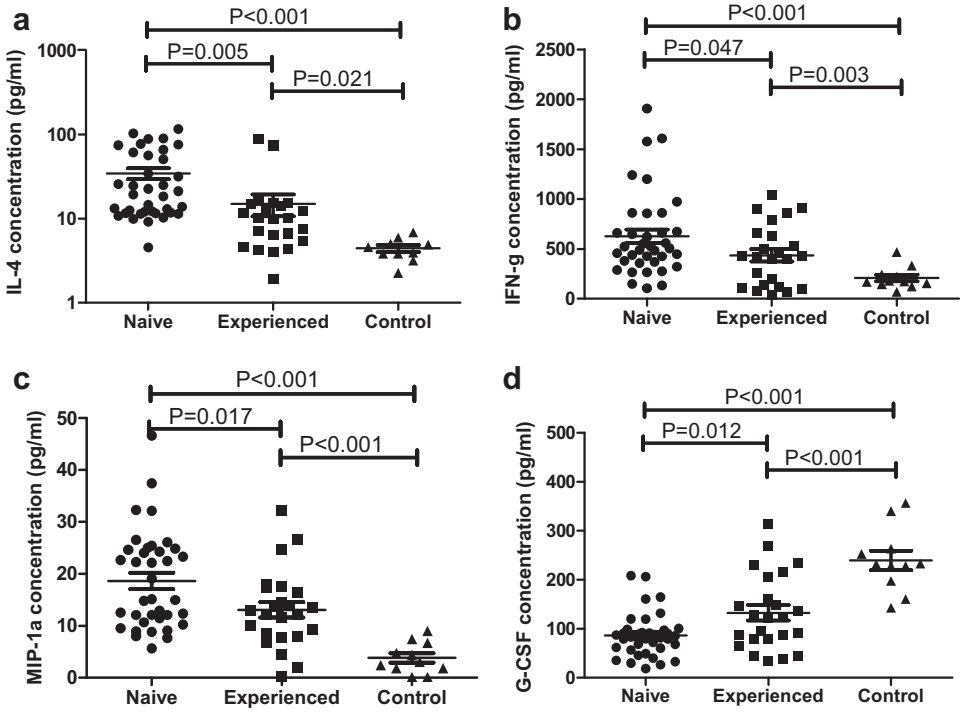
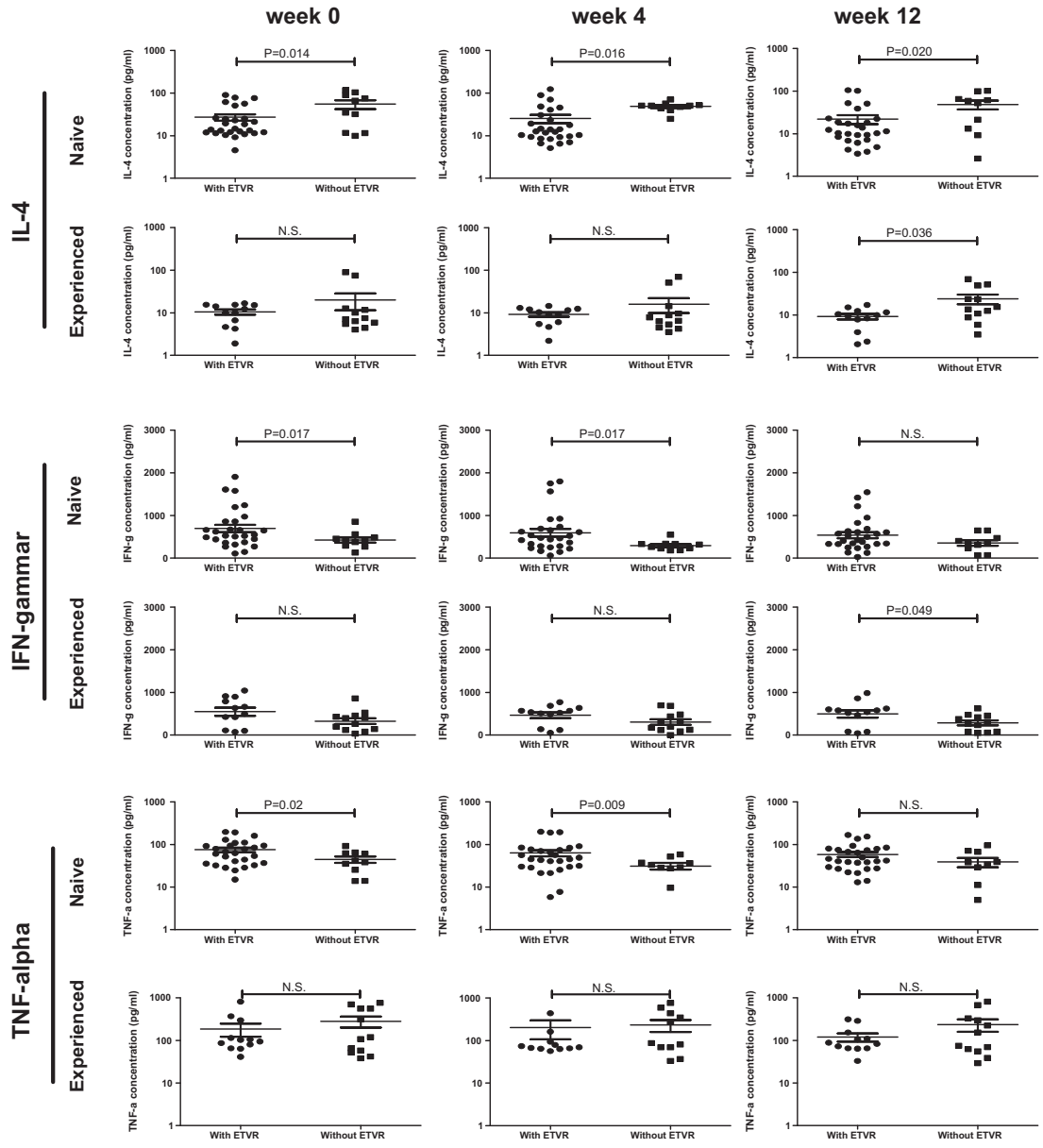


Fig. 4. Profiling of immune mediators before therapy between naïve and experienced patients. Twenty-seven immune mediators were measured using the multiplex Biorad 27 plex assay between 37 antiviral-naïve patients, 24 experienced patients and 11 healthy controls before combination therapy. A significant distinction between antiviral-naïve and -experienced patients was identified; IL-4 (4a), IFN-γ (4b) and CCL-3 (MIP-1a) (4c) in naïve patients were higher than those in experienced patients, while G-CSF in naïve was lower than that in experienced patients (4d).



		Week 0	Week 4	Week 12
Antiviral Naïve	IL-4	+	+	+
	IFN-γ	+	+	-
	TNF-α	+	+	-
Antiviral Experienced	IL-4	-	-	+
	IFN-γ	-	-	+
	TNF-α	-	-	-

Fig. 5. Dominant Th1-polar cytokines were associated with a favorable viral response. A significant trend of preferential shift towards a Th1-polar cytokines associated with good outcome of antiviral therapy was presented, in which IL-4 was significantly lower in patients with ETVR than in patients without ETVR, IFN-γ and TNF-α were higher in patients with ETVR than those without ETVR. This trend was also correlated with the dynamics of HCV viral load, which appeared in the early of antiviral therapy (week 0 and 4) in naïve patients, and at week 12 of therapy in experienced patients.

5. Conclusions

Analysis of the immune mediators involved in the host response to HCV during combination therapy in antiviral-naïve and -experienced chronic HCV infections revealed the role of the host immune response in the success of combination therapy, especially in antiviral-experienced patients. Immune tolerance in antiviral-experienced patients may provide an explanation for the failure in

the first round of antiviral therapy. The coincidence between dominant Th1-polar cytokines and favorable viral response in both antiviral-naïve and -experienced patients supports the important role of host immune response during combination therapy.

Disclosure statement

The authors declare no financial or commercial conflict of interest.

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