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Restoration of Innate Immune Responses may be a Novel Therapeutic Strategy for Treatment of Chronic Hepatitis C Virus Infection

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Abstract

HCV persistence results from inefficient innate and adaptive immune responses. We investigated whether an induction approach (IA) with natural (n) IFN-beta followed by Peg IFN-alpha and ribavirin (Standard of Care; SOC) (novel combination treatment; NCT) increased the virologic response and restored innate and adaptive immune responses in chronic hepatitis C (CHC) patients with HCV genotype 1b and a high viral load. Seven CHC patients were treated with NCT. NCT overcame viral escape and breakthrough, resulting in persistent viral clearance. Early virologic responders (EAVR, n=5) with undetectable HCVRNA before the end of IA showed a sustained virologic response (SVR). Late VR (LAVR, n=2) with undetectable HCVRNA after the end of IA showed a transient VR. IL-15 was increased at the end of IA in both EAVR and LAVR. CXCL-8, CXCL-10, CCL-4, and CCL-11 levels were significantly decreased ($p<0.05$) in EAVR, but were not in LAVR during NCT. IL-12 increased significantly ($p<0.05$) and CXCL-8 decreased significantly ($p<0.05$) after the end of NCT in EAVR, but did not in LAVR. The present study suggested that the initial early virologic clearance induced by CPIT before the use of SOC induced the restoration of DC function and improvements in the activation of NK cells, as indicated by the up-regulation of IL-12 and IL-15 and down-regulation of CXCL-8, CXCL-10, CCL-4, CCL-11. Early virologic clearance by IA with nIFN-beta induced the restoration of innate immune responses linked to adaptive immune responses, which resulted in SVR. NCT (n=8) achieved a higher SVR rate than that of SOC (n=8) in difficult-to-treat CHC patients. The results showed the safety of the nIFN-beta treatment and supported the use of nIFN-beta as a safe and alternative option. NCT is more effective and have less adverse effects than SOC in difficult-to-treat CHC patients with genotype 1b and a high viral load.

Keywords: Chronic Hepatitis C; Innate Immune Response; Cytokine; Chemokine; Interferon

Introduction

Approximately 170 million individuals are chronically infected with hepatitis C virus (HCV) worldwide, all of whom are at risk of the long-term complications of cirrhosis, liver failure, and hepatocellular carcinoma [1,2]. Complications associated with chronic HCV may be prevented by viral eradication. The standard of care (SOC) treatment for the past decade has been the combination of Pegylated interferon-alpha (PegIFN-alpha) and ribavirin (RBV). Unfortunately, this treatment only cures approximately 50% of individuals infected with HCV genotype 1, which is the most prevalent HCV genotype [3]. The triple combination of PegIFN-alpha, RBV, and a protease inhibitor (telaprevir or boceprevir) failed to eradicate HCV in approximately 20-30% of treatment-naïve patients, 50-60% of treatment-experienced patients, and 70-80% of patients with no response to SOC. Furthermore, resistance-associated variants were detected in approximately 50% of patients who failed to achieve SVR [4,5]. Therapies in addition to Direct-acting Antiviral Agents (DAA) under evaluation for Chronic Hepatitis C (CHC) include host targets such as cyclophilin inhibitors and immunomodulators, Toll-like receptor agonist and micro-RNA, RNA interference, and entry inhibitors. Passive therapeutic vaccines hold promise for the future. New interferon agents including peg IFN-lambda, IFN-omega, and Alb-IFN are currently being developed, and RBV variants are still under consideration [6]. However, at this point in HCV drug development, no drug is expected to be potent enough or have a high enough barrier to resistance to be used in immunotherapy. Thus, more effective, tolerable, and/or tailored therapies are required.

HCVRNA serum concentrations have been shown to promptly decrease to undetectable levels with successful antiviral treatment, and remain negative throughout treatment and thereafter. The faster the virus becomes undetectable during therapy, the better the chance of achieving a sustained virologic response (SVR). Accumulating evidence has suggested that an early response to treatment is best determined by serum levels of HCVRNA after 4 and 12 weeks of therapy [7,8].

Recent advances in the understanding of innate immunity show that activation of the innate immune system is essential for subsequent adaptive immune responses including specific antibody production and CTL activation, which play key roles in protection against viral infections [9].

HCV can interfere with innate immune activation at multiple levels. The non-structural proteins of HCV, particularly NS3-4A, have

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been found to interact with various host adaptor molecules to disrupt type I IFN induction pathways. Foy and colleagues reported that NS3-4A serine protease blocked the HCV-induced activation of IRF3 [10]. Absolute numbers of myeloid DC (mDC)s, plasmacytoid DC (pDC)s, and DC progenitors in the periphery were significantly lower in patients with CHC than in healthy volunteers. Myeloid and plasmacytoid DCs from patients exhibited impaired abilities to stimulate allogenic CD4 T cells and produce interleukin (IL)-12 p70 and IFN-alpha, relative to those from healthy volunteers. In chronic HCV infection, both types of blood DCs are reduced and have impaired abilities to polarize T helper cells [11]. IFNs represent the first line of defence against viral pathogens and act both directly on viral replication and indirectly through the activation of host immune response genes [12].

Small amounts of IFN-beta are initially produced upon viral infection through the activation of unidentified factors. IFN-beta then stimulates IFNAR to activate ISGF3. IRF-3 is critical for both the early and late phases of IFN-alpha/beta gene induction. The biphasic IFN-alpha/beta gene induction mechanism, regulated by IRF-3 and IRF-7, ensures the transcriptional efficiency and diversity of genes for an efficient antiviral response (Figure 1). The expression and or function of IRF family members are regulated by immunomodulatory cytokines. They participate in regulating the development and function of the immune system, in addition to antiviral responses (Figure 2) [13].

IFN-beta was shown to have different signaling and biological activities from those of IFN-alpha, and achieved a higher rate of viral clearance than IFN-alpha [14-19]. Adverse events (AEs) including depression, alopecia, general fatigue, and hematological changes were less frequently reported during treatment with natural IFN-beta (nIFN-beta) than with treatment with IFN-alpha [20-22]. In contrast to the actions of IFN-alpha, IFN-beta and IFN-lambda signaling in the liver does not become refractory during repeated stimulation of the IFN signaling transduction pathway. The sustained efficacy of IFN-beta and IFN-lambda could be important for the treatment of patients who do not respond to PegIFN-alpha through a pre-activated endogenous IFN system [23].

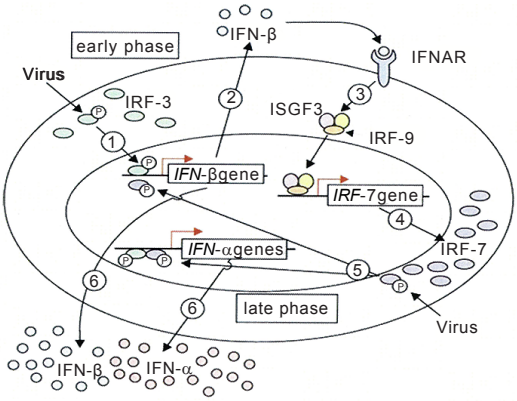

Figure 1: Schematic representation of the biphasic mechanism for IFN-alpha/beta gene induction, mediated by IRF-3 and IRF-7. In the early phase, constitutively expressed IRF-3 is activated by viral infection, resulting in the weak activation of the IFN-beta gene. In this phase, IRF-7 is expressed only at very low levels by spontaneous IFN-alpha/beta signaling. This initially produced IFN-beta strongly induces IRF-7 expression through activation of the ISGF3. In the late phase, IRF-3 and IRF-7 cooperate with each other to amplify IFNalpha/beta gene induction, resulting in the full procurement of the normal mRNA induction profile of the IFN-alpha gene subfamily. Circle numbers indicate the sequence of events. [Tadatsugu Taniguchi, Kouetsu Ogasawara et al. IFR FAMILY OF TRANSCRRIPTION FACTORS AS REGULATORS OF HOST DEFENCE. Annu. Rev. Immunol. 2001. 19: 623-55 (modification)].

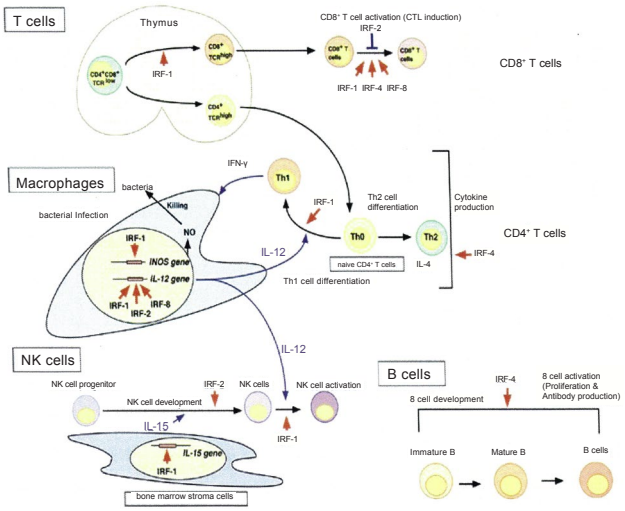

Figure 2: Regulation of the immune system by IRF family members. IRFs regulate lymphocyte development and function. Only known target genes in this system are shown. IRF-1, IRF-2 and IRF-8 contribute to IL-12p40 gene induction and have crucial roles in Th1-type immune response. IRF-1 regulates NK cell development through the induction of the IL-15 gene in bone marrow stroma cells. The iNOS gene is induced by IFN-gamma through IRF-1 in macrophages for anti-bacterial infection. Although critical target genes are unclear, the other processes regulated by IRFs are indicated by red arrows. It was shown that IRF-2 is involved in activation (red arrow) or repression (blue line) in these processes. [Tadatsugu Taniguchi, Kouetsu Ogasawara et al. IRF FAMILY OF TRANSCRIPTION FACTORS AS REGULATORS OF HOST DEFENSE. Annu. Rev. Immunol. 2001. 19:623-55 (modification)].

The resolution of a HCV infection may restore impairments in innate and adaptive immunities [24-26]. However, the issue of how to increase the initial virologic response rate has not yet been resolved and is complicated by viral breakthrough and AEs. In a previous study, we showed that cyclic and periodic IFN treatment (CPIT) consisting of induction treatment (IT) with nIFN-beta for 2 weeks followed by maintenance treatment (MT) with nIFN-alpha for 2 weeks overcame virologic breakthrough, and achieved an early virologic response (EVR) and an End Treatment Virologic Response (ETVR). We treated a patient with CHC [60 years old, female, serotype 1 (genotype 1b), HCVRNA 4240 Kcopies/ml, and Stage 2 and Grade 2], who was obese (BMI 35.5 kg/m^2) and being treated for diabetes mellitus and hypertension, with CPIT for 24 weeks followed by IFN-alpha 2b, 6MU/day, percutaneous inj., three times weekly plus RBV, 200-600mg/day, per os, every day for 26 weeks, and subsequently with Nifn alpha 6MU/day, percutaneous inj., two times weekly for 22 weeks after stopping RBV due to anemia as AEs (total 72 weeks). HCV viral titers markedly decreased with RVR and complete EVR after CPIT. The subsequent IFN alpha and RBV treatments caused persistent virological clearance resulting in SVR and SBR. This case suggested that CPIT restored innate immune responses, as indicated by the up-regulation of serum IL-15, RANTES, and MCP-1 (Figure 3). In addition to the improvement in innate immunity due to virologic clearance by CPIT during the initial course of therapy, persistent virologic clearance and the restoration of innate and adaptive immune responses by SOC were more likely to result in a higher rapid virologic response (RVR), EVR, ETVR, and SVR. On the basis of these findings, we conducted a pilot study in 7 CHC patients with genotype 1b, high viral loads, and a wild or intermediate type IFN sensitivity

determining region (ISDR) to assess the efficacy, tolerability, and safety of treatment with RBV plus PegIFN-alpha 2b for 48 weeks (SOC) using an induction approach with initial virologic clearance induced by CPIT for 24 weeks (novel combination treatment: NCT) [27].

Little is known about the chemokine and cytokine response to HCV infection before, during, and after IFN treatment. With an aim to better understand the immunological determinants of the protective immune response to HCV infection, we performed an extensive analysis of the innate and adaptive immune responses in CHC patients with genotype 1b and high viral loads. We evaluated serum levels of cytokines and chemokines that mediate humoral and cellular immunities and inflammation, correlated them with disease activity, and characterized the immunomodulatory effects of therapy. We also compared the efficacy and safety of NCT versus SOC in CHC patients with genotype 1b and high viral loads. The rate of SVR was significantly higher among patients receiving NCT than those receiving SOC [27]. NCT was shown to be more effective and have less adverse effects (AEs) than SOC among difficult-to-treat CHC patients with genotype 1b and high viral loads [28].

Patients and Methods

Study 1

Patients: Seven patients [3 males and 4 females, mean age 53.3+/-8.5 years (range 39-66)] with CHC, genotype1b (serotype 1), ISDR (3 wild-type, 3 intermediate-type, and 1 not determined), and a viral load of 2144.3 ± 1701.2 KIU/ml (range 536->5000 KIU/ml) were enrolled in this open-label, prospective study. Patients underwent liver biopsy before the IFN therapy, and the severity [inflammation (Grade) and fibrosis (Stage)] of liver disease [29] was evaluated as chronic hepatitis (Grades 1-3, Stage 1-2) (Table 1). Serum was collected from five healthy donors, ranging in age from 28 to 58 years. Written informed consent was obtained from all patients according to the Declaration of Helsinki.

Exclusion criteria: The following were considered as exclusion criteria: Refusal by women of child – bearing age or by sexually active patients to use a safe contraceptive, pregnancy or breast-feeding, cirrhosis with signs of decompensated liver diseases, coronary heart diseases, the presence of overt psychiatric diseases, active alcohol or drug abuse, uncontrolled diabetes mellitus, uncontrolled hypertension, uncontrolled retinopathy, autoimmune disorders, or any other unstable medical condition not because of liver disease. All patients were negative for the hepatitis B surface antigen, and frequent causes of chronic liver diseases were excluded.

Study design: Cyclic and periodic IFN treatment (CPIT): Patients were treated with 6 cycles (24 weeks) of Cyclic and Periodic IFN Treatment (CPIT). One cycle of CPIT consisted of IT with nIFN-beta (Feron®, Toray, Chiba, Japan) at 3-6 MU/day, intravenously by drip infusion in 100 ml of saline solution, daily for 2 weeks followed by MT with nIFN-alpha (Sumiferon®, Sumitomo, Osaka, Japan) at 6 MU/day, subcutaneously, three times weekly for 2 weeks.

CPIT was followed by treatment with RBV plus PegIFN-alpha 2b (SOC) (Novel combination treatment: NCT): We investigated the efficacy, tolerability, and safety of CPIT for 24 weeks as induction therapy followed by RBV (Rebetol®: Schering Plough, Kenilworth, NJ, USA; 200-800 mg/day, per os, daily) plus PegIFN-alpha 2b (Pegintron®, Schering Plough, Kenilworth, NJ, USA; 60-120 micro-g/day, percutaneously inj., once weekly) (SOC) for 48 weeks (total 72 weeks) in a pilot clinical trial as a potential treatment for 7 difficult-to-treat CHC patients with genotype 1b, high viral loads (more than 100 KIU/ml), and wild or intermediate type ISDR [27].

Measurements: All patients were monitored with clinical, biochemical, and virologic assessments before and every 1 to 4 weeks during the entire 72-week treatment period, and were followed for an additional period of more than 24 weeks. Serum levels of HCVRNA were determined using the quantitative COBAS AMPLICOR HCV MONITOR test, ver. 2.0 (Roche Diagnostic Systems, Tokyo, Japan; sensitivity <50 IU/ml).

Assessments of serum cytokines and chemokines (multiplex cytokine assay) were performed. A multiplex biometric Enzyme-linked Immunosorbent Assay (ELISA)-based immunoassay [30,31], with dyed microspheres conjugated to a monoclonal antibody specific for a target protein, was used according to the manufacturer's instructions (Bio-Plex Human Cytokine assay; BioRad Inc., Tokyo, Japan). The cytokines measured were Th1 cytokines [IFN-gamma, TNF-alpah, IL-2, IL-12 (p70), and IL-15], Th2 cytokines [IL-4, IL-6 and IL-10], hematopoietic cytokines [GM-CSF], CXC chemokines [CXCL-8 and CXCL-10] and CC chemokines [CCL-2, CCL-3, CCL-4, CCL-5, and CCL-11].

Study 2

We investigated whether induction therapy using CPIT with nIFN-beta increased SVR rates in CHC patients with genotype 1b and high viral loads. We compared the efficacy and safety of NCT (n=8) versus SOC (n=8) in CHC patients with genotype 1b and high viral loads. All patients were monitored with clinical and biochemical assessments and

Patient NO	Age/gender	Body wt (Kg)	BMI (kg/m²)	Liver Histology (Stage grade)	Alt(IU/ml)				
					Baseline	24 weeks (End of NCT)	72 weeks (end of NCT)	96 weeks (24 weeks after end of NCT)	Outcome of treatment
Early Virologic responders									
1	61/F	46.1	20.4	1/2	197	37	15	13	SBR
2	47/M	67.0	21.1	1/1	37	28	22	22	SBR
3	66/M	78.0	24.5	3/2	57	29	29	17	SBR
4	49/M	60.0	19.8	1/2	50	32	12	11	SBR
5	61/F	48.5	20.9	1/2	38	9	14	10	SBR
Late Virologic responders									
6	39/F	73.0	27.1	1/2	48	331	264	161	NBR
7	56/F	55.0	20.6	-/-	33	19	13	49	TBR
Mean ± SD	53.3 ± 8.5	61.1 ± 12.1	22.1 ± 2.7	1-3/1-2	65.7 ± 58.5	69.3 ± 115.8	52.7 ± 93.3	39.0 ± 59.9	

Table 1: Characteristics of chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b), and wild or intermediate type in ISDR before, during and after ribavirin plus PegIFN-alpha 2b using an "induction" therapy with CPIT (NCT). CPIT: Cyclic and Periodic IFN Treatment, NCT: Novel Combination Treatment, IFN: Interferon, BMI: Body Mass Index, ISDR IFN sensitivity determining region, F: Female, M: Male, SBR: Sustained Biochemical Response, NBR: No Biochemical Response, TBR: Transient Biochemical Response.

virologic responses assessed by TaqMan PCR (limit of detection, 15 IU/ml) before and every 1 to 4 weeks during the 48-72-week treatment period, and were followed for at least an additional 24 weeks after the cessation of treatment. The primary efficacy end point was the achievement of SVR 24 weeks after the cessation of treatment (Table 2).

Assessment of safety

Safety was assessed with laboratory tests and an evaluation of adverse events (AEs) every 1-4 weeks during and after the end of NCT. A reduction in the RBV dosage from 800 to 200-600 mg per day and reduction in the PegIFN-alpha 2b dosage from 60-120 mg to 50-100 mg without virologic breakthrough were allowed to manage AEs or laboratory abnormalities that had reached predetermined thresholds of severity. If AEs were resolved or improved, a return to initial dosing levels was permitted.

Statistics

Data were expressed as the mean ± standard deviation and a paired-t test was used to evaluate differences in the means between groups, with a p value of <0.05 being considered significant.

Results

Study 1

HCV viral titers decreased in all patients after 4 weeks of CPIT, which highlighted the efficacy of this treatment modality. None of the patients showed virologic breakthrough. Serum HCVRNA [2144.3 ± 1701.2 (range536 ≥ 5000) KIU/ml at baseline] decreased significantly to 1.5 ± 2.4 KIU/ml (p=0.0157) at the end of CPIT. The rates of RVR and EVR [partial EVR (pEVR), complete EVR (cEVR), and RVR plus cEVR (extended RVR)] were 7/7(100%) and 7/7 [100%; 4/7 (57.1%), 3/7 (42.9%), and 3/7 (42.9%)], respectively. Viral titers dropped below detectable levels in 5 patients before the end of CPIT, and in 2 patients after the end of CPIT (after beginning SOC). The rates of ETVR at the end of CPIT and NCT were 5/7 (71.4%) and 7/7 (100%), respectively. The rate of SVR was 5/7 (71.4%). A transient virologic response was found in 2 patients with undetectable HCVRNA in the serum after the end of CPIT (Table 3).

To refine our understanding of the heterogeneity of therapeutic responses, patients were classified into two distinct groups based on the time of clearance of viremia. Of note, early virologic responders (EAVR) with undetectable HCVRNA in the serum before the end of CPIT, which included 5 patients (pt. NO 1-5), showed SVR. Late virologic responders (LAVR) with undetectable HCVRNA in the serum after the end of CPIT, which included 2 patients (pt. NO 6 -7), showed TVR. The viral titer values in LAVR were very high (>5000 and 4400 KIU/ml). Serum ALT decreased at the end of NCT and after the end of NCT. The rate of the sustained biochemical response (SBR) was 5/7 (71.4%) (Table 1).

Serum cytokines and chemokines at baseline (Figure 4)

CXCL-8, CXCL-10, CCL-4, and CCL-11 levels were significantly higher (p<0.05); IFN-gamma, TNF-alpha, IL-2, IL-6, IL-15, GM-CSF, and CCL-2 levels were higher; and IL-10, IL-12, and IL-13 levels were significantly lower (p<0.05) in all CHC patients than in the controls. IL-6, IL-15, CXCL-8, CXCL-10, and CCL-11 levels were significantly higher (p<0.05) and IFN-gamma, TNF-alpha (p<0.1), IL-2, GM-CSF, CCL-2, and CCL-4 levels were higher in EAVR than in the controls. IL-10 and IL-13 levels were significantly lower (p<0.05), and IL-12 levels were lower (p<0.1) in EAVR than in the controls. GM-CSF, CXCL-10, and CCL-4 levels were significantly higher (p<0.05) and TNF-alpha (p<0.1), IFN-gamma, IL-2, IL-15, IL-6, IL-4, CXCL-8, and CCL-11 levels were higher in LAVR than in the controls.

3-1-2 Serial values of serum cytokines and chemokines during NCT (Figures 5-14): At the end of CPIT:

The levels of CCL-4 decreased significantly in all CHC patients (p<0.05), IFN-gamma, TNF-alpha, IL-2, IL-4, and GM-CSF levels decreased, and IL-10, IL-13, IL-15, and CCL-2 levels increased from baseline.

Patient NO	Age/gender	Body wt(Kg)	BMI(kg/m²)	Liver Histology (Stage grade)	HCV-RNA(taqman PCR LogIU/ml)	ALT (IU/ml)	Hb(G/dl)	Platelet (10^4 /ml)	outcome
NCT									SVR
1	61/F	48.5	20.9	A1/F1	5.9	38	12.1	17.7	SVR
2	61/F	46.1	20.4	A1/F1	6.2	197	9.2	17.5	SVR
3	47/M	67.0	21.1	A1/F1	5.9	37	14.8	143	SVR
4	49/M	60.0	19.8	A2/F1	6.6	50	15.0	22.0	SVR
5	66/M	78.0	24.5	A2/F3	5.8	19	15.7	9.6	SVR
6	40/F	73.O	27.1	A2/F1	7.7	48	13.8	39.1	TVR
7	58/F	55.0	20.6	-/-	6.4	32	12.6	17.7	TVR
8	73/M	53.4	21.0	A1/F1	6.4	20	12.1	22.4	SVR
Mean ± SD	58.9 ± 10.8	60.1 ± 11.6	21.9 ± 2.5	1-2/1-3	6.36 ± 61	55.1 ± 58.4	13.2 ± 2.1	18.9 ± 5.5	
SOC									
1	62/M	61.O	19.7	A2/F3	6.1	65	14.2	9.6	NVR
2	56/M	61.3	19.6	A2/F2	6.8	96	16.0	16.4	TVR
3	69/F	48.2	21.4	A2/F2	7.4	54	12.0	12.9	NVR
4	54/M	77.9	24.0	-/-	6.8	79	15.8	7.9	NVR
5	64/M	71.4	25.0	A3/F2	7.3	273	14.2	13.9	SVR
6	69/F	58.1	23.9	A1/F1	5.7	31	15.7	13.4	TVR
7	56/M	75.7	24.4	-/-	6.5	63	14.0	22.4	SVR
8	51/M	49.5	165.5	A1/F0	6.1	56	13.2	18.2	SVR
Mean ± SD	60.1± 6.9	62.9 ± 11.2	22.1 ± 2.7	1-3/0-3	6.59 ± 0.60	89.6 ± 76.5	14.4 ± 2.0	17.8 ± 6.1	

Table 2: Baseline characteristics of chronic hepatitis C patients with serotype 1(genotype 1b) and high viral loads treated with the NCT or the SOC. NCT: Novel Combination Treatment, SOC: Standard of Care, SVR: Sustained Virologic Response, NVR: No Virologic Response, TVR: Transient Virologic Response.

Patient NO	Sero type	ISDR Number of Mutations in NS5A GNE	HCV RNA in serum (KIU ML)							
			baseline	4weeks(RVR)	12 weeks (EVR)	24 weeks (end of CPIT)	72 weeks (end of NCT) 24	96 weeks (after end of NCT)	Virologic break through	outcome
Early Virologic responders										
1	I	nd	1480	<5.0	(-)	(-)	(-)	(-)	(-)	SVR
2	I	Wild(0)	824	<5.0	(-)	(-)	(-)	(-)	(-)	SVR
3	I	Wild(0)	536	<5.0	(+)	(-)	(-)	(-)	(-)	SVR
4	I	Wild(0)	3600	<5.0	(+)	(-)	(-)	(-)	(-)	SVR
5	I	Intermediate(1)	770	<5.0	(-)	(-)	(-)	(-)	(-)	SVR
Late virologic responders										
6	I	Intermediate(2)	>5000	<5.0	(+)	(+)(-)[4]	(-)(+)<	3400	(-)	TVR
7	I	Intermediate(1)	4400	<0.5	(+)	(+)(-)<	$_{(-)600}\delta_{4500}\delta$	850	(-)	TVR
Mean<SD			2298<1523	RVR 7/7	EVR 7/7	ETVR(CPIT) 5/7	ETVR(NCT)7/7	SVR 5/7 TVR2/7	WITHOUT BT 7/7	

Table 3: Effect of NCT on serum HCVRNA in chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b), and wild or intermediate type in ISDR.ISDR; IFN sensitivity determining region, IFN; interferon, RVR; rapid virologic response, EVR: Early Virologic Response, cEVR: complete EVR, pEVR: partial EVR, SVR: Sustained Virologic Response, TVR: Transient Virologic Response, NCT; Novel Combination Treatment, n.d.: Not Detected, (-) in HCVRNA; undetectable HCVRNA, (+) in HCVRNA; detectable HCVRNA, $; 29 weeks of NCT, δ; 4 weeks after end of NCT, £; 30 weeks of NCT,§; 8 weeks after end of NCT, BT; break through.

In EAVR, CCL-4 levels decreased significantly ($p<0.05$) from baseline, but to a lesser extent than that in LAVR. CCXL-8 levels decreased in EAVR, but increased significantly ($p<0.05$) in LAVR. CXCL-10 and CCL-3 levels decreased in EAVR, but increased in LAVR at the end of CPIT. CPIT induced the up-regulation of IL-15 expression, whereas SOC did not.

At the end of NCT: In all CHC patients, CCL-4 levels decreased significantly ($p<0.05$) and the levels of IFN-gamma, TNF-alpha, IL-2, IL-4 ($p<0.1$), IL-6, IL-15, CXCL-8, CXCL-10 ($p<0.1$), CCL-11, and GM-CSF decreased from baseline.

In EAVR, IFN-gamma, CCL-4 and CXCL-8 levels decreased significantly ($p<0.05$) and CXCL-10 levels decreased ($p<0.1$) from baseline. In LAVR, IFN-gamma, and CCL-4 levels decreased and CXCL-8 increased. The level of CXCL-10 ($p<0.1$) decreased in EAVR, but did not in LAVR. IL-6, IL-12, IL-15, and CCL-3 levels ($p<0.1$) decreased in LAVR, but did not in EAVR. The level of IL-10 increased in LAVR, but was unchanged in EAVR.

Four weeks after the end of NCT: In all CHC patients, IL-12 level increased significantly ($p<0.05$), and IL-10 ($p<0.1$) and CCL-2 levels increased from baseline. IFN-gamma, TNF-alpha, IL-2, IL-4, IL-6, CXCL-8, CXCL-10 ($p<0.1$), CCL-4 ($p<0.1$), CCL-11 and GM-CSF levels decreased from baseline. The level of IL-12 significantly increased ($p<0.05$) in EAVR, and increased to a lesser extent in LAVR. IL-15 and CCL-2 levels increased in EAVR, but decreased in LAVR. The level of IL-13 increased ($p<0.1$) in LAVR, and increased to a lesser extent in EAVR. The level of CXCL-10 ($p<0.1$) decreased in EAVR, and decreased to a lesser extent in LAVR. 3-1-3-3 Correlation between serum cytokine and chemokine levels and therapeutic responses (Figures 5-14).

The level of IL-15 increased at the end of CPIT in EAVR and LAVR and 4 weeks after the end of NCT in EAVR, but did not in LAVR. The level of CXCL-8 decreased significantly ($p<0.05$) in EAVR, but did not in LAVR during NCT. After the end of NCT, the IL-12 level increased significantly ($p<0.05$) and CXCL-8 level decreased significantly in EAVR, but not in LAVR ($p<0.05$). CXCL-8 increased in LAVR at the end of CPIT and NCT. At baseline, IFN-gamma and TNF-alpha levels were higher than those in the control. At the end of CPIT, at the end of NCT, and 4 weeks after the end of NCT, IFN-gamma and TNF-alpha levels were decreased (Figures 11 and 12). At the end of NCT and after the end of NCT, the level of CXCL-10 significantly decreased ($p<0.05$) in EAVR, but did not in LAVR. At the end of CPIT and the end of NCT, the level of CCL-4 significantly decreased ($p<0.05$) in EAVR, but did not in LAVR.

Case No 3: A patient with CHC [66 year-old, male, serotype 1 (genotype 1b), HCVRNA 536 Kcopies/ml, Stage 3 and Grade 2, and BMI 24.6 kg/m^2] with thrombocytopenia was treated with NCT. HCV viral titers markedly decreased with RVR and cEVR after CPIT for 24 weeks, and subsequent SOC for 48 weeks showed persistent virological clearance and the restoration of innate immune responses, as indicated by the up-regulation of serum IL-12, IL-15 and down-regulation of CXCL-8, which resulted in SVR, SBR, an improvement in thrombocytopenia from 9.6×10^4 /microl before NCT to 19.1×10^4 / microl after cessation of NCT, and improvement in hepatic histological findings from Stage 3 and Grade 2 before NCT to Stage 2 and Grade 1, 15 months after the cessation of NCT (Figure 15).

Study 2

HCV viral titers significantly decreased ($p<0.05$) from baseline in NCT and SOC after the beginning of treatment. HCV RNA levels decreased more in NCT than in SOC. The rates of virologic responses differed in the initial 4 and 12 weeks, and ETVR and SVR in CHC patients with genotype 1b and high viral loads treated with NCT and SOC. The rates of RVR in week 4, pEVR and cEVR in week 12, virological response in week 24, and ETVR and SVR among CHC patients with genotype 1b and high viral loads receiving SOC and NCT were 87.5 versus 100%, 50 versus 25%, 50 versus 75%, 50 versus 75%, 50 versus 100% ($p=0.0764$), and 37.5 versus 75% ($p=0.0435$), respectively (Figure 16).

Discussion

Hepatitis C virus (HCV) persistence in the host results from inefficiencies in innate and adaptive immune responses [32]. Innate immunity controls adaptive immune responses through a direct interaction and through the exchange of signals between immune cells belonging to both compartments [33,34].

This study investigated the hypothesis that an induction approach using CPIT with nIFN-beta may increase the initial virologic response rate and restore innate and adaptive immune responses in CHC patients with genotype 1b and a high viral load. Study 1 showed that NCT with an induction approach with nIFN-beta overcame the emergence of viral escape and breakthrough, resulting in the persistent

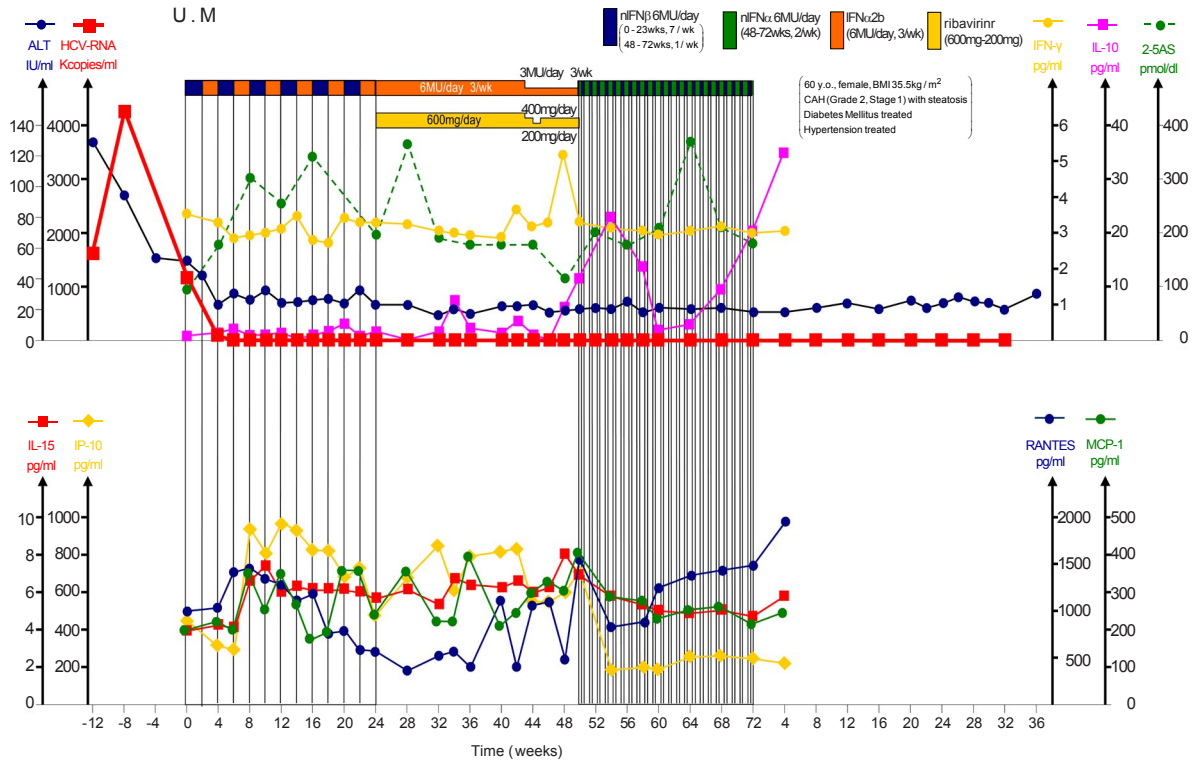

Figure 3: A patient with chronic hepatitis C [63 years old, female, serotype 1(genotype 1b), HCVRNA 4240 K copies/ml, Stage 2 and Grade 2] was treated with CPIT for 24 weeks followed by IFN-alpha 2b three times weekly plus ribavirin for 26 weeks and subsequent nIFN-alpha two times weekly for 22weeks after stopping ribavirin due to anemia. CPIT; Cyclic and Periodic IFN Treatment.

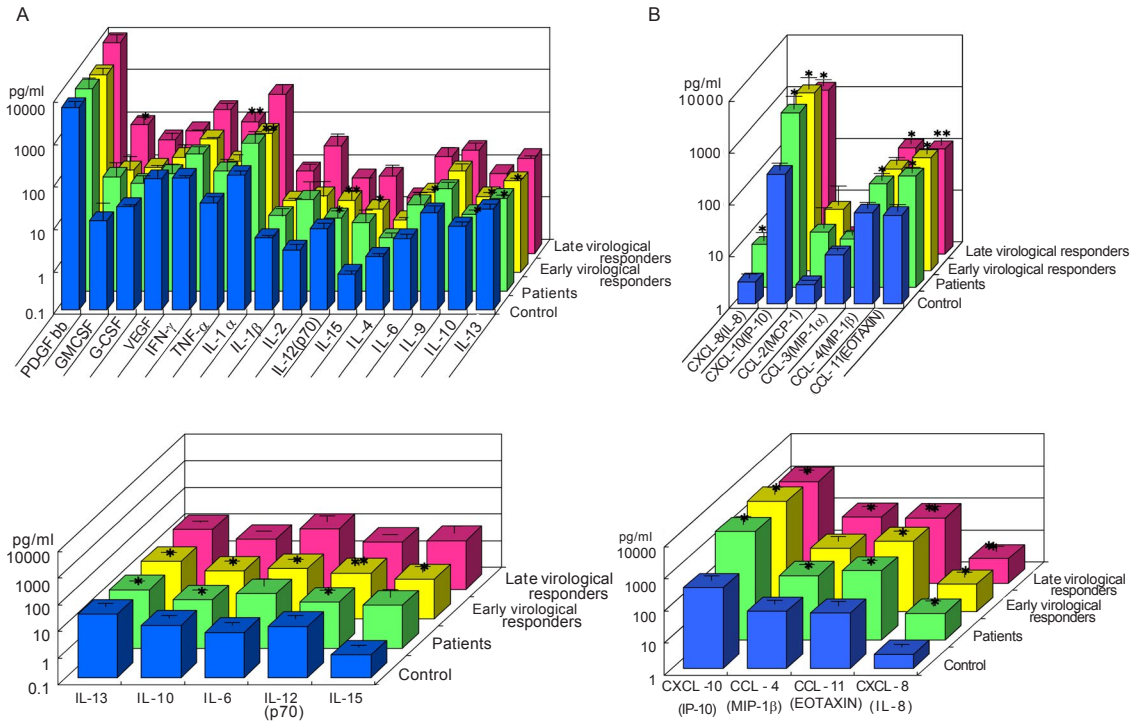

Figure 4: Levels of serum cytokines (A) (A') and chemokines (B)(B') at baseline in chronic hepatitis C patients with high viral loads, serotype 1 (genotype 1b), and wild or intermediate types of ISDR. ISDR; IFN sensitivity determining region. Significant difference: * p<0.05, ** p<0.1.

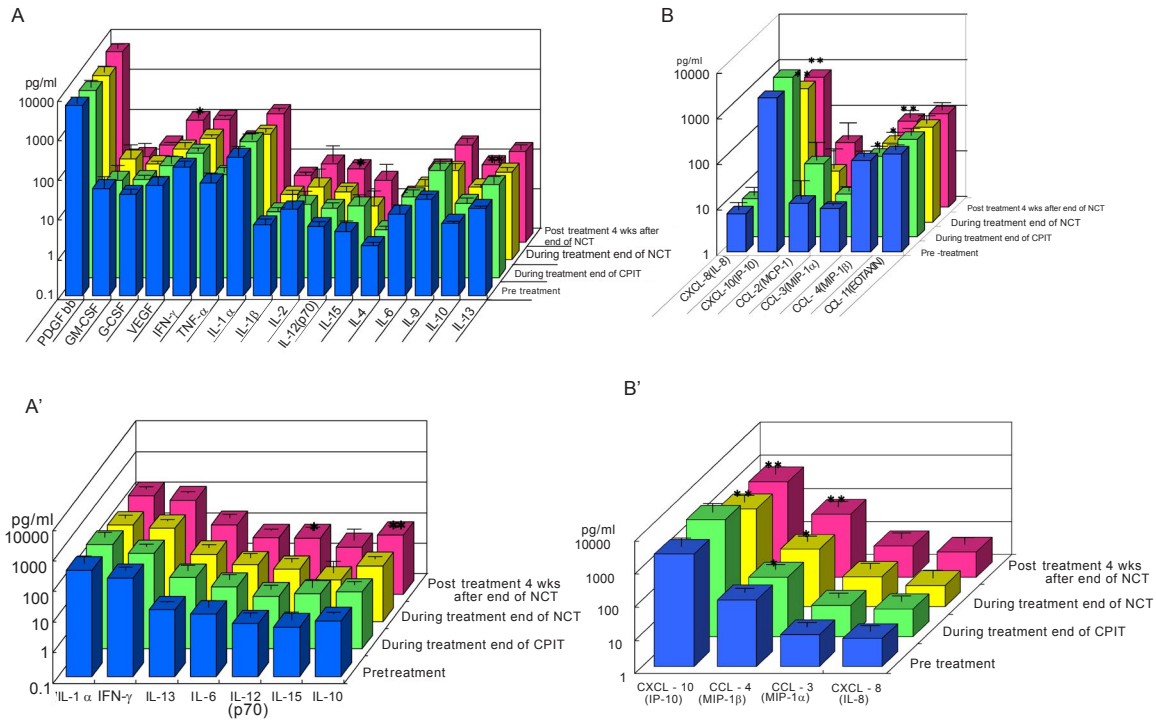

Figure 5: Effect of NCT on serum cytokines (A)(A') and chemokines (B)(B') in chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b) and wild or intermediate type of ISDR (all patients). NCT: Novel Combination Treatment. Significant difference: * p<0.05, ** p<0.1.

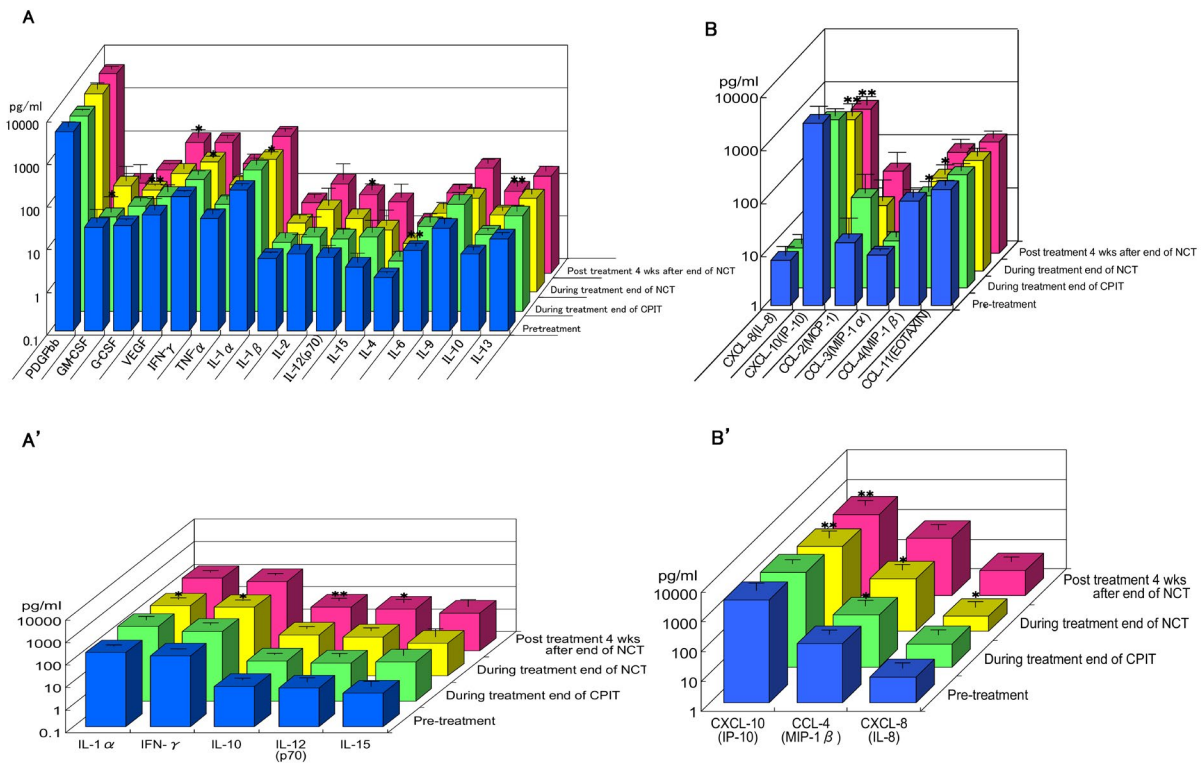

Figure 6: Effect of NCT on serum cytokines (A)(A') and chemokines (B)(B') in chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b) and wild or intermediate type of ISDR (early virologic responders). NCT: Novel Combination Treatment. Significant difference: * p<0.05, ** p<0.1.

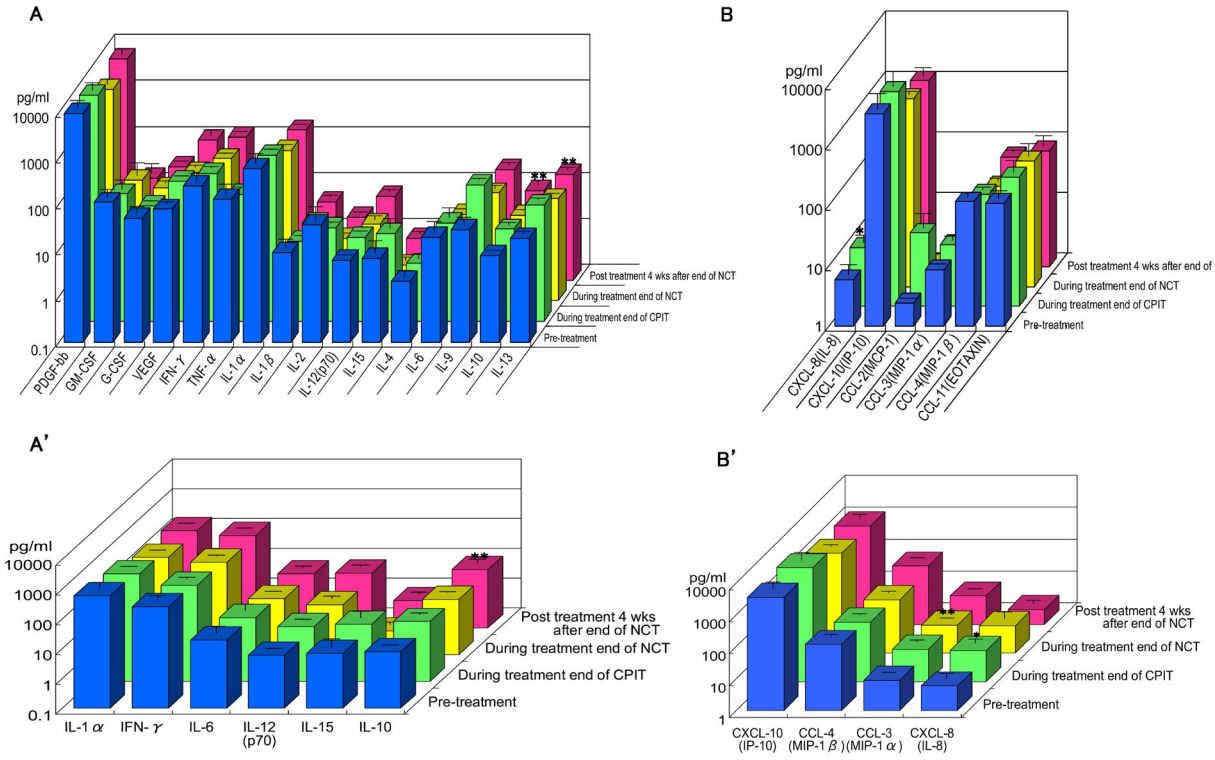

Figure 7: Effect of NCT on serum cytokines (A)(A') and chemokines (B)(B') in chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b) and wild or intermediate type of ISDR (late virologic responders). NCT: Novel Combination Treatment. Significant difference: * $p<0.05$, ** $p<0.1$.

viral clearance of HCVRNA, which lead to an improvement in innate and adaptive immune responses in difficult-to-treat CHC patients with genotype-1b, high viral loads, and wild or intermediate types of ISDR. The current results (Figures 4-15) showed that (1) the significantly lower levels ($p<0.05$) of IL-12 and significantly higher levels ($p<0.05$) of IL-10, CXCL-8, CXCL-10, CCL-4 and CCL-11 in CHC patients than in the controls at baseline suggested an impairment in innate and adaptive immunities in CHC patients, (2) the level of IL-15 was increased at the end of CPIT in both EAVR, and LAVR, CXCL-8, CXCL-10, and CCL-4 levels were significantly decreased ($p<0.05$) in EAVR, but not in LAVR during NCT, (3) the level of IL-12 increased significantly ($p<0.05$) and the level of CXCL-8 decreased significantly ($p<0.05$) after the end of NCT in EAVR, but not in LAVR, and (4) levels of IFN-gamma and TNF-alpha at baseline were higher than those in the controls. At the end of CPIT, at the end of NCT, and 4 weeks after the end of NCT, IFN-gamma and TNF-alpha levels were decreased (Figures 10 and 11). The results obtained in the present study suggest that initial early virologic clearance induced by CPIT before the use of SOC induced the restoration of DC function and improvements in the activation of natural killer (NK) cell as indicated by the up-regulation of IL-12 and IL-15 and down-regulation of CXCL-8, CXCL-10, CCL-4, and CCL-11.

As a key component of innate immunity in the liver, NK cells perform critical roles in the host defense response against a pathogen directly or indirectly through their natural cytotoxicity and cyotkine production, and they also act as regulatory cells by engaging in reciprocal interactions with DCs, Kupffer cells, macrophages, T cells, B cells, and endothelial cells through cell-to-cell contact and the production of cytokines, including IFN-gamma and TNF-alpha, which can directly inhibit viral replication and prime the adaptive immune response and chemokines, and growth factors or through innate immune recognition [35]. Among these cytokines, IFN alpha/beta is believed to be the most potent activator of NK cell cytotoxicity with IL-12 and IL-18 being strong inducers of NK cell production of IFN-gamma, and IL-15 promoting NK cell proliferation. IFN-alpha/beta produced by HCV-infected hepatocytes, or treatment with exogenous IFN-alpha/beta play critical roles in inducing NK cell activation and controlling HCV infection. NK cells interact with dendritic cells (DCs) in a reciprocal manner, leading to increased NK cell activation as well as the maturation of DCs. NK cells play an important role in controlling viral hepatitis. NK cell activity is stringently controlled by inhibitory NK receptors (NKRs) [36]. The function of NK cells is known to be tightly orchestrated by a balance between signals derived from inhibitory and activating receptors [37]. NK cell functions are also greatly influenced by the presence of polarizing cytokines such as interleukin (IL)-12 and IL-4 [38]. NK cells can produce IFN-gamma when stimulated with IL-12, a cytokine critical for inducing T helper (Th) 1-type immune responses [13]. In the case of TH1 cells, IL-12 produced by DCs is essential [39]. The infection of DCs with HCV was associated with the impaired expression of IL-12 and TNF-alpha [10].

NK cells are activated during acute infection (Figure 17). With persistent HCV infection (Figure 18), activated NK cells may contribute to liver damage or produce regulatory cytokines such as IL-10 and TGF-beta, which dampen liver inflammation, as well as adaptive immune responses. Upon the initiation of IFN therapy, NK cells are further activated and may participate in both the first- and second-phase decline in the viral load via direct cytotoxicity and TRAIL-mediated killing of infected hepatocytes [40,41]. HCV inhibits NK cell functions and escapes from the immune surveillance of NK cells, leading to

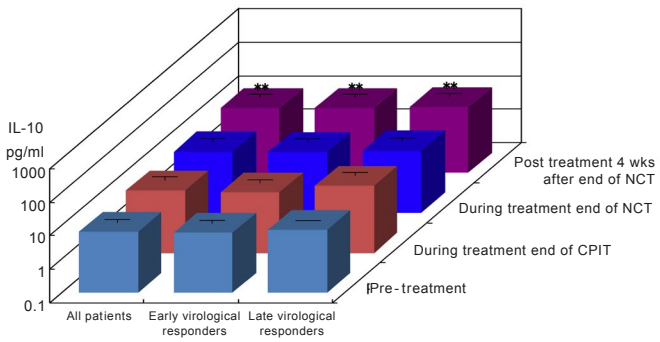

Figure 8: Effect of NCT on serum IL-10 in chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b) and wild or intermediate type of ISDR. NCT: Novel Combination Treatment. Significant difference: * p<0.05, ** p<0.1.

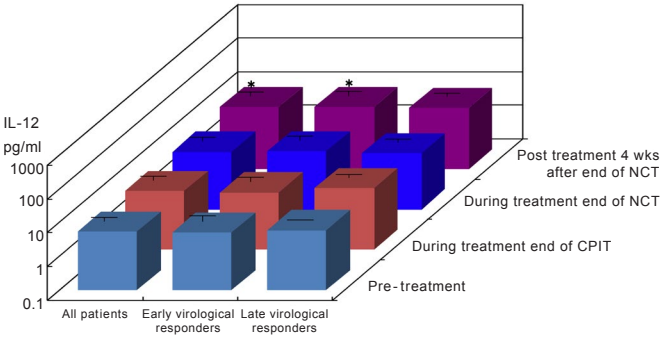

Figure 9: Effect of NCT on serum IL-12 in chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b) and wild or intermediate type of ISDR. NCT: Novel Combination Treatment. Significant difference: * p<0.05, ** p<0.1.

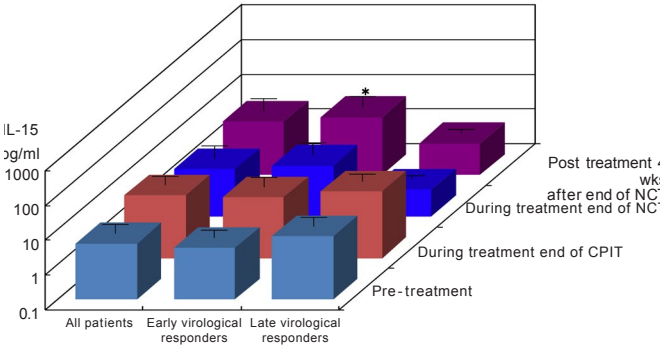

Figure 10: Effect of NCT on serum IL-15 in chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b) and wild or intermediate type of ISDR. NCT: Novel Combination Treatment. Significant difference: * p<0.05, ** p<0.1.

chronic infection [35]. NK cell frequencies are lower in patients with chronic HCV infection than in healthy individuals. In addition, the impaired production of the TH1 polarizing cytokine, IFN-gamma, and increased production of immunoregulatory cytokines, such as IL-10 and TGF−beta, has been reported [42]. The inhibition of IL-10/TGF-beta appears to be the most promising condition to restore CD4+ T cells [43]. Chronic HCV infection showing impaired NK cytolytic function is associated with a down-regulation in TRAIL expression, which may in part explain HCV persistence in the host liver [32].

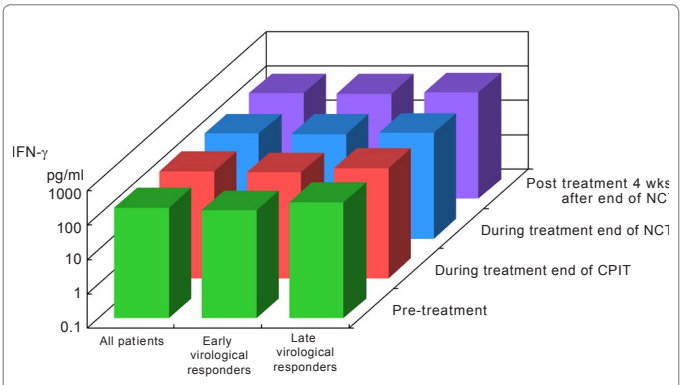

Figure 11: Effect of NCT on serum IFN-gamma in chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b) and wild or intermediate type of ISDR. NCT: Novel Combination Treatment. Significant difference: * p<0.05, **p<0.1.

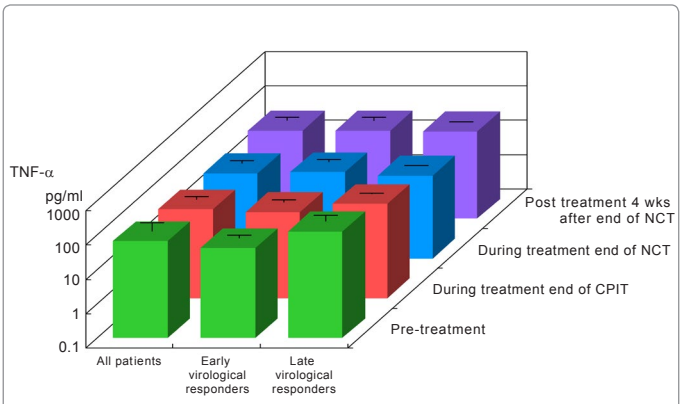

Figure 12: Effect of NCT on serum TNF-alpha in chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b) and wild or intermediate type of ISDR. NCT: Novel Combination Treatment. Significant difference: * p<0.05, ** p<0.1.

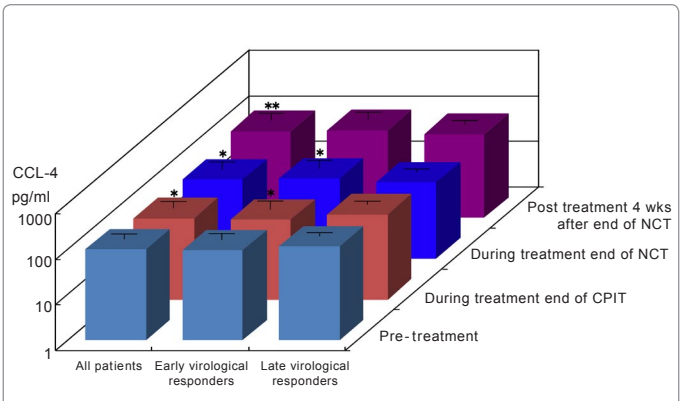

Figure 13: Effect of NCT on serum CCL-4 in chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b) and wild or intermediate type of ISDR. NCT: Novel Combination Treatment. Significant difference: * p<0.05, ** p<0.1.

NK cells are activated by IFN-alpha/beta and other cytokines such as IL-12, IL-15, IL-18, and IFN-gamma and play critical roles in controlling viral hepatitis and liver fibrosis [35]. IFN activates NK cells early after the treatment is initiated. Their cytotoxic function, in particular, is strongly induced, which correlates to the virologic response. Therefore, NK cell activation indicates responses to IFN-

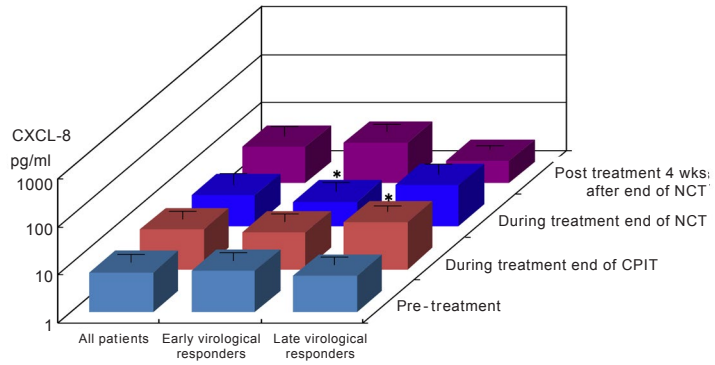

Figure 14: Effect of NCT on serum CXCL-8 in chronic hepatitis C patients with high viral load, serotype 1 (genotype 1b) and wild or intermediate type of ISDR. NCT: Novel Combination Treatment. Significant difference: * p<0.05, ** p<0.1.

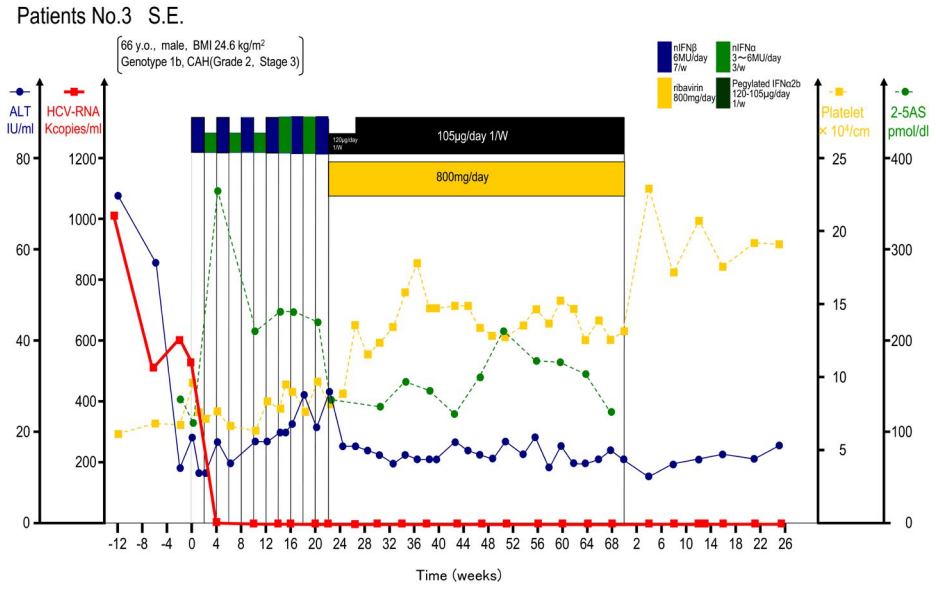

Figure 15: Case 3. A patient with chronic hepatitis C [66 year old, male, serotype 1(genotype 1b), HCVRNA 536 K copies/ml, Stage 3 and Grade 2] was treated with NCT for 72 weeks resulting in RVR, EVR, ETVR and SVR. NCT; novel combination treatment, RVR; rapid virologic response, EVR; early virologic response, ETVR; end treatment virologic response, SVR; sustained virologic response.

alpha based treatment and suggests the involvement of innate immune cells in viral clearance [41]. NKp46^{High} NK cells are "bifunctional", in that they are capable of producing IFN-gamma and yet have higher cytolytic activity than that of NKp46^{Dim} subset. Nkp46^{Dim} NK cells from HCV (+) patients displayed significantly lower IFN-gamma secretion than that of NKp46^{Dim} NK cells obtained from healthy controls. NKp46^{High} NK cells were significantly more effective in blocking HCV replication in vitro than that of the NKp46^{Dim} NK cell subset [37]. The up-regulation of NKp46 expression in response to interferon-alpha (IFN-alpha) is predictive of SVR in chronic HCV infection [36]. High-dose IFN-alpha treatment is known to enhance NK cell polarization toward cytotoxicity and TRAIL expression during the first week Figure 18 [40]. A correlation was previously shown between NK cell responsiveness, specifically the induction of cytotoxic NK cell function and first-phase (48 hours) virological response, as well as the early (12 weeks) virological response of IFN-alpha based therapy [41]. Thus, it is conceivable that NK cells are activated early during IFN-based therapy, but that the NK-cell-mediated clearance of HCV-infected cells may take longer to occur [41]. T-cell responses were more likely to peak late in the course of treatment. Combination therapy for HCV has a transient effect on host virus-specific T cells in the blood. The induction of sustained T-cell responses may require additional immune modulation later in therapy [44].

The results of our study suggested that CPIT restored innate immune responses, as indicated by the up-regulation of IL-12, and IL-15 and down-regulation of CXCL-8, CCL-4, and CXCL-10, however, there was an insufficient improvement in adaptive immune responses, as indicated by the down-regulation of IFN gamma and TNF-alpha in CHC patients during NCT. Therefore, more effective NCT for the up-regulation of the NKp46^{High} NK cell subset, which is effective in polarizing Th1 type immune responses and induction of sustained T-cell responses, may be necessary to improve the rate of SVR in difficult-to-treat CHC patients.

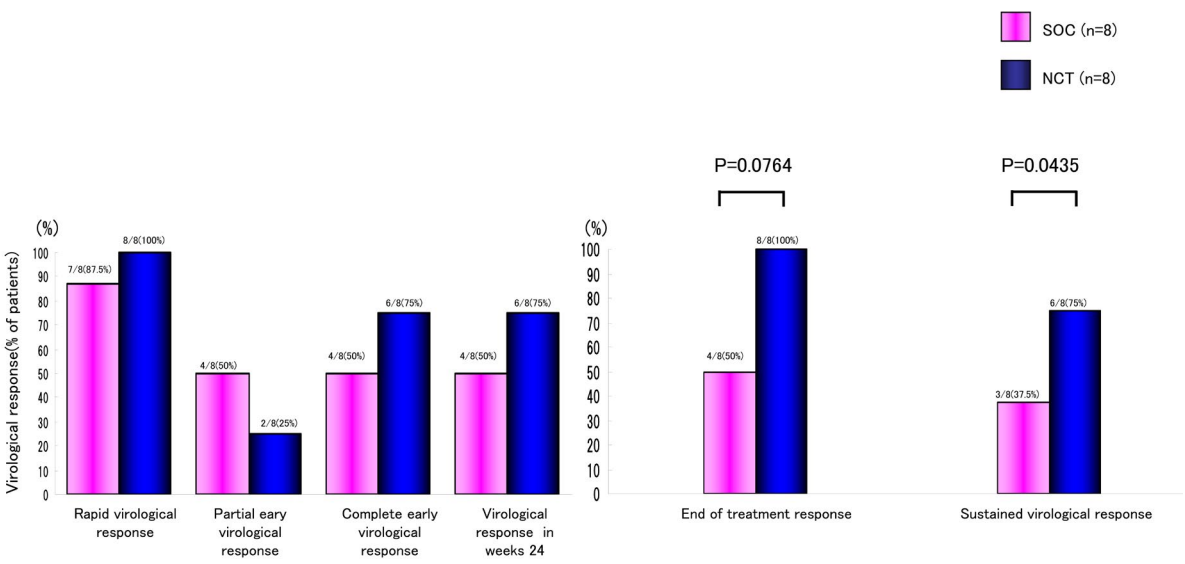

Figure 16: Rate of early virologic responses in the 4, 12, and 24 weeks (A) and end-of –treatment virologic response, and sustained virologic response (B) in chronic hepatitis C patients with serotype 1 (genotype 1b) and high viral load treated with the NCT or the SOC according to intention-to-treatment. NCT: novel combination treatment, SOC: standard of care.

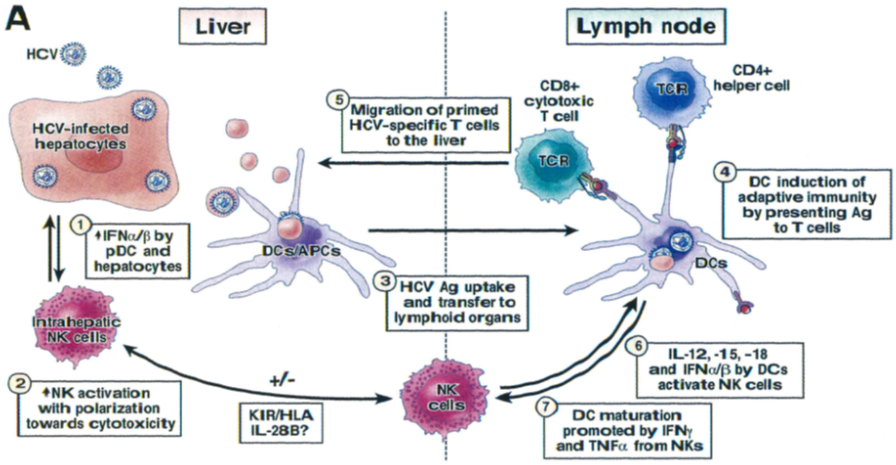

Figure 17: Model of NK cell activation during acute and chronic HCV infection.During acute infection, (1) IFN-alpha/beta is produced by plasmacytoid DCs,infected hepatocytes or other intrahepatic cells upon sensing HCV-RNA, leading to (2) activation of NK cells toward cytotoxicity to kill HCV-infected hepatocytes via perforin/granzyme or TRAIL-dependent mechanism. This increased NK-lysis of infected hepatocytes can (3) enhance the uptake of HCV antigens by DCs and other antigen-presenting cells and transfer of HCV antigens to the draining lymph nodes where (4) HCV-specific CD4+ and CD8+ T cells are primed and (5) remigrate to the liver to efficiently mediate viral clearance. NK cells are (6) further activated by various cytokines including IL-12, -15 and -18, and IFN-alpha/beta, where (7) NK-derived IFN-gamma and TNF-alpha can promote DC maturation. Although the precise mechanisms are not yet defined, several host factors may also modulate NK cell activation or NK/DC interactions, including polymorphism in the KIR/HLA loci and IL28B. [GOLD AHLENSTIEL, BIRGIT EDLICE et al. Early Changes in Natural Killer Cell Function Indicate Virologic Response to Interferon Therapy for Hepatitis C. GASTROENTEROLOGY. 2011; 141: 1230-1239 (modification)].

The timing of these responses is not the same for early and late virologic responders. There is a distinct shift at the point at which viral replication begins to decrease in individual HCVRNA titers. One of the key characteristics of a HCV infection is a delayed immune response in spite of an early increase in the HCV titer and induction of ISGs. A delay in the induction of the innate immune response that caused this decrease resulted in continued viral replication, which may account for the higher peak in HCVRNA titers seen in the non SVR group. This delay may lead to immune escape or exhaustion of the induced response due to the high numbers of infected cells [45].Chemokines and cytokines are critical regulators of liver inflammation and also innate and adaptive immunities to HCV, the complex orchestration of which is suggested to determine the outcome of HCV infections [46,47]. Both maturation and functional differentiation of cDCs are altered during a HCV infection with decreased IL-12 [48] and increased IL-10 production in vitro [49,50]. The HCV core protein has been shown to bind to the globular domain of the complement receptor of macrophages and DCs and down-regulate IL-12 production [51]. Since IL-12 is a key cytokine in the induction of CD4 T cell activation, whereas IL-10 has complex inhibitory effects, the HCV-induced modulation of

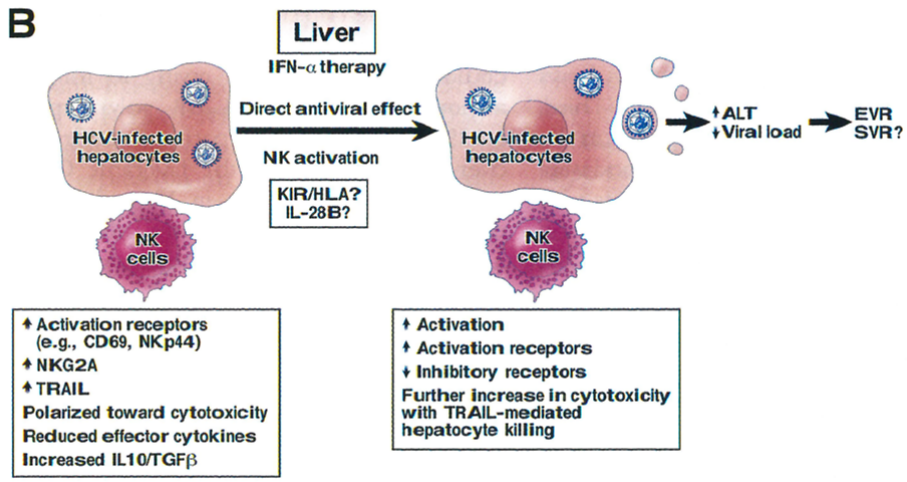

Figure 18: Model of NK cell activation during acute and chronic infection. During chronic HCV infection, NK cells generally activated, but with increased expression of the inhibitory receptor NKG2A. They are polarized toward cytotoxicity with reduced expression of IFN-gamma and increased expression of regulatory cytokines such as IL-10 and TGF-beta. Upon initiation of IFN-alpha therapy, the first-phase decline of plasma viral load is mediated by direct inhibition of viral replication by IFN-alpha. NK cells are activated by exogenous IFN-alpha and may participate in both first- and second-phase declines of viral load via direct cytotoxicity and TRAIL-mediated killing of infected hepatocytes. NK cell activation during the first phase virologic response correlates with EVR.The relevance of genetic polymorphisms including IL28B polymorphism in NK activation remains undefined. [GOLD AHLENSTIEL, BIRGIT EDLICE et al. Early Changes in Natural Killer Cell Function Indicate Virologic Response to Interferon Therapy for Hepatitis C. GASTROENTERLOGY. 2011; 141: 1231-1239 (modification)].

these cytokines may have special importance in the altered the HCV-specific T cell responses observed in chronic HCV infections [46]. IL-12 is an interleukin produced primarily by antigen-presenting cells (monocytes, macrophages, and DCs) that play an essential role in the interaction between innate and adaptive immunities acting on T and NK cells to generate cytotoxic lymphocytes. IL-12 acts on the NK receptor NKG2D and promotes NK cell function. IL-12 was shown to enhance the cytotoxicity of NK cells toward different solid and haematological tumor cell lines and promote IFN-gamma secretion by NK cells [52]. IL-12 governs the Th1-type immune response, which affects spontaneous and treatment-induced recovery from HCV infection [53].

Increased levels of IL-15 at the end of CPIT suggested that the initial viral clearance, induced by CPIT before the beginning of SOC, improved the innate immune response to HCV. IL-15 plays an important role in the innate immune system and is a stimulatory cytokine for DCs impaired in CHC. IL15 is induced by IFN-alpha and/or IFN-beta and stimulates the proliferation and accumulation of NK cells. IL-15 is required for the maturation and survival of NK cells. NK cells play a role in both innate and adaptive immunities [33]. Therefore, IL-12 and IL-15 are essential cytokines for innate and adaptive immune responses. The activation of NK cells, as well as the timing, breadth, and robustness of subsequent antigen-specific T cell immunity, is likely to be markedly shaped by early events in the innate response to the pathogen. NK cells exhibit beneficial effects by inhibiting viral hepatitis. The activation of NK cells may be a novel therapeutic strategy for the treatment of chronic HCV infection. IFN-alpha/beta is one of the most potent NK cell activators. NCT induced the activation of NK cells, as indicated by the up-regulation of IL-12 and IL-15.

High serum levels of IL-10 have been associated with an incomplete response to IFN therapy. Chronic HCV infection is characterized by a poor cellular immune response, which may be due in part to the production of immune suppressive cytokines such as like IL-10 [54-57]. IL-10 inhibits IFN-alpha production, promotes the apoptosis of pDC, and down regulates effector T cell responses [46]. IL-10-inhibiting peptides may have important applications to enhance anti-HCV immune responses by restoring the immuno-stimulatory capabilities of DCs. Our results showed that IL-10 levels were slightly lower in CHC patients than in the controls. At the end of NCT, IL-10 levels increased in CHC patients (Figure 7). IL-10 can up-regulate B-cell survival and maturation into plasma cells and increase the activity of CD 8 T cells [58]. These results suggest that an incomplete cellular immune response to the IFN treatment.

CXCL-10 and CCL-3 levels decreased in EAVR, but increased in LAVR at the end of CPIT. CXCL-8 levels were significantly higher in CHC than in the controls. Because the production of CXCL-8 is stimulated by HCV NS5A, it is able to directly inhibit the antiviral activity of IFN-alpha, and higher CXCL-8 levels in non-responders may partly contribute to the poor response to IFN-alpha therapy [59,60]. CCXL-8 levels decreased in EAVR, but increased significantly in LAVR. These results suggested the restoration of antiviral activity of type 1 IFN inhibited by CXCL-8 in EAVR, but not in LAVR. Serum CXCL-10 levels at baseline were higher in CHC patients than in the controls. Serum CXCL-10 levels were significantly decreased in EAVR, but not in LAVR. CXCL-10 is a chemotactic CXCL chemokine that targets the CXCR 3 receptor and attracts T lymphocytes, NK cells, and monocytes. Low CXCL-10 levels both in the liver and plasma before the onset of treatment have been associated with SVR and pronounce the first phase reduction in the HCV viral load for all viral genotypes [61-63]. CXCL-10 levels have been correlated with elevated numbers of circulating CXCR3+ cells. A previous study proposed that high CXCL-10 levels in patients who did not respond to anti-HCV therapy could act as an antagonist of T cell migration [64].

Serum CCL-4 and CCL-11 levels at baseline were significantly higher in CHC patients than in the controls. Serum CCL-4 and CCL-11 levels significantly decreased in EAVR, but not in LAVR. CCL-4 mediated T-cell infiltration is essential for the delivery of IFN-gamma to mediate protective downstream responses against HCV infection in the liver. The intra-hepatic gene expression profiles of chimpanzees

showed that CCL-4 was up-regulated during acute infection at the time of viral clearance, but was not in those who failed to eradicate the virus [10]. CCL-11 is a chemokine that is thought to selectively attract eosinophils by activating CCR3 receptors. Several studies have shown that CCL-11 is also involved in the pathogenesis of inflammatory processes during liver diseases [65]. Harvey et al. recently analyzed the association between chemokines and virologic responses to IFN and RBV in HIV and HCV co-infected patients [66]; plasma CCL-11 levels before therapy were significantly higher in patients achieving SVR than in non-responders [10].

Study 2 revealed that NCT was well tolerated and enhanced RVR, cEVR, ETVR, and SVR rates in difficult-to-treat CHC patients with genotype 1b and high viral loads, and showed less AEs than those in SOC. These higher virologic response rates highlight the benefit of NCT with an induction approach using nIFN-beta in CHC patients. These results suggest that (1) early virological clearance by CPIT before the beginning of SOC induced the restoration of innate immune responses and to anti-viral responses, (2) persistent virologic clearance for more than 48 weeks with subsequent SOC induced the restoration of innate immune responses linked to adaptive immune responses resulting in SVR and SBR, and (3) CPIT improved the innate immune response; however, there was an insufficient improvement in the adaptive immune response in CHC during NCT. T cell responses were more likely to peak late in the course of treatment. Combination therapy for HCV has a transient effect on host virus-specific T cells in the blood. The induction of sustained T cell responses may require additional immune modulation later in therapy [45]. Most antiviral compounds can induce a good end-of-therapy response rate that is often not sufficient to result in the effective immune-mediated elimination of the virus, which is required for a curative sustained virologic response [44]. The findings of this study support the concept that viral clearance early in the course of therapy with reduced virologic resistance is linked to the restoration of innate and adaptive immune responses, which suggests that agents providing the greatest viral suppression leading to extended RVR including nIFN-beta, IFN-lambda, DAAs and new developing agents may be preferable for the initial early induction approach. Initial viral clearance induced by CPIT in combination with those agents may lead to an improvement in innate and adaptive immune responses, resulting in a higher rate of SVR in difficult-to-treat CHC patients with genotype 1b and a high viral load. Restoration of innate immune responses including the activation of NK cell activity may be a novel therapeutic strategy for chronic HCV infection. In previous studies, dose reductions in or treatment discontinuations of PegIFN-alpha, which were often required to manage adverse hematological events, have been associated with a reduction in therapeutic efficacy. No serious AEs were found in NCT, and good tolerance of NCT was confirmed by high compliance rates. The results observed in this study agree favorably with findings on the safety of nIFN-beta treatment in CHC patients and support the use of nIFN-beta as a safe and alternative option.

Conclusions

An induction approach with nIFN-beta for 24 weeks followed by SOC for 48 weeks (NCT) was well tolerated without discontinuation. NCT overcame viral breakthrough with viral clearance, leading to an enhanced early virologic response and improved SVR rates in difficult-to-treat CHC patients with genotype 1b and high viral loads. Early virologic clearance by CPIT for 24 weeks before beginning SOC induced the restoration of innate immune responses linked to adaptive immune responses and resulting in SVR and SBR. SVR rates in CHC patients with genotype 1b and high viral loads were higher in patients receiving NCT than in those receiving SOC. NCT was more effective and caused less adverse effects than SOC in difficult-to-treat CHC patients with genotype 1b and a high viral load.

Conflict of Interests

The authors declare that they have no conflict of interests.

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