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CD81 down-regulation on B cells is associated with the response to interferon-alpha-based treatment for chronic hepatitis C virus infection

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Abstract

The lymphocytic CD81 molecule, capable of modulating type-1/-2 T-helper responses and serving as a putative receptor for hepatitis C virus (HCV), might influence the outcome of anti-HCV treatment. This study characterized the interferon-alpha-induced alteration of lymphocytic CD81. The CD81 levels in healthy subjects and naïve chronic HCV patients were compared, with the results showing that the two groups had comparable surface CD81 levels for total peripheral blood lymphocytes, subpopulation-B, -T, and -NK cells. In vitro interferon-alpha treatment could suppress the CD81 expression from both groups. Subsequently, we compared the in vitro interferon-alpha modulatory effects on lymphocytic CD81 from patients having received anti-HCV therapy with either sustained virological response (SVR) or without SVR. There was a significant down-regulation of the B-cell's CD81 only in the SVR group. The CD81 modulation was further investigated using Daudi lymphoid cell line, showing declined surface CD81 levels following treatment with interferon-alpha, interferon-beta or polyI:C. Thus, interferons could directly decrease CD81 expression. The interferon-alpha effect could be restored by 2-aminopurine, suggesting that double-stranded RNA activated kinase might be involved in the suppression of CD81. In conclusion, CD81 down-regulation is a primary host response to interferon-alpha-based therapy and an immunophenotype associated with anti-HCV SVR.

Keywords: CD81; Interferon-alpha; Hepatitis C virus

1. Introduction

Hepatitis C virus (HCV) infection causes life-threatening disease due to its persistence in the liver, over time leading to cirrhosis and hepatocellular carcinoma. Therapeutic interventions with interferon (IFN)-alpha combined with ribavirin are aimed at viral clearance (McHutchison et al., 1998), which might discontinue the virus-induced hepatocyte damage, thus presumably slowing down the progress to end-stage liver disease (Davis et al., 1998; Poynard et al., 2000). Current evidence has demonstrated that the combination regimen could exert apparent eradication of HCV viremia in 46–78% of the treated patients (Fried et al., 2002; Manns et al., 2001), with the therapeutic efficiency depending upon the viral genotype and initial circulating viral load. In addition to these known virological factors, host factors associated with antiviral immunity seem to affect the outcome of the combination therapy since both IFN-alpha and ribavirin have shown immuno-modulatory activities in the clearance of HCV (Lau et al., 2002). Sufficient HCV-specific T cells function to elicit intrahepatic cytotoxicity (Lohr et al., 1999; Nelson et al., 1998), to produce type-1 cytokines, such as IFN-gamma, and to suppress type-2 cytokines, such

Abbreviations: 2-AP, 2-aminopurine; ALT, alanine transaminase; E, envelope; FACS, fluorescence-activated cell scan; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; MFI, mean fluorescence intensity; NK, natural killer; PBLs, peripheral blood lymphocytes; PKR, double-stranded RNA activated kinase; S.D., standard deviation; SVR, sustained virological response; TSA, trichostatin A

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as interleukin (IL)-4 and IL-10 (Cramp et al., 2000; Kamal et al., 2002; Tsai et al., 2003), and most likely favor a successful response to the current anti-HCV treatment. Additionally, genetic polymorphisms which affect the function or expression of chemokines and MxA protein may be associated with therapeutic responses against chronic HCV infection (Hellier et al., 2003; Konishi et al., 2004; Promrat et al., 2003; Suzuki et al., 2004).

It has been demonstrated that HCV virion, via major structural envelope (E) protein 2 (Pileri et al., 1998) or E1E2 complex (Cocquerel et al., 2003) could specifically bind to the human CD81 molecule, thereby altering the cellular activities in B cells (Cocquerel et al., 2003; Flint et al., 1999), T cells (Wack et al., 2001), and natural killer (NK) cells (Crotta et al., 2002; Tseng and Klimpel, 2002). CD81, a surface protein belonging to the tetraspanin family, facilitates B–T cell interaction in the process of antigen presentation (Mittelbrunn et al., 2002; Secrist et al., 1996). Evidence has revealed that CD81 on B and T cells substantially enhanced T helper IL-4 secretion (Deng et al., 2002; Maecker et al., 1998; Maecker, 2003), and NK-cell CD81 enabled E2-mediated reduction of the IFN-gamma levels (Crotta et al., 2002; Tseng and Klimpel, 2002). Those data suggest that CD81 might activate the pathway leading to a predominant type-2 immune response, and as such be prone to cause a strong antibody production but weak or insufficient cytotoxic activities to clear the virus (Cramp et al., 2000; Kamal et al., 2002; Tsai et al., 2003). Down-regulation of CD81 has been demonstrated in association with a decrease in the HCV viral load in IFN-alpha recipients. This gives rise to the possibility for evaluating the CD81 status in correlation with the HCV pathogenesis and therapeutic responsiveness (Curry et al., 2003; Kronenberger et al., 2001; Zuckerman et al., 2002, 2003). This study was aimed to investigate the clinical relevance and the possible mechanism of CD81 down-regulation under IFN-alpha treatment. We examined the CD81 expression on the subpopulations of peripheral blood lymphocytes (PBLs) in healthy subjects and chronic hepatitis C patients who were naïve, responsive, and non-responsive, respectively, to antiviral treatment. Next, Daudi lymphoid cells were then exploited to investigate the possible mechanism of CD81 modulation by IFN-alpha, showing that double-stranded RNA activated kinase (PKR) might be involved in this intracellular regulatory function.

2. Materials and methods

2.1. Healthy subjects and chronic hepatitis C patients

The study was approved by the Institutional Review Board, and all patients signed a written informed consent prior to entering into the study. Regarding the patients, they were categorized into (a) naïve patients with no previous anti-HCV therapy and (b) patients having received anti-HCV therapy.

2.1.1. Healthy subjects and naïve chronic hepatitis C patients

Two groups of subjects, including healthy subjects and naïve chronic hepatitis C patients were studied. Group 1 enrolled 15 (6 males and 9 females, age by mean ± standard deviation (S.D.) = 52.1 ± 13.8) healthy subjects and 12 (5 males and 7 females, mean age ± S.D. = 52.8 ± 10.4) naïve chronic hepatitis C patients for examination of CD81 on the surface of freshly isolated PBLs. Group 2 enrolled 17 (6 males and 11 females, mean age ± S.D. = 36.8 ± 17.9) healthy subjects and 22 (14 males and 8 females, mean age ± S.D. = 53.6 ± 11.3) naïve chronic hepatitis C patients to compare the IFN-alpha effect on CD81 expression of cultivated PBLs. Naïve chronic hepatitis C patients were diagnosed as having their values of serum alanine transaminase (ALT) elevated above the normal range for at least 6 months, having detectable anti-HCV antibodies and serum HCV RNA, and who did not previously receive anti-HCV therapy. All healthy subjects and naïve chronic hepatitis C patients were serologically negative for surface antigen of hepatitis B virus.

2.1.2. Patients with sustained virological response (SVR) and non-SVR of combination therapy

Twenty-seven adult patients who had received combination therapy of IFN-alpha with ribavirin for 24 weeks were recruited to study the association of IFN-alpha modulation on CD81 linked to therapeutic response. Pretreatment evaluation showed no significant differences in age, gender, initial ALT values and viremic titers between the patient groups of SVR ($n = 12$) and non-SVR ($n = 15$). The rate for achieving SVR was lower in patients infected with HCV genotype 1 (4/12, 33.3%) than those with genotype non-1 (8/15, 53.3%); however, the differences were not statistically significant. IFN-alpha was incubated in vitro with the cultured PBLs taken from these patients during follow-up after cessation of therapy for at least 3 months. There was no difference in duration of post-treatment between SVR and non-SVR groups (7.25 ± 1.91 months versus 6.40 ± 1.84 months, $p = 0.253$). SVR was defined as patients having undetectable HCV RNA at the end of treatment and at follow-up visit 24 weeks after treatment. HCV genotyping was performed by real-time PCR with type-specific primers and probes which could identify genotypes 1 and 2 of HCV (Schroter et al., 2002). Circulating HCV RNA titers were absolutely measured by reverse transcription coupled to real-time PCR analysis with a LightCycler® instrument (Roche, Germany). The reaction included the sense primer: 5′-CCC TGT GAG GAA CTA CTG TCT TCA CG-3′, the antisense primer: 5′-CGG AAC CGG TGA GTA CAC-3′, the sensor probe: 5′-LC Red 640-CCC GGG AGA GCC ATA GTG GTC TG-3′ and the anchor probe: 5′-GCA GCC TCC AGG ACC CCC C-fluorescein-3′. The concentration of HCV RNA was calculated based on an external standard curve constructed with cloned plasmid containing HCV sequences in serial dilutions.

2.2. Materials

Antibodies to human CD81 (clone JS-81), CD19 (clone HIB19), CD3 (clone UCHT1), CD56 (clone B159), HLA-ABC (clone G46-2.6), and isotypic IgG_1, κ mouse antibody (clone MOPC-21) for fluorescence-activated cell scan (FACS) analysis were purchased from Pharmingen (San Diego, CA). IFN-alpha

2b and IFN-beta 1a used in in vitro treatment of cells was obtained from Schering-Plough Corporation (Kenilworth, NJ) and Serono Corporation (Aubonne, Switzerland), respectively. PolyI:C was purchased from InvivoGen (San Diego, CA). Trichostatin A (TSA) and 2-aminopurine (2-AP) were purchased from Sigma (Steinheim, Germany).

2.3. FACS analysis

PBLs were freshly isolated from whole blood samples by gradient centrifugation (Ficoll-Hypaque, Pharmacia Biotech, Sweden). PBLs or cultured cells were washed twice in phosphate-buffered saline, and were directly immunostained with R-phycoerythrin-conjugated anti-CD81 monoclonal antibody for 15 min. After the second washing, the excited fluorescent signals were recorded by FACsort instrument (Becton Dickinson, NJ). PBLs sub-populations of B cell, T cell and NK cell were gated according to $CD19^+$, $CD3^+$, and $CD3^-CD56^+$, respectively, by immunostaining with fluorescein isothiocyanate-conjugated anti-CD19 in one tube, fluorescein isothiocyanate-conjugated anti-CD3 and allophycocyanin-conjugated anti-CD56 antibodies in the other tube. Cognate fluorophore-labelled isotypic mouse IgG antibodies were included in each parallel sample to determine the level of background fluorescence of the negative cells. The mean fluorescence intensity (MFI) of stained cells was determined according to the fraction of positive cells only.

2.4. Cell culture

Isolated PBLs and Daudi lymphoid cell line (ATCC CCL-213) in a density of (5–7) $\times 10^5$ cells/mL were cultured at 37 °C in RPMI 1640 medium (Gibco-BRL, MD) supplemented with 10% fetal bovine serum, L-glutamine, non-essential amino acids, penicillin and streptomycin. IFN-alpha at a concentration of 100 U/mL was used for the in vitro treatment of PBLs from patients.

2.5. Quantification of CD81-RNA by real-time RT-PCR

Total RNA was isolated from cells with a single-step method modified from the acid guanidinium–thiocyanate–phenol–chloroform extraction procedure. A total of 1×10^6 cells were mixed with REzol™ C&T reagent (PROtech Technology Co., Taiwan) as per manufacturer's recommendation. After extraction and centrifugation for removal of DNA and protein, the total RNA was precipitated with isopropanol and then re-dissolved in RNase-free water. Three micrograms of total RNA were reversely transcribed with M-MLV reverse transcriptase (Promega, Madison, WI). Then, absolute CD81-cDNA was quantified online by hybridization probe method based on an external standard curve with the LightCycler® instrument (Roche, Germany). The reaction included the sense primer 5′-ACCTTCCACGAGACGCTT-3′, the antisense primer 5′-CAGGATCATCTCGAAGATCATG-3′, the sensor probe: 5′-LC Red 640-CTGATGATGTTGCTGCCCGAGG-3′ and the anchor probe: 5′-GTGGCAGTCCTCCTTGAAGAGGTT-fluorescein-3′. The cellular levels of CD81 expression were normalized to glyceraldehyde-3-phosphate dehydrogenase, which was quantitated with LightCycler® h-G6PDH Housekeeping Gene Set (Roche, Germany), prior to the statistical comparison.

2.6. Statistical analysis

Mean ± S.D. was used to describe the distribution of continuous variables. The statistical methods used to analyze the data sets were Pearson chi-square test, Fisher's exact test, independent samples *t*-test and paired samples *t*-test. These analyses were performed using SPSS software (Version 9.0 for Windows; SPSS Inc., Cary, NC, USA). The statistical significance level was $p < 0.05$.

3. Results

3.1. CD81 expression on freshly isolated and IFN-alpha-treated PBLs from healthy subjects and naïve chronic hepatitis C patients showed no significant difference

We first characterized the overall magnitude of CD81 in relation to HCV infection. PBLs were freshly isolated from 15 healthy subjects and 12 naïve chronic hepatitis C patients. Table 1 (group 1) shows the CD81 expression by MFI on total PBLs and subpopulations, including B cells, T cells and NK cells. The results showed that expression of CD81 was not significantly different in any tested cell populations between healthy subjects and naïve chronic hepatitis C patients.

We then assessed the modulation of the surface CD81 expression by in vitro IFN-alpha treatment on PBLs from 17 healthy subjects and 22 naïve chronic hepatitis C patients. The histograms from one representative PBLs sample regarding modulation of surface CD81 in response to in vitro IFN-alpha treatment are shown in Fig. 1, exhibiting down-regulation of CD81 on total PBLs (A), B cells (B), T cells (C) and NK cells (D) after incubation with IFN-alpha for 24 h. Overall, most healthy subjects (82.3%, 14 of 17) and naïve chronic hepatitis C patients (90.9%, 20 of 22) showed a slight reduction in CD81 expression on PBLs after IFN-alpha treatment in comparison to the matched untreated controls. Further analysis of CD81 response to in vitro IFN-alpha treatment between healthy subjects and naïve chronic hepatitis C patients, total PBLs ($p = 0.468$) and subpopulations of B cells ($p = 0.804$), T cells ($p = 0.156$) and NK cells ($p = 0.536$), showed a comparable down-regulation of CD81 as expressed by the alteration of MFI (Table 1, group 2). Taken together, our data demonstrated that IFN-alpha might induce a general decline of PBL's CD81, with no dependency on chronic HCV infection.

We also examined the in vitro effects of IFN-alpha on the surface HLA-ABC expression of total PBLs and subpopulations from selected normal subjects ($n = 7$) (Table 1, group 3).

Table 1
Comparison of surface CD81 expression on freshly isolated PBL, and the change of CD81 and HLA-ABC by in vitro IFN-alpha treatment between healthy subjects and naïve chronic hepatitis C patients

	Healthy subjects ($n = 15$)	Naïve chronic hepatitis C patients ($n = 12$)	p value
Group 1: surface CD81 expression on freshly isolated PBL, expressed as MFI			
Total	554.59 ± 25.93[a]	542.50 ± 24.09	0.741
B cells	459.51 ± 34.64	405.59 ± 25.05	0.240
T cells	583.67 ± 33.98	570.36 ± 26.24	0.769
NK cells	603.35 ± 41.31	511.52 ± 45.90	0.150
	Healthy subjects ($n = 17$)	Naïve chronic hepatitis C patients ($n = 22$)	p value
Group 2: effect of in vitro IFN-alpha treatment on CD81 expression, expressed as %[b]			
Total	87.63 ± 3.52	91.20 ± 3.29	0.468
B cells	94.85 ± 2.82	93.79 ± 3.01	0.804
T cells	85.55 ± 3.46	93.26 ± 3.84	0.156
NK cells	86.43 ± 8.30	89.77 ± 3.99	0.536
	Healthy subjects ($n = 7$)	Naïve chronic hepatitis C patients	p value
Group 3: effect of in vitro IFN-alpha treatment on HLA-ABC expression, expressed as %[b]			
Total	106.59 ± 3.74	–	–
B cells	116.63 ± 5.10	–	–
T cells	103.95 ± 6.90	–	–
NK cells	119.50 ± 10.59	–	–

[a] Mean ± standard error.
[b] Data shown as percentage values of (IFN-alpha treated sample − matched untreated control)/matched untreated control.

In contrast to the decline patterns of CD81, our results revealed up-regulation of HLA-ABC molecules after treatment with IFN-alpha for 24 h, with a level of 106.59 ± 3.74%, 116.63 ± 5.10%, 103.95 ± 6.90%, 119.5 ± 10.59% to the untreated counterparts for total PBLs, B cells, T cells and NK cells, respectively. It suggested that IFN-alpha treatment might not induce a general reduction of total surface protein expression.

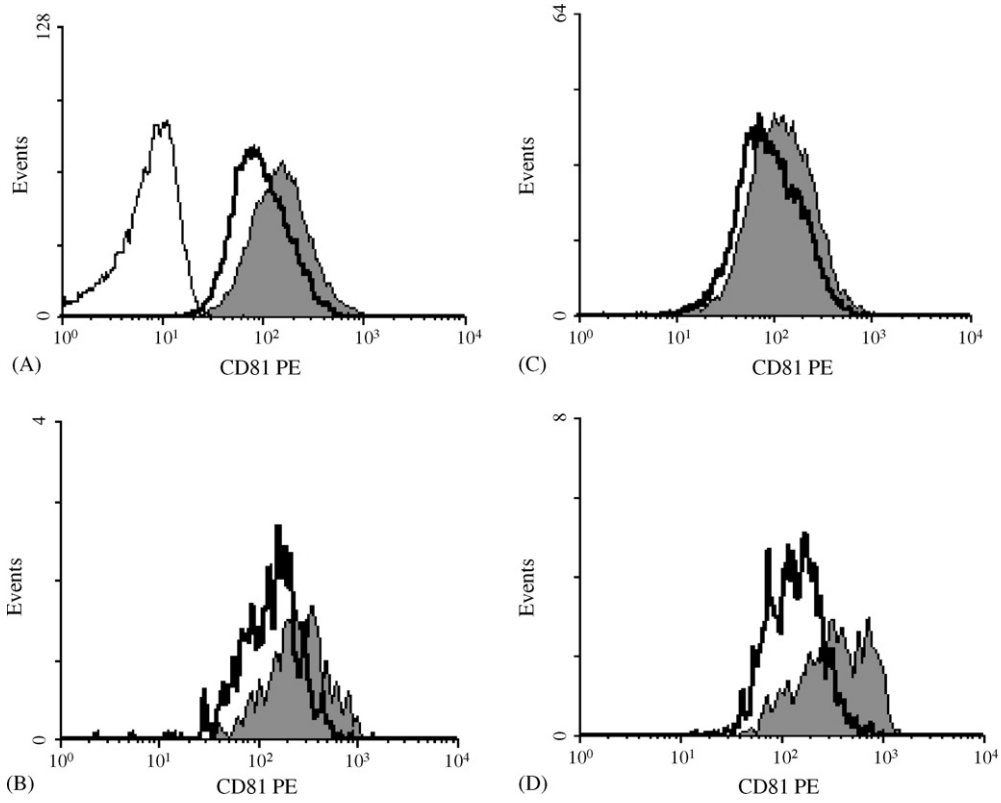

Fig. 1. Modulation of surface CD81 in response to in vitro IFN-alpha treatment. The fluorescent intensity of CD81 on total PBLs (A) $CD19^+$ (B), $CD3^+$ (C), and $CD3^-CD56^+$ (D) lymphocytes were determined by FACS analysis after in vitro incubation in the presence (bold line) or absence (filled curve) of 100 U/mL IFN-alpha for 24 h. The fluorescent intensity from cells stained with isotypic antibody is shown as a thin line.

Table 2
Comparison of CD81 down-regulation by in vitro IFN-alpha treatment on PBL from patients with SVR and non-SVR

Characteristics	SVR ($n=12$)	Non-SVR ($n=15$)	p value
Total	92.43 ± 4.72[a,b]	90.29 ± 3.56	0.716
B cells	84.53 ± 3.20	97.40 ± 4.02	0.023
T cells	98.26 ± 6.11	99.80 ± 9.23	0.896
NK cells	97.21 ± 8.69	84.01 ± 4.16	0.154

[a] Mean ± standard error.
[b] Data shown as percentage values of (IFN-alpha treated sample − matched untreated control)/matched untreated control.

3.2. In vitro treatment of IFN-alpha down-regulated the expression of CD81 on B cells in SVR

We then tested the hypothesis that CD81 might be a host cellular factor related to therapeutic response, and that therefore the patients with SVR displayed a better susceptibility to IFN-alpha modulation than did those of non-SVR. PBLs from 27 patients who had finished combination therapy for at least 3 months were collected to assess the in vitro IFN-alpha modulation of CD81. The PBLs were incubated with IFN-alpha for 24 h and subsequently the CD81 expression was detected according to each of B, T and NK subpopulation. There were no significant differences in CD81 expression on untreated PBLs between the patient groups of SVR and non-SVR. Table 2 showed the comparison of CD81 modulation by IFN-alpha treatment. When compared to the corresponding untreated samples, the percentage of CD81 expression on B cells was significantly more decreased in the SVR group than in the non-SVR group (84.53 ± 3.20% versus 97.40 ± 4.02%, $p=0.023$). However, the IFN-alpha-induced surface CD81 alteration showed no significant difference in T cells and NK cells between the two groups. We examined additional hosts and virological factors between patient groups in the presence or absence of CD81 down-regulation by 10% reduction as a cutoff value. The results indicated that the decreased regulatory effect of CD81 on B cells was only associated with a therapeutic response, but not related to gender, age, baseline ALT level, initial HCV viral titer or HCV genotype (Table 3).

3.3. PKR might be involved in the IFN-induced CD81 down-regulation

The results shown above suggested that IFN-alpha might exert a direct regulatory effect on the CD81 expression of B lymphocytes. To further evaluate how IFN-alpha could possibly induce the decreasing of CD81 expression, we employed a B lymphoid cell line to undergo in vitro IFN-alpha treatment. In the literature, the Daudi lymphoid cell line has been broadly employed in the study of IFN-alpha responses. Preliminary results showed that IFN-alpha down-regulated surface CD81 on Daudi cells in a time-dependent manner within 36 h post-treatment. Then, an interval of 24 h of IFN-alpha treatment was applied for the further study.

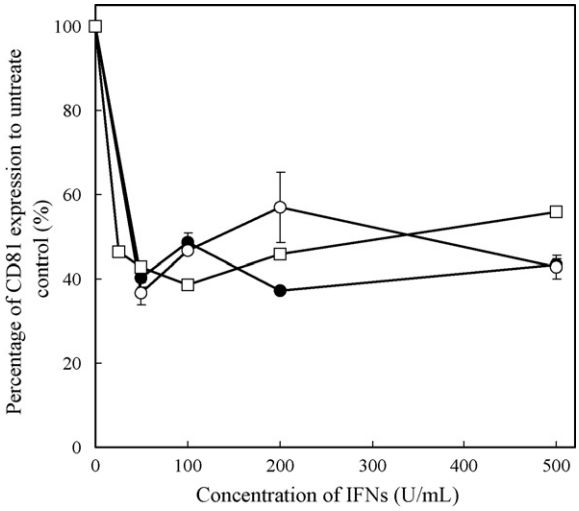

Fig. 2. Type I IFNs, both IFN-alpha and IFN-beta, down-regulated surface CD81 expression on Daudi cells. Daudi cells were treated with IFN-alpha (●), IFN-beta (◯) or combination of IFN-alpha and IFN-beta (□) at various concentrations for 24 h. The surface CD81 expression was assayed by FACS analysis. Results were shown as the percentage to the corresponding untreated control without IFN treatment, by the mean ± S.D. from two independent experiments and two determinations performed in each experiment.

We then tested whether IFN-beta, which interacts with the same cognate receptor as IFN-alpha, could modulate CD81 expression. Fig. 2 demonstrates that either single or combination treatment of IFN-alpha/IFN-beta down-regulates surface CD81 levels in similar dynamics, with a maximal decreased level of up to 40% of the untreated controls. It is therefore suggested that IFN-alpha and IFN-beta might induce the same signaling output in this modulatory event. In addition, we further tested if polyI:C, a stimulator of endogenous IFNs, could exhibit CD81 modulation. Daudi cells were treated with polyI:C at various concentrations in the presence or absence of 100 U/mL of IFN-alpha for 24 h. In the absence of exogenous IFN-alpha, polyI:C treatment could also decrease CD81 expression in a dose-dependent manner (Fig. 3). Meanwhile, the CD81 modu-

Table 3
Comparison of clinical characteristics from hepatitis C patients with or without CD81 down-regulation on B cells by in vitro IFN-alpha treatment

	Presence of CD81 down-regulation ($n=18$)	Absence of CD81 down-regulation ($n=9$)	p value
Gender (M/F)	11/7	3/6	0.236
Age (medium; range)	53.1 ± 10.7 (55.5; 30–69)	58.3 ± 11.9 (63; 28–66)	0.262
Baseline ALT (IU/L)	196.2 ± 115.3	252.8 ± 192.6	0.347
Initial HCV RNA titer in log 10/mL serum (medium; range)	6.08 ± 0.93 (6.34: 3.70–7.52)	5.98 ± 0.71 (6.32; 4.82–6.61)	0.789
HCV genotype (1/non-1)	7/10	5/4	0.683
Therapeutic response (SRV/non-SVR)	11/7	1/8	0.019

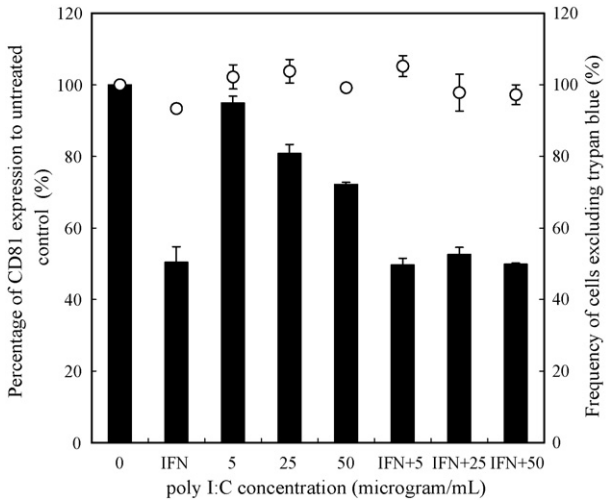

Fig. 3. Down-regulation of CD81 expression on Daudi cells by polyI:C treatment. Daudi cells were treated with polyI:C at concentrations of 0, 5, 25 and 50 μg/mL in the presence or absence of 100 U/mL of IFN-alpha for 24 h. The surface expression of CD81 was measured by FACS analysis (bar). The frequency of cells excluding trypan blue was shown (◯). Results are shown as the percentage to the corresponding control sample without treatment, by the mean ± S.D. from two independent experiments and five determinations performed in each experiment: CD81 expression (*Y* axis at left); the frequency of cells excluding trypan blue (*Y* axis at right).

lation by polyI:C was saturated in the presence of 100 IU/mL IFN-alpha, as evident by exogenous IFN-alpha, alone or in combination with various concentrations of polyI:C, showing maximal decreased responses up to 50% of the untreated controls.

We next examined the possible mechanisms by which IFN-alpha decreased the surface expression of CD81 protein. CD81-RNA quantification was performed in Daudi cells in the presence or absence of IFN-alpha. Our data revealed that the CD81-RNA levels in Daudi cells after treatment with IFN-alpha for 24 h at 100, 200, 500 U/mL were 103.1 ± 6.5%, 100.6 ± 5.6% and 98.0 ± 2.7%, respectively, of the untreated control. Thus, incubation with IFN-alpha decreased the surface CD81 protein expression, but it did not change the total CD81-RNA, suggesting that the IFN-alpha regulation of CD81 expression might take place post-transcriptionally. Subsequently, the regulatory steps by which IFN-alpha could decrease CD81 expression were further clarified in comparison with the presence and absence of inhibitors. As shown in Fig. 4, an IFN-alpha-induced CD81 decline could be restored (+IFN-alpha group) by 2-AP, an inhibitor of PKR, where 2-AP treatment showed slight alteration on CD81 expression (−IFN-alpha group). These results suggested that PKR might participate in the IFN-alpha-induced CD81 down-regulation. In contrast, TSA, a histone deacetylase inhibitor in regulation of RNA-transcription steps, had no obvious function in IFN-alpha regulation of CD81 protein. Finally, to test if CD81 down-regulation by IFN-alpha was also PKR-dependent in patient-based samples, we performed an in vitro rescue experiment with 2-AP by using PBLs from four naïve hepatitis C patients, who had responded to IFN-alpha-mediated suppression of CD81. As a result, 2-AP could restore the modulatory effect in the four patients with significant

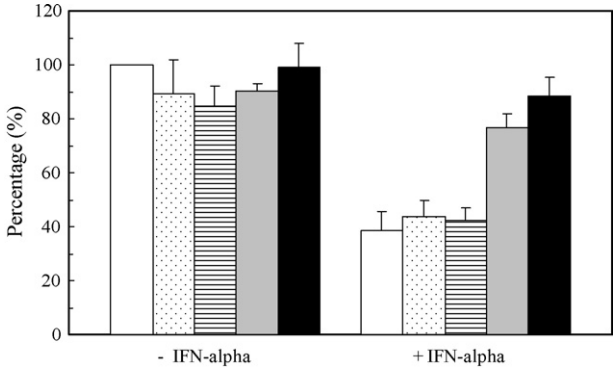

Fig. 4. Effects of TSA and 2-AP on IFN-alpha regulation of CD81 expression. Daudi cells were treated with TSA and 2-AP at various doses in the absence or presence of 200 U/mL of IFN-alpha for 24 h, then the surface expression of CD81 was measured by FACS. Results are shown as the percentage to the control without any treatment by the mean ± S.D. from two independent experiments and four determinations performed in each experiment. No inhibitor (white), TSA at 1 ng/mL (dotted) and 5 ng/mL (striped); 2-AP 5 mM (grey) and 10 mM (black).

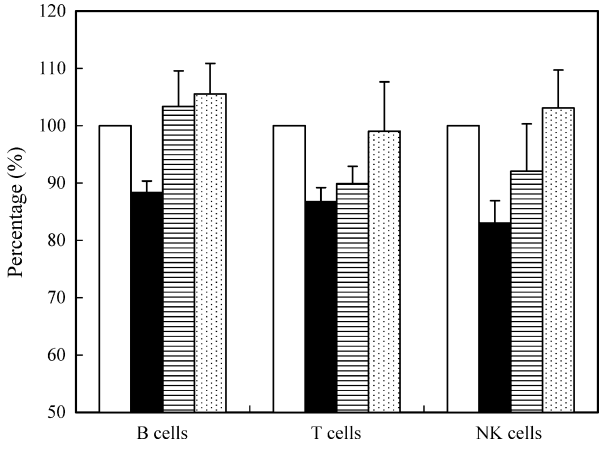

Fig. 5. IFN-alpha-induced down-regulation of CD81 on PBMCs could be restored by 2-AP in isolated PBLs. PBLs from four naïve chronic hepatitis C patients were treated with 100 U/mL of IFN-alpha combined with 2-AP at 0 mM (black), 5 mM (striped) and 10 mM (dotted) for 24 h. Results are shown as the percentage to the matched control sample without treatment (white) by the mean ± S.D.

CD81 down-regulation by IFN-alpha on B cells, T cells and NK cells (Fig. 5).

4. Discussion

IFN-alpha in combination with ribavirin is effective in treating chronic hepatitis C and leads to sustained undetectable viremia and normalized liver enzymes in SVR. However, the issues dealing with the efficacy of the anti-HCV therapy remained, as approximately 50% of patients with HCV genotype 1 infection and 20% with genotype non-1 infection failed to achieve a sustained response (Pawlotsky, 2003). IFN-alpha-based therapy starts with a rapid phase of blocking viral production in the first day of initial injection and proceeds to a slower phase of gradually clearing the infected cells on day 2 and thereafter (Lam et al., 1997; Layden et al., 2002; Neumann

et al., 1998). The second phase includes the destruction of the infected hepatocytes, which is probably due to an increased host immune action, associated with SVR.

Kronenberger et al. have demonstrated a slight but significant reduction of cellular CD81 expression after in vitro IFN-alpha treatment for 48 h, and that the IFN-alpha-induced CD81 down-regulation was correlated with a positive virological response during the initial 4 week treatment of patients with chronic HCV infection (Kronenberger et al., 2001). During the full-term period of anti-HCV treatment and follow-up, lymphocyte subsets showed a different pattern of CD81 response (Kronenberger et al., 2006). Previous studies demonstrated that HCV infection facilitated CD81 over-expression in peripheral B cells (Kronenberger et al., 2001), and the amount of B-cell CD81 correlated with HCV viral load (Zuckerman et al., 2002), implying that IFN-alpha-induced CD81 down-regulation could be secondary to the eradication of HCV (Zuckerman et al., 2003). A higher expression of CD81 on PBLs in patients with naïve chronic HCV when compared to healthy individuals has been reported (Kronenberger et al., 2001; Zuckerman et al., 2002), although no such observation was noted in one study (Curry et al., 2003) and our present results (see Table 1). The reasons for this discrepancy will require further investigations, such as different clinical characteristics of patients. Our data further revealed that IFN-alpha could induce down-regulation of CD81 expression on PBLs and subpopulations at a similar extent among the majority of both healthy controls and naïve-treatment patients with chronic HCV infection (see Table 1).

It is interesting to note that we found patients with SVR to combination treatment of IFN-alpha and ribavirin had significantly more prominent down-regulation of CD81 expression on B cell as compared to patients with non-SVR. Prominent CD81 modulation of patients might play a crucial role in the response to anti-HCV treatment from virological and immunological aspects. Firstly, CD81 has been considered as a putative HCV (co)receptor through interaction with the E2 protein, which might be attributed to virion attachment and entry into susceptible cells (Pileri et al., 1998). IFN-alpha treatment did reduce surface CD81 expression on peripheral lymphocytes and primary hepatocytes in patients as shown in previous studies (Kronenberger et al., 2001) and ours. It then probably further decreased de novo HCV infection and subsequently enhanced the response rate to IFN-alpha treatment. Secondly, CD81 also participates in the coordination of the immune response between B and T cells, as less CD81 expression can reduce Th2-responsive antibody production (Deng et al., 2002; Maecker et al., 1998; Maecker, 2003; Secrist et al., 1996), indicating the Th1/Th2 balance might be prone to shift toward the Th1 cytokine profile. This resulting predominant Th1 response is of great importance for the efficient induction therapy of anti-HCV (Kamal et al., 2002; Tsai et al., 2003). Robust hepatocyte killing by cytotoxic effector cells have a significant impact on HCV clearance. In addition, IFN-gamma has been demonstrated to also inhibit HCV RNA replication in a cultivated replicon system (Frese et al., 2002), suggesting that a Th1 cytokine in capable of curing HCV infection even without massive hepatocyte kill. The second phase of the viral decline during IFN-alpha treatment is associated with the host immune action and sustained response. According to our study, a decreased B-cell CD81 level might represent an immuno-modulatory response to anti-HCV treatment.

The mechanism by which IFN-alpha suppresses CD81 expression was investigated in the present study. Our data showed that IFN-alpha exerted no obvious effect on CD81-RNA quantitation, but that it decreased the surface CD81 protein in the Daudi B-lymphoid cell line. Furthermore, the IFN-alpha-induced CD81 down-regulation could be restored by 2-AP, implying PKR participation in the down-regulation of CD81. PKR is one of the best characterized IFN-induced gene products. This activated protein kinase can inhibit translation through its ability to phosphorylate protein synthesis initiation factor eIF2, which might possibly account for the IFN-mediated CD81 suppression. Enhanced intracellular PKR expression was demonstrated in the PBLs of hepatitis C patients with IFN-based therapy (Asahina et al., 2003). Thus, inhibitory effects on CD81 via IFN-alpha inducible PKR might exist in vivo. Since a replicative intermediate of the RNA virus can induce the production of endogenous IFNs, HCV replication per se was expected to lower CD81 expression. However, the naïve chronic hepatitis C patients in the present study showed levels of CD81 expression on PBLs comparable to those of the healthy subjects. The results suggest that a HCV replicative intermediate in chronic infection might not produce adequate endogenous IFNs to activate PKR (MacQuillan et al., 2002), or that HCV-encoding proteins might impair IFN-signalling proteins, among which the kinase activity of PKR could be inhibited by E2 (Taylor et al., 1999) and NS5A proteins of HCV (Gale et al., 1997).

5. Conclusions

Our data suggests that the primary CD81 levels were indistinguishable on PBLs between healthy subjects and naïve chronic hepatitis C patients, and that IFN-alpha-mediated down-regulation of CD81 expression was independent of chronic HCV infection. However, the sufficient decline of surface CD81 on B cells by IFN-alpha-based therapy correlated with SVR in the treatment of chronic hepatitis C. Furthermore, PKR mediates the pathway of the IFN-alpha effect on CD81 modulation.

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