Human Interferon Regulatory Factor 2 Gene Expression is Induced in Chronic Hepatitis C Virus Infection—A Possible Mode of Viral Persistence

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Background: **The interferon regulatory factors (IRFs) are a family of transcription factors known to be involved in the modulation of cellular responses to interferons (IFNs) and viral infection. While IRF-1 acts as a positive regulator, IRF-2 is known to repress IFN-mediated gene expression. The increase in the IRF-1/IRF-2 ratio is considered as an important event in the transcriptional activation of IFN-α gene toward development of the cellular antiviral response.** ***Objective:*** **This study was performed to assess the expression of IRF mRNAs along with the expression level of IFN-α, its receptor (IFNAR-1), and the signal transduction factor (STAT-1) in treatment naive hepatitis C virus (HCV)-infected subjects.** ***Materials:*** **Thirty-five chronically infected (CHC) patients and 39 voluntary blood donors as controls were included in the study. Quantification of HCV-RNA (ribonucleic acid) and genotyping were done by real-time polymerase chain reaction (PCR) and hybridization assays, respectively, using patient's serum/plasma. In both controls and patients, the serum level of IFN-α and IFN-γ was measured by flow cytometry. Target gene expressions were studied by retro-transcription of respective mRNAs extracted from peripheral blood mononuclear cells (PBMCs) followed by PCR amplification and densitometry. Minus-strand HCV-RNA as a marker of viral replication in PBMCs was detected by an inhouse PCR assay.** ***Results:*** **Both IRF-1 and IRF-2 genes were significantly enhanced in CHC than in control subjects ($P<0.001$). A significant positive correlation ($r^2=0.386$, $P<0.01$) was obtained between higher IRF-2 gene expression and increasing level of HCV-RNA. Chronically infected subjects (13%) harboring replicating HCV in PBMCs showed no significant differences in gene expressions than the subjects without HCV in PBMCs.** ***Conclusion:*** **Our findings indicate that HCV modulates host immunity by inducing IRF-2 gene to counteract IRF-1-mediated IFN-α gene expression. Since the IRF-2 gene is known to encode oncogenic protein, the role of IRF-2 in CHC patients developing hepatocellular carcinoma warrants further studies. (J Clin Exp Hepatol 2012;2:27–34)**

Hepatitis C virus (HCV) infection is a major health concern in India constituting about 10–15% of chronic liver disease (CLD) and hepatocellular carcinoma (HCC)[1] with a median rate of fibrosis progression of 0.25 fibrosis units annually.[2] While about 30% of acute HCV-infected subjects are able to clear the virus, the remaining subjects develop chronic infection (CHC) which can lead to cirrhosis and HCC, thus requiring interferon (IFN)-based therapy.[3] The mechanisms of high rate of HCV persistence are still not understood adequately although attributed to involvement of altered IFN-mediated antiviral defense.[4] Hepatitis C virus, although primarily a hepatotrophic virus, detection of HCV-RNA (ribonucleic acid) in peripheral blood lymphocytes suggests the lymphotrophic nature of the virus also.[5,6] This secondarily lymphotrophic nature of HCV has been suggested to be responsible for impaired immune response of the host favoring viral persistence.[7,8] The HCV infection activates host signaling pathways which induce the generation of endogenous type I IFNs as major antiviral cytokines.[9,10] Type I IFNs, mainly the IFN-α which acts via the Jak-STAT signaling pathway upon high-affinity binding to specific cell surface receptors (IFNAR), are currently being used to treat CHC infection. Binding of type I IFN to IFNAR activates the receptor-associated tyrosine kinases Jak1 and Tyk2 to phosphorylate activating signal transducer and activator of transcription-1 (STAT-1) homodimers. The activated STATs upon translocation to nucleus bind with specific DNA elements in promoters of IFN-stimulated genes (ISGs). It has been reported that HCV-encoded proteins hamper the optimum expression of ISGs by

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Abbreviations: CHC: chronic hepatitis C; CLD: chronic liver disease; HBsAg: hepatitis B virus surface antigen; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; IFN: interferon; IRES: internal ribosomal entry site; IRF: interferon regulatory factors; PBMC: peripheral blood mononuclear cells; PCR: polymerase chain reaction; SVR: sustained virological response; VCAM: vascular cell adhesion molecule
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modulating induction of IFN and consequent signaling mechanisms.[11,12] Human IFN-α gene expression has been shown to be downregulated in the liver but upregulated in peripheral blood mononuclear cells (PBMCs) of CHC patients.[13] Interferon regulatory factors (IRF-1 and IRF-2) belong to a family of DNA-binding proteins which regulate IFN genes and IFN-inducible genes[14] where IRF-1 acts as a positive regulator and IRF-2 as a negative transcription regulatory factor.[15] The increase in the IRF-1/IRF-2 ratio is considered an important event in the transcriptional activation of IFN-α gene toward the development of the cellular antiviral response.[16] In CHC, the predictors of an unfavorable response to IFN therapy known so far include serum HCV-RNA level, viral genotypes, pretherapeutic expression status of ISGs,[17]and IL-28B genotype of the infected subject.[18] On the other hand, the generation of functional IFN system, binding of IFN molecules to specific receptors on target cells followed by the functional intracellular signal transduction mechanisms are considered the crucial steps in the generation of antiviral state. Taking into account that HCV can infect lymphoid cells,[5,6] and since there is no data regarding pretherapeutic endogenous IFN-mediated signaling in Indian CHC patients, we attempted to analyze IFN-α, IRF-1, IRF-2, IFNAR-1, and STAT-1 mRNA expressions in PBMCs of treatment naive CHC subjects. In addition, we evaluated serum level of IFN-α and IFN-γ in such patients in comparison to healthy controls. Furthermore, in order to ascertain the specific influence of HCV on the expression of abovementioned genes, attempts have been made in this study to detect minus-strand HCV-RNA in PBMCs as a marker of viral replication. In this study, we found that HCV can effectively suppress endogenous INF-α through the induction of a negative transcription factor IRF-2, which has not been reported so far. Our study provides important information to reveal the role of IRF-2 in the course of HCV-mediated disease progression.

MATERIALS AND METHODS

Study Subjects

Thirty-five treatment naive subjects with CHC having anti-HCV antibody but devoid of hepatitis B virus surface antigen (HBsAg) and antibody against human immunodeficiency virus (HIV1/2) who attended the outpatient's department of the Asian Institute of Gastroenterology, Hyderabad, AP, India, during the period March 2010 to September 2010 were enrolled for the study. Healthy voluntary blood donors ($n=39$) with no previous or present history or evidence of liver disease and negative for hepatitis B virus (HBV), HCV, or HIV infection were included as controls. Peripheral blood mononuclear cells were separated from whole blood immediately after collection and processed as follows. The serum was separated from parallel sample of clotted blood and immediately stored at −80°C until further use. The study protocol was approved by the institutional ethics committee. Informed consent was obtained before enrolling the patients for conducting various blood tests including studies on mRNA expressions. All the patients and controls belonged to the same ethnic group.

Biochemical and Serologic Tests

Biochemical tests were done using standard automated analyzers. Anti-HCV was measured by third-generation enzyme-linked immunosorbent assay (ELISA) (Bio ELISA HCV 4.0 kit, Biokit SA, Spain).

Quantification of Serum Hepatitis C Virus-ribonucleic Acid

Total RNA was isolated from 500 µL serum of individual subjects using High Pure Viral Nucleic Acid Kit (Roche Molecular Systems, Inc., NJ, USA) as per manufacturer's instructions. Serum HCV-RNA was amplified using Cobas® Taqman® HCV Test, v2.0 kit (Roche Molecular Systems, Inc; NJ, USA) as per manufacturer's instructions and was quantified using Roche Cobas® TaqMan® 48 Analyzer with lower limit of detection being 25 IU/mL of serum.

Hepatitis C Virus Genotyping

The Linear Array HCV Genotyping Test (Roche Molecular Systems, Inc., NJ, USA) based on reverse hybridization was used for the determination of HCV genotypes as per manufacturer's instructions.

Serum IFN-α and IFN-γ Assay

The level of IFN-α and IFN-γ in serum was estimated by flow cytometry using HU IFN-ALP CBA FLEX SET 100TST B8 and HU IFN-GMA CBA FLEX SET 100TST E7 kits, respectively (BD Biosciences, USA), according to the manufacturer's protocol. The values were expressed in picograms per milliliter (pg/mL) of serum.

Isolation of Total Ribonucleic Acid from Peripheral Blood Mononuclear Cell

Peripheral blood mononuclear cells were immediately isolated from ethylenediaminetetraacetic acid (EDTA) containing whole blood using histopaque-1077 (Sigma chemicals, USA) by recommended protocol. The cells were subjected to RNA isolation by Trizol (Life Technologies, USA) method and the extracted RNA was dissolved in diethyl pyrocarbonate (DEPC) treated water.

Preparation of cDNA

A common cDNA pool was generated by reverse transcription from total RNA using random hexamers and MuLV-H reverse transcriptase (Fermantas Life Sciences, Germany). Beta-actin as the housekeeping gene was used as internal control for normalization of all the samples used to study

the target genes IFN-α, IRF-1, IRF-2, IFNAR-1, and STAT-1. Before the reverse transcription, 1 µg of total RNA was treated with 1 U of deoxyribonuclease (DNase I amplification grade, Gibco-BRL, USA) to remove all the contaminating DNA. The presence of traces of DNA was further excluded by performing control reactions without reverse transcriptase enzyme. The RNA was reverse-transcribed (60 minutes at 37°C) with 200 U of M-MuLV reverse transcriptase (Fermantas Life Sciences, Germany) in 20 µL volume of 5X RT buffer (250 mM Tris-HCl pH 8.3, 375 mM KCl, 15 mM $MgCl_2$) supplemented with 5 mM dithiothreitol (DDT), 0.5 mM deoxynucleoside triphosphates (dNTPs, Fermantas Life Sciences, Germany), 25 U ribonuclease inhibitor (Promega Corporation, Madison, WI, USA), and 200 ng random hexamers (Fermantas Life Sciences, Germany).

Polymerase Chain Reaction Amplification of Candidate Genes

After heating (95°C, 1 minute) and quick-chilling on ice, an aliquot of 5 µL (0.3 µg) of the cDNA pool was used for polymerase chain reaction (PCR) amplification in 50 µL of 10X buffer solution (100 mM Tris–HCl pH 9.3, 500 mM KCl, 1% Triton X-100) containing 0.08 mM dNTP, forward and reverse primers (40 ng each), 1.5 mM $MgCl_2$, and 2 U of Taq DNA polymerase (Fermantas Life Sciences, Germany). Blank reactions without cDNA template were performed in all experiments as negative reaction control. Interferon-alfa cDNA fragments were amplified by 20 cycles (94°C, 60°C, and 72°C, 1 min/step) using forward and reverse primers (5′-3′) d(TCCATGAGATGATCCAGCAG) and d(ATTTCTGCTCTGACAACCTCCC), whereas beta-actin fragments were amplified by 20 cycles (94°C, 55°C, and 72°C, 1 min/step) using forward and reverse primers (5′-3′) d(TCTACAATGAGCTGCGTGTG) and d(GGTGAGGATCTTCATGAGGT) generating amplicons of 274 bp and 314 bp, respectively.[19] IRF-1 and IRF-2 cDNA fragments were amplified by 19 and 28 cycles, respectively (94°C, 55°C, and 72°C, 1 min/step), using upstream and downstream primers (5′-3′) d(CTGATACCTTCTCTGATGG), d(TCCAGGTTCATTGAGTAGG) to yield a product of 353 bp for IRF-1 while primers d(TTTTCAGATCCCCTGGATGC) and d(TCAGTGGTGACCTCTACAAC) were used to derive 525 bp amplicon of IRF-2 cDNA, respectively[19]; IFNAR and STAT-1 cDNA fragments were amplified by 35 cycles each (94°C, 55°C, and 72°C, 1 min/step) using upstream and downstream primers (5′-3′) d(AGTGTTATGTGGGCTGGATGGTTTAAGC), d(TCTGGCTTCACACAATATACAGTCAGTGG) for IFNAR and d(CAGTTCTCCCAAGGGAGTTAG), d(GTATGCAGTGCCACGGAAAGC) for STAT-1 to yield amplicons of 765 bp and 649 bp, respectively.[20] Each amplified product (10 µL) was subjected to 2% agarose gel electrophoresis (100 V, 45 minutes) along with a 100-bp DNA ladder and visualized by ultraviolet (UV) fluorescence after staining with ethidium bromide. Semiquantification of the DNA bands was performed by densitometry upon capturing the gel image by Bio-Capt (Vilber Lourmat, France) using the software Image J 1.42 (Broken Symmetry Software, USA). The integrated density obtained from respective DNA bands was normalized against beta-actin and converted to units nanograms per milliliters (ng/mL) to define the expression of respective genes.

Detection of Minus-strand Hepatitis C Virus-ribonucleic Acid in Peripheral Blood Mononuclear Cells

Trizol extracted PBMC-RNA (5 µL) was reverse-transcribed using a sense primer[21] to generate cDNA from minus-strand HCV-RNA followed by subsequent nested PCR amplification using primers and reaction conditions as mentioned earlier.[21] The second PCR product was finally clarified upon 2% agarose gel electrophoresis to resolve an amplicon of 144 bp after staining with ethidium bromide.

Statistical Analysis

Descriptive statistics (mean and standard deviations [SD]), Student's *t*-test, coefficient of determination (R^2), linear regression analysis, and Fisher's exact tests were performed as and where applicable using GraphPad Prism software, USA. A value of $P<0.05$ was considered statistically significant.

RESULTS

All the 35 CHC patients (mean age 50.9±9.44 [SD] years, M:F ratio=24:11) were anti-HCV-positive having mean alanine aminotransferase (ALT) value of 62.15±38.5 and mean serum log HCV-RNA value of 5.65±1.75 IU/mL. Raised ALT (>40 IU/mL) was observed in 21 subjects with corresponding mean log viral load of 5.83±1.86 IU/mL, while 14 showed normal (<40 IU/mL) values of ALT having mean log viral load of 5.13±1.63 IU/mL. Genotypes 3 and 1 were detected in 20 (57.2%) and 15 (42.8%) patients, respectively.

Serum Level of IFN-α and IFN-γ

The serum IFN-α and IFN-γ levels were not altered significantly in CHC subjects compared to the controls (Figure 1).

Expression of IRF-1 and IRF-2 Genes in Chronic Hepatitis C Subjects

The INF-α gene expression was significantly decreased in CHC subjects when compared to control subjects (Figure 2). While no significant differences were seen in the expression level of IFNAR and STAT-1 genes between the study groups, the expression of both the IRF-1 and IRF-2 genes

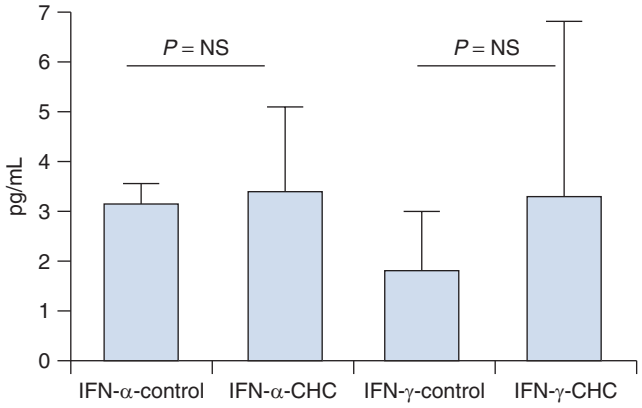

Figure 1 Serum level of IFN-α and IFN-γ in control and CHC subjects. Estimated by flow cytometry and expressed as pg/mL of serum.
CHC: chronic hepatitis C; IFN: interferon; NS: nonsignificant; vertical bar: sd.

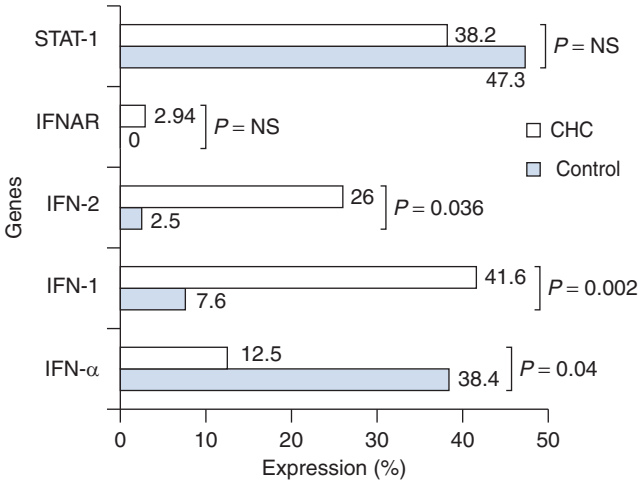

Figure 2 Percentage expression of five candidate genes in control and CHC subjects. Messenger RNAs extracted from PBMC lysates were subjected to RT-PCR, amplified and resolved by agarose gel electrophoresis. Expression of β-actin was used as an internal control for sample loading. The signals were quantified by densitometry analysis after normalization against β-actin.
CHC: chronic hepatitis C; IFN: interferon; NS: nonsignificant; PBMC: peripheral blood mononuclear cells; RT-PCR: real-time polymerase chain reaction; STAT-1: signal transducer and activator of transcription-1.

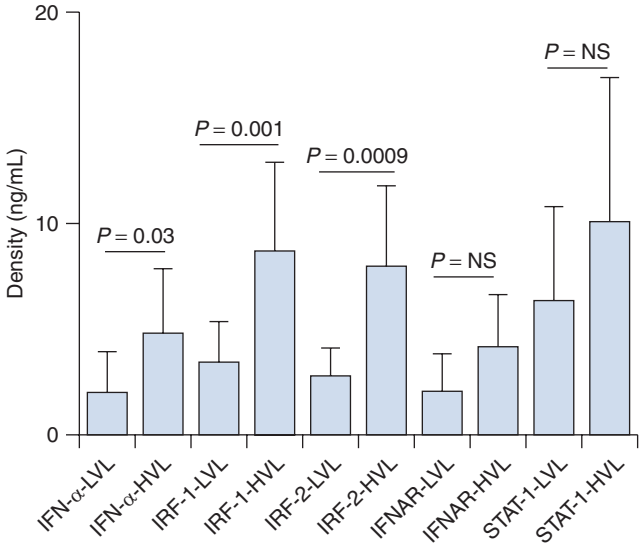

Figure 3 IRF-1 and IRF-2 genes are significantly elevated in CHC subjects having higher viral load. Chronic hepatitis C subjects were categorized into two groups having lower and higher serum viral load (LVL=lower viral load; <5 log IU/mL; HVL=higher viral load; >5 log IU/mL), respectively. Messenger RNAs extracted from PBMC lysates were subjected to RT-PCR, amplified and resolved by agarose gel electrophoresis. Expression of β-actin was used as an internal control for sample loading. The signals were quantified by densitometry analysis after normalization against β-actin and expressed as ng/mL.
CHC: chronic hepatitis C; IFN: interferon; NS: nonsignificant; PBMC: peripheral blood mononuclear cells; RT-PCR: real-time polymerase chain reaction; STAT-1: signal transducer and activator of transcription-1; vertical bar: sd.

was found to be significantly elevated in CHC subjects than in controls (Figure 2). No association was found between HCV genotypes and the pattern of gene expressions studied.

Hepatitis C Virus-induced IRF-2 Gene Expression in Chronic Hepatitis C Subjects

When grouped on the basis of viral load, significant elevation of IFN-α (4.73±3.16 vs 1.88±2.04), IRF-1 (8.55±4.31 vs 3.35±1.99), and IRF-2 (7.84±3.91 vs 2.68±1.41) genes was observed in CHC subjects having higher viral load (>5 log IU/mL) than those with lower viral load (<5 log IU/mL) while IFNAR-1 and STAT-1 gene expressions were not significantly different between these groups (Figure 3). Expression of IRF-2 gene was found to be significantly correlated (r^2=0.386; P=0.01) with increasing serum level of HCV-RNA (Figure 4).

Expression of Interferon Regulatory Factor Genes in Chronic Hepatitis C Subjects with Elevated Alanine Aminotransferase

Figure 5 depicts the comparative expression level of five candidate genes in control and CHC subjects with normal (<40 IU/mL) and raised (>40 IU/mL) ALT levels. Interferon-alfa expression was less in CHC subjects than in controls without any difference between the normal and raised ALT groups. Highly significant elevation of both IRF-1 (0.80±0.51 vs 0.35±0.63; P=0.0005) and IRF-2 (0.77±0.45 vs 0.34±0.58; P=0.0002) gene expressions was seen in raised ALT group compared with controls. In comparison to IRF-1 (0.80±0.51 vs 0.50±0.33; P=0.05), increased expression of IRF-2 gene was more pronounced (0.77±0.45 vs 0.39±0.29; P=0.008) in raised ALT groups than in CHC subjects having normal ALT levels.

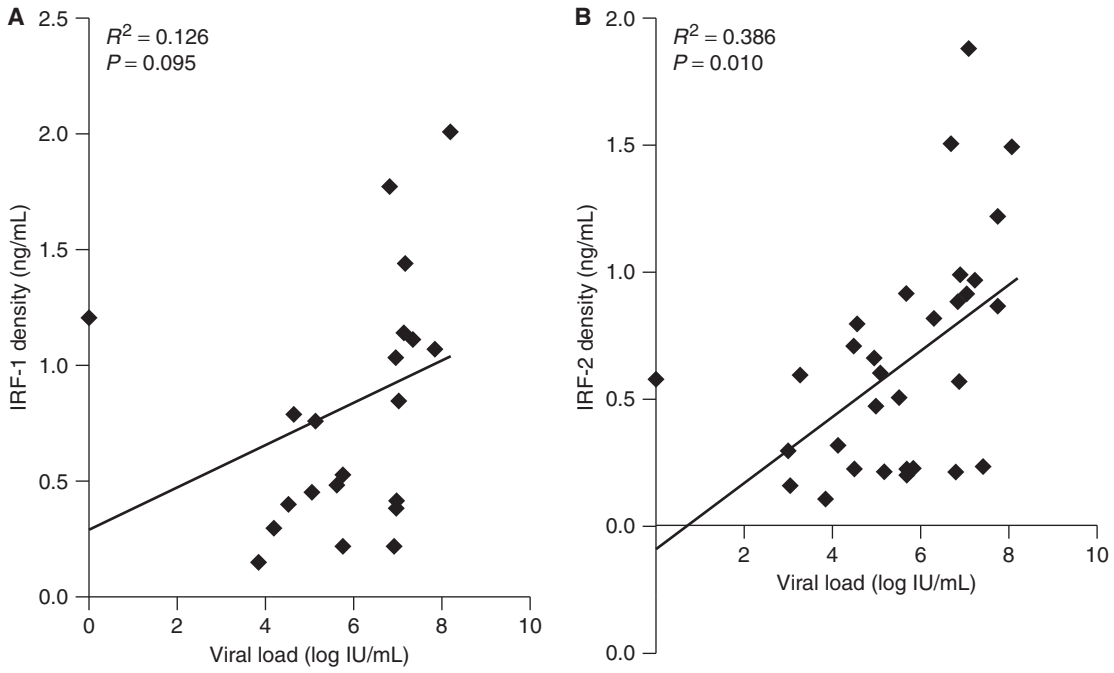

Figure 4 Viral load correlates with IRF-2 gene expression but not with IRF-1 in CHC subjects. (A) No correlation was found between viral load and IRF-1 gene expression. (B) Significant correlation was found between viral load and IRF-2 gene expression (P=0.01) as monitored by coefficient of determination (R^2) and regression analysis.
CHC: chronic hepatitis C; IRF: interferon regulatory factor.

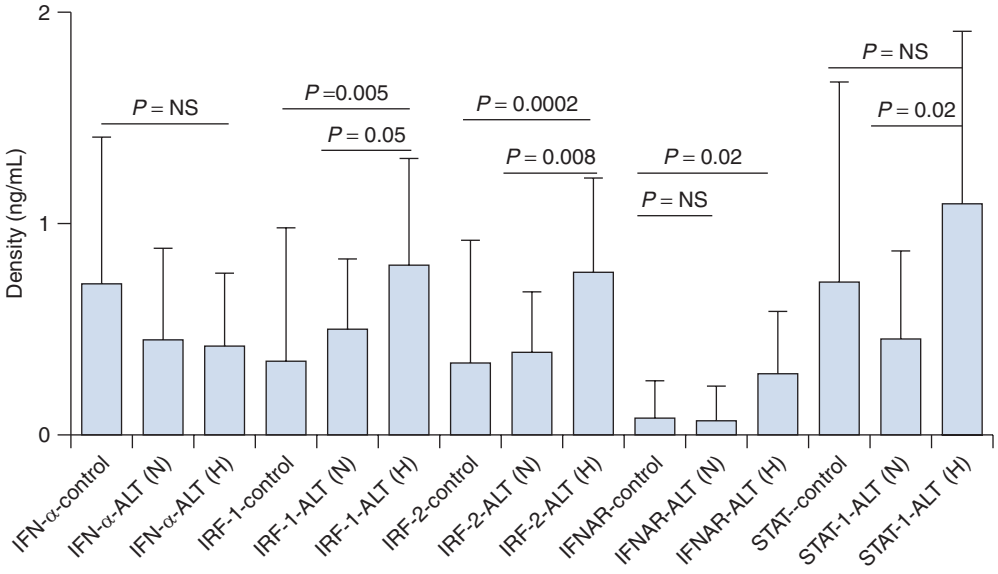

Figure 5 IRF-2 gene expression more significantly increased in CHC subjects with elevated ALT. Chronic hepatitis C subjects were grouped into normal ALT (N≤40 IU/mL) and high ALT (H≥40 IU/mL) levels. mRNAs extracted from PBMC lysates were subjected to RT-PCR, amplified and resolved by agarose gel electrophoresis. Expression of β-actin was used as an internal control for sample loading. The signals were quantified by densitometry analysis after normalization against β-actin and expressed as ng/mL.
ALT: alanine aminotransferase; CHC: chronic hepatitis C; IFN: interferon; IRF: interferon regulatory factor; NS: nonsignificant; PBMC: peripheral blood mononuclear cells; RT-PCR: real-time polymerase chain reaction; STAT-1: signal transducer and activator of transcription-1; vertical bar: sd.

The IFNAR gene expression was found to be increased in CHC with raised ALT group (0.29±0.30) than in CHC with normal ALT (0.07±0.17) as well as the controls (0.08±0.18). Interestingly, the level of STAT-1 expression dropped significantly (P=0.02) in CHC subjects with normal ALT (0.45±0.42) in comparison to controls (0.71±0.95) which upregulated again in raised ALT group (1.04±0.84) subjects (Figure 5).

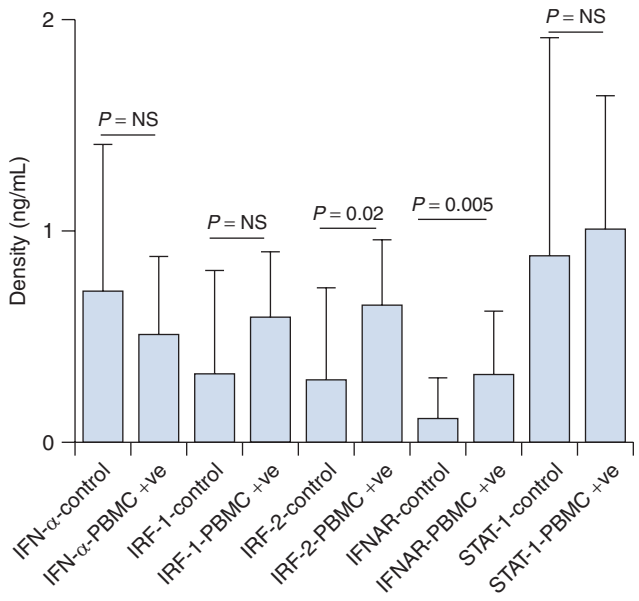

Figure 6 Replicating HCV in PBMC induces expression of IRF-2 gene in CHC subjects. mRNAs extracted from PBMC lysates were subjected to RT-PCR, amplified and resolved by agarose gel electrophoresis. Expression of β-actin was used as an internal control for sample loading. The signals were quantified by densitometry analysis after normalization against β-actin and expressed as ng/mL.
CHC: chronic hepatitis C; HCV: hepatitis C virus; IFN: interferon; IRF: interferon regulatory factor; NS: nonsignificant; PBMC: peripheral blood mononuclear cells; RT-PCR: real-time polymerase chain reaction; STAT-1: signal transducer and activator of transcription-1; vertical bar: sd.

Comparative Gene Expressions in Chronic Hepatitis C Subjects with or without Replicating Hepatitis C Virus in Peripheral Blood Mononuclear Cells

Of 35 serum HCV-RNA-positive subjects, minus-strand HCV-RNA was detected in 13 (37.1%) by nested PCR assay. There was no significant difference observed in the mean log viral load and mean ALT levels between patients with and without HCV-RNA in PBMCs, respectively. Among the candidate genes assayed, IFNAR-1 expression was significantly elevated in CHC subjects with additional presence of HCV-RNA (0.314±0.31) than in those without HCV in their PBMCs (0.11±0.31). Significant elevation of IFNAR-1 (0.314±0.31 vs 0.10±0.20; P=0.005) and IRF-2 (0.64±0.32 vs 0.29±0.44; P=0.01) was evident in CHC subjects having additional presence of HCV in their PBMCs in comparison to the healthy controls (Figure 6).

DISCUSSION

The host–virus interaction outcome in persistence HCV and replication of HCV are largely unknown but are supposed to involve the disruption of components mediating the innate intracellular antiviral response. Interferon therapy mimics endogenous type I IFN signaling, which plays a crucial role in the innate antiviral response through the regulation of ISGs. The ISGs in turn encode antiviral effector proteins that limit viral replication and spread. Human IFN-α comprises a family of genes which encode extracellular signaling cytokines that involve resistance to viral infection[22] and have antiproliferative as well as immunomodulatory functions.[23,24] On the other hand, IRFs are DNA-binding proteins that regulate IFN gene expression. While IRF-1 functions as an activator of IFN and IFN-inducible genes, IRF-2 negatively regulates IRF1-mediated transcriptional activation of IFN-α and presumably other genes that employ IRF1 for transcription activation.[25] In this study, we found that IFN-α mRNA is downregulated in PBMCs of CHC subjects along with inappropriate increase of IFN-α protein in sera. This indicates HCV as weak inducer or suppressor of IFN-α although elevation of IFN-γ to some extent in CHC subjects is indicative of prevailing innate immune response. The IFNAR-1 and STAT-1 gene expressions were not induced appropriately in the CHC subjects. The reason might be the absence of the respective ligand (IFN-α), thus showing suboptimum involvement of the receptor and the signaling pathway. Significant elevation of IRF-1 gene was noticed in our CHC subjects which although is indicative of positive host response for generation of IFN-mediated basal antiviral state, failed to induce IFN-α gene expression in those subjects. Interestingly, IRF-2 gene, which has known negative regulatory role on IFN-α gene expression, was found to be significantly elevated in the same CHC subjects. From this finding, one can envisage that elevated IRF-2 might have countered the positive influence of IRF-1 on IFN-α gene and thus limit the expression of the latter. The role of HCV in inducing IRF-2 was further corroborated by significant positive correlation of IRF-2 gene expression with the serum level of HCV-RNA. Since a repressed state of endogenous IFN-α is supposed to be advantageous for the virus to survive and replicate within the host, our data strongly suggest that HCV suppresses endogenous INF-α by repressing the positive regulator IRF-1 through induction of the negative regulator IRF-2 as a survival strategy. This might have some implications regarding the role of pretherapeutic lower level of IRF-2 in predicting sustained virological response (SVR) in previously treated CHC subjects.[26] Our data are in contrast to the finding that IFN-α gene expression is downregulated in the liver but upregulated in PBMC of CHC subjects.[13] However, our data are in accordance with a recent report where IRF-2 expression was shown to be upregulated with enhanced IRF-2 oncogenic protein expression in CD19-positive blood B cells obtained from CHC subjects.[27] The involvement of potentially oncogenic IRF-2 protein has been implicated in the stimulation of vascular cell adhesion molecule (VCAM) and regulation of histone H4 genes under various conditions.[28,29] IRF-2 gene expression has shown to be controlled by the internal ribosomal entry site (IRES) and is able to encode IRF-2 protein effectively independent of cap-dependent translation.[30] This raises the caveat that

our CHC subjects having raised ALT level with elevated IRF-2 expression might be at risk of developing HCC. Interestingly, STAT-1 expression was found to be significantly ($P<0.05$) reduced in CHC subjects with normal ALT in comparison to the healthy controls as well as to the CHC subjects having raised ALT level. This might have implications to the inhibited IFN signaling by an HCV-induced upregulation of the protein phosphatase 2A resulting STAT-1 hypomethylation[31] as well as inhibition of STAT-1 activation through upregulation of the negative regulator SOCS3 by HCV core protein.[32] In our study, significant ($P<0.05$) elevation of STAT-1 gene expression in raised ALT group than CHC subjects with normal ALT but not more than control subjects appears somewhat intriguing. One possible explanation might be that in our CHC subjects with raised ALT, the STAT-1-encoded protein instead of serving type I IFNs acts as a transcription factor for other pro-inflammatory cytokines like IL-6, IL-12, and IL-27 which are known to mediate through the same Jak-STAT signaling pathway. However, studies on assessment of the phosphorylation status of STAT-1 protein will help to resolve the issue further. It has been shown that STAT-1 activation has positive correlation with elevated level of serum AST level and liver injury while showing negative correlation with hepatocyte proliferation in HCV-induced cirrhosis of liver.[33] This might have implications to our group of CHC subjects having elevated level of serum ALT and higher expression of STAT-1 gene than of controls indicative of their potential vulnerability to develop cirrhosis. High pretherapeutic intrahepatic mRNA levels of IFNAR-1and mRNA ratio of IFNAR-1 to IFNAR-2 have been suggested to be associated with a favorable response to IFN therapy.[34] In this study, the group of CHC subjects harboring replicating HCV as revealed by the detection of minus-strand HCV-RNA in PBMCs showed significant elevation of IFNAR-1 than of controls as well as CHC subjects devoid of replicating HCV in PBMCs. Extrapolation of this finding to infected hepatocytes, though suggestive of better response to exogenous IFN therapy because of increased expression of IFNAR-1, time raises the possibility of post-treatment relapse in these subjects harboring replicating HCV in their PBMCs as reported earlier.[21] However, the limitations of this study are small sample size, IL-28B genotyping of the study subjects has not been done, and the histological gradations of liver fibrosis has not been evaluated. Hence, to our consideration, the data presented in this study appear preliminary that require validation using larger cohort of patients undergoing treatment with IFN, comparing responders with nonresponders. Considering that substantial differences exist between gene expression patterns of liver cells and PBMCs, the predictors of outcomes obtained from PBMC transcriptome can be taken as a reflection of host response rather than the viral factors-dependent processes as demonstrated earlier.[17]

In conclusion, our data indicated HCV-associated suppression of basal IFN-α-mediated antiviral state in CHC subjects through induction of negative regulatory factor IRF-2 gene of the host which has not been reported before. Whether the increased IRF-2 expression is directly responsible for the observed reduction of serum IFN-α in CHC patients warrants further quantitative follow-up studies. This, along with the identification of the specific HCV component(s) responsible for upregulation of the IRF-2 gene, might help to develop interventional strategies for boosting the generation of endogenous IFN-α in the better management of HCV disease burden.

CONFLICTS OF INTEREST

All authors have none to declare.

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