Role of Endogenous Gamma Interferon in Chronic Hepatitis C Virus Infection among Egyptian Patients Treated with Peginterferon Alpha 2a plus Ribavirin

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Abstract: Hepatits C virus (HCV) infections remain an increasingly prevalent and dreadful health problem worldwide that causes a wide spectrum of liver diseases. Combination therapy with pegylated interferon (peginterferon) plus ribavirin is currently recognized as the standard treatment of chronic hepatitis C. Several immunological mechanisms are involved during the course of treatment of HCV via interferon. Data are scattered in the literature on the role of endogenous interferon gamma (IFNγ) in Egyptian patients with chronic hepatitis C treated with pegylated interferon and ribavirin therapy. The present study was thus conducted to evaluate the role of endogenous IFNγ in the response of chronic hepatitis C (CHC) patients following treatment with pegylated plus ribavirin. Forty patients with chronic hepatitis C (CHC) infection were included in the study. Twenty healthy blood donors were used as healthy control. It was found that enhanced IFNγ production would be predicted to favor HCV clearance. The magnitude of pretreatment of interferon alpha (IFNα)-driven IFNγ responses correlates with initial response to therapy is certainly consistent with this prediction. In addition, the results suggested that the single nucleotide polymorphism (SNP) of the MxA gene is one of the important host factors that independently influenced the response to IFNα in patients with CHC infection, especially those with a low viral load.
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1. Introduction

Hepatitis C virus (HCV) is an enveloped positive-stranded RNA virus of the genus *Hepacivirus* within the family *Flaviviridae*. HCV is classified into eleven major genotypes genotypes (designated 1-11), many subtypes (designated a, b, c, etc.), and about 100 different strains (numbered 1, 2, 3, etc.) based on the genomic sequence heterogeneity (Zein, 2000). The lowest sequence variability between genotypes is found in the 5`UTR, where specific sequences and RNA secondary structure are required for replication and translation function. HCV genotypes have been further classified into more than 80 subtypes (Simmonds et al., 2005). The World Health Organization reports that approximately 170 million people worldwide are infected with HCV (Thomas et al., 2007).

HCV infected patients develop chronic infection, manifested by the persistence of detectable HCV in the serum more than 6 months, and this has been primarily attributed to the ability of HCV to mutate and evade host defenses (Seeff, 2002). The major complication of chronic HCV infection is progressive hepatic fibrosis leading to cirrhosis, which develops in about 20% of those with chronic HCV (Alberti et al., 1999). Several strategies can be employed to detect HCV infection. In clinical practice, the usual approach is to test initially for antibodies to HCV (anti-HCV), followed by the use of HCV ribonucleic acid (RNA) to document viremia (Pawlotsky, 2004).

HCV RNA could be detected in the blood using amplification techniques such as polymerase chain reaction (PCR) or transcription-mediated amplification (TMA) (Pawlotsky, 2003). The most commonly used qualitative NATs (Nucleic acid Amplification, tests) use reverse transcription PCR to detect viral RNA (Ferreira-Gonzalez and Shiffman, 2004). Many determinants of the immune response have been implied in the pathogenesis of chronic hepatitis C (Gigi et al., 2008). The critical role of innate as well as adaptive immunity has been reported in HCV persistence and liver injury. Long-lasting memory of CD4+ or CD8+ T cells against HCV in the absence of viremia and antibody is confirmed by observations on health-care workers and healthy family members of acute or chronic hepatitis C patients (Kamal et al., 2004).

The recent discoveries of pathogen-associated molecular patterns (PAMPs) that are recognized by specific toll-like receptors (TLRs) have dramatically advanced the understanding of the innate host response to viral infection (Sumpter et al., 2005). Production of type I IFN and other cytokines (including IL-12, IL-15, and IL-18) from hepatocytes activate NK cells and induce IFNγ production from these cells.(Sumpter et al., 2005) Type I interferons activate the expression of more than 300 interferon-stimulated genes that also have antiviral functions. The best characterized include the RNA-dependent protein kinase (PKR), 2′5′-oligoadenylate synthetase, RNase L, adenosine deaminase (adenosine deaminase, RNA-specific), and the Mx protein GTPases (Wohnsland et al., 2007, Blackard et al., 2008). A growing body of evidence indicates that the spontaneous clearance of HCV is associated with a strong HCV-specific CD4+ T cell response (Folgori et al., 2006). A number of studies have indicated that successful cellular immune responses in recovered patients appear to be multispecific and sustained, with CD4+ T cells playing major roles (Shata et al., 2002). However, CTLs are traditionally thought to be the main effector cells that eliminate HCV-infected cells, it is clear that HCV-specific CD4+ T cells also play a critical role (Gremion and Cerny, 2005).

Hepatitis C virus (HCV) infection is curable. The current standard antiviral therapy for chronic HCV infection consists of administration of peginterferon and ribavirin for 24 or 48 weeks (Hadziyannis et al., 2004). The main end point of treatment is a sustained virological response (SVR), defined as undetectable HCV RNA in peripheral blood 24 weeks after the end of treatment which generally corresponds to permanent cure (Poynard et al., 2000). Measurement of the viral load (quantitative test) at the start of therapy and after 12 weeks of treatment is needed to decide about the usefulness of further treatment (stopping rule) (de Bruijne et al., 2008, Desombere et al., 2005, Jensen et al., 2006). Factors affecting the response to current therapy should be studied and evaluated, specifically host genetic factors including interferon-stimulated genes in addition to immune elements as INFγ.

In the present study we investigate the role of endogenous IFNγ in the response of chronic hepatitis C virus patients to IFNα therapy.

2. Material and Methods

Subjects:

Forty patients with chronic hepatitis C (CHC) infection were included in the study. Twenty healthy blood donors were used as healthy control. Ethical approval for the research was obtained from the review boards of the Faculty of Medicine, Zagazig University. Informed consents were obtained from all subjects. All patients were selected according to the Egyptian protocol of treatment of HCV patients with the following criteria:

1. Positive HCV RNA of serum samples as confirmed by qPCR-based assay more than six months ago.
2. Positive HCV antibodies of serum samples as confirmed by ELISA assay.
3. Elevated ALT level in patients with F1 Metavir score of liver biopsy.
4. F2 and F3 Metavir score of liver biopsy without esophageal gastric varices.

The clinical protocol called for patients participants to be treated for up to 12 weeks with peginterferon-2α and ribavirin (Copious; Roche). All patients were subjected to HCV RNA qualitative and quantitative PCR-based assay and ALT assay pretreatment and on 12th week after initiation of the treatment). According to the results of the second quantitative PCR on 12th week after initiation of treatment, the patients would be classified into:

A. Group I (Responders) was defined as patients who had a decrease of HCV RNA levels of more than 2 $\log_{10}$ IU/ml 12 week after initiation of treatment.

B. Group II (Non- responders) was defined as patients who had persistent viremia at 12th week.

C. Group III (Healthy controls) was defined as healthy blood donors with negative HCV RNA and negative HCV antibodies of their serum samples. All groups I, II &III were subjected to h**uman peripheral blood samples.** Sodium heparin -anti-coagulated whole blood sample (5ml) was obtained from each subject in this study by venipuncture and single peripheral blood samples were obtained from healthy adult volunteers. Double peripheral blood samples were obtained from 40 patients with chronic hepatitis C; the first sample was obtained before starting therapy and the second one was obtained 12 weeks after starting the combined therapy.

Human peripheral blood mononuclear cells (PBMCs) preparation and culture condition:

Whole Whole blood was diluted with an equal volume (5 ml) of phosphate-buffered saline (PBS), carefully layered over a 10-ml ficoll-hypaque gradient (Amersham/Pharmacia, Piscataway, New Jersy, USA), and centrifuged at 100 *x g* for 20 min at room temperature. The buffy coat layer was transferred to a 50 ml RNase-free tube diluted with PBS, centrifuged at 100 *x g* for 15 min at room temperature, the supernatants were discarded, and the PBMCs were retained (Taylor et al., 2007).

PBMC culture

Cell pellets were recovered into RPMI 1640 as previously described. (Thomas et al., 2007)

Stimulation of PBMC or lymphocyte subsets was performed using freshly isolated PBMC which were seeded at 2×10^6/ml (2 ml) into 96-well microtiter plates, phytohemagglutinin (PHA, 10 µg/ml, Sigma, MO, USA) was added to each well. For the stimulation, the final ratio between PBMC and PHA was 10:1. For control treatment, PHA (1 µg/ml) or culture medium alone was added to the PBMC suspension. To determine the cytokine expression by PBMC, the samples were incubated for 24 hr at 37^0C in presence of 5% CO_2. Subsequently, PBMC were collected, washed in cold PBS, and centrifuged.

Cytokine measurement in culture supernatant

Cell culture supernatants were collected separately. IFNγ secretion from PBMC/ml was determined in 24 hr culture supernatants, using standard protocols for sandwich enzyme linked immunoadsorbant assay (ELISA) (OptEIA, Pharmingen), according to the manufacturer's instructions. The lower limit of IFNγ detection is 7.8 pg/ml.

MxA restriction fragment length polymorphism

DNA extraction and PCR were performed to all cell pellets using AxyPrep Blood Genomic DNA Miniprep kit (Axgen Biosciences, USA). The biallelic G/T polymorphism in the promoter region of MxA at position –88 from the transcription start site (Hadziyannis et al., 2004) was genotyped using restriction fragment length polymorphism (RFLP) using the enzyme HhaI (New England Biolabs) to digest the PCR fragment of 351 bp. PCR reactions were all performed using Taq PCR master mix Kit (Bioron, Germany).

Amplification was carried out in a final volume of 20 µl containing 10–100 ng DNA, 2.5 mM $MgCl_2$, 500 nM of each primer, 500 M dNTPs, 1µl PCR buffer, 1 U Taq DNA polymerase, 0.16 ul Taq Start Antibody (BD Clontech). A forward primer, whose sequence was 5′-TGAAGACCCCCAA TTACC AA -3′, was designed to anneal to nucleotides 269 to 287 of the MxA sequence. A reverse primer, whose sequence was 5′- CTCTCGTTCGCCTCTTTCAC-3′, was designed to anneal to nucleotides 619 to 600. These primers produced a predicted ampilicon of 351 bp. The reaction conditions were: denaturation at 94^0C for 5 min, subsequently 35 cycles of denaturation at 94^0C for 30 sec; annealing at 58^0C for 30 sec; and extension at 72^0C for 1 min. This was followed by a final extension step at 72^0C for 7 min. For the HhaI restriction digest 8 µl of the amplicon were digested for at least 4 hr in a volume of 20 µl with 5 U of HhaI according to the manufacturer's specifications. Digested PCR products of 10 µl were visualized on 2% agarose gel containing ethidium bromide. In the presence of the G allele, the 351 bp amplicon is cut into fragments of 261, 51, 23 and 16 bp, and in the presence of the T allele into fragments of 312, 23 and 16 bp (Knapp et al., 2003).

Statistical analysis

Comparisons between stimulation conditions in PBMC from healthy donors were analyzed using the paired t-test. Associations between baseline characteristics and outcome group in the longitudinal study were examined using t-tests and X^2 tests. Statistical analyses for data of responders and nonresponders were conducted using SAS version 9.1 (SAS Institute, Cary, NC, USA). The criterion of statistical significance was $P < 0.05$. For data management and statistical analysis the Prism V3.0 statistical software was used (Graphpad, San Diego, USA). All data are given as means ± standard deviation unless indicated differently. Significance of differences between patients and controls was calculated using the Mann–Whitney test and Wilcoxon test. Correlation was done using Spearman rank.

3. Results

The median of ALT level in HCV patients was higher than that of the healthy control (125.5 U/ml vs 45.0 U/ml). The difference between the two groups was statistically significant as $P< 0.05$ as shown in table (1).

Table 1. Distribution of ALT, IFN- and MxA promotor genotypes among the chronic HCV patients before treatment and the Control groups

		Patients	Control	X^2	P-value
ALT U/ml	**Median**	125.5	45.0	1.87	0.02 **P< 0.05**
	Range	100-232	38-55		
IFN- pg/ml	**Median**	453.0	869	2.92	0.001 **P< 0.05**
	Range	102- 1220	371-1298		
MxA promotor genotypes	**G.G homozygos**	18 (45%)	6(30%)	2.85	0.24 **P>0.05**
	G.T heterozygos	14 (35%)	6(30%)		
	T.T homozygos	8 (20%)	8 (40%)		

The median of IFN level in chronic HCV patients was lower than that of the healthy control (453 vs 869 pg/ml). The difference between the two groups was statistically significant (P< 0.05) as shown in table (1). MxA 1 promotor genotype was determined using PCR amplification in both test and control individuals. Table 1 showed that T.T homozygosity was less frequently found in patients group than in healthy control group (20% vs 40%). The reverse was true for GG homozygosity. These differences were of no statistical significance as P >0.05.

After 12-week therapy, patients were classified into responders and non responders based on the result of HCV genome PCR. In the responders group, table 2 presents that the median of the basal viral load was significantly higher than the median of the viral load at 12th week of the therapy (440,000 IU/ml vs 33,00 IU/ml) P<0.005. In the non-responders group, there was no statistical difference between the median of the basal viral load and that 12 weeks therapy as P >0.05.

Mann–Whitney U test that compared the two groups showed that the basal and 12 weeks viral load in the responders was significantly lower than that in the non responders P <0.05 as shown in table 2. In the responders, the median of the basal level of ALT was significantly higher than the median of the ALT level at 12 weeks of the therapy (154 U/ml vs 50 U/ml) P<0.005 as shown in table (2).

Table 2. Viral load at start and after 12-weeks therapy, basal and post treatment ALT and basal IFN among responders and non-responders groups

Study groups	Basal Viral load IU/ml	Viral load at 12 wks IU/ml	Wilcoxon test	P value	ALT Pre U/ml	ALT Post U/ml	Wilcoxon test	P value	IFN-re pg/ml	IFN-ost pg/ml	Wilcoxon test	P value
The responders Median Range	440,000 3.2×10^3- 2.2×10^4	33,00 -80,00	3.92	0.002	154 100-232l	50 40-60	3.92	0.002	585.5 250-1100	3778.5 2000-7510	3.92	0.002
Non-responders Median Range	5.9×10^5 5×10^4- 4×10^5	6×10^5 4.6×10^4- 3.8×10^5	1.91	0.06	119 100-155	179 98-354	3.5	0.002	313.5 102-1220	310 100-1300	1.77	0.07
Mann-Whitney U test	1.67	5.4			3.7	5.4			2.23	5.4		
P value	0.04	0.0001			0.002	0.000			0.02	0.000		

P< 0.05 significant

In the non-responders, there was no statistical difference between the median of the basal ALT level and that at 12 weeks therapy as P >0.05. Mann–Whitney U test that compared the two groups showed that the basal and 12 weeks ALT level in the responders was significantly lower than that in the non responders P <0.05 as shown in table (2).

Regarding IFN level, the median of IFN level after 12 weeks of therapy was significantly higher than the median at initial therapy (3778.0 pg/ml vs 585.5 pg/ml) P<0.005 as shown in table (2). In the non-responders, there was no statistically difference between the median of the basal viral load and that at 12 weeks therapy (P >0.05) as shown in table (2). Mann–Whitney U test that compared the two groups showed that the basal and 12 weeks IFN in the responders was significantly higher than that in the non responders P <0.05.

Statistical analysis was used to determine if there was association between basal IFN level and the response to treatment. It was found that there was significant association between basal IFN level and the median fold increase of IFN level from one side and the response to treatment on the other side. The median-fold increase of IFN was significantly higher in responder group than non-responders group (6.64 vs 1.0) P< 0.005.

Spearman rank was used to determine the correlation of the median fold increase of IFN level and each of basal IFN level, baseline plasma viral load and basal ALT level. There were significant correlations between median fold increases of IFN level and both basal IFN level and basal ALT level as shown in table (3).

Table 3. Correlation between basal IFN level, basal viral load, basal ALT, and Median-fold increase in IFN

	IFN- Median-Fold Increase	
	R	P
Basal IFN level	0.3	0.002
Basal viral load	0.39	0.08
Basal ALT level	0.27	0.04

P> 0.05 Insignificant

Using PCR-RFLP of MxA 1 promotor of both responders and non responders, it was found that GG homozygosity was significantly less frequently found in the responders than in non-responder group (25% vs 65%) P< 0.005. The reverse was true for TT

homozygosity as TT homozygosity was significantly more frequently found in the responders than in non-responder group (40% vs 0%) P< 0.005. T allelic frequency was calculated for responder group (0.6) and for non responder group (0.2) and it was found that the T allele was significantly more frequently found in the responders than in the non-responder group. The reverse was true for G allele where for responder group (0.4) and for non responder group (0.8). It was found that MxA promoter polymorphism and the response to treatment and basal viral load were independent determinants of the outcome of combined therapy. However, there was a true significant correlation between MxA promoter polymorphism and the basal viral load as shown in table (4).

Table 4: Spearman rRank correlation between MxA promoter polymorphism and the response to treatment

	MxA Promoter Polymorphism	
	R	**P**
The Response to treatment	0.8	0.03
Basal Viral Load	0.67	0.004

P< 0.05 significant

4. Discussions

Antiviral treatment should be considered in all chronically HCV-infected patients. Current antiviral treatment is a long-term process and is accompanied by a number of patient-related side effects. When a starting treatment is decided, the chance of being successful is variable (80% with hepatitis C genotype 2 and 3 after 12 to 24 weeks treatment, 50% with hepatitis C genotype 1 and 4 after 24 to 48 weeks treatment (de Bruijne et al., 2008). The level of IFN in PBMC culture supernatant of both patients and control was quantified using ELISA technique.

Results in the present study were contrary to those found by Najafizadeh et al (Najafizadeh et al., 2007). In the present study the median of IFN level in HCV patients was lower than that of the healthy control (453 vs 869) as previously shown in table (3). However, Hassan et al found that there was no significant difference between patients and healthy control regarding IFN level in sera (Hassan, et al., 2004). In addition, it was found that Th1 cytokine IFN level was not significantly changed during HCV infection (Fan et al., 1998). Furthermore, the elevated levels of Th2 cytokines were greater than Th1 cytokines in HCV infection. Thus, their study indicated that enhanced Th2 responses are present during chronic HCV infection, which may partly be responsible for the persistence of HCV infection.

Although treatment of CHC has significantly been improved by the introduction of pegylated alpha interferon/ribavirin combination therapy, it is associated with considerable side effects and many patients especially those with genotype 4 do not respond to therapy (Fried et al., 2002). Therefore, the need for improved or alternative treatment regimens continues to be a requirement. The antiviral activities of IFNα superfamily genes formed the rationale for their therapeutic use in viral hepatitis. In addition to antiviral activities, however, these cytokines are pleiotropic immune regulators (Karp et al., 2000). The complicated immuno-regulatory functions of the type I IFNs are likely to be important determinants of their therapeutic efficacy. Positive and negative effects on the IFNγ may be of particular importance (Byrnes et al., 2007).

The data shown in the present study provided further insights into the complex effects of IFNα on the IFNγ by PBMC in responders and non-responders to IFNα therapy. The results were consistent with other findings that type I IFNs can up-regulated IFNγ production, augmenting NK cell IFNγ production, and promoting CD8+ T cell production of IFNγ (Cousens et al., 1999, Matikainen et al., 2001).

Previous studies of naive human CD4+ T cells demonstrated that IFNα /ß drived IFNγ secretion by memory human CD4+ T cells (Byrnes et al., 2007). The likely biological relevance of such IFNα amplification of IFNγ responses is underscored by the finding that hepatitis C patients with a virological response to IFNα therapy were marked by greater IFNα-driven IFNγ, the paradigmatic effector cytokine of cell-mediated immunity, production by PBMC after starting therapy.

In the present study, the role of IFNα in amplifying IFNγ productive capacity in response to mitogenic (PHA) stimulation was examined, previous studies determined the effect of IFNα therapy on HCV protein-specific CD4+ T cell proliferation and IFNγ production (Kamal et al., 2004, Kamal et al., 2002). In the current study, it was found that IFNα /ß can enhance IFNγ secretion by PBMC immune cells, which is consistent with other studies (Cousens et al., 1999, Matikainen et al., 2001, Parronchi et al., 1996, Rogge et al., 1997, Wenner et al., 1996, Hunter et al., 1997, Jewett et al., 1996). However, it was revealed that at least in CD8+ T cells and NK cells, IFNα /ß can also suppress IFNγ secretion.(Gil et al., 2006, Nguyen et al., 2000, Nguyen et al., 2002) In addition, it was suggested that high-dose IFNα induction therapy caused a profound decline in IL-2- and IFNγ secreting HCV- and CMV-specific T cells which was explained by that restoration of T cell responses was unlikely to be causally linked to an early response or SVR to therapy (Barnes et al., 2009). The outcome

(enhancement *vs*. suppression) depends on variable use of STAT proteins in the signaling cascade downstream of the type I IFN receptor, something regulated by changes in STAT protein expression and accessibility (Gil et al., 2006).

There is a mechanism for cross-talk between types I and II IFN pathways as the archetypal type I IFN signaling molecule ISGF3 is also activated by IFNγ. ISGF3 is able to induce expression of type I IFN, thereby further amplifying the response of IFNα /ß-induced genes. Conversely, type I IFN can elicit classic type II IFN signaling molecules such as active Stat1 homodimers, which are able to bind to GAS sites to activate transcription of target genes (Byrnes et al., 2007).

The present study found that the magnitude of the basal and post treatment level of IFNγ responses is associated with initial responses to therapy because the pretreatment level of IFN was significantly higher in the responder group than the non-responders group as presented in table (3) These results were consistent with the finding that enhanced IFNγ production would be predicted to favor HCV clearance (Lempicki et al., 2006).

In addition, this study showed that IFNγ expression level was increased to levels higher than the basal after IFNα therapy in responders group (Median-fold increase of IFNα was significantly higher in responder than non-responders (6.64 vs 1.0) P< 0.005. The current results were similar to the data presented others (Kamal et al., 2002) who found that in both acute and chronic HCV infection, successful IFNα therapy was associated with the development of robust, multispecific Th1 responses to HCV proteins. IFNγ is a major product of Th1 cells and further skews the immune response toward a Th1 phenotype.

It was found that IFNγ inhibited the replication of subgenomic and genomic hepatitis C virus (HCV) RNAs in vitro and noncytolytically suppressed hepatitis B virus (HBV) replication in vivo (Shin et al., 2005). It is also suggested that IFNγ also had immunomodulatory effects and as a marker of a successful cellular immune response to HCV as the present study followed the effect of IFNγ in vivo. INFγ is induced by *IFNα* may mediate the response by several ways. First, IFNγ enhances NK cell activity and induces the expression of inflammatory and potentially antiviral cytokines such as tumor necrosis factor alpha, TNF*α* (Paludan, 2000). Second, IFNγ facilitates induction and effector function of T cells via upregulation of major histocompatibility complex class I and II proteins and promotes antigen processing via induction of immunoproteasomes (Fruh and Yang, 1999). In this respect, it is noteworthy that frequency and repertoire of HCV-specific, IFNγ-producing Th1 and Tc1 cells correlate closely with HCV clearance (Lechner et al., 2000). Third, IFNγ facilitates T-cell homing from lymph nodes and peripheral blood to the site of infection via induction of T-cell-recruiting chemokines such as IFN-inducible protein 10 (CXCL10), IFN-inducible T-cell chemo attractant (CXCL11) and monokine induced by IFN-γ (CXCL9*)* (Pham et al., 2009).

In this study, it was suggested that there was no significant correlation between basal-line viral load and IFNα driven IFNγ (r=0.39, P=0.08). These results coincide with that of Byrnes et al. who found that IFNα driven IFNγ was not significantly affected by the basal viral load (Byrnes et al., 2007). On the other hand, Pham et al. found that lower transcription of IFNγ was associated with a more robust HCV replication in immune cells and HCV replication in T lymphocytes could be completely eliminated by activation of endogenous IFNγ in CHC, but of IFNα in occult (Pham et al., 2009). This work revealed a significant correlation between the basal ALT level and mean fold increase in the IFNγ (r= 0.27, P<0.05) which was consistent with other results (Taylor et al., 2007, Najafizadeh et al., 2007). In spite of these finding, Byrnes et al found that IFNα driven IFN*γ* not correlates with basal ALT level (Byrnes et al., 2007).

It was found that the expression of IFN*γ* by PBMC was shown to parallel closely serum transaminase activities during IFNα 2a therapy (Mihm et al., 1996). With regard to MxA 1 promoter genotype distribution, it found that T.T homozygosity was less frequently found in the patients than in control group (20% vs 40%). The reverse was true for GG homozygosity where, the differences were of no statistical significance as presented in table (1) which was consistent with results by Knapp et al (Knapp et al., 2003).

IFNs mediate their effects by binding to cell surface receptors activating members of the JAK kinase family of proteins (Zhou et al., 1999). Activated JAK kinases phosphorylate the signal transducers and activators of transcription (STAT) family of transcription factors, which form complexes with other transcription factors to activate transcription of IFN-stimulated genes (ISGs) (de Veer et al., 2001). Type I interferon (IFNα/β) is also induced by virus infection among innate immunity against viral infection and generally play an important role in the first line of defense, inducing intracellular antiviral proteins, such as 20, 50- oligoadenylate synthetase 1 (OAS-1), myxovirus resistance-A (MxA), and dsRNA-dependent protein kinase (PKR) (Hamano et al., 2005).

The IFN-induced proteins and enzymatic pathways involved in establishing the antiviral state are not entirely defined. The numerous candidates, which probably act together, include the 2´-5´-

oligoadenylate synthetase system, Mx proteins, and double-stranded RNA-dependent protein kinase, as well as other, less well characterized or unknown, IFN-induced intracellular pathways (Pawlotsky, 2003).

The present study highlighted the important role of host genetic factors, in particular variation in the interferon-induced MxA gene in the modulation of the response of HCV to IFNα therapy. In agreement with Knapp et al (Knapp et al., 2003), we found that GG homozygosity was significantly less frequently found in the responders than in non-responder group (25% vs 65%) P< 0.005. The reverse was true for TT homozygosity where TT homozygosity was significantly more frequently found in the responders than in non-responder group (40% vs 0%) P< 0.005.

From PCR-RFLP of MxA promoter, it is also clear that the T allele was significantly more frequently found in responders (0.6, P<0.05) than in non-responder group (0.2, P< 0.05). The reverse was true for G allele table (1). In addition, it was found that MxA T-positive patients were more likely to show initial response compared with MxA-T-negative patients (Knapp et al., 2003). The present study suggested that the SNP of the MxA gene is one of the important host factors that independently influenced the response to IFNα in patients with chronic HCV infection, especially those with a low viral load.

It was found that MxA-T-positive patients were more likely to show a sustained response compared with MxA-T-negative patients (2.87 (1.3-6.3); 62%vs 36%; P = 0.0075) (Suzuki et al., 2004). In addition, it was revealed that MxA expression is significantly induced after *IFNα* therapy and associated with the response (Gilli et al., 2002). It was found that the rate of GG homozygosity was 31% in the SR patients, significantly lower than in the NR patients (62%, p = 0.0009), while that of healthy controls was between the two groups (48%) GG genotype was found to be more frequently in non-responders of IFN treatment in hepatitis C, and a luciferase reporter assay revealed that the MxA promoter sequence of G haplotype had lower promoter activity than that of T haplotype (Gilli et al., 2002).

GG genotype expressed the lower amount of MxA mRNA than GT or TT genotype in IFN-treated peripheral blood mononuclear cells in vitro (Antonelli et al., 1999). These findings may due to SNP was involved in a genetic element highly homologous to the IFN-stimulated response element consensus sequence, and the G-to-T change makes this homology a little greater.

The Mx proteins belong to the dynamin family (a family of large guanosine triphosphatases (GTPases) and inhibit the replication of some RNA viruses by binding to viral ribonucleoprotein structures and preventing transcription of viral RNA or movement of viral subparticles within the cell by the array experiments (de Veer et al., 2001). The apparent effect of the T-allele is supported by in vitro functional work, suggesting that this variant has higher transcriptional activity than the G allele when stimulated with IFNα (Hijikata et al., 2001). This is because the polymorphism at position -88 lies within a sequence element similar to an interferon sensitivity response element (ISRE), and the T-allele increases this similarity (Antonelli et al., 1999). Consequently, patients with the GG genotype at position -88 may produce a suboptimal MxA response when given IFN*α*. Clinical studies have also suggested that those who respond to interferon- *α* treatment express increased amounts of MxA mRNA during treatment (Antonelli et al., 1999). A second SNP was described at position -123 from the transcription start site, which is in close linkage disequilibrium with the SNP at position -88, and was therefore ignored in analysis (Hijikata et al., 2001). The present study analysis showed that among all patients, HCV RNA level and the SNP of the MxA gene were independent and significant determinants of the outcome of IFN therapy which was in agreement with another (Suzuki et al., 2004).

In general, enhanced IFNγ production would be predicted to favor HCV clearance. The present results suggested the magnitude of pretreatment of IFNα-driven IFNγ responses correlates with initial response to therapy is certainly consistent with this prediction. It was found that the SNP of the MxA gene is one of the important host factors that independently affected the response to IFNα in patients with chronic HCV infection, especially those with a low viral load.

Conflict of interest:

The authors declared no competing interests.

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