Cell Death and Differentiation (2003) 10, S22–S24

www.nature.com/cdd

News and Commentary

HCV and interferon: viral strategies for evading innate defence mechanisms in the virus-host battle

MR Capobianchi*,[1], I Abbate[2], G Cappiello[2] and M Solmone[1]

[1] National Institute for Infectious Diseases L. Spallanzani, Rome, Italy
[2] Sandro Pertini Hospital, Rome, Italy
* Corresponding Author: Dr MR Capobianchi PhD, National Institute for Infectious Diseases L. Spallanzani, Via Portuense, 292, 00149-Rome, Italy. Tel: +390655170434; Fax: +39065582346; E-mail: mrcapobianchi@libero.it

Cell Death and Differentiation (2003) **10,** S22–S24. doi:10.1038/sj.cdd.4401142

HCV infection develops, in a substantial proportion of instances (estimated to be higher than 80%), as a chronic infection, despite the elicitation of both humoral and cellular immunity. To be able to establish such a high proportion of persistent infections, it is clear that mechanisms developed by HCV to circumvent antiviral defences are a key issue.

The interferon (IFN) system is a pivotal factor involved in the innate mechanisms of defence against virus infections, responsible both for direct activity, aimed at restricting viral replication, and enhancing immune responses.[1] Type I IFNs, comprising the multi-gene cluster of IFN-α/ω proteins plus the unique IFN-β glycoprotein, are a first line defence, acting immediately after virus infection, whereas type II IFN (IFN-γ) is likely to be a later player, whose activation is parallel to the intervention of a specific immune response. Even though the two IFN types have a temporally different role in the antiviral defence, the intracellular mechanisms activated by the two types of IFN are redundant and partially overlapping. IFN-receptor binding triggers a cascade of signal transduction and transcriptional activation, involving both the Janus kinases (JAK) and the signal transducers and activators of transcription (STATs), ultimately leading to the induction of an array of IFN-response genes, which are, in turn, responsible for the establishment of the antiviral state.[2]

The complete elucidation of the antiviral mechanisms triggered by IFNs has not been accomplished. However, it is well understood that a variety of pathways are involved, capable of blocking virus replication virtually at each step, including uncoating, transcription, translation, assembly and maturation. One of the most important pathways involves the transcriptional activation of dsRNA-dependent protein kinase (PKR), a 68 kDa serine–threonine protein kinase, which, after interaction with viral dsRNA, becomes activated and phosphorylates the protein synthesis initiation factor-2α (eIF-2α), rendering it unable to initiate protein synthesis. Due to the necessity of dsRNA for its activation, the PKR-driven inhibition of protein synthesis selectively occurs in virus-infected cells, where the abnormal form of the nucleic acid is very common. In addition to the e-IF2α phosphorylation-dependent protein synthesis inhibition, PKR is also able to initiate a DNA transactivation cascade, mediated by the phosphorylation of either IκB, the inhibitor of the transcriptional activator NF-κB, or of the interferon regulatory factor (IRF)-1. Based on these multifaceted mechanisms, PKR is a key regulator of several cellular activities, including IFN induction *per se*, calcium mediated signal transduction, apoptosis, cell growth and malignant transformation.

Several viruses, including influenza virus, vaccinia virus, adenoviruses, reoviruses, EBV and HIV,[3] have developed efficient escape strategies to circumvent or IFN antiviral action, in particular involving efficient blocking of PKR activation or catalytic functions.

In this contest, the possible existence of efficient and specific mechanisms exerted by HCV to circumvent IFN action is particularly meaningful in view of the fact that IFN, alone or in combination with ribavirin, is the only available treatment for chronic HCV infection. As a matter of fact, combined regimens of IFN plus ribavirin and the new pegylated IFN formulations have increased the rate of sustained response in HCV-infected patients. Nevertheless, a substantial proportion of patients still remain non-responsive to IFN therapy. Several factors, including viral load, viral dynamics, virus variability and quasispecies evolution, are involved in determining the response to IFN therapy (Table 1).

Molecular mechanisms underlying the different sensitivity to IFN treatment are not completely understood, and are the object of intense investigation, in particular those focused on the non-structural (NS) 5A region of HCV genome. The first indication of a genetically-encoded intrinsic resistance of HCV to IFN came from the observations made by Enomoto and colleagues.[4] By comparing the sequence of HCV isolates from patients displaying different response patterns to IFN treatment with that of a reference strain, they identified a 40 aminoacid stretch in the N terminal region of NS5A gene (codons 2209–2248, numbered according to reference HCV-J D90208) whose sequence was associated with the therapeutic outcome. This region was called the interferon sensitivity determining region (ISDR). Patients, before treatment, infected with genotype 1b HCV with more than three aminoacid substitutions in ISDR, with respect to reference region, showed a higher probability of being sustained responders than those harbouring HCV with fewer mutations. These assumptions led to the classification of ISDR into three types: no mutation (wild type, WT); 1–3 mutations (intermediate type, IT); >3 mutations

Table 1 Viral determinants of HCV response to IFN

- HCV genotype
- Viral load (VL)
- Dynamic changes, quantitative (VL decrease)
- Dynamic changes, qualitative (viral quasispecies evolution)
- Mutations in NS5A
- Mutations in E2
- Interference with signalling pathways

(mutant type, MT). These results were subsequently confirmed by a number of other studies, mostly performed in Japanese patients, and were reinforced by the finding that the ISDR region can undergo selection-driven changes in individual patients. The importance of ISDR as a predictive factor of IFN therapeutic outcome in Japan has recently been underlined by its inclusion in a decisional analysis model for therapeutic intervention in genotype-1b infection.[5] However, no correlation between the ISDR pattern and the IFN response has been found for non-genotype 1b-infected patients. Furthermore, several studies, mainly performed in European or American patients, did not show any clear association between the presence of multiple mutations in ISDR and the IFN response.[6]

Recently, in a meta-analysis of all available studies, Witherell *et al*.[7] analyzed a database of 675 individual ISDR sequences, and demonstrated a positive correlation between ISDR substitutions and IFN response. The authors indicated that previous failure to show such a correlation was most likely due to the low frequency of MT ISDR type in patients from western countries, and to the resulting inadequate sample size in the individual studies. In this analysis, most of the identified mutations in ISDR have been reconsidered, taking into account their association with response to therapy, and have been classified as detrimental or tolerated with respect to their virtual effect on NS5A function.

On the whole, the intense debate on the putative predictive role of NS5A on therapeutic outcome indicates that this issue remains controversial. The recent availability of pegylated IFN, whose higher effectiveness is linked to a more constant antiviral pressure, supports the need to re-evaluate this issue, after the elimination of confounding factors such as sub-optimal regimens or intermittent activation of the intracellular antiviral pathways. As a matter of fact, in a recent study performed on genotype 1b-infected Italian patients, we found that the baseline pattern of ISDR is unrelated to treatment outcome, and selection towards a dominant IFN-resistant strain does not occur under treatment with either standard or pegylated IFN plus ribavirin.[8]

Recently, ISDR-driven molecular mechanisms of IFN evasion exerted by HCV have been partially elucidated, showing that PKR is a central focus of HCV defensive strategies.

In a fundamental study, Gale and colleagues have shown that the product of the NS5A gene can bind PKR *in vitro*, and localized the binding region in the ISDR domain.[9] After binding with NS5A protein, PKR becomes incapable of dimerization, and e-IF2 phosphorylation-dependent inactivation no longer takes place. Furthermore, in the same laboratory it has been shown that NS5A, through interaction with the growth factor receptor bound protein 2 (GRB2), can determine reduced phosphorylation of the extracellular signal-regulated protein kinase 1 and 2 (ERK1/2), leading to reduced IFN induction.[10]

Overall, studies performed in *in vitro* systems based either on sub-genomic replicon, or on binary transfection systems, have shown that HCV, like many other viruses, may be sensitive to interferon (IFN)-α antiviral action.[11] However, in the sub-genomic replicon system it has been shown that a cluster of mutations usually develop around the PKR-binding domain of NS5A, giving a strong replicative advantage to the mutated HCV replicon quasispecies.[12] Subsequent work by Gale and colleagues has shown that efficient replication of HCV *in vitro* may involve a block in PKR-dependent signalling, leading to impaired IRF-1 activation.[13] IRF-1 binding to DNA, alone or in cooperation with IRF-3 and IRF-7, regulates the expression of several antiviral genes, including those involved in type I IFN induction and action. Therefore, the authors suggest that suppression of PKR-dependent pathways is naturally developed by HCV to relieve the constraints to viral replication exerted by endogenously activated antiviral mechanisms.

Several other studies on *in vitro* replication systems, performed in either liver-derived or non-hepatocytic cell lines, support the existence of NS5A-mediated mechanisms of IFN resistance, capable of rescuing *in trans* the replication of different viruses, such as EMCV or VSV, from the antiviral effect of IFN.[14] However, from these studies it is evident that ISDR is not essential for the ability of NS5A to promote IFN resistance, and that sequences outside this region might also be involved. The existence of non-ISDR-dependent mechanisms indicated by *in vitro* studies are supported by *ex vivo* findings.[15] These studies indicated that a variable region in the C-terminus region of the NS5A gene, called V3, is under selective pressure during IFN treatment, and can be involved in resistance mechanisms. Recently, Polyak *et al*.[16] have suggested that the NS5A-driven IFN resistance may be mediated also through the induction of IL-8, a cytokine capable of counteracting the antiviral activity of IFN.[16] The overall conclusion from the above mentioned studies is that the NS5A protein, whose function in HCV replication is still obscure, can help HCV in evading a broad spectrum of IFN-activated pathways.

Recently, it has been reported that another region of the HCV genome is associated with molecular mechanisms of IFN resistance, lying in a 12 aminoacid domain of the E2 glycoprotein.[17] This region is highly homologous to the autophosphorylation domain of PKR, and to the phosphorylation target region of eIF2α, and therefore has been designated PKR – eIF2α phosphorylation homology domain (PePHD). The E2 protein of the IFN-resistant HCV genotype 1 is capable of blocking the PKR-driven inhibition of protein synthesis and cell growth, while E2 from the IFN-sensitive genotypes 2 and 3 exerts only a weak effect, providing a partial explanation for the intrinsic differences in IFN sensitivity of the different HCV genotypes.

Other HCV proteins may be involved in IFN system interference by altering signal transduction for early events (IFN production) or delayed events (effector mechanisms). *In vitro* studies have revealed that HCV may also interfere with STAT 1. In fact, expression of HCV proteins in cultured cells determined, after IFN exposure, the impaired DNA binding of ISGF3, the pivotal type I IFN-induced transcriptional activator, deriving from the association of STAT1, STAT2 and ISGF3γ-p48.[18]

In conclusion, as IFN exerts its antiviral effects through multiple pathways, HCV appears to be able to circumvent its antiviral activity through multiple mechanisms, targeted at both upstream and downstream steps involved in IFN system activation. It is not surprising that such mechanisms are necessary to allow the virus to evade the innate mechanisms of antiviral defence, leading to the successful establishment of persistent infection (Figure 1). However, the clinical value of such mechanisms as factors able to predict resistance or sensitivity to exogenously administered IFN, either alone or in combination with other drugs, remains mainly speculative at present. Moreover, we believe that these factors should be considered in a wider context including host and environmental factors, as additional bidirectional players in the game.

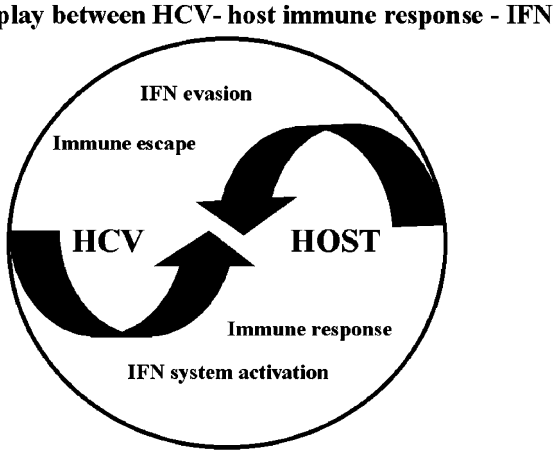

Figure 1

Acknowledgements

This work has been partially supported by grants from the Italian Ministry of Health, Fondi Ricerca Corrente e Finalizzata.

1. Baron S *et al.* (1994) Antiviral. Res. 24: 97–110
2. Samuel CE (2001) Clin. Microbiol. Rev. 14: 778–789
3. Grandvaux N *et al.* (2001) Curr Opinion Infect Dis 16: 259–267
4. Enomoto N *et al.* (1996) New Engl. J. Med. 334: 77–81
5. Moriguchi H *et al.* (2002) Hepatology 36: 177–185
6. Zeuzem S *et al.* (1997) Hepatology 25: 740–44
7. Witherell GW *et al.* (2001) J. Med. Virol. 63: 8–16
8. Cappiello G *et al.* (2001) Hepatology 34: 415 A
9. Gale MJ *et al.* (1997) Virology 230: 217–227
10 Tan SWL *et al.* (1999) Proc. Natl. Acad. Sci. USA 96: 5533–5538
11. Castes V *et al.* (2002) J. Virol. 76: 8189–8199
12 Guo JT *et al.* (2001) J. Virol. 75: 8516–8523
13 Pflugheber J *et al.* (2002) Proc. Natl. Acad. Sci. USA. 99: 4650–4655
14 Podevin P *et al.* (2001) Hepatology 33: 1503–1511
15 Nousbaum JB *et al.* (2000) J. Virol. 74: 9028–9038
16 Polyak SJ *et al.* (2001) J. Virol. 75: 6095–6106
17 Taylor DR *et al.* (1999) Science. 285: 107–110
18 Heim M *et al.* (1999) J. Virol. 73: 8469–8475