

Hepatitis C Virus, the E2 Envelope Protein, and α -Interferon Resistance

Taylor *et al.* (1) showed that the hepatitis C virus (HCV) envelope protein E2 inhibits the activity of the interferon-inducible protein kinase PKR in cell systems. PKR is an antiviral protein that blocks protein synthesis by phosphorylating the translation initiation factor eIF2 α (2). HCV E2 binds to PKR through a 12-amino acid sequence similar to the PKR autophosphorylation site and the eIF2 α phosphorylation site, the PKR-eIF2 α phosphorylation homology domain (PePHD). This binding and inhibition may account for the intrinsic resistance to interferon (IFN) therapy of chronic hepatitis C patients infected with genotype 1, but not of those with genotypes 2 or 3, in agreement with clinical data (3).

To assess whether the E2 PePHD sequence may be specifically associated with a pattern of response to α -IFN that may have diagnostic and prognostic significance, we studied 15 patients with chronic hepatitis C. Response to α -IFN was defined as lack of serum HCV RNA (by qualitative RT-PCR; Amplicor, Roche, Switzerland) after 12 weeks of treatment at a dose of 3 MU three times a week. Eight patients—two responders and six nonresponders—belonged to HCV genotype 1; seven patients—five responders and two nonresponders—belonged to genotype 3 (Fig. 1). Complementary DNA derived from total liver RNA was PCR-amplified using *Pfu* polymerase and primers encompassing the COOH-terminal E2-encoding region of HCV. Purified PCR products were directly sequenced and analyzed by multiple sequence

alignment with hierarchical clustering (4).

All eight HCV genotype 1 isolates, irrespective of the pattern of response to α -IFN, had the same consensus sequence, corresponding to that of the IFN-resistant patient belonging to genotype 1b in the Taylor *et al.* study [figure 1 of (1)]. The consensus sequences of HCV genotype 3 isolates were also identical in all but two patients, again irrespective of the response to α -IFN. These results show that the PePHD sequence may not necessarily account for the pattern of response to α -IFN *in vivo*. The failure to respond of two patients infected with HCV genotype 3 may have stemmed from an insufficient dose of α -IFN (5). By contrast, the response to treatment of two patients infected with HCV genotype 1, despite a PePHD sequence identical to that of α -IFN-resistant HCV isolates, suggests that other factors may have had a greater influence on the response to α -IFN. The interaction between HCV-E2 and PKR may well affect the PKR activity, but that activity may not be entirely suppressed if the baseline state is highly activated.

We do not dismiss the elegant data obtained by Taylor *et al.* in cell systems (1). Steadfast application of those results to the human model may be premature, however, until the appropriate correlative studies have been made.

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pt. #	eIF2 α PKR	S	E	L	S	R	R	R	S	T	T	T	T	T	T	T	T	T	
1	HCV-1	R	S	E	L	S	P	L	L	L	S	T	T	T	T	T	T	T	responder
2		1	R	S	E	L	S	P	L	L	L	S	T	T	T	T	T	T	responder
3		1	R	S	E	L	S	P	L	L	L	S	T	T	T	T	T	T	NR
4		1	R	S	E	L	S	P	L	L	L	S	T	T	T	T	T	T	NR
5		1	R	S	E	L	S	P	L	L	L	S	T	T	T	T	T	T	NR
6		1	R	S	E	L	S	P	L	L	L	S	T	T	T	T	T	T	NR
7		1	R	S	E	L	S	P	L	L	L	S	T	T	T	T	T	T	NR
8		1	R	S	E	L	S	P	L	L	L	S	T	T	T	T	T	T	NR
9		3	R	S	E	Q	H	P	L	L	H	S	T	T	T	T	T	T	responder
10		3	R	S	E	L	H	P	L	L	H	S	T	T	T	T	T	T	responder
11		3	R	S	E	Q	H	P	L	L	H	S	T	T	T	T	T	T	responder
12		3	R	S	E	Q	H	P	L	L	H	S	T	T	T	T	T	T	responder
13		3	R	S	E	Q	H	P	L	L	H	S	T	T	T	T	T	T	responder
14		3	R	S	E	Q	H	P	L	L	H	S	T	T	T	T	T	T	NR
15		3	R	S	E	Q	H	P	L	L	H	S	T	T	T	T	T	T	NR

Fig. 1. Alignment of the PePHD sequences from 15 HCV isolates of genotypes 1 and 3, derived from patients who responded to α -IFN therapy (responder; $n = 7$) and who failed to respond (NR; $n = 8$). Sequences of the PKR phosphorylation site (amino acids 48 to 54) of eIF2 α and of the autophosphorylation domain (amino acids 79 to 90) of PKR are shown for comparison.

References and Notes

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6. Supported by the Swiss National Science Foundation (contract #32-52193.97) and the Fondation Cancer-Solidarité.

9 December 1999; accepted 4 February 2000

Response: Our report (1) showed that the E2 protein of genotype 1 hepatitis C virus (HCV) can inhibit the cellular double-stranded RNA-activated protein kinase PKR, whereas the E2 protein from other genotypes cannot. This property correlates with the extent of sequence similarity between the PePHD sequence of E2 and PKR and eIF-2 α among the different genotypes. We believe that this finding can explain the resistance to IFN of genotype 1 viruses relative to that of other genotypes. At the same time, as we noted (1), although all viruses within a genotype have the same, highly conserved PePHD sequences, some are sensitive to IFN, whereas others are resistant to it. Thus, this property cannot explain the sensitivity or resistance of different isolates within the same genotype.

HCV uses a multipronged approach to develop resistance to IFN, including the NS5A protein, which also inhibits PKR activity (2). Heim *et al.* (3) showed that another, unknown HCV protein inhibits the IFN-induced JAK-STAT signaling pathway. Study of PKR-, RNase L-, and Mx-triple-deficient mice has shown that there are multiple alternative antiviral pathways for IFN (4). The E2-PKR interaction can only explain the generally higher resistance of the genotype 1 viruses relative to that of other genotypes. As the sequence conservation of the PePHD sequence within the same genotype implies, this molecular-mimicry mechanism cannot explain the difference in IFN sensitivity between individual isolates within the same genotype—nor did we claim that it does. Other mechanisms must explain the variations among viruses within the same genotype.

Nevertheless, the E2-PKR interaction is clearly one of the mechanisms by which HCV evades the actions of IFN. Moreover, the sequence analysis submitted by Abid *et al.* supports our hypothesis that the genotype 1 PePHD sequence is associated with a higher percentage of IFN resistance than genotype 3. This is consistent with the interpretation that this sequence contributes to IFN resistance, although IFN sensitivity or resistance within a genotype cannot be predicted from the PePHD sequence.

We agree with Abid *et al.* that the PePHD sequence within the same genotype does not vary with the clinical resistance or sensitivity of the individual HCV isolates. Our preliminary analysis of 43 patients from various genotypes led us to reach the same conclusion. Nevertheless, our original observation that E2-PKR homology can explain the relative resistance of different HCV genotypes is still valid and offers a potential strategy for improving the efficacy of IFN—particularly for viruses of genotype 1, the most prevalent genotype.

TECHNICAL COMMENT

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- 14 January 2000; accepted 4 February 2000

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Science **287** (5458), 1555

DOI: 10.1126/science.287.5458.1555a

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