Letter to the Editor

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Analysis of Sequence Configurations of the PKR-Interacting HCV Proteins from Plasma and PBMC as Predictors of Response to Interferon-Alpha and Ribavirin Therapy in HIV-Coinfected Patients

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To the Editor

Interferon (IFN) and ribavirin combination therapy for chronic hepatitis C virus (HCV) infection yields a sustained response rate of only ~40%. Previous studies have linked IFN responsiveness to viral sequence variation in parts of the structural envelope 2 (E2) and NS5A genes. It has been proposed that mutations in the RNA-dependent protein kinase (PKR)-binding domain (PKR-BD) within the HCV viral NS5A gene disrupt NS5A-PKR interactions and are important factors contributing to IFN sensitivity and repression of viral function. The proposed double-stranded RNA-dependent PKR-BD (codons 2209–2274) within the NS5A gene contains the putative IFN-sensitive determining region (ISDR, codons 2209–2248) which represses its function [1]. Similarly, the double-stranded RNA-activated protein kinase-eukaryotic initiation factor 2 alpha (PKR-eIF2) phosphorylation homology domain (PePHD, codons 659–670) within the E2 gene has been shown to interact with the IFN-induced PKR as a pseudosubstrate, interfering with its inhibitory effect on protein synthesis [2]. The role of these sequence variations is still controversial in HCV-monoinfected patients, and no data have been reported in HIV/HCV-coinfected patients. A comprehensive understanding of IFN treatment response or failure is still lacking. However, it is well recognized that both HCV genotype and HCV RNA levels are major determinants for response to IFN therapy in HCV-monoinfected patients. The rate of response according to viral genotype could imply a differential role for those proteins with capability to affect the success of IFN-based therapy.

The peripheral blood mononuclear cells (PBMCs) of a significant proportion of HCV-monoinfected subjects harbor viral variants ascribed to genotypes that are not found in plasma, likely acquired through superinfections [3]. The prevalence and the mechanisms of this compartmentalization, and their implications for anti-HCV therapy in HIV-coinfected patients are unknown.

In order to study HCV-related genomic factors associated with the response to IFN therapy in patients coinfected with HIV, we analyzed the sequence configurations in the PKR-interacting viral proteins (NS5A and E2) of HCV and linked them with virological response in order to explore their value in predicting therapeutic response. Considering that HCV compartmentalization might influence IFN-based treatment outcome [4], the analysis of viral proteins was carried out in plasma and PBMCs for each patient.

As HCV strategies to evade PKR-mediated antiviral actions imply direct interactions with viral domains, the characterized sequences were compared with a prototypic sequence named HCV-J (GeneBankD90208), and the 1b genotype was previously reported to respond least well to IFN [5]. This approach was chosen because there are no published NS5A or E2 prototype sequences derived from HCV-non-1b-infected patients characterized as nonresponders to IFN-based therapy.

Samples from a small homogeneous cohort of 10 HCV/HIV-coinfected patients who completed IFN-based treatment were retrospectively identified. This material was collected immediately before initiating the IFN-based treatment (baseline). Written informed consent was obtained, and the study protocol was approved by the Texas University Institutional Review Board. All patients were on treatment for HIV-1 infection with combination antiretroviral therapy. HCV and HIV RNA levels were determined with commercial assays (Cobas® Amplicor HCV Monitor, v2.0,

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Table 1. Immune and HCV- and HIV-related characteristics of the patients at baseline

Patient	Compart-ment	HCV genotype	Mutations in PKR-BD	Mutations in PePHD	Treatment outcome	HCV viral load log_{10} copies/μl	HIV viral load log_{10} copies/μl	CD4 n/mm^3	CD8 n/mm^3
A	Plasma	1a	7	0	NR	>5.7	<2.6	462	849
	PBMCs	1a	9	ND					
B	Plasma	1a	6	0	NR	>5.7	<2.6	491	766
	PBMCs	1a	6	0					
C	Plasma	1a	8	0	NR	>5.7	<2.6	593	855
	PBMCs	1a	8	ND					
D	Plasma	1b	2	0	SVR	>5.7	<2.6	511	4,649
	PBMCs	1b	ND	0					
E	Plasma	1b	6	0	SVR	>5.7	2.9	547	ND
	PBMCs	1b	6	0					
F	Plasma	1a	11	1	NR	5.5	<2.6	422	1,258
	PBMCs	1a	10	1					
G	Plasma	1a	10	0	SVR	5.6	<2.6	431	2,167
	PBMCs	1a	10	0					
H	Plasma	2a	ND	3	NR	5.2	<2.6	1,449	ND
	PBMCs	2a	ND	3					
I	Plasma	1a	6	0	NR	>5.7	<2.6	728	1,381
	PBMCs	1a	6	0					
J	Plasma	4a	ND	1	SVR	5.3	<2.6	550	1,127
	PBMCs	4a	ND	ND					

ND = Not done.

and Cobas® Amplicor HIV-1 Monitor, v1.5, both from Roche Diagnostic Corporation) and CD4+ and CD8+ T lymphocyte counts were done by flow cytometry and were not significantly different ($p > 0.05$ by Fisher's two-tailed test) among patients (table 1). The analysis of variance, Fisher's exact test, and Student's t test were used where appropriate to determine the association of mutations in the NS5A and E2 regions with IFN efficacy. A p value of <0.05 was considered statistically significant.

All patients received peginterferon alfa-2a, 180 mg/week, or peginterferon alfa-2b, 1.5 mg/kg/week, plus ribavirin, 1,000–1,200 mg/day based on body weight, for 48 weeks as the recommended treatment regimen unless they had an adverse event or did not respond after 12 weeks. Sustained virological response (SVR) was defined on the basis of the absence of plasma HCV RNA 6 months after treatment withdrawal. The nonresponders (NR) included those who did not respond at all and those who relapsed during the follow-up period. Four of the studied patients achieved SVR and 6 were NRs. HCV genotype (based on 5′-NCR + NS5B phylogenetic relatedness), E2 (PePHD) and NS5A (PKR-BD) genomic analyses (after direct sequencing using an ABI Prism 377 DNA sequencer) were performed in both compartments: plasma and PBMCs. Phylogenetic sequence analysis was carried out using the DNADIST and NEIGHBOR programs of the Phylip program package, version 3.53. Phylogenetic trees were constructed by the neighbor-joining method. Bootstrap values were determined on 1,000 resamplings of the datasets to assess the stability of the nodes. HCV intergenotype recombination was ruled out by bootscanning, using the SimPlot program, version 2.5 (Stuart Ray, http://www.med.jhu.edu).

The HCV genotype in HIV/HCV coinfected patients was predominantly (80%) type 1 as reported by others [6]. Among patients with SVR, the genotypes were Gt-1 (n = 3, including 1a n = 1 and 1b n = 2) and Gt-4a (n = 1). NRs were infected with Gt-1a (n = 5) and Gt-2a (n = 1). In all cases, the HCV genotype was coincident for each compartment analyzed (plasma or PBMCs). The HCV genotype ascribed to each isolate was coincident between compartments irrespectively of the partial genomic region considered, with no evidence of intergenotype homologous recombination (fig. 1).

Table 1 shows that at baseline, all characterized HCV isolates displayed E2-PePHD and/or NS5A-PKR-BD mutations unrelated to IFN therapy outcome. In general, the total number of mutations was significantly higher in NS5A-PKR-BD than in E2-PePHD ($p < 0.002$, Fisher's exact test). Separately, each genomic region exhibited no significant differences ($p > 0.05$, Student's t test) in the mean number of mutations between the SVR and NR groups. Similarly, the number of mutations was conserved in a given isolate when intrapatient analysis between plasma and PBMC compartments was performed (fig. 2). Likewise, the quantitative (number) or qualitative (amino acid change) mutation distribution between plasma and PBMC compartments for each analyzed genomic region was almost equal. The qualitative analysis was also similar across NR or SVR patients (fig. 2).

We presume that in the absence of specific antiviral therapy pressure, HCV exhibits homogeneous genomic variants in different compartments. As previously re-

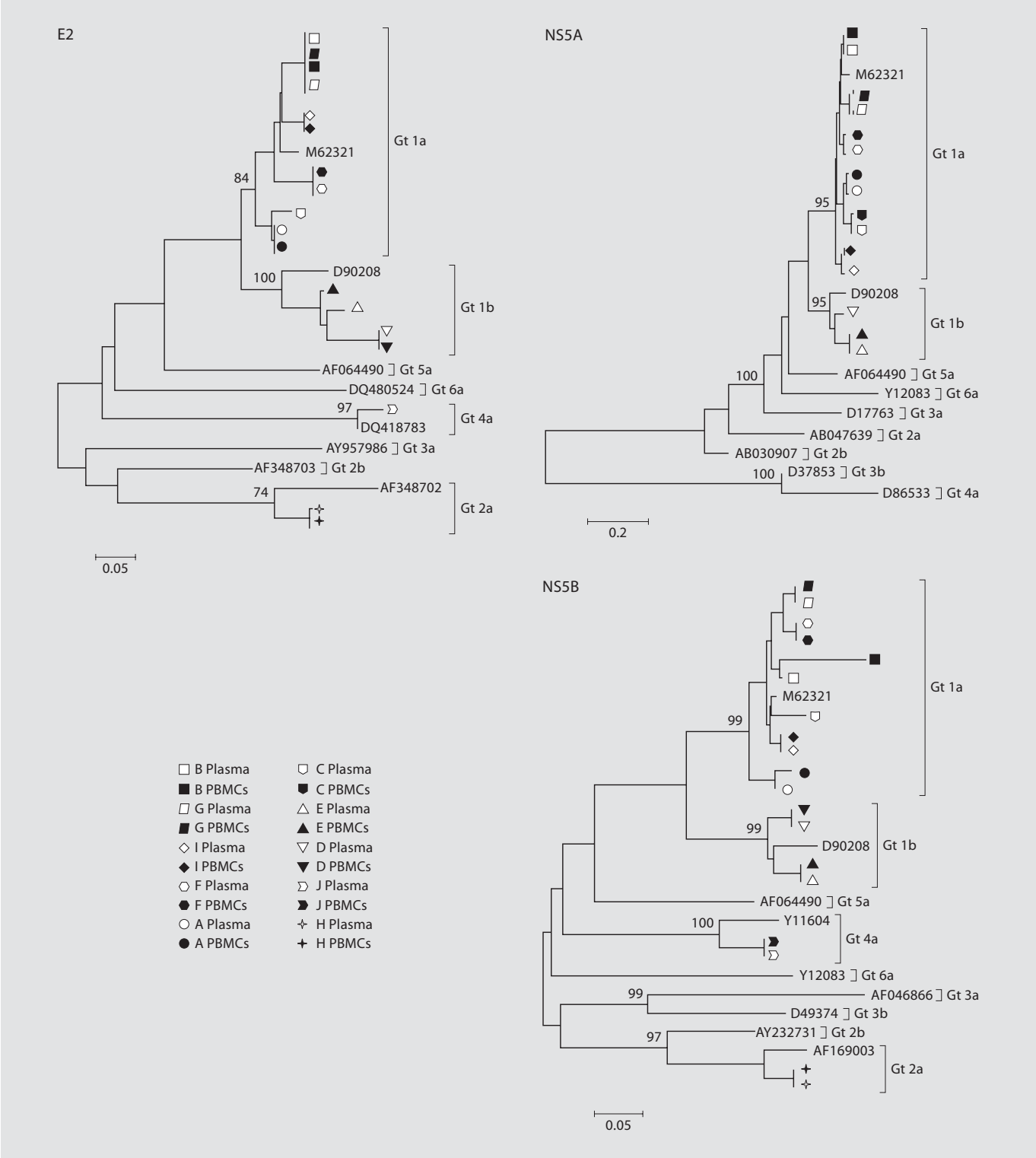

Fig. 1. Phylogenetic tree of the HCV partial E2, NS5A and NS5B sequences from HIV-coinfected patients. Branch lengths are drawn to scale. Prototype HCV sequences are included from each genotype. Numbers represent bootstrap proportions in support of the adjacent node based on 1,000 resampling iterations. Each symbol (square, triangle, diamond, circle, star) corresponds to a given HCV isolate from plasma (white) or PBMCs (black) from a given patient. Distance scale: 10% sequence dissimilarity. Internal node numbers represent bootstrap values.

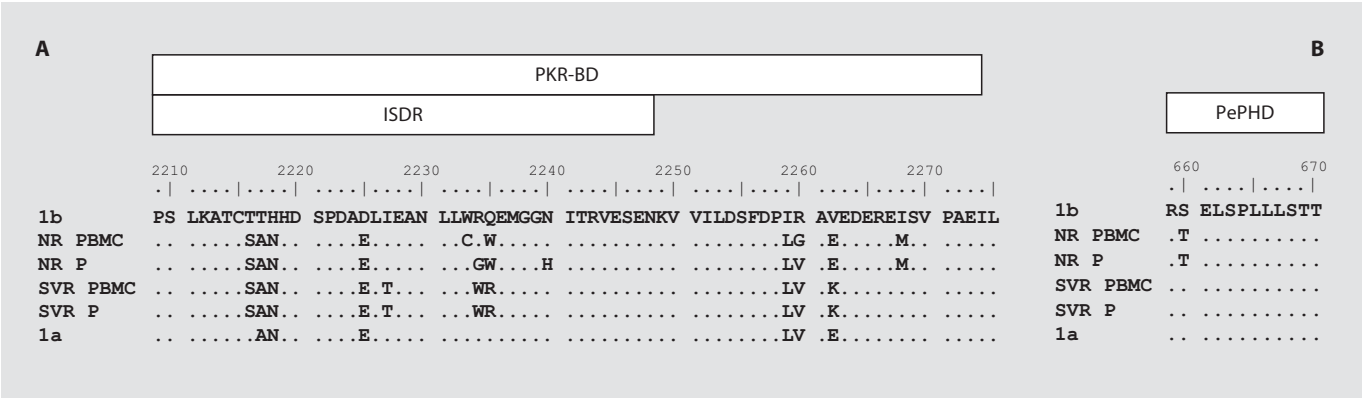

Fig. 2. Deduced amino acid sequence for HCV. NS5A-RNA-dependent PKR-BD including the ISDR (**A**) and the E2 including PKR-eIF2 PePHD in two representative chronically coinfected HIV-HCV-1a patients (**B**). Response to IFN and ribavirin combination therapy: NR (patient F); SVR (patient G); P = plasma. Two HCV prototype sequences are included belonging to genotypes 1b (D90208) and 1a (M62321).

ported, taking into account that all patients included in the present study were at a similar stage of HIV infection as well as under antiretroviral therapy, diminished HCV quasispecies variability is expected when HIV is also present [7], which may explain this similarity in the consensus sequences for both serum and PBMC compartments. However, it is likely that this approach underestimates the true probability of detecting PBMC-specific variants because this is a very conservative approach without follow-up during and after therapy. In addition, the compartmentalization phenomena should be analyzed in other genomic regions where the viral tropism is more critically influenced, such as in E1-HVR1. Because PBMCs could serve as a critical site for direct interactions between HIV and HCV, further examination of this compartment is warranted.

Our results suggest that in HCV/HIV-coinfected patients, the overall number of amino acid substitutions in the PKR-BD and/or PePHD at baseline, relative to an NR prototype 1b-sequence, did not have predictive value for sustained virological response to IFN-based therapy. Therefore, although the presence of higher NS5A-PKR-BD mutational frequency might influence the ability of IFN to inhibit virus production, other factors might be necessary to facilitate a sustained IFN effect for SVR in patients with HCV/HIV coinfection.

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