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Evaluating the Role of Cellular Immune Responses in the Emergence of HCV NS3 Resistance Mutations During Protease Inhibitor Therapy

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Abstract

The efficacy of protease inhibitor drugs in hepatitis C virus (HCV) treatment is limited by the selection and expansion of drug-resistant mutations. HCV replication is error-prone and genetic variability within the dominant epitopes ensures its persistence. The aims of this study are to evaluate the role of cellular immune response in the emergence of HCV protease resistance mutations and its effects on treatment outcome. Ten chronically HCV-infected subjects were treated with boceprevir (BOC)-based triple therapy. HCV-RNA was tested for BOC resistance-associated viral variants. HCV protease resistance mutations were investigated pretreatment and 24 weeks post-treatment. Synthetic peptides representing the wild-type and the potential nonstructural (NS)3 variants were used to evaluate T cell responses and human leukocyte antigen binding. Sustained viral response was achieved in 70% of patients, two patients were treatment nonresponders (NRs) and one was classified as a relapse. Pretreatment, the proportion of drug-resistant variants within individuals was higher in sustained viral responders (SVRs) than in NR patients. However, resistance-associated variants increased in NRs after BOC combined triple therapy. In contrast to NR patients, significant stronger cell-mediated immune responses were observed at the baseline among those who achieved sustained viral response for all T cell epitopes tested. Despite the increase in cell-mediated immune responses at week 24 in NRs, they failed to control the virus replication, leading to development of overt drug-resistant variants. Our data suggest that strong NS3-specific T cell immune responses at the baseline may predict a positive outcome of directly acting antiviral-based therapy, and the presence of pre-existent resistance mutations does not play a significant role in the outcome of anti-HCV combined therapy.

Introduction

Recent advances in molecular biology have led to the development of several novel small molecules that target specific viral proteins in the hepatitis C virus (HCV) life cycle. These directly acting antiviral (DAA) drugs, which include a range of inhibitors targeting nonstructural (NS) 3/NS4A protease and NS5B polymerase, are at various stages of clinical development. However, the rapid replication rate of HCV, along with the low fidelity of its polymerase, leads to the emergence of drug-resistant mutations that limit the overall efficacy of DAA drugs (2,9,21). In this study, we focused on the protease inhibitor (PI), boceprevir (BOC), as a model PI that has Food and Drug Administration (FDA) approval in the United States for the treatment of HCV in conjunction with pegylated interferon (PEG-IFN) and ribavirin (RBV).

The overall clinical efficacy of BOC combined therapy may be limited by the development of drug-resistant HCV quasispecies during treatment. Furthermore, some studies suggest that pre-existing mutations may limit DAA effectiveness in some settings (2). For example, pretreatment Q80 mutations limit sustained viral response in strains of HCV genotype 1a patients treated with simeprevir/PEG-IFN/RBV (8). Resistance mutations frequently result in a decrease in overall viral replicative fitness (4,5,15).

Another selective force that continues to shape HCV diversity throughout the course of infection is the host human leukocyte antigen (HLA)-restricted immune response and the presence of T cell receptors (TCRs) specific to these epitopes. HCV-specific T cells are stimulated by the presentation of processed viral epitopes in the context of the HLA molecules. Substitutions in viral epitopes may alter their HLA binding or their recognition by TCRs and result in

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the emergence of escape mutation. Therefore, the selection of HCV sequences targeted by the immune response is dependent on the HLA and T cell repertoires of the host (10,19).

There are numerous examples in which mutations within or flanking HLA-restricted HCV epitopes allow the virus to evade the host's immune response (7,11,17). However, variability within the immunodominant cytotoxic T lymphocyte (CTL) epitopes of the NS3 protease is limited by viral fitness. Consequently, not all mutations at critical CTL-recognized epitopes are preserved during HCV infection. In fact, some mutations may reduce protease activity and RNA replication (viral fitness). Therefore, viral fitness can limit the variability of HCV within immunological epitopes. This helps to explain why certain immunological escape variants never appear as a major viral quasispecies during infection (16).

The overall goal of this study was to examine the relationship between the host immune responses and the development of PI resistance mutations. We screened plasma from treatment-resistant chronically HCV-infected patients receiving triple-based therapy containing PEG-IFN, RBV, and BOC for their susceptibility to the emergence of PI mutants using HLA information and published data on the strength of binding of their HLAs with the HCV epitopes.

Patients and Methods

A cohort of 10 HCV-infected patients was enrolled in this prospective pilot study. Informed consent was collected from all enrolled subjects under the University of Cincinnati review board number (IRB #2012-3388) and registered at www.clinicaltrials.gov (NCT01517529). Detailed demographic information was collected for all the patients. All the parameters of disease activity were assessed in the clinic or in the hospital, depending on the setting, including viral load, and liver panel (ALT, aspartate transaminase [AST], and bilirubin). Treatment-experienced chronically HCV-infected patients were recruited from the University of Cincinnati hospital and clinics at University of Cincinnati Physicians, a tertiary referral center utilizing both inpatient and outpatient settings. Patients started the lead-in phase of treatment with 4 weeks on PEG-IFN + RBV; on week 5, BOC was added to the regimen. The duration and the doses of therapy were determined according to the FDA product insert.

For this study, successful treatment was defined as undetectable HCV viral load at week 24 after the start of the treatment since almost all the patients who failed the therapy would have PI mutants by week 24 (1). Inclusion criteria were chronically HCV-infected patients with persistent viremia who failed prior -IFN and RBV therapy and were eligible for combined treatment with PI therapy. Briefly, this included male or female, age 18–65, chronic HCV infection as evidenced by liver biopsy and/or persistent HCV viremia for >6 months, and treatment experienced who were classified as nonresponder or relapser to prior interferon-based therapy. Exclusion criteria were treatment naïve chronically HCV-infected patients, patients with a history of inflammatory bowel diseases (IBDs) or suspected IBD, autoimmune diseases, including rheumatoid arthritis, any patients on systemic immunomodulators, and patients who were pregnant or HIV infected. Thirty to 50 mL of blood was drawn from each subject pretreatment (time 0) at weeks 4, 8, 12, and 24.

Characterization of HLA of the enrolled subjects

The HLA typing was done at the core facility of Transplant Immunology Division, Hoxworth Blood Center, University of Cincinnati, using high-resolution sequence-specific oligonucleotide probes. Allele-level HLA typing (four numerals) was performed by sequence-based typing.

HLA binding with predictions to wild and mutant NS3 peptides

The affinity of HLA class A and B binding for each patient to the dominant wild and mutant NS3 PI epitopes was determined using a computational method provided by the Immune Epitope Database and Analysis Resource (IEDB, www.immuneepitope.org) (12). In this HLA prediction/consensus method, a low percentage indicates a stronger binder epitope. Data are presented as binding affinity by subtracting 100 from each predicted binding affinity value to make the highest value, which denotes stronger binding.

Analysis of the NS3 PI mutants at pretreatment and at the end of therapy

HCV RNA was extracted from patients' plasma using the QIAamp viral RNA mini kit (Qiagen. Valencia, CA). A 508–628-bp-long cDNA fragment encompassing the HCV NS3 serine protease domain genotype 1a and 1b was amplified with a pair of HCV-specific oligonucleotides at pretreatment and after 24 weeks of BOC combined treatment. Samples were tested for 11 key NS3 PI-resistant mutations using assays designed for pyrosequencing. The pyrosequencer, PyroMark Q96 MD (Qiagen), was used for SNP detection. Two external primers were used to amplify the HCV 1a and 1b genotype NS3 region. The reverse primers were labeled with biotin to facilitate its binding to streptavidin-coated sepharose beads. Three internal assays were designed using PyroMark Assay Design SW 2.0 (Qiagen) for sequencing primers to detect 11 mutations. The quantitative analysis of nucleotide incorporation allows for an accurate SNP analysis with a sensitivity of detecting minor viral variants as low as 2%.

Peptide synthesis of wild-type and mutant NS3 epitopes

Eight to 10 amino acid peptides corresponding to the wild-type sequences of genotype 1a or 1b of the NS3 dominant epitopes, in which PI mutants are present, as well as epitopes with PI mutant sequences were synthesized by Sigma-Genosys with 85%–95% purity and solubilized to a concentration of 1 mg/mL according to the manufacturer's recommendations (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/vim).

In vitro *stimulation of T cells with wild-type and PI mutant peptides using ELISPOT assay*

The ELISPOT assay was performed according to the manufacturer's instructions in the IFN-(ELISPOT kit with modifications (MABTECH, Mariemont, OH) and as previously described (13,14,20). Briefly, $1–3 \times 10^5$ PBMCs were plated onto a 96-well plate and stimulated with HCV synthetic peptides representing regions within the NS3 active PI site at 1 (g/mL (for HCV-specific $CD8^+$ T cells responses) for 18 h. Irrelevant peptides were used as negative controls.

Phytohemagglutinin (Difco/Becton Dickinson, Sparks, MD) and CEF control peptide pools (Mabtech, Cat No 3615-1) were used as positive controls. The number of IFN-γ-secreting cells (ISCs) were enumerated using an automated ELISPOT 3B (CTL, Cleveland, OH) designed to detect spots using predetermined criteria based on size, shape, and calorimetric density and expressed per 10^6 cells. Analysis of the data was done using the following equation:

Ag-specific ISC/10^6 cells = number of ISCs in response to antigen per10^6 cells—number of ISCs in the presence of media alone per 10^6 cells.

The cutoff level is calculated as the average number of ISCs in the presence of medium or negative peptides + two standard deviations (SDs) and it is approximately ≥37.5 spots/10^6 cells, according to our preliminary data.

Statistical analysis

All patients were classified as SVRs, nonresponders (NRs), or relapsers (Rs) at the end of the study. SVR is defined as the undetectable plasma HCV viral load at week 24 after completion of treatment. NR is defined as either viral breakthrough (achievement of an undetectable plasma HCV RNA level and subsequent occurrence of HCV RNA level >1,000 IU/mL) after the end of treatment or incomplete virological response and rebound (an increase of 1 $\log_{10}$ IU/mL in the plasma HCV RNA level from the nadir with an HCV RNA level >1,000 IU/mL) during treatment. Relapsing is defined as undetectable HCV RNA levels at completion of treatment, but becoming detectable during follow-up.

Data are given as range (minimum, maximum); mean ± SD. Student's t test was used to examine the difference between the two groups with a significance value at $p \leq 0.05$. Nonparametric Wilcoxon rank sum tests were used to compare means of primary measures between SVR and NR patients and rank a significant difference between T cell responses to peptides at $p \leq 0.05$. Statistical significance of the frequency of T cell response to peptide tested was calculated using Poisson statistics for the number of responders to each peptide within the dataset. Poisson-based determination was used, $1 - \sum_i^x = 0(\lambda^x e^{-\lambda}/x!)$ where λ = the median responses to the peptide tested in the dataset and x = the

Table 1. Demographic and Clinical Characteristics of Patients

		Responders, N = *7*	*Nonresponders,* N = *3*	*OD*	p
Age (years) (mean ± SD)		55.3 ± 14.3	55.3 ± 39.1		ns
Gender					
Male		6 (86%)	2 (67%)		
Female		1 (14%)	1 (33%)		
Race					
Black		5 (71%)	1 (33%)		
Caucasian		2 (29%)	2 (67%)		
Ethnicity					
Hispanic		0	0		
Non-Hispanic		7 (100%)	3 (100%)		
IDU history		4 (57%)	2 (67%)	1.5	ns
Previous HCV treatment		2 (29%)	NA		
Responses to last/prior therapy			NA		
Relapse		1 (14%)	NA		
Response		1 (14%)			
Log_{10} HCV viral load at baseline/IU (mean ± SD)		6.3 ± 0.4	6.5 ± 0.1		ns
HCV genotype					
1a		7 (100%)	1 (33%)		
1b		0 (0%)	2 (67%)		
Platelets thousands/μL (mean ± SD)	Baseline	206.0 ± 40.6	224.0 ± 23.4		ns
	24 weeks	135.2 ± 55.2	160.0 ± 83.0		ns
HB g/dL (mean ± SD)	Baseline	14.0 ± 1.9	14.4 ± 1.1		ns
	24 weeks	11.5 ± 2.0	12.2 ± 2.6		ns
ALT U/L (mean ± SD)	Baseline	82.8 ± 41.3	69.0 ± 32.8		ns
	24 weeks	39.0 ± 14.6	55.3 ± 23.0		ns
AST U/L (mean ± SD)	Baseline	62.6 ± 38.9	84.7 ± 48.1		ns
	24 weeks	35.0 ± 14.9	74.3 ± 32.9		0.0261[a]
Bilirubin total mg/dL (mean ± SD)	Baseline	0.8 ± 0.2	0.9 ± 0.3		ns
	24 weeks	0.7 ± 0.2	0.8 ± 0.3		ns
Alkaline phosphatase U/L (mean ± SD)	Baseline	97.8 ± 36.7	102.3 ± 17.7		ns
	24 weeks	97.0 ± 53.8	81.0 ± 27.4		ns
Albumin (mean ± SD)	Baseline	4.5 ± 0.2	4.2 ± 0.4		ns
	24 weeks	4.4 ± 0.2	4.2 ± 1.0		ns

[a]Significant.
AST, aspartate transaminase; HCV, hepatitis C virus; IDU, injection drug users; SD, standard deviation.

number of SVR patients who had a T cell response to the tested peptide. The response to a peptide was considered significant if the number of patients responding to the peptide was different from the Poisson distribution defined by the dataset with a $p \leq 0.05$.

Results

Demographic characterization

Ten chronic HCV-infected patients were recruited to this study from University of Cincinnati Medical Center. Table 1 shows the demographic clinical characteristics of patients. Eight males and two females of non-Hispanic ethnicity were enrolled. Six patients were African American and four of Caucasian origin. All patients were positive for HCV, eight with genotype 1a virus and two were infected with genotype 1b. Eight patients responded to treatment initially, then one of the eight (CTL003) relapsed at the end of treatment. Two of the patients did not respond to BOC triple-based treatment. Patients were categorized as responders (SVRs) (seven patients) and NRs (three patients) to compare differences in immune response and mutation frequency. There was no significant difference in age, gender, or clinical parameters between SVR and NR patients, except in the level of the liver enzyme AST after 24 weeks of treatment ($p = 0.03$).

Cell-mediated immune responses to NS3 peptides

HCV-specific cell-mediated immune (CMI) responses were measured using Ag-specific IFN-γ ELISPOT assay. A significantly ($p \leq 0.05$) stronger CMI response to wild and mutant T cell epitopes (Supplementary Table S1) was observed in SVR patients pretreatment than in NR patients (mean ± SD: 90 ± 131 ISC/10^6 cells and 7.9 ± 9 ISC/10^6 cells, respectively) (Fig. 1A). This difference in CMI was not observed following 24 weeks of treatment in the SVR patients compared with the NR patients (mean ± SD: 16.6 ± 14.8 ISC/10^6 cells and 20.4 ± 22.8 ISC/10^6 cells, respectively) (Fig. 1B). A significant ($p = 0.01$) increase in the NS3-specific CMI response was noticed after 24 weeks of treatment in the NR patients compared with pretreatment (mean ± SD: 16.6 ± 14.8 ISC/10^6 cells and 7.9 ± 9.1 ISC/10^6 cells, respectively) (Fig. 1). Patient CTL003 who relapsed after treatment had the worst immune response before and after treatment (mean ± SD: 1.61 ± 2.62 ISC/10^6 cells and 6.88 ± 5.47 ISC/10^6 cells, respectively) (Fig. 2). Poisson distribution and probability were calculated to determine the frequency and the probability of CMI to different peptides tested among patients. Among SVR patients, the T54A/S variant epitope region induced the strongest CMI response before treatment (mean ± SD: 162.3 ± 211.8 ISC/10^6 cells, $p < 0.0001$, and 133.7 ± 162.0 ISC/10^6 cells, $p < 0.0001$, respectively), followed by A156T (mean ± SD: 131.8 ± 175.4

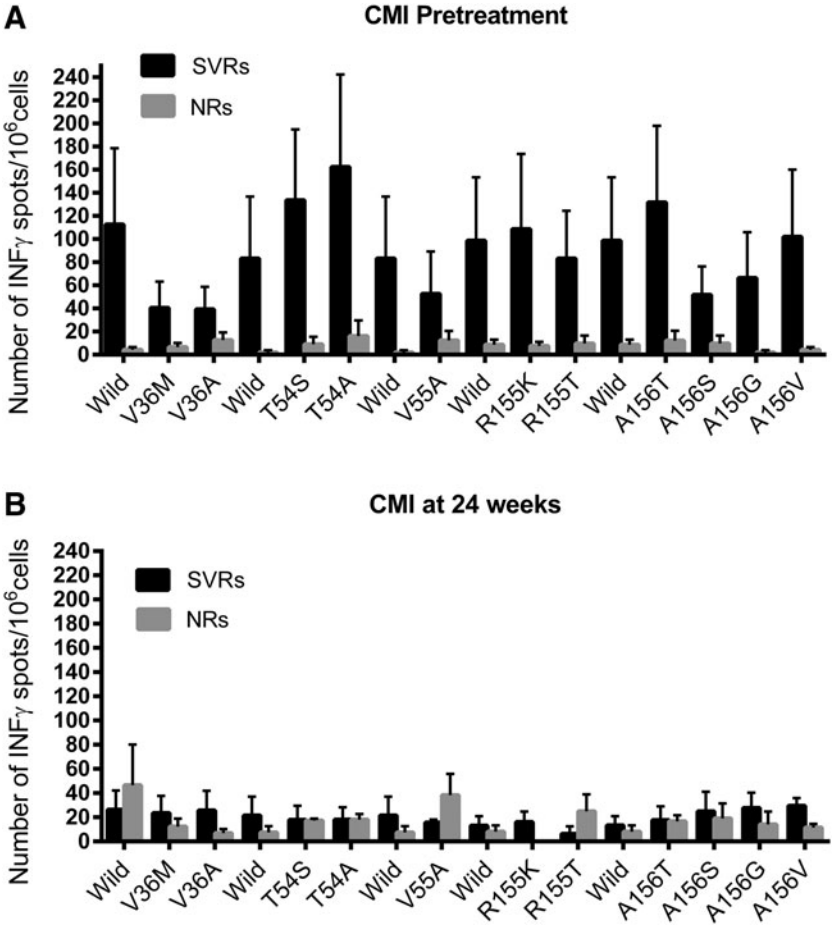

FIG. 1. Cumulative cell-mediated immune (CMI) response in sustained viral responder (SVR) and nonresponder (NR) patients. **(A)** The pretreatment CMI response to wild-type and mutant T cell epitopes tested in both groups of patients. **(B)** The cell-mediated response to all T cell epitopes tested in both group of patients after 24 weeks of boceprevir-based triple therapy.

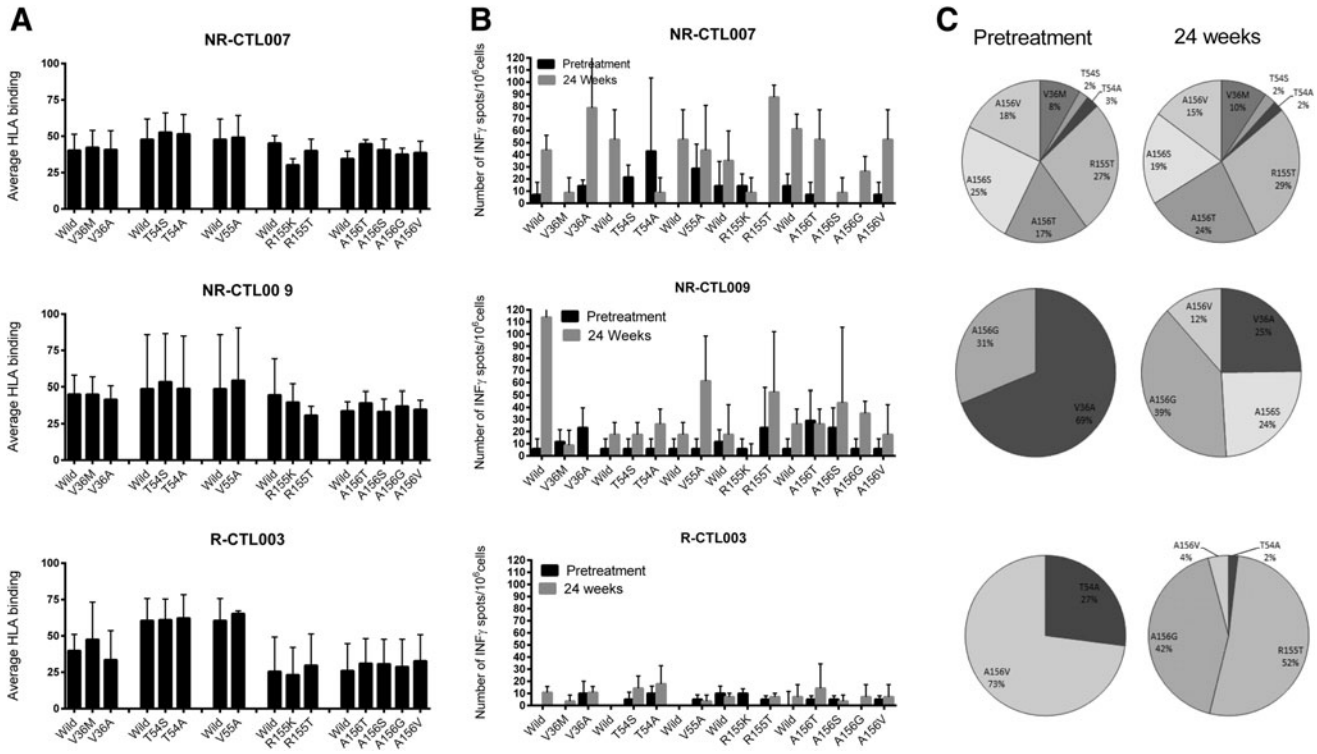

FIG. 2. Human leukocyte antigen (HLA) binding rank, CMI response, and protease inhibitor (PI) resistance variants in NR patients. **(A)** Average of HLA binding capacity to wild and variant peptides in nonresponder patients: NR-CTL007, NRCTL009, and relapser patient (R-CTL003). **(B)** CMI response to nonstructural (NS)3 wild and variant peptides for nonresponder patients before and after 24 weeks of PI treatment. **(C)** Resistance-associated variants detected in NR patients before and after PI treatment.

ISC/10^6 cells, $p<0.0001$) and R155K (mean ± SD: 108.7 ± 171.7 ISC/10^6 cells, $p<0.0001$). The weakest immunogenic epitopes were associated with the V36M/A variants (mean ± SD: 40.5 ± 60.08 ISC/10^6, $p<0.0001$, and 39.1 ± 50.0 ISC/10^6 cells, $p<0.0001$, respectively), the difference in CMI response in the SVR group to all peptides studied was significantly different ($p<0.0001$) before and after treatment. In NR patients, the NS3 peptides displaying V55A and R155T variants showed that they have induced the strongest CMI after treatment (mean ± SD: 38.3 ± 30.6 ISC/10^6 cells, $p=0.001$, and 24.9 ± 24.2 ISC/10^6 cells, $p<0.001$, respectively).

HCV NS3 protease-associated variants

To confirm the validity of the pyrosequencing method, viral RNA isolated from five chronic HCV patients from UC IRB-approved (#2012–3689) collection bank were tested for PI-resistant mutations using clonal sequencing methods. These samples were also examined using the pyrosequencing assay as described above. The results were comparable within each patient.

NS3 protease-associated variants at amino acid positions V36, T54, V55, R155, and A156 were investigated before and after 24 weeks of combined triple BOC treatment in all patients. At pretreatment, resistance-associated variants (RAVs) were present at higher frequency in those who achieved sustained viral response versus NRs. In general, the pattern changed after 24 weeks of treatment where NRs carried more variants than SVRs at the baseline (Tables 2 and 3). The number of variants at pretreatment in the SVR patients ranged from 4 to 10 detectable RAVs, while in the three NRs, the numbers ranged from 3 to 7 RAVs ($p=0.342$). This number of variants increased in the NRs to 5–7 RAVs after treatment, however, the difference was not significant ($p>0.05$). The proportion of variants within the patient was higher at pretreatment in SVRs than in the NRs (Tables 2 and 3). V36M, T54S, and A156T were the most frequent (100%) mutations in SVRs at pretreatment, while V36A and A156S were the least frequent (28.6%, respectively) in the same group. The most frequent mutations in NRs at pretreatment were T54A and A156V (28.6% each). After 24 weeks of treatment, A156V was 100% frequent in NRs (Tables 2 and 3).

HLA binding and association with HCV NS3 variants

HLA typing (A and B alleles) for each patient was identified to evaluate the role of HLA binding in selection of resistant mutations (Supplementary Table S2). The effect of HLA binding on the emergence of resistance variants was examined using the IEDB (12) to predict HLA binding rank to NS3 protease epitopes. As each patient's HLA alleles are unique, we compared the binding rank within HLA class A and B allele-restricted epitopes for each patient and compared that with their CMI and the emerged mutations. Figure 2 shows a combined description of the HLA binding rank, T cell immune response to tested peptides, and BOC-RAVs in the three patients who did not respond to treatment. Overall, peptides that carried the variation at positions T54

Table 2. Characterized Resistance-Associated Variants in SVR Patients Before Treatment

RAV	*CTL001 %*	*CTL002 %*	*CTL004 %*	*CTL005 %*	*CTL006 %*	*CTL008 %*	*CTL0010 %*	*RAVs pretreatment % (n)*
V36M	50.9	11.8	18.7	20.3	16.1	0.3	2.6	100 (7)
V36A	10	—	—	1.8	—	—	—	29 (2)
T54S	5.8	3.1	6.6	2.7	2.5	4.5	1.9	100 (7)
T54A	5.6	—	3.4	7.5	7.9	7.6	7.9	86 (6)
V55A	0.2	2.4	6.7	—	—	5.6	0.8	71 (5)
R155K	—	—	—	—	—	68.2	65.2	29 (2)
R155T	29	—	59.5	68	4.1	6.9	8.9	86 (6)
A156T	22.7	12.5	53.7	50	4.2	14.8	13.1	100 (7)
A156S	—	—	—	—	—	85.1	86.8	27 (2)
A156V	37	—	—	—	5.2	95.3	93.8	57 (4)
A156G	26	—	100	—	6	4.7	6.2	71 (5)
No. of RAVs within patient pretreatment% (*n*)	81 (9)	36 (4)	64 (7)	60 (6)	64 (7)	90 (10)	81 (9)	

CLT, cytotoxic T lymphocyte; RAV, resistance-associated variants; SVR, sustained viral responder.

and V55 were strongly recognized by the HLA molecules (rank >50) in the NR patients with increases in binding rank in the relapser patient. Other tested peptides showed low binding rank to the HLA molecules (rank <50).

Discussion

The present pilot study supports the notion of a significant role of the pretreatment NS3-specific CMI response in the outcome of combined triple therapy, which includes BOC. Furthermore, it debates the clinical relevance of pre-existent drug-resistant mutations on the outcome of anti-HCV combined triple therapy.

BOC-based triple therapy regimen was used for treatment of 10 chronically HCV-infected patients and sustained viral response was achieved in 70% of the enrolled patients. Two patients were classified as treatment NRs and one patient relapsed following HCV RNA clearance at the end of therapy. Overall, the results demonstrate that SVR patients had T cells capable of recognizing conserved and mutant sequences of the HCV NS3 protein, despite the presence of baseline BOC RAVs. In contrast, NR patients had a lower overall T cell response that was more pronounced in the patient who relapsed after treatment. In the NR patients, including the patient who relapsed after treatment, a selection of the highly BOC-resistant variants, which binds poorly to the patients' HLA molecules, was detected after treatment. These data suggest that HLAs of NR patients may play a significant role in the selection of highly resistant variants during treatment.

Our results showed a higher frequency of pretreatment RAV proportion in SVR patients compared with NRs; however, the difference was not significant (ranges from 36% to 90% in SVRs and 27%–63% in NRs, $p > 0.05$) and did not significantly affect the response to BOC triple therapy, which is comparable with a previous study by Howe *et al.* who reported that 64.6% of BOC recipients achieved sustained viral response when baseline RAVs were detected and 64.3% when baseline RAVs were not detected (6).

Table 3. Characterized Resistance-Associated Variants in Nonresponder Patients Before and After Treatment

	CTL003 %		*CTL009 %*		*CTL007 %*		*RAVs pretreatment % (n)*
RAV	*Pretreatment*	*24 W*	*Pretreatment*	*24 W*	*Pretreatment*	*24 W*	
V36M	—	—	—	—	18.5	19.3	33 (1)
V36A	—	—	19	8.8	—	—	33 (1)
T54S	—	—	—	—	4.4	4.9	33 (1)
T54A	4.3	2.6	—	—	5.6	4.3	67 (2)
V55A	—	—	—	—	—	—	0.0 (0)
R155K	—	—	—	—	—	—	0.0 (0)
R155T	—	72.9	—	—	58.8	58	33 (1)
A156T	—	—	—	—	38.2	47.9	33 (1)
A156S	—	—	—	8.6	53.7	37.7	33 (1)
A156V	11.6	5.8	—	4.2	39.4	30.4	67 (2)
A156G	—	58.9	8.5	13.9	—	—	33 (1)
RAVs within patient pretreatment % (*n*)	18 (2)		18 (2)		63 (7)		
RAVs within patient at 24 weeks % (*n*)		36 (4)		36 (4)		63 (7)	

Our data are in agreement with a previous report, which showed that HCV quasispecies in chronically infected chimpanzees acquired mutations during acute infection in multiple dominant epitopes that impaired class I MHC binding and/or CTL recognition (3). In contrast, most HLA-binding dominant epitopes remain intact during acute infection in chimpanzees who resolved infection. Consequently, CTLs exert positive selection pressure against the HCV quasispecies, and the outcome of infection may be predicted by mutations in class I MHC-restricted epitopes (20). Additionally, the role of CTL escape mutations in HCV persistence was confirmed by inoculation of a chimpanzee with a virus inoculum containing a highly conserved NS3 epitope, which suggested that CTL escape mutations significantly impact viral persistence (18).

One of the limitations of our study is the small number of patients enrolled. This constraint was due to the unavailability of qualified chronically HCV-infected patients who accepted the combined triple BOC therapy especially after FDA approval of new DAA drugs with a higher success rate.

In conclusion, virus clearance versus persistence after BOC treatment may not be explained primarily by the occurrence of viral escape mutations, but by initially weak virus-specific T cell responses that make viral escape possible. Additional large-scale studies with different members of anti-HCV drugs are needed to further explore these results.

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Author Disclosure Statement

No competing financial interests exist.

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