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Evolutionary dynamics of the E1–E2 viral populations during combination therapy in non-responder patients chronically infected with hepatitis C virus subtype 1b

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ABSTRACT

Half of the patients chronically infected with hepatitis C virus (HCV) genotype 1 fail to respond to pegylated interferon alpha (PEG-IFN) and ribavirin (RBV) therapy. This study assesses the effects of treatment on the evolution of the E1–E2 viral region in non-responder patients infected with HCV-1b. Twenty-three HCV-1b chronically infected patients were studied retrospectively, including 19 non-responders to PEG-IFN/RBV therapy (11 null-responders and 8 relapsers) in the study group, and 4 untreated patients in the control group. Genetic and phylogenetic analyses of the E1–E2 viral populations were performed at baseline and at the time of treatment failure to assess changes in genetic variability and evolutionary dynamics during treatment. Baseline virological characteristics were similar in null-responders, relapsers and controls. E1–E2 genetic variability decreased during treatment in non-responders, with a more pronounced decline in relapsers than in null-responders. A specific evolutionary pattern was not observed in null-responders, while a complete substitution of viral variants found at baseline characterised relapser patients. No specific E1–E2 amino acid substitution involved in treatment failure could be identified. In conclusion, although diverse evolutionary patterns with no apparent common adaptive changes were observed during therapy, treatment failure was characterised by a decline in genetic diversity.

1. Introduction

Hepatitis C virus (HCV), with an estimated 170 million people infected worldwide, is the major causative agent of chronic liver disease, cirrhosis and hepatocellular carcinoma (World Health Organization, 2010). HCV possesses a positive-sense, single-stranded RNA genome that exhibits significant levels of genetic variability (Simmonds et al., 2005). A high replication rate and lack of proofreading activity of the viral RNA-dependent RNA polymerase generate a dynamic mosaic of closely related variants, usually referred to as quasispecies, within an infected individual. This phenomenon allows the establishment of chronic infection and may also have important implications in pathogenicity and resistance to antiviral drugs (Farci and Purcell, 2000).

For almost a decade, the standard of care against chronic hepatitis C consisted of the combined administration of pegylated interferon alpha and ribavirin (PEG-IFN/RBV). This treatment regimen is not effective in about 50–60% of patients infected with HCV genotype 1 (Manns et al., 2001; Fried et al., 2002), the most common genotype worldwide (World Health Organization, 2010). To

Abbreviations: T_f, final time point; HCV, hepatitis C virus; HVR, hypervariable region; T_i, initial time point; π, nucleotide diversity corrected by the Jukes–Cantor method; nHap, number of haplotypes; Ka, number of nonsynonymous substitutions per nonsynonymous site; S, number of segregating sites; Ks, number of synonymous substitutions per synonymous site; PEG-IFN, pegylated interferon alpha; RBV, ribavirin; η, total number of mutations.

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improve treatment response rates, the standard of care for chronic HCV-1 infections has recently switched to novel therapeutic strategies, including a protease inhibitor. However, these new drugs should be administered in triple therapy together with PEG-IFN/RBV, in order to prevent HCV resistance (Ghany et al., 2011). It is important to note that HCV-1b is the most prevalent subtype in Southern and Eastern Europe (approximately ranging from 30% to 70% of HCV infections) (Esteban et al., 2008), as well as in Japan and other countries (Sy and Jamal, 2006), and is associated with a higher risk for hepatocellular carcinoma development (Raimondi et al., 2009).

Response to PEG-IFN/RBV treatment is influenced by the rate of viral load decline, which helps predicting treatment outcome (Ghany et al., 2009). In addition, qualitative changes in viral populations may be involved in treatment outcome through the selection of viral variants able to escape the effects of the antiviral therapy. Along this line, previous studies have suggested that changes in the genetic heterogeneity present in the E1–E2 region during combination therapy in chronically infected patients may be an important determinant of treatment failure (Ueda et al., 2004; Abbate et al., 2004; Cuevas et al., 2008b; Torres-Puente et al., 2008b; Cuevas et al., 2009a,b; Fan et al., 2009). Hypervariable region (HVR)-1 is the most variable portion of the HCV genome (Weiner et al., 1991), and during the course of infection escape variants are generated in response to the selective pressure from the host's immune system (Weiner et al., 1992; Farci et al., 1994). While several HCV proteins have been shown to block IFN-α effects (Wohnsland et al., 2007), specific mutations associated with non-response to PEG-IFN/RBV therapy have not been identified.

In a previous study comparing baseline characteristics between HCV-1b–infected responder and non-responder patients to PEG-IFN/RBV therapy, a combination of viral and host factors allowed the treatment outcome to be predicted with a high accuracy (Saludes et al., 2010). Among other variables, higher values of several variability estimators in the E1–E2 region were associated with treatment failure. The goal of the present study was to assess the effect of PEG-IFN/RBV treatment on the evolutionary dynamics of viral populations in this genetic region in HCV-1b non-responder patients as compared to untreated control patients.

2. Materials and methods

2.1. Patients and specimens

Twenty-three Caucasian persons with chronic HCV-1b infection were selected retrospectively and included both treated and untreated patients. Exclusion criteria were: previous interferon-based treatment, HIV or HBV coinfection, having other causes of liver disease or alcohol abuse. The study group included 19 non-responder patients who received PEG-IFN (180 μg/week) and RBV (1000–1200 mg/day) combination therapy for 48 weeks at the Hospital Universitari Germans Trias i Pujol. Non-response was defined as the continued presence of HCV RNA during therapy (null-response at week 12 of treatment, $n = 11$) or detection of HCV RNA 24 weeks after showing an end-of-treatment response (relapse, $n = 8$). Good treatment adherence was defined as having received $\geqslant$80% of total maximum dose prescribed of both drugs for $\geqslant$80% of the expected duration of therapy (McHutchison et al., 2002). All non-responder patients were treated for the complete scheduled time, and adherence to both drugs was good overall, with >80%; only two patients had a slightly lower adherence, of 76% to ribavirin. Serum specimens included those drawn at baseline (initial time point, T_i) and at the time when lack of response was established (final time point, T_f), i.e., after 12 weeks of treatment in null-responders, and 24 weeks after the end of treatment in relapsers. For two relapser patients, additional follow-up specimens were available at four weeks of treatment. The baseline specimens corresponding to the non-responder group were analyzed in a previous study (Saludes et al., 2010). The control group included a total of four untreated patients, two of them followed at the Hospital Universitari Germans Trias i Pujol and the other two at the Hospital del Mar, for the same period of time as the non-responder group, with sera drawn at baseline ($n = 4$), 12 weeks (short-term follow-up, $n = 2$), and 48 or 72 weeks (long-term follow-up, $n = 2$ and $n = 1$, respectively). One control person was used both as a short-term and long-term control, since the respective specimens at both time-points were available. All serum specimens had been preserved at −80 °C until testing. All data were analyzed anonymously. The study was approved by the Clinical Research Ethics Committee at the Hospital Universitari Germans Trias i Pujol, and performed according to the World Medical Association Declaration of Helsinki.

2.2. Baseline clinical parameters

Gender, age, body mass index and stage of fibrosis according to the Scheuer scoring system (Scheuer, 1991) were considered in order to compare the study and control groups and to test for differences between null-responders and relapsers.

2.3. HCV subtyping

HCV-1b infection was confirmed through NS5B sequencing followed by phylogenetic analysis, as previously described (Saludes et al., 2010).

2.4. HCV serum viral load

HCV RNA had been previously quantified by RT-PCR (Cobas® Amplicor HCV Monitor test, Roche Molecular Systems, Pleasanton, CA, USA) or by real-time RT-PCR (Abbott RealTi*me* HCV assay, Abbott Molecular Inc., Des Plaines, IL, USA), according to manufacturer's instructions.

2.5. RNA extraction, reverse transcription, amplification, cloning and sequencing

All virological analyses were carried out in each patient for each time-point sample. Due to the high level of genetic variability in the E1–E2 region, PCR products were cloned before sequencing. RNA extraction, reverse transcription, PCR amplification, cloning and sequencing are described in detail elsewhere (Saludes et al., 2010). A 532-bp sequence encompassing the E1 C-terminal region and the E2 N-terminal region (nucleotides 1322–1853 and amino acids 328–504 in the H77 reference sequence, accession number AF009606) was obtained and is referred to as the E1–E2 region. This region includes HVR-1, -2 and -3 subregions (H77 positions 1491–1571, 1761–1787, and 1632–1739, respectively). A total of 1078 sequences were obtained.

2.6. Genetic variability analysis

Codon-based multiple alignments for the E1–E2 region, as well as the HVR-1, -2 and -3 subregions, were generated for the sequences derived for each patient and time-point using ClustalW as implemented in the MEGA 4 package (Tamura et al., 2007). The following estimates of genetic variability were obtained for each multiple alignment with DnaSP v4.50 software (Rozas et al., 2003): number of segregating sites (S), total number of mutations (η), nucleotide diversity corrected by the Jukes–Cantor method