Vertical transmission of hepatitis C virus: A tale of multiple outcomes

Alejandro Escobar-Gutiérrez[a], **Hugo Soudeyns**[b,c], **Ariane Larouche**[b,c], **Juan Carlos Carpio-Pedroza**[a], **Armando Martinez-Guarneros**[a], **Carlos A. Vazquez-Chacon**[a], **Salvador Fonseca-Coronado**[d], **Lilian H.T. Yamasaki**[e], **Karina Ruiz-Tovar**[d], and **Mayra Cruz-Rivera**[f,*]

[a]Instituto de Diagnóstico y Referencia Epidemiológicos, Mexico City, Mexico

[b]Unité d'immunopathologie virale, Centre de Recherche du CHU Sainte-Justine, Montreal, Canada

[c]Department of Microbiology, Infectiology & Immunology, Faculty of Medicine, Université de Montréal, Montreal, Canada

[d]Laboratorio de Inmunobiología de Enfermedades Infecciosas, Unidad de Investigación Multidisciplinaria, Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Estado de México, Mexico

[e]Department of Biology, Institute of Bioscience, Language and Exact Science, São Paulo State University, São José do Rio Preto, SP, Brazil

[f]Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, Mexico

Abstract

Globally, hepatitis C virus (HCV) infection affects approximately 130 million people and 3 million new infections occur annually. HCV is also recognized as an important cause of chronic liver disease in children. The absence of proofreading properties of the HCV RNA polymerase leads to a highly error prone replication process, allowing HCV to escape host immune response. The adaptive nature of HCV evolution dictates the outcome of the disease in many ways. Here, we investigated the molecular evolution of HCV in three unrelated children who acquired chronic HCV infection as a result of mother-to-child transmission, two of whom were also coinfected with HIV-1. The persistence of discrete HCV variants and their population structure were assessed using median joining network and Bayesian approaches. While patterns of viral evolution clearly differed between subjects, immune system dysfunction related to HIV coinfection or persistent HCV seronegativity stand as potential mechanisms to explain the lack of molecular evolution observed in these three cases. In contrast, treatment of HCV infection with PegIFN, which did not lead to sustained virologic responses in all 3 cases, was not associated with commensurate variations in the complexity of the variant spectrum. Finally, the differences in the degree of divergence suggest that the mode of transmission of the virus was not the main factor driving viral evolution.

*Corresponding author. Address: Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de Mexico, Av. Universidad 3000, Col. Copilco Universidad, México DF 04510, Mexico. Tel.: +52 55 56232466; fax: +52 55 56232382. mayracr@yahoo.com (M. Cruz-Rivera).

1. Introduction

Globally, hepatitis C virus (HCV) infection affects approximately 130 million people, in addition to 3 million new infections occurring annually (Alter, 2007). The HCV RNA-dependent RNA polymerase lacks proofreading and error correction mechanisms, and, as a consequence, the viral replication process is highly error prone (Moradpour et al., 2007). The high mutation rate of HCV favors a high degree of intrahost genetic diversity, allowing rapid adaptation that ultimately facilitates evasion from host immune responses (Cruz-Rivera et al., 2013; Escobar-Gutierrez et al., 2012; Fonseca-Coronado et al., 2012; Forns et al., 1999). HCV evolution is determined by a combination of evolutionary processes, including mutation and replication rates, natural selection, and genetic drift (Gray et al., 2012). This plasticity is a key biological property of HCV that enables it to reshape the structure of the intra-host viral population.

In several developed countries, HCV has become an important cause of chronic liver disease in children (Bortolotti et al., 2007; Slowik and Jhaveri, 2005). Viral transmission commonly occurs from mother to child *in utero* or during the *peripartum* period, but not as result of breastfeeding (Conte et al., 2000; Jhaveri et al., 2006). Reported rates of spontaneous HCV clearance in children vary greatly between studies (Ceci et al., 2001; Palomba et al., 1996; Tovo et al., 2000). Though pediatric HCV infection associated with mother-to-child transmission is thought to be generally asymptomatic (Farmand et al., 2011), the natural history of chronic hepatitis C in children is not completely understood (Le Campion et al., 2012). Molecular evolution of HCV in chronically-infected infants is characterized by a high degree of divergence that correlates with the development of immunity against the virus (Farci et al., 2006). In turn, high diversity has been associated with progression to chronic infection and poor response to treatment (Farci et al., 2000; Morishima et al., 2006). Thus, viral evolution is a critical factor that in many ways influences the outcome of HCV infection. Here, we compare the molecular evolution of HCV among three unrelated children who were infected via mother-to-child transmission, including two subjects who were co-infected with human immunodeficiency virus type 1(HIV-1).

2. Materials and methods

Patients' characteristics are summarized in Table 1. Detailed case reports were described elsewhere (Canobio et al., 2004; Larouche et al., 2012). None of the children responded satisfactorily to treatment with pegylated interferon alfa-2b (PegIFN) and ribavirin (RBV) (Larouche et al., 2012; Quesnel-Vallieres et al., 2008) (Table 1). Serum was separated from venous blood samples by centrifugation. Viral RNA was isolated using the QIAamp Viral RNA Mini Kit (Qiagen, Mississauga, Canada). Hypervariable region 1 (HVR1) of the HCV E2 protein was amplified using previously-described primers and PCR conditions (Farci et al., 2000, 2002). Amplicons were sized on agarose gels, extracted, and subcloned into

pCR2.1 using the Topo TA Cloning method (Invitrogen, Mississauga, Canada). Approximately 20 independent recombinants were randomly selected and subjected to unidirectional Sanger sequencing using an ABI 3730xl automated DNA sequencer (Applied Biosystems, Foster City, CA). Sequences were visualized and manually edited using Chromas v. 1.45 (Technelysium, Southport, Australia). The intra-host viral population structure, complexity and variant persistence were assessed using median joining network (MJN) analysis as implemented in Network v. 4.611, using an epsilon value of zero an calculating a full MJN (Bandelt et al., 1999; Fonseca-Coronado et al., 2012), assigning weights to individual nucleotide positions as described by Henikoff and Henikoff (1994), and map color analysis, respectively. Multiple nucleotide alignments were generated using MAFFT v. 7, using a subgenomic region of 264 nucleotides in lenght (Katoh and Standley, 2013). The structure of the viral population for each patient was assessed using a Bayesian approach as described elsewhere (Cheng et al., 2013). The clustering was performed with increasing levels in the hierarchy (2–20;   = 2) and 20 as the upper bound for number of clusters. Communities were defined as the number of genetically diverged groups in population as random variables.

3. Results

Patient 1, a female born to a mother infected with HCV genotype 1a, showed long-term HCV seronegativity in absence of evidence of HIV-1 infection (Larouche et al., 2012). MJN analysis showed that the viral population was composed of two distinct (1–2) but closely related subpopulations (Fig. 1A). Interestingly, and despite the extended duration of the follow-up, the viral variants obtained from the mother when the child was 9.9 years of age were closely related to these two subpopulations, indicating a very limited molecular evolution in the mother. One of the viral variants from the mother was the center of the network and directly connected both subpopulations. In addition, all viral variants from the mother were grouped within subpopulation 1, with no viral variants found in subpopulation 2. All viral variants from the first time point (T01) in the child were also grouped within subpopulation 1. In contrast, variants identified at later time points were generally grouped within subpopulation 2. Viral variants from T02, T04 and T05 were found intermixed in both sub-populations, whereas viral variants from time points T03 and T06 were found exclusively in subpopulation 2. The complexity of the variant spectrum and the persistence of individual variants were assessed using a color map approach. As shown in Fig. 1B, the viral populations from the mother and the patient's earlier time points (T01–T03) showed considerably less complexity in comparison to the later time points (Fig. 1B). In general, the nucleotide distances were rather small (mean = 1.47, range = 0–7).

Patient 2, a male who was infected with HCV genotype 1b and co-infected with HIV-1, was treated with zidovudine from birth, with zidovudine-lamivudine combination starting at 6 weeks of age, and with combination antiretroviral therapy (cART; lamivudine, stavudine, and ritonavir) starting at 1.16 years of age (Canobio et al., 2004). The structure of the viral population was considerably more complex than the one observed in Patient 1 (Fig. 1C). Two major viral variants were identified and grouped viral sequences from the first four time points. Interestingly, one variant identified at the second time point was considerably distant from the two major variants and to some degree closer to sequences identified at time point

T05 (Fig. 1C; indicated by "*"). Later time points were highly divergent and occupied larger extensions of the sequence space. The viral variants identified through time points T01–T04 showed important similarities in terms of nucleotide sequence (Fig. 1D). Likewise, the profiles of the viral populations observed in the later time points (T05–T07) also exhibited considerable similarities.

Patient 3, a male who was infected with HCV genotype 1a and also co-infected HIV-1, showed moderate immunosuppression and was repeatedly found to be HCV-seronegative. The subject was treated with zidovudine monotherapy until age 6 and various cART regimens were subsequently introduced (Canobio et al., 2004). At least five different major variants were identified among samples obtained from this patient (Fig. 1E). The major variants were genetically similar and included viral sequences that were identified throughout the follow-up period. Major sequence 1 and 3 comprised variants isolated from ten different time points, including early and late time points. Conversely, major variants 2 and 4 primarily comprised sequences identified in the later time points. The remaining major sequence (5) included viral variants from the first time point. Several viral variants persisted throughout the follow-up period (Fig. 1F). Later time points (T07–T11) showed a higher degree of complexity in comparison to the early time points.

HCV circulates within the infected host as co-evolving viral communities or subpopulations (Ramachandran et al., 2011). Therefore, the structure of the viral population for each patient was assessed using a Bayesian approach (Cheng et al., 2013). Patient 1 exhibited three different viral communities (Fig. 2A). Communities 1 and 2 were present throughout the follow-up period. However, a third viral community arose at 9.25 years of age and became the predominant population at the late time points. Community 1 was the predominant subpopulation throughout the observation period. In the case of Patient 2, 4 HCV communities were identified. Communities 1 and 2 represented the major subpopulations at early time points. However, community 1 was no longer present at late time points. Two extra communities (3 and 4) arose at the late time points accounting for more than 50% of the entire viral population. Viral variants found in Patient 3 formed one unique community (data not shown).

4. Discussion

In this study, we have shown that HCV evolution can exhibit profound differences among children who were infected as a result of mother-to-child transmission. Importantly, these 3 patients exhibited some degree of immunologic dysfunction: Patient 1 was persistently seronegative, while Patient 2 and Patient 3 were co-infected with HIV-1 and were born at a time when highly active antiretroviral therapy was not readily available. Our study does not include cases with no preexisting conditions, and therefore, the long term molecular evolution of HCV under normal immunological pressure could not be assessed. Importantly, limited genetic diversity has been previously reported among pediatric cases associated with vertical transmission (Gismondi et al., 2009). In general, molecular evolution of HCV is heterogeneous (Cruz-Rivera et al., 2013; Ray et al., 2005). However, under specific circumstances, HCV molecular evolution can be significantly slow. Patient 1 showed no anti-HCV antibody response and few IFN-γ producing cells in the peripheral blood

(Larouche et al., 2012). The weak immune response exhibited by the subject presumably exerted a limited pressure over the prevailing viral population, consequently allowing limited divergence for an extended period of time. This situation is reminiscent of what was reported in HCV-infected children suffering from X-linked agammaglobulinemia, a hereditary deficit that interferes with the development of humoral immune responses (Gaud et al., 2003). Interestingly, the viral population from the mother, which was sampled 9.91 years after the birth of the child, also remained in close proximity to the viral variants from the subject. While the limited divergence could be explained at least partially by development of tolerance in the child, the slow molecular evolution of HCV in the mother is a remarkable finding (Larouche et al., 2012).

HIV co-infection has been reported to be associated with limited genetic complexity of HCV variant spectra (Lopez-Labrador et al., 2007). Consistent with this, the diversity of HCV quasispecies is influenced by seroconversion to HIV-1 and varies as a function of CD4 cell counts in co-infected subjects (Mao et al., 2001; Roque-Afonso et al., 2002). Conversely, patient 2 exhibited a high degree of HCV intra-host genetic variation. In general, the composition of the viral population changed over time, occupying different regions of the sequence space (Fig. 1D). Patient 3 also exhibited a rather high degree of HCV complexity that was associated with initiation of antiretroviral therapy (Canobio et al., 2004), a phenomenon that was previously reported in co-infected adults and which is thought to relate to the rapid restoration of immune pressure as a result of effective control of HIV-1 replication and associated immune suppression (Blackard et al., 2004; Shuhart et al., 2006).

In contrast, treatment of HCV infection with PegIFN, which did not lead to sustained virologic responses in all 3 cases, was not associated with commensurate variations in the complexity of the variant spectrum (Table 1). This lack of variation in the quasi-species following introduction of IFN treatment was previously shown to predict non-response to IFN treatment in adults chronically infected with HCV (Farci et al., 2002). However, the restricted sample size and the heterogeneity of the cases described in the present study limit the formulation of any strong conclusions, and further studies with larger number of patients will be required to confirm these observations.

The extended timeframe in this follow-up study (i.e. 6–11 years) allowed for a comprehensive depiction of the landscape of the sequence space in which these HCV strains evolved. It is important to mention that in the two patients displaying high degree of HCV genetic diversity, it was possible to identify viral variants from the early points in the late time points. This indicates that certain variants can persist for long periods of times. We hypothesize that the entirety of the variant spectra was not sampled in all time points because of the limited sensitivity of the methodology. Recent studies have shown the advantages of using next generation sequencing platforms for the analysis of the HCV intra-host variability (Fonseca-Coronado et al., 2012).

Examination of HCV molecular evolution has shown the coexistence of genetically distinct viral lineages that are not detected at all time points in infected patients (Alfonso et al., 2005; Ramachandran et al., 2011; Smith et al., 2010). Here, the Bayesian analysis showed a relative small number of communities circulating among these cases. Patients 1 and 2

showed a predominant community at the early time points that was significantly reduced in the later time points. In both patients, the rise of new communities at the late time points was the characteristic feature. Interestingly, Patient 3 only exhibited one community throughout the follow-up. Adaptation to late stages of infection has been previously reported, although, the participating mechanisms responsible for this adaptation have not been elucidated (Ramachandran et al., 2011). However, temporal cooperation between viral subpopulations at different stages of chronic infection has been proposed as a plausible scenario. Thus, a particular viral population at a given stage elicits a specific effect on the host and reduces the host selection pressure for the next viral population until the final population achieves a stable state (Ramachandran et al., 2011).

In summary, HCV exhibits a varied array of genetic variability and heterogeneity among children who acquired HCV infection via mother-to-child transmission. The characteristic plasticity and dynamic processes responsible for the genetic variation displayed by HCV appears to allow for a rapid adaptation to changes in the microenvironment where the viral population resides. This genetic variation represents an important challenge for the development of prophylactic measures.

Acknowledgments

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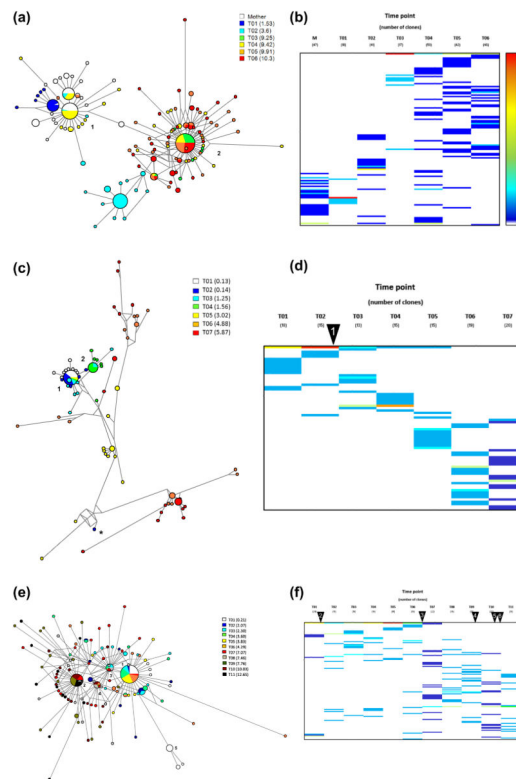
Fig. 1.

HCV intrahost molecular evolution. Median-joining network (A, C and E) and color map (B, D and F) approaches were used to assess the architecture of the viral population at each time point in all patients. In the network, each node represents a unique haplotype within the viral population. The size of the node reflects the frequency, in absolute numbers, of each viral variant. The length of the link represents the nucleotide differences between the two different haplotypes. Numbers depict the major variants in each network. Viral persistence of individual viral variants was assessed using color maps. Each line represents a unique variant during the follow up, and the percentages are color coded. Number of clones analyzed at each time point is shown between brackets. Warmer colors represent higher frequencies, while cooler colors depict lower frequencies. Arrowheads represent introduction of or modifications to regimens of antiretroviral therapy administered to coinfected patients. Arrowhead 1 (D): introduction of combination treatment with zidovudine, lamivudine and ritonavir; Arrowhead 2 (F): introduction of zidovudine monotherapy; Arrowhead 3 (F): combination treatment with zidovudine, lamivudine and saquinavir; Arrowhead 4 (F): combination treatment with amprenavir, didanosine and stavudine; Arrowhead 5 (F): combination treatment with lamivudine, stavudine, ritonavir and nelfinavir; Arrowhead 6 (F): combination treatment with didanosine, stavudine, lopinavir and ritonavir (Canobio et al., 2004).
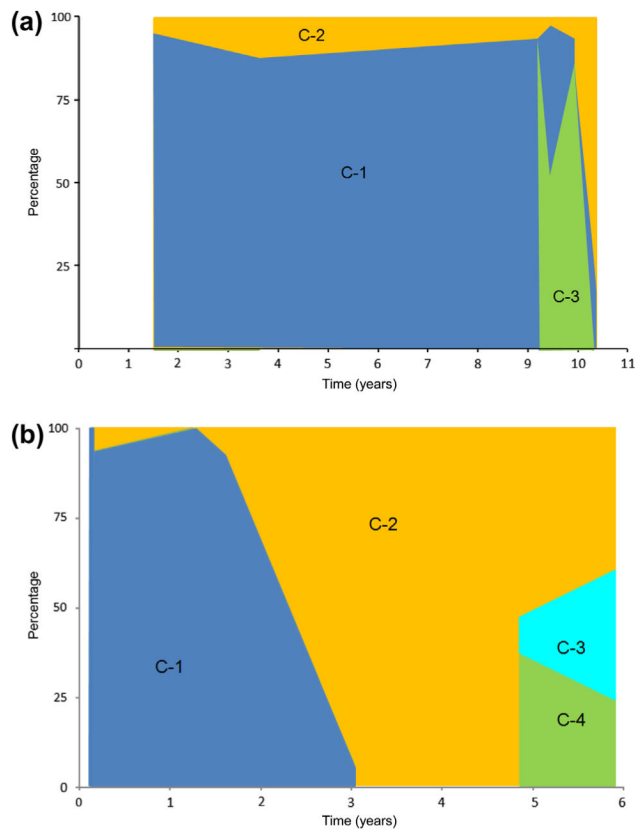
Fig. 2.
Population structure analysis. All viral variants from Patient 1 (A) and Patient 2 (B) were analyzed used using a Bayesian approach to identify the communities within the population. The frequency of any given community at different time points is color coded for each community.

Table 1

Clinical characteristics of study subjects.

Characteristics	Patient 1	Patient 2	Patient 3
Gestational age (weeks)	35.7	39.0	39.0
Birth weight (g)	2560	3270	3160
Mode of delivery	Caesarean section[a]	Vaginal	Vaginal
HCV genotype	1a	1b	1a
Co-infection with HIV-1	No	Yes (clade B)	Yes (clade B)[b]
PegIFN alfa-2b and ribavirin treatment	PegIFN: 50 μg/week ribavirin: 600 mg/day	PegIFN: 3×10^6 IU/m^2 three times/week (BSA = 0.95m2) ribavirin: 200 mg BID (15 mg/[kg day])	PegIFN: 80 mg once/week ribavirin: 400 mg BID (14 mg/[kg day])
Duration of PegIFN alfa-2b and ribavirin treatment (weeks)	30 (9.42–10.00 years of age)	42 (6.4–7.2 years of age)	12 (16.6–16.8 years of age)
SVR	No	No	No

[a] Membrane rupture for 24 h; cephalo-pelvic disproportion.

[b] HIV-1 strain that carried mutations associated with major resistance to multiple antiretroviral agents (zidovudine, lamivudine, abacavir and atazanavir). PegIFN, pegylated interferon; BSA, body surface area; SVR, sustained virologic response.