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Short communication

Consecutive infections and clearances of different hepatitis C virus genotypes in an injecting drug user

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Abstract

Background: The hepatitis C virus (HCV) causes significant morbidity and mortality worldwide, and is highly prevalent among injecting drug users (IDUs). Whether initial HCV infection and clearance provides protection from reinfection has not been established, but is an important question for vaccine development.
Objective: To elucidate an unusual history of HCV infection and clearance in an IDU.
Study design: The subject was interviewed and gave blood samples at approximately three-month intervals; all samples were tested for anti-HCV and HCV RNA, genotyped if RNA detected, and checked for mixed genotypes; phylogenetic analysis performed on the subject's and injecting partners' core HCV sequences.
Results: We observed consecutive infections with HCV genotypes 3a, 1a and 6l, and intervening clearances, in a young IDU over 449 days. Genotypes 1a and 6l were probably acquired from the subject's injecting partners, who had genetically related infections.
Conclusion: This case illustrates (1) the ease with which IDUs can acquire HCV, (2) that prior HCV infection does not protect against reinfection with heterologous strains, and (3) that IDUs can clear consecutive HCV infections. Our subject's history of HCV infection and clearance offers hope for vaccine development, yet demonstrates that HCV vaccines must have cross-genotypic effectiveness.

Keywords: Hepatitis C virus; Reinfection; Clearance; Prevention; Vaccine

1. Introduction

The hepatitis C virus (HCV) infects an estimated 170 million people worldwide, and is a significant cause of morbidity and mortality due to cirrhosis and hepatocellular carcinoma (Shepard et al., 2005). The level of protection from reinfection conferred by HCV infection is at best uncertain, with various authors reporting widely differing findings (Aitken et al., 2004a; Grebely et al., 2006; Micallef et al., 2007). In this brief communication, we describe the pattern of HCV reinfection observed in a single heroin and cocaine injector between November 2005 and July 2007, and discuss its relevance to future HCV prevention efforts.

2. Methods

The subject is one of 396 people recruited into an ongoing, multi-disciplinary, longitudinal study of HCV in the social networks of injecting drug users (IDUs) in Melbourne, Australia. At recruitment our subject was just over 19 years old and had been injecting illicit drugs for 4 years. All participants are interviewed about their risk behaviour and injecting partners at intervals of approximately 3 months, and supply

Abbreviations: HCV, hepatitis C virus; IDU, injecting drug user; RNA, ribonucleic acid; PCR, polymerase chain reaction; LiPA, line probe assay; ALT, alanine aminotransferase.

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up to 50 ml of blood for virological and immunological analyses. Informed consent for participation is obtained after the nature and possible consequences of the studies have been fully explained.

Venous blood samples are screened for anti-HCV by a third-generation enzyme immunoassay (Abbott Laboratories, Chicago, IL) and anti-HCV positive specimens are tested again by Murex anti-HCV Version 4.0 (Murex Biotech, Kyalami, South Africa) for confirmation. All samples are tested for HCV RNA by the COBAS AMPLICOR HCV test Version 2.0 (Roche, Branchberg, NJ). PCR-positive blood samples are genotyped by a reverse hybridisation line probe assay (LiPA, Versant HCV Genotype Assay, Bayer, Tarrytown, NY) as previously described (McCaw et al., 1997). PCR amplification was performed using a nested in-house PCR with core-specific primers (Dev et al., 2002) and the PCR product sequenced using the Applied Biosystem (ABI) PRISM^{TM} Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Version 3.1 (Applied Biosystems, Foster City, CA, USA). ClustalW alignment was performed using MacVector^{TM} 8.0 (Accelrys, San Diego, CA, USA). Phylogenetic analysis was performed using the SEQBOOT, DNADIST and Neighbour-Joining programs available in the Phylip package (Felsenstein, 1993). The phylogenetic tree was generated using Treeview (Page, 1996). Mixed genotype detection was conducted using LiPA and melting curve analysis (Bullock et al., 2002).

3. Results

Table 1 shows that our subject cleared an initial infection of HCV genotype 3a in the 128 days between test 1 and test 2, then acquired genotype 1a in the 75 days to test 3. ClustalW alignment showed that the subject's genotype 1a core sequence was 99.7% homologous (one nucleotide difference in 332 base pairs) to that of an injecting partner (5029 in Fig. 1) with whom he reported injecting twice (but not sharing needles) in the month prior to test 1. The two sequences were part of a strongly related 1a clade (see Fig. 1), the other member of which (1033) reported injecting and sharing needles with 5029, but not with our subject (4069). The 1a infection was cleared in the 79 days to test 4, and the subject returned a second consecutive RNA-negative result 89 days later.

Between tests 4 and 5, the subject reported injecting cocaine daily and sharing needles twice, and injecting (but not sharing needles) 30 times—20 of which were in the presence of an injecting partner (1043 in Fig. 1) in whose sample HCV genotype 6l had been detected previously. At test 6 our subject tested HCV RNA-positive for the third time; ClustalW alignment showed the subject's genotype 6l core sequence was 99.7% homologous (one nucleotide difference in 331 bp) to that of the aforementioned individual, and the 6l clade received strong bootstrap support (see Fig. 1). The subject was then imprisoned for 5 months before test 7, at which time HCV RNA titre was too low to allow genotyping. No evidence of mixed infection in the three HCV RNA-positive samples was detected with either LiPA or melting curve analysis.

4. Discussion

Three different HCV genotypes, punctuated by HCV RNA-negative results, were detected in our subject's first six blood samples taken over 449 days. This case, which we believe is unique in the literature, illustrates three distinct points—that;

- IDUs are at high risk of HCV infection even with low rates of risk behaviour
- previous HCV infection does not confer immunity to reinfection with heterologous strains
- IDUs can acquire and clear multiple consecutive HCV infections.

It is well established that IDUs are the population subgroup at greatest risk of HCV infection in more developed countries. Despite the successful containment of HIV in IDUs in Australia, the UK, the Netherlands and elsewhere through widespread adoption of needle–syringe programs and other harm reduction measures (Mateu-Gelabert et al., 2007), HCV incidence rates in IDUs of 25% per annum or greater are common (Hahn et al., 2002; Judd et al., 2005; Maher et al., 2006). Moreover, the vast majority of new infections continue to occur in IDUs (Dore et al., 2003). Development of prophylactic or therapeutic vaccines seems the best hope for stemming the HCV epidemic. The ability of our subject to repeatedly clear infection, and of other IDUs (Grebely et al.,

Table 1
HCV genotype history and self-reported risk behaviour of subject

Test no.	Test date	Days since last test	Cumulative days	No. of times injected, past month	No. of times shared needle, last 3 months	No. of injecting partners, last 3 months	Genotype detected	ALT
1	15 November 2005		0	5	1	2	3a	68
2	23 March 2006	128	128	0	0	0	0	20
3	06 June 2006	75	203	3	0	0	1a	255
4	24 August 2006	79	282	8	1	3	0	11
5	21 November 2006	89	371	30	2	5	0	13
6	07 February 2007	78	449	3	0	3	6l	39
7	31 July 2007	174	623	10	4	5	[a]	69

[a] Too low to genotype.