Hepatitis C Virus (HCV) Diversity

A hallmark of RNA viruses is their extreme genetic diversity. Although viral replication is extremely robust, producing an estimated 10 trillion viral particles per day [1], the HCV NS5B protein is an RNA-dependent RNA polymerase that lacks a proofreading mechanism. This results in a population of distinct but closely related viral variants, termed the “viral quasispecies,” within a single individual. These viral variants may display divergent phenotypic properties, as viral diversity is a key determinant of replication capacity or fitness, cell tropism, immunologic escape, and antiviral-drug resistance. The associations between HCV heterogeneity and liver-transplantation outcome, disease progression, chronicity of infection, and treatment outcome have been explored previously (reviewed in [2]).

At the population level, HCV consists of multiple genotypes and subtypes that differ by >30% at the nucleotide level. Multiple HCV genotypes may circulate within a particular population, although there is some geographic restriction in genotype distribution. For instance, the predominant genotype within the United States is genotype 1, although other genotypes are also common. Despite remarkably similar genome structures and replication strategies, HCV genotype is an important determinant of the virologic response to HCV treatment [3], whereas differences in disease pathogenesis among genotypes may also exist [4, 5].

Reinfection

The ability of a previously cleared or ongoing HCV infection to protect against subsequent infection with a distinct HCV may reflect the robustness of the adaptive immune response to provide adequate cross-protection. Initial studies demonstrated that rechallenge of infected chimpanzees with the same or a different HCV strain resulted in the reappearance of viremia [6, 7]. A recent report by Prince et al. found that 9 of 9 recovered chimpanzees that were subsequently rechallenged with homologous HCV experienced acute, resolving infection [8]. In contrast, 4 of 11 chimpanzees rechallenged with heterologous HCV experienced chronic infection, suggesting that the immunity induced after primary infection was insufficient to prevent chronic infection in all chimpanzees.

There is the widespread assumption that HCV reinfection and treatment non-compliance occur frequently among injection-drug users (IDUs). As recently as 1997, the National Institutes of Health recommended that IDUs have a drug-free period of abstinence for at least 6 months prior to initiating HCV treatment. Despite some evidence of favorable treatment outcomes among IDUs [9–16], only a minority currently receives HCV treatment [17], even though this recommendation was recently modified to include active IDUs [18]. Among IDUs, Mehta et al. found that incident HCV infections were more frequent in persons with no previous HCV infection than in those who had previously been infected but were not currently infected with HCV [19], suggesting that repeat exposure could partially protect against reinfection. Nonetheless, fears of HCV reinfection are reasonable, on the basis of several case reports of patients...
reinfecting themselves by injection-drug use after successful HCV treatment [20, 21].

DEFINING MIXED INFECTIONS

The issue of superinfection is frequently fraught with misused terminology. Adapting definitions from the HIV field [22], one can say that HCV reinfection refers to a primary infection that is completely cleared virologically prior to a subsequent, secondary infection with either a homologous or a heterologous HCV. This is distinct from dual infection, which occurs when an individual is infected with HCV derived from 2 or more individuals, such as occurs in individuals with hemophilia who have been given blood products from multiple individuals. Dual infections can further be divided into coinfections and superinfections (Figure 1). Coinfection is defined as infection with 2 or more homologous HCVs either simultaneously or within a very narrow window period before infection with the first HCV has resulted in an immunologic response to that virus. Superinfection is defined as infection with a second HCV after the establishment of persistent HCV infection and development of an immunologic response to the first virus. Although these terms have been previously defined in the HIV field to refer to infection with distinct types and/or variants of HIV, coinfection and superinfection have also been utilized to refer to the simultaneous presence of distinct viruses (e.g., HIV/HCV, HIV/HBV, or HCV/HBV) within an individual.

The recent documentation of recombinant HCVs [23–25] is de facto evidence that dual infection has occurred, as the generation of a recombinant virus requires that a cell must first be infected simultaneously with 2 or more distinct HCVs. Moreover, dual HCV infections have been documented extensively [21, 26–34]. Preliminary data suggest that HCV viral-load levels are not significantly different among persons infected with a single HCV genotype than among those infected with multiple HCV genotypes [31]. However, one study has suggested that dual HCV infection may result in acute exacerbation of chronic HCV infection [35], whereas another has found that dual HCV infection is associated with immunologic progression of HIV disease in HIV/HCV coinfected IDUs [33].

To date, there are no published reports on dual HCV infection and rates of response to HCV treatment, although small preliminary studies have suggested that failure of HCV treatment is not frequently associated with a shift in the infecting HCV genotype [36]. Nevertheless, the infrequent sampling of HCV genotype and the lack of viral sequences from various compartments, including peripheral-blood mononuclear cells (PBMCs) and the liver, somewhat limit the interpretability of these data. For instance, 2 recent studies documented a significant proportion of HCV-infected persons who harbor in their PBMCs highly divergent viral variants that are not detectable in the plasma [37, 38]. Moreover, compartmentalization of HCV was found to be correlated with IDU. These data suggest that frequent exposure to HCV could contribute significantly to increased viral diversity and cell tropism within an individual and might also result in variants that would not be readily detected should only serum/plasma be evaluated by a clinical HCV genotype assay and not by a robust, multicompartment sequencing approach.

SUPERINFECTION

Although it is possible for individuals to be infected with multiple distinct variants of a virus simultaneously, superinfection refers to a primary infection with hepatitis C virus followed by a secondary reinfection with a distinct hepatitis C virus at a later time point. This phenomenon has been well documented in the HIV literature (reviewed in [22]) and has a significant impact on the development of antiretroviral resistance, immunologic escape, and disease progression. Thus, determining the frequency and clinical consequences of HCV superinfection is also of great interest for the immunopathology and clinical management of chronic HCV infection.

As shown in Table 1, a small number of case reports have demonstrated that HCV superinfection does indeed occur within chimpanzees and humans [29, 39–45]. Interestingly, reinfection with HCV during or after successful treatment or spontaneous clearance has also been documented [6, 19–21, 46–49], suggesting that virologic clearance does not provide 100% protection against reinfection. However, these cases should not be classified as HCV superinfection, because establishment of superinfection, by definition, requires extensive evaluation of HCV diversity, at multiple time points, to distinguish it from coinfection and does not apply to cases in which HCV viremia has been cleared prior to subsequent reinfection. Replacement of 1 HCV genotype with a distinct genotype over time also provides evidence of likely HCV superinfection. For instance, Eyster et al. documented a change in genotype among 18 of 32 individuals with hemophilia [49]. Interestingly, coinfection with...
analyses may have more difficulty in ac-
genotype 1–infected person becomes su-
tect HCV superinfection within a partic-
tensive longitudinal sequence analysis.
proof of superinfection may require ex-
distinct genotype cannot be formally
HIV was associated with changes in HCV
genotype, and HCV RNA was elevated in
patients whose genotypes changed. How-
without extensive sequencing analysis
at multiple time points, virologic
selection and its clinical consequences have not
baseline samples, these cases were con-
change in the predominant HCV genotype
over time, and this change could indicate
HCV superinfection. This has been ob-
erved in several high-risk populations, in-
cluding patients undergoing hemodialysis
transfusion recipients [45, 52], IDUs
and individuals with hemophilia [49,
although clearance of the first HCV
prior to reinfection with another HCV,
rather than true HCV superinfection,
cannot be ruled out in these studies. More-
over, it is important to emphasize that
commercial HCV genotyping assays, such
as LiPA, likely underestimate the true
prevalence of dual infection with heter-
ologous virus [27] and do not provide
sufficient data on dual infection or
superinfection with homologous virus.
Moreover, transient infections may be
missed if sampling is infrequent and/or
the superinfecting virus replicates only at
low levels. Genotype-specific polymerase
chain reaction (PCR), restriction-fragment
length–polymorphism analysis, primer-
specific and mispair extension analysis,
and heteroduplex tracking/mobility assays
also have been utilized to examine dual
infection, with varying degrees of sensi-
tivity and specificity [27, 34, 54–57]. The
gold standard for rigorous documentation
of HCV superinfection involves sequenc-
ing of multiple HCV variants over time,
to demonstrate the absence of the super-
infecting virus at baseline, followed by the
appearance of multiple distinct viruses at

HIV, hypervariable region 1; PCR, polymerase chain reaction.
* Superinfecting HCV was a highly divergent strain of the same genotype.
a subsequent time point. Concomitant analysis of HCV from the donor is also highly desirable, although samples are not readily available in most cases. This approach is costly and requires protocols to ensure that PCR cross-contamination does not occur. Antibody-profile assays may be employed to further confirm the origin of plasma samples used for such analyses [29].

In vitro systems that are used to examine interactions between distinct hepatitides C viruses are severely limited. However, a recent report described the simultaneous selection of different HCV subgenomic replicons within the same cell [58]. Competition between replicons was also observed, such that the presence of one replicon reduced the replicative capacity of a second replicon. Although these data might suggest the presence of an outgrowth of a single HCV after dual superinfection, such systems do not recapitulate the in vivo environment, because adaptive immunologic selection pressures are absent. Further experimentation will be necessary to evaluate any potential replicative-fit differences among distinct HCV genotypes, as well as among homologous viruses of the same genotype. Furthermore, determining why superinfection can lead to coexistence of 2 divergent HCVs in some cases, whereas in other cases the superinfecting HCV may replace the primary HCV, is necessary. Finally, the recent development of tissue-culture systems capable of producing infectious HCV particles [59–63] may ultimately aid in the development of more-relevant models of dual HCV infection and/or superinfection.

**FUTURE RESEARCH DIRECTIONS**

To date, the impact that HCV superinfection has on clinical disease progression has not been fully elucidated. Nonetheless, several potential complications may be implied from the available literature, including (1) reduced efficacy of HCV treatment, (2) limited immunologic cross-protection, which may reduce the efficacy of future HCV vaccines, (3) elevated levels of transaminase and/or hepatitis flares, (4) alterations in levels of HCV RNA, and (5) development of recombinant viruses with enhanced pathogenic potential. Thus far, there is little data to support or refute any of these possible consequences of HCV superinfection, although there is some preliminary data to suggest that HCV superinfection is associated with elevated levels of transaminase [6, 39, 40, 42]. At least one group has suggested that HCV superinfection may result in both decreased probability of spontaneous clearance and reduced rates of response to treatment [29], although this has not been formally tested in studies using multiple antirisk groups.

In light of the limitations in our understanding of HCV superinfection, several key areas of research should be investigated; these include (1) determining the effectiveness that an immunologic response against the first virus has in conferring protection against subsequent superinfection with heterologous or homologous virus; (2) assessing the effects that dual infection and/or superinfection has the rate of response to treatment; (3) establishment of more-sensitive and -specific techniques for assessment of superinfection; (4) establishment of natural-history cohorts, to examine whether dual infection and/or superinfection has a deleterious impact on HCV viral load, transmissibility, immunologic escape, resistance to treatment, and/or development of cirrhosis and/or hepatocellular carcinoma; and (5) identification and characterization of the immunologic, virologic, and genetic factors that influence the incidence of HCV superinfection. From a public-health perspective, the limited available data would appear to suggest that, until such time as the true extent of HCV superinfection is more clearly defined, HCV-infected patients (as well as those individuals who have already experienced a complete virologic response to HCV treatment) should protect themselves against the possibility of HCV superinfection.

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**References**

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