The majority of acute HCV infections progress to chronicity, implying that the immune response is unable to clear virus in most instances. Reinfection with a second strain of HCV after clearance of an initial infection has been reported in several recent studies. Moreover, individuals with HCV infection may be at risk of HCV superinfection with a second strain of HCV even after the establishment of persistent infection and the development of an immunological response to the initial virus. In vivo and in vitro data regarding HCV reinfection and superinfection, including the clinical consequences of these phenomena and the impact they have on vaccines require consideration in future studies.

It is estimated that the global prevalence of HCV is 2.35% and that 160 million individuals are chronically infected with HCV [1]. A common feature of RNA viruses, including HCV, is their possession and usage of an RNA polymerase that lacks proofreading capacity. Thus, RNA viruses typically exhibit significant diversity. For instance, a number of HCV genotypes have been identified that differ from one another by approximately 30% at the nucleotide level. Despite similar replication strategies, HCV genotype is an important predictor of treatment response [2,3] and possibly of disease progression [4,5]. Additional variability is observed within an individual in whom a population of viral variants – termed the quasispecies – exists. The consequences of quasispecies variability have been reviewed elsewhere and include immune escape, altered virulence and the development of drug resistance mutations [6,7].

HCV diversity

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HCV clearance and reinfection

As much as 85% of those with acute infection develop chronic disease [8]. Nonetheless, reinfection – defined as infection with either a homologous or a heterologous virus subsequent to a first viral infection that has previously cleared – can occur [9], implying that the immune response to one infection is frequently inadequate to clear a subsequent exposure. Previous studies of HCV reinfection in chimpanzees reported that all chimpanzees that initially cleared infection had the reappearance of viraemia when rechallenged [10]. Other researchers rechallenged chimpanzees that had previously cleared HCV infection and reported more complex findings. For instance, all 9 animals rechallenged with a homologous genotype of HCV developed self-limited infections, whereas 4 of 11 animals developed chronic infection when challenged with heterologous genotypes [11]. T-cell responses during rechallenge showed that an early and strong recall response to viral non-structural proteins was associated with viral clearance [12]. However, proliferative T-cell responses were not observed in persistently infected chimpanzees, and only a weak response was observed in 1 of 2 animals during acute self-limited infection.

Studies of HCV reinfection have more recently been conducted in humans. Mehta et al. [13] examined HCV-uninfected and previously infected injection drug users (IDUs) for new or incident cases of HCV. Among HCV-uninfected individuals, the HCV incidence was 8.6 per 100 person-years of observation (pyo). By contrast, amongst IDUs who were previously infected but subsequently cleared infection, the incidence of a new infection was 5.4 per 100 pyo. Grebely et al. [14] subsequently reported a similar decrease in incident HCV infections among previously infected individuals. Although these data may imply partial protective immunity associated with repeat exposure, similar findings were not observed in other studies in which an increased HCV incidence was reported among previously infected individuals [15,16]. Other studies have reported incident HCV infections ranging from 0.47 to 30.1 per 100 pyo in previously infected individuals but did not include an HCV-uninfected group for comparison [17,18]. Additional
population-based studies have reported frequent HCV reinfection among high-risk individuals [19–21]. In addition to study design issues such as sampling interval and assay sensitivity that could partially explain such divergent findings, biological factors should be considered, including risk profiles, genetic factors that impact immune responses and coinfections.

In addition to individuals who experienced spontaneous clearance, reinfection has been evaluated in individuals who achieved treatment-induced viral clearance. Backmund et al. [22] followed 18 IDUs after treatment-induced clearance of HCV RNA and identified 2 patients who were later reinfected for an estimated incidence of 0–4.1 cases per 100 pyo. In a prospective study of HCV natural history and treatment of recent infection, reinfection occurred in 5 of 88 individuals (4.7 cases per 100 pyo) following treatment-induced viral suppression [23]. Among 30 untreated individuals who experienced spontaneous clearance, reinfection was observed in 2 of 30 (7%). Similarly, Grebely et al. [24] included 35 IDUs who cleared HCV upon treatment and observed 2 cases of reinfection upon follow-up for an estimated incidence of 3.2–5.3 cases per 100 pyo. These studies indicate that reinfection is possible; however, large prospective analyses of reinfection in populations with distinct risk profiles are generally lacking.

**Dual/mixed HCV infections**

A number of studies have reported dual (or mixed) HCV infections. As illustrated in Figure 1, dual infection occurs when an individual is infected with HCV derived from at least two individuals (for example, haemophiliacs given HCV-infected blood derived from multiple individuals prior to when screening of the blood supply for HCV was implemented [9]). Population studies of dual infection have reported dual infection rates of <5% [25–27]. However, dual infection has been reported in >10% of individuals in other studies [20,28–32], implying that repeat exposures to HCV are common or that there is limited immunological capacity to clear multiple HCV infections. As with HCV clearance and reinfection, multiple study design and biological factors may contribute to considerable variation in the prevalence of dual infection among distinct populations. Dual infections can be further divided into coinfections and superinfections. Coinfection is defined as infection with at least two heterologous HCVs simultaneously or within a narrow period of time [9].

**Superinfection and recombination**

As represented in Figure 2, superinfection is defined as infection with a second strain of HCV after the establishment of persistent infection and the development of an immunological response to the first virus [9]. Cases of HCV superinfection have been reported in IDUs, transfused or transplanted patients, patients undergoing colonoscopy and during perinatal transmission [33–42]. HCV superinfection has also been reported in experimentally infected chimpanzees [43]. Recently, several population-based studies examined the occurrence of HCV superinfection. Dove et al. [44] observed no switches in HCV genotype that might be indicative of reinfection or superinfection among eight individuals despite ongoing injection drug use. Van de Laar et al. [19] examined HCV seroconversion among IDUs. Dual/multiple HCV infections were detected in 23 (39%), whereas 14 (24%) experienced HCV superinfection. Grebely et al. [23] studied superinfection in a prospective cohort of recent infection. Among 37 with persistent infection, superinfection was observed.

![Figure 1.](image1.png)  
HCV dual infection occurs when an individual is infected with HCV derived from at least two strains.

![Figure 2.](image2.png)  
Superinfection refers to infection with a second strain of HCV after establishment of persistent infection and the development of an immunological response to the first virus.
in 3 treated and 3 untreated individuals. Reinfection or superinfection occurred more frequently among participants with poorer social functioning at enrolment and more often in those with ongoing IDU. A similar study of 87 IDUs with incident HCV infection identified 15 cases of superinfection or reinfection at follow-up [20]. Recently, HCV reinfection and superinfection have been examined among HIV-positive men who have sex with men (MSM) experiencing acute HCV infection. Lambers et al. [45] studied 56 HIV-positive MSM who became HCV-RNA-negative during treatment of acute HCV infection; 11 became reinfected and the cumulative incidence was 33% within 2 years of follow-up. Similar findings rates of reinfection have been reported elsewhere [46]. Thus, HCV superinfection occurs in vivo, although its clinical consequences have not been adequately examined in longitudinal studies. Nonetheless, it is reasonable to suggest that HCV superinfection contributes to reduced treatment response, limited immunological cross-protection that reduces efficacy of future vaccine strategies, elevated liver transaminase levels and fluctuations in HCV RNA levels in serum, and altered pathogenic potential (reviewed in [9]).

By definition, HCV superinfection involves at least two HCVs; thus, viral recombination is also a possibility. HCV recombination involves the exchange of genetic material between two or more HCV strains during coinfection of the same host cell. Several cases of HCV recombination have been reported in humans and experimentally infected chimpanzees [47–67]. Although large studies are relatively uncommon, HCV recombination has been reported in several cohorts based on discordant genotype results from at least two genomic regions [59,65,68–70]. Analysis of over 17,000 sequences from 111 patients also revealed recombination events among 18% of patients [71]. Similarly, a separate study reported that 1 of 6 patients undergoing HCV therapy had detectable recombination [72]. By contrast, a preliminary study of IDUs who became superinfected with a distinct strain of HCV showed no evidence of intra- or intergenotypic recombination [73]. A study of Chinese IDUs and haemodialysis patients also found no evidence of recombination despite high rates of dual/multiple infections. These data may suggest that recombination events do occur; however, the resulting recombinant viruses are rarely viable in vivo.

Probing HCV pathogenesis

Studies of HCV reinfection, superinfection and recombination have greatly facilitated our understanding of HCV pathogenesis. In vitro investigation of HCV superinfection suggested that acutely infected hepatocytes were resistant to infection with another strain of HCV [74]. However, replicon-containing cells were permissive to reinfecion after treatment with an HCV-specific protease inhibitor. A separate study reported that simultaneous infection of hepatocytes with two HCVs resulted in replication of both within the same cell [75]. Conversely, when the infections were performed sequentially, the secondary infection was impaired significantly. Interestingly, this superinfection exclusion was due to a functional block of virion entry, but rather was likely mediated by interference at the level of RNA translation. A subsequent analysis implicated the tight junction proteins claudin-1 and occludin in the prevention of superinfection in vivo. Nonetheless, it is clear that superinfection does occur in vivo, albeit at a low frequency. HCV recombination has also been examined in vitro [76–78]. Despite its infrequent occurrence in an experimental system, Reitter et al. [77] suggested that recombination might be clinically relevant given the high HCV replication rate and large number of infected hepatocytes, particularly when strong selection pressures are present. However, HCV recombination in patients receiving highly potent, direct-acting antiviral agents has not been reported to date.

What are we still missing?

Reinfection and superinfection clearly occur in vivo, thus generating considerable concern about the ability of the immune system to protect against repeated HCV exposure and the ability to develop a highly efficacious vaccine in the future. The inability to protect against homologous and heterologous HCV strains strongly suggests that a future HCV vaccine will need to evoke protective immunity against multiple distinct HCV genotypes/subtypes to be highly efficacious. Moreover, careful examination of the multiple immunological, virological and genetic factors associated with viral clearance – or reinfection – will greatly facilitate vaccine design and development, as well as suggest more effective therapeutic strategies. Nonetheless, for the most part, they have been limited to observational cohorts and convenience sampling. Moreover, whenever possible, studies of HCV reinfection should include HCV sequencing during original infection and after recurrence of viraemia to distinguish reinfection from re-emergence of the same virus.

A recent study of immune responses during reinfection in active IDUs reported a high level of spontaneous clearance in reinfected patients [17]. The duration and peak virus level were significantly lower during reinfection compared with initial infection in the same individuals. Moreover, reinfection was associated with an increase in the breadth of T-cell responses and increased detection of neutralizing antibodies against heterologous virus compared with individuals who progressed to chronic infection. Innate antiviral responses also play
an important role in regulating HCV clearance and reinfection. For instance, the IL28B allele has received considerable attention recently as an important predictor of HCV clearance (reviewed in [79]). Thus, both innate and adaptive immune responses are critical elements in controlling – and possibly eliminating – HCV replication. Nonetheless, in the absence of additional in vivo data, HCV-positive individuals should be cautioned about the risks of HCV reinfection and superinfection.

Larger, population-based studies have only recently been performed, so the consequences of HCV reinfection and superinfection are largely unknown. Nonetheless, identifying and characterizing the factors associated with reinfection and superinfection will greatly facilitate vaccine design and development, as well as suggest more effective therapeutic strategies. By analogy to HIV, HCV superinfection could impact overall HCV RNA levels, disease progression, innate and adaptive immune responses to HCV, spontaneous viral clearance, the development of drug resistance mutations and treatment response rates, transmission dynamics and biological phenotype. However, more detailed virological and immunological studies are clearly warranted to examine the possible association of HCV superinfection with these clinical outcomes. Careful consideration must also be given to other possible determinants of HCV clearance, such as the endogenous interferon response, IL28B genotype, size of the viral inoculum, frequency of exposure, time elapsed since previous exposure, demographic and behavioural characteristics, viral diversity and other coinfections. The study design must also include prospective sampling (rather than convenience sampling), highly sensitive HCV RNA assays, appropriate definitions of reinfection or superinfection, as well as data regarding possible behavioral and risk modifications, or treatment-related optimism.

Acknowledgements

The author would like to thank Eleanor Powell and Mohamed Tarek Shata and Kenneth Sherman for helpful discussions and review of this manuscript.

Disclosure statement

The author declares no competing interests.

References


