Hepatitis C virus clearance, reinfection, and persistence, with insights from studies of injecting drug users: towards a vaccine

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Hepatitis C virus (HCV) was discovered more than two decades ago, but progress towards a vaccine has been slow. HCV infection will spontaneously clear in about 25% of people. Studies of spontaneous HCV clearance in chimpanzees and human beings have identified host and viral factors that could be important in the control of HCV infection and the design of HCV vaccines. Although data from studies of chimpanzees suggest that protection against reinfection is possible after spontaneous clearance, HCV is a human disease. Results from studies of reinfection risk after spontaneous clearance in injecting drug users are conflicting, but some people seem to have protection against HCV persistence. To guide future vaccine development, we assess data from studies of HCV reinfection after spontaneous clearance, discuss flaws in the methods of previous human studies, and suggest essential components for future investigations of control of HCV infection.

Introduction

Two decades have passed since the discovery of hepatitis C virus (HCV),[1] and although understanding of the virus has greatly increased and major advances in therapeutic development have been made, no effective vaccine exists to prevent new infections. Spontaneous viral clearance occurs in about 25% of individuals, generally in the first 6 months of infection.[2] Researchers are interested in whether spontaneous viral clearance (host immune-mediated clearance) confers protection against reinfection, particularly against reinfection followed by viral persistence.

Studies of chimpanzees[3–8] and human beings[9,10] have shown that, after HCV reinfection, control of viral replication is better, duration of infection is shorter, and the likelihood of viral clearance is higher than in primary infection. These findings suggest that previous clearance of an HCV infection could provide some protection against persistent reinfection. In chimpanzees, rapid virological control after reinfection is associated with HCV-specific T-cell responses.[5,7,8] Cohort studies of injecting drug users (IDUs)[10–19] have assessed whether previous spontaneous HCV clearance provides protection against HCV reinfection, with inconsistent results. Immunological correlates of improved clearance after reinfection might identify potential targets for vaccine development.[20]

Acute HCV infection and clearance

HCV virus is present in blood 2–14 days after initial exposure. Concentrations of alanine aminotransferase and aspartate aminotransferase increase and HCV-specific antibodies are produced 20–150 days after exposure.[21–23] Primary infection with HCV is generally asymptomatic, although 15–30% of individuals develop symptomatic acute hepatitis illness within 5–12 weeks of exposure lasting 2–12 weeks.[24,25] Symptomatic primary HCV infection is often mild, with non-specific symptoms such as lethargy and myalgia, but individuals can present with jaundice.[24,25] In about 25% of patients, acute infection is followed by viral clearance, defined as undetectable concentrations of HCV RNA in blood.[2] Most of these individuals clear infection by 6 months (73–86%) or 12 months (87–95%).[26–28] However, spontaneous HCV clearance after 1 year has been reported.[29,30] Most patients do not have viral clearance and viraemia persists after 6 months, leading to chronic infection and progression to cirrhosis in 5–10% of individuals within 20 years.[31]

Whether HCV infection spontaneously clears or persists is affected by a complex set of interactions between virus and host that is only partly understood. Host factors such as female sex,[2,18,32] initial immune response,[33–37] virus-specific neutralising antibodies,[38,39] and host genetics[40–42] have been associated with clearance in prospective studies of acute HCV infection. Pathogen-associated factors, such as diversity of HCV viral quasispecies[43,44] and HCV genotype,[45] might also be linked with clearance. In large cross-sectional studies of people infected with HCV for an unknown period, viral clearance is associated with several factors: female sex,[2,46,47] young age,[48,49] indigenous Canadian ethnic origin,[46] non-black ethnic origin,[50–52] absence of alcohol-use disorder,[53] no tobacco use,[50] HIV-negative status,[46–48,51,53] and chronic hepatitis B infection.[47,48,50,52,54,55] However, these cross-sectional studies of individuals who tested positive on tests for HCV antibodies—ie, have been exposed to the virus at some point—are subject to selection bias, in view of the potential for HCV reinfection in people with initial spontaneous clearance during long-term follow-up.

Host polymorphisms of proteins such as HLA class I and II, natural-killer-cell receptors, chemokines, interleukins, and of interferon-stimulated genes have been associated with control of HCV.[40] However, the genetic associations identified have not been confirmed in independent cohorts, differ in diverse populations, and studies are limited by small sample size or varying definitions of HCV outcome; moreover, little is known about their functional basis.[40]

Perhaps the strongest genetic association with HCV clearance is with *IL28B*.[41,42,56,57] This gene encodes interferon-λ 3, which is involved in viral control.[58]

Individuals with unfavourable *IL28B* genotypes are less likely to clear HCV infection than are those with favourable alleles.[41,42,56,57] This association is independent of both sex and symptomatic HCV infection with jaundice.[42] Although the mechanism by which interferon-λ 3 acts during HCV infection is unknown, this cytokine has direct antiviral actions in vivo and readily inhibits HCV replication in hepatoma cells.[58]

A strong host immune response (innate and adaptive) is important for spontaneous HCV clearance.[33–36] During acute infection, HCV persistence can occur through evasion of the innate immune response.[37] HCV could partly or completely counter the innate immune response by disrupting cellular signalling pathways that lead to interferon synthesis, and by subverting cellular signalling to restrict expression of interferon-stimulated genes and block their antiviral effects.[37] The response of interferon-stimulated genes seems to be important since findings from chimpanzee studies suggest that their expression in the liver during acute HCV correlates with spontaneous clearance.[59]

Available evidence indicates that individuals with primary infections that later clear have strong, broadly specific, and sustained adaptive cellular immune responses, whereas many of those who develop persistent infection have weak cellular immune responses that do not last.[38,39] Strong cellular immune responses have also been noted in high-risk individuals who do not have HCV antibodies, suggesting that clearance can occur rapidly, before antibodies are produced.[60,61]

Virus-specific neutralising antibodies can drive sequence evolution and might affect the outcome of infection[62] and protection against reinfection.[10] The best available assay systems for HCV neutralising antibodies use virus-like particles or envelope sequences incorporated within pseudotyped viruses that maintain the native configuration of the HCV envelope glycoproteins. Initial studies with this method showed that neutralising antibodies were rare in individuals who went on to resolve infection,[63–65] although this finding was not universally reported.[66] However, a longitudinal study with homologous viral pseudoparticles showed that clearance of infection was associated with rapid development of neutralising antibodies.[67]

HCV reinfection

Occurrence

Studies of HCV reinfection provide insight into factors important for protection against persistent infection, which is a central issue for vaccine design. However, study of HCV reinfection in people has been difficult. Studies in chimpanzees have generated the most robust data on HCV reinfection because experiments can be carefully designed to study re-exposure and reinfection. Despite apparently efficient immune responses in primary infection resulting in viral clearance, reinfection can occur in chimpanzees with both homologous and heterologous viruses.[68,69] However, reinfection episodes have been linked with improved control of viral replication, a short course of infection, and an increased likelihood of viral clearance compared with primary infection.[3–8] Rapid virological control after chimpanzees are reinfected is connected to HCV-specific T-cell responses.[5,7,8] When CD4 T cells are depleted in vivo before reinfection, persistent HCV infection ensues.[70] Similarly, depletion of CD8 T cells extended HCV viraemia, which was controlled only when this subset of cells recovered in the liver.[8] In this context, cross-genotype immunity has been recorded,[6] but viral persistence seems more likely in the setting of heterologous reinfection.[7]

Nevertheless, HCV is a uniquely human disease, and investigations of HCV reinfection in people have improved understanding of protective immunity. In an early case series,[71] reinfection was recorded in five children with thalassaemia that were re-exposed to HCV after spontaneous clearance. Reinfection has also been reported in case studies of IDUs[9,12,13,17,28,72–75] and men who have sex with men.[76] Several observational cohort studies of IDUs with continuing risk behaviours for HCV acquisition have been done, assessing HCV reinfection after spontaneous clearance (tables 1, 2).[10,12–19,77] Collectively, these studies of IDUs are valuable because they give a human model for protection against HCV infection. Specifically, these investigations enable measurement of the incidence of HCV reinfection (and how it compares with incidence of primary HCV infection), the proportion who develop persistent HCV reinfection (and hence incidence of persistent infection), and the natural history of HCV reinfection.

Similar rates of primary infection and reinfection after adjustment for potential differences in risk behaviour would suggest that previous clearance of HCV infection does not provide sterilising immunity against reinfection. However, the proportion of persistent HCV reinfections should be measured. For example, if most reinfections spontaneously cleared, there would be a strong argument for some level of protection. Measurement of the size and duration of HCV viraemia during reinfection as compared with primary infection helps to establish whether protection is genetic or immunological. A reduction in the degree or duration of viraemia would suggest that acquired protective immunity has a role, because fixed genetic factors would not adapt and become more efficient as does the immune response. Studies of HCV reinfection in IDUs (tables 1, 2) further understanding of all three parameters and have implications for HCV vaccines.

Researchers in Baltimore (MD, USA) investigated whether previous clearance reduces the risk of HCV reinfection in a cohort study.[12] After adjustment for risk behaviour, individuals with previous HCV clearance were half as likely to be infected during follow-up as were those who had not been infected previously (table 2).[12] Further data supporting these findings came

from a prospective cohort of IDUs in Vancouver (BC, Canada).[13] Importantly, the median time between HCV RNA testing was long in both studies (table 2).[13]

Data from other cohorts, however, suggest that previous spontaneous clearance of HCV infection might not reduce risk of new infection.[10,14,15,17,18] A retrospective cohort study of young, high-risk IDUs from Sydney (NSW, Australia)[14]—with more frequent testing than in the other studies[12,13]—showed no difference between incidence of HCV infection in individuals with no previous infection and in those with previous HCV clearance (table 2). A prospective cohort study in Melbourne (VIC, Australia),[15] also showed high reinfection rates in IDUs who had previously cleared HCV infection (table 2). Previously infected IDUs with HCV clearance were 2·5 times more likely to become infected than were those who had not been previously infected. Similar findings have been reported in the USA.[10,18] Frequent monitoring of HCV infection status in a study of young IDUs from Baltimore[10] showed infection rates of individuals who had no previous infection and of those with previous clearance were similar (table 2).

In the Netherlands, van de Laar and colleagues[17] noted that HCV reinfection was at least as common as initial infection in their cohort (table 2). Although testing intervals for HCV reinfection were long, they recorded a decline in the incidence of HCV reinfection from 20·4 per 100 person-years in 1985–1995, to 4·2 per 100 person-years in 1995–2005. Incidence of initial HCV infection fell from 27·5 per 100 person-years in the late 1980s to roughly 2·0 per 100 person-years in 2005. Collectively, these cohort studies suggest that rates of infection and reinfection are similar when short testing intervals are used. Thus, HCV infection in people does not confer sterilising immunity.

Clearance of reinfection

Although reinfection is common, it does not always lead to persistent infection. Spontaneous clearance of HCV reinfection has been frequently recorded (table 2); data suggest that some individuals can clear HCV after one exposure more efficiently than can others. Overall, clearance of the reinfection strain is fairly common, with some individuals able to spontaneously clear HCV with different genotypes from that of the initial infection.[10,14,15,18]

A high rate of clearance of HCV reinfection is not surprising, because, by definition, individuals at risk have had clearance of primary infection, and host characteristics are associated with clearance. Furthermore, it does not indicate that previous HCV infection with clearance changes the course of reinfection. Rates of clearance after reinfection are probably underestimated in most studies, because HCV RNA testing intervals longer than 1 month could cause many cases of clearance to be missed, and will therefore be biased to detection of HCV reinfections with viral persistence.[80] Furthermore, longitudinal follow-up of HCV reinfection cases with

	Country	Cohort design	HCV virological assessments	Study period	Study populations	Age (years)	Men	Ethnic origin	Infected with HIV at baseline	Injection drug use at baseline	Frequent injecting*
Mehta[12]	USA	Prospective	Retrospective	1988–95	Not infected (n=164) *vs* HCV clearance (n=98)	32 (7·0) *vs* 41 (6·3)	121 (74%) *vs* 58 (59%)	African-American: 146 (90%) *vs* 87 (90%)	17 (10%) *vs* 36 (37%)	129 (79%) *vs* 64 (65%)	35 (21%) *vs* 32 (33%)
Grebely[13]	Canada	Retrospective	Retrospective	1992–2005	Not infected (n=926) *vs* HCV clearance (n=152)	44 (7·7) *vs* 41 (11·3)	628 (67%) *vs* 93 (61%)	White: 541 (58%) *vs* 69 (45%)	68 (7%) *vs* 35 (23%)	241 (26%) *vs* 73 (48%)	129 (14%) *vs* 38 (25%)
Micallef[14]	Australia	Retrospective	Retrospective	1993–2002	Not infected (n=423) *vs* HCV clearance (n=18)	23 (15–54)† *vs* 23 (16–32)†	166 (39%) *vs* 7 (39%)	NA	NA	NA	61% *vs* 56%‡
Aitken[15]	Australia	Prospective	Prospective	2005–07	Not infected (n=55) *vs* HCV clearance (n=50)	25 *vs* 27	19 (35%) *vs* 22 (44%)	White: 37 (74%) *vs* 45 (82%)	0 (0%) *vs* 0 (0%)	55 (100%) *vs* 50 (100%)	29 (58%) *vs* 20 (36%)
van de Laar[17]	Netherlands	Prospective	Retrospective	1985–2005	Not infected (n=168)§ *vs* HCV clearance (n=24)	29 *vs* 27	112 (67%) *vs* 9 (38%)	Western European: 139 (83%) *vs* 20 (83%)	4 (2%) *vs* 2 (8%)	100 (60%) *vs* 23 (96%)	26 (16%) *vs* 12 (50%)
Page[18]	USA	Prospective	Prospective	2000–08	Not infected (n=380) *vs* HCV clearance (n=22)	23 *vs* 22	253 (67%) *vs* 10 (46%)	White: 290 (77%) *vs* 16 (73%)	6 (2%) *vs* 0 (0%)	380 (100%) *vs* 22 (100%)	122 (33%) *vs* 4 (24%)
Osburn[10]	USA	Prospective	Prospective	1997–2008	Not infected (n=179) *vs* HCV clearance (n=22)	23 *vs* 25	80 (45%) *vs* 10 (45%)	White: 134 (75%) *vs* 22 (100%)	NA *vs* 1 (4%)	NA *vs* 22 (100%)	NA
Dove[77]	USA	Prospective	Prospective	NA	HCV clearance (n=6)	46 (37–70)†	4 (67%)	African-American: 2 (33%)	0 (0%)	6 (100%)	6 (100%)
Currie[16]	USA	Prospective	Prospective	1997–2001	HCV clearance (n=29)	47 (7·5)	16 (55%)	African-American: 7 (24%)	12 (41%)	17 (59%)	NA
Grebely[19]	Australia	Prospective	Prospective	2004–07	HCV clearance (n=30)	33	20 (67%)	White: 28 (93%)	7 (23%)	5 (17%)	2 (7%)

Data are n (%) or mean (SD when available), unless otherwise stated. Percentages taken directly from relevant reports. HCV=hepatitis C virus. NA=not available. *Frequent use is classed as use more than once every day at the baseline visit. †Median (range). ‡Data taken from Dore and Micallef.[78] §Data taken from van den Berg et al.[79] ¶Data taken from Cox et al.[22]

Table 1: **Characteristics of injecting drug users assessed for HCV infection and reinfection in longitudinal studies**

	Study populations	Number of new infections during follow-up	Median follow-up (years)	Incidence rate per 100 person-years	Crude incidence rate ratio	Adjusted ratio (95% CI)	p value	Median HCV RNA testing interval for patients previously infected (months)*	Clearance of reinfection in patients whose infection had previously cleared†	Reinfection in prevalent or incident cases?
Mehta[12]	Not infected (n=164) *vs* HCV clearance (n=98)	35 *vs* 12	2·4 *vs* 2·1	8·6 *vs* 5·4	0·63	0·45 (0·23–0·88)†	0·02	6·3 (6)	6 of 9 (67%)‡	Prevalent
Grebely[13]	Not infected (n=926) *vs* HCV clearance (n=152)	172 *vs* 14	2·8 *vs* 5·2	8·1 *vs* 1·8	0·22	0·23 (0·10–0·51)§	<0·001	15·6	4 of 14 (29%)	Prevalent
Micallef[14]	Not infected (n=423) *vs* HCV clearance (n=18)	114 *vs* 13	1·0 *vs* 1·2	17·0 *vs* 42·0	2·47	1·1¶	0·80	5·0 (6)	3 of 7 (43%)	Incident
Aitken[15]	Not infected (n=55) *vs* HCV clearance (n=50)	10 *vs* 23	NA	15·5 *vs* 46·8	3·02	2·54 (1·11–5·78)‡	0·027	3·8 (3)	9 of 22 (41%)	Prevalent and incident
van de Laar[17]	Not infected (n=168)|| *vs* HCV clearance (n=24)	58 *vs* 9	3·6 *vs* 10·5	6·7 *vs* 9·9	1·5	NA	NA	7·3 (4–6)	3 of 9 (33%)	Incident
Page[18]	Not infected (n=380) *vs* HCV clearance (n=27)	132 *vs* 7	NA	26·7 *vs* 24·6	0·92	NA	NA	3·0 (3)	7 of 7 (100%)	Incident
Osburn[10]	Not infected (n=179)** *vs* HCV clearance (n=22)	62 *vs* 11	NA	27·2 *vs* 30·1	1·11	NA	NA	1·0 *vs* 1·0 (1)	10 of 12 (83%)	Incident
Currie[16]	HCV clearance (n=29)	0	5·5	0·0	NA	NA	NA	NA (6)	0 of 29	Prevalent
Grebely[19]	HCV clearance (n=30)	2	1·1	6·1	NA	NA	NA	3·0 (3)	2 of 2 (100%)	Incident

HCV=hepatitis C virus. NA=not available. *Scheduled interval given in parentheses when available. †Hazard ratio. ‡Restricted to HIV-negative participants. §Odds ratio. ¶Incidence rate ratio. ||Data taken from van den Berg et al.[79] **Data taken from Cox et al.[22]

Table 2: **Infection and reinfection in injecting drug users in longitudinal studies**

long intervals between tests will mean clearance cases are misclassified as persistent cases. As such, caution must be used in interpretation of results of studies with long intervals between tests or short follow-up time.

Natural history of reinfection

As recorded in chimpanzees, evidence indicates that HCV RNA concentrations after reinfection in people are lower, generally more transient, and shorter in duration than during initial infection.[10] In a longitudinal study of IDUs,[10] median duration of HCV viraemia was four times longer during initial infection than during reinfection (232 days *vs* 77 days) and peak median log HCV RNA concentration was lower (3·1 log IU/mL *vs* 6·7 log IU/mL),[10] suggesting people develop adaptive protective immunity (figure).

The emergence of a new dominant virus during chronic infection (without a period free of viraemia) does not elicit an increased number of new HCV-specific T-cell responses,[10] potentially because of virus-induced immune tolerance or exhaustion.[81,82] By contrast, different responses of HCV-specific T cells during reinfection have been documented.[10] Additionally, a response of neutralising antibodies to heterologous HCV pseudoparticles was noted in 60% of reinfected IDUs. Although neutralising antibodies do not generally neutralise heterologous HCV pseudoparticles during the acute phase of infections that progress to chronicity,[62,64] their presence in reinfected individuals was independent of the sequence divergence between the stimulating virus and the test HCV pseudoparticle sequence.[10] These data suggest that reinfection is associated with the generation of cross-reactive neutralising antibodies.

However, Osburn and colleagues[10] detected new HCV-specific T-cell responses and cross-reactive neutralising antibodies in reinfected individuals who did not clear reinfection. Therefore, although improved cellular and humoral immune responses play a part in control of reinfection, they are probably not sufficient for protection against HCV reinfection with persistence in all cases. Further longitudinal investigation of adaptive immunity during primary infection and reinfection is necessary for reliable identification of the characteristics of protective immunity associated with repeated clearance of HCV infection and hence for future vaccine research.

Study limitations

The substantial heterogeneity of studies of HCV reinfection in people has an important effect on interpretation, particularly on cross-study comparison. Apart from differences in study design (eg, follow-up of cohorts with previous infection and clearance *vs* cohorts with incident infection and subsequent reinfection) and statistical analyses, clear variation in age, sex, ethnic origin, injecting risk behaviours, and presence of viral co-infections between the cohorts exists (table 1). Risk behaviours of individuals with no previous infection and of those who have cleared an infection might differ and change over time; hence, an analysis without adjustment for time-updated risk behaviour as a proxy for re-exposure to HCV might have misleading findings.[78] Risk behaviour information needs to be collected accurately and regularly.

Definitions of viral clearance and reinfection vary between studies, as do the testing intervals and HCV RNA assays (table 2). The type of assay used is important because

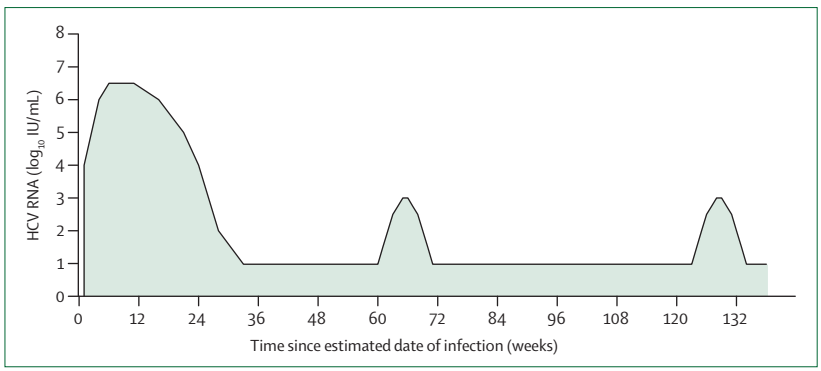

Figure: **HCV infection, clearance, and reinfection**
HCV reinfection events after spontaneous clearance have lower HCV RNA concentrations and shorter infection durations than initial HCV infection. HCV=hepatitis C virus.

Search strategy and selection criteria

We searched Medline with the terms "hepatitis C", "HCV", "reinfection", "re-infection", "drug users", "epidemiology", "diagnosis", "natural history", and "spontaneous clearance" to identify reports published in English before Sept 15, 2011. The reference lists of identified reports were manually searched for further relevant papers. Key abstracts at international meetings were also included. We selected reports on the basis of relevance to design, implementation, and analysis of studies related to HCV reinfection in injecting drug users and then assessed them for quality of methods and relevance of results.

HCV seroconversion reliably allows detection of almost all initial infections, but HCV reinfection necessitates detection of HCV RNA. Mathematical modelling has shown that studies with long HCV RNA testing intervals underestimate the incidence of HCV reinfection and probability of spontaneous HCV clearance after reinfection.[83]

Future studies

Investigations into spontaneous HCV clearance of infection and reinfection and into correlates of protection could provide crucial insights into HCV vaccine design. Understanding of host factors essential for control of HCV—particularly after several exposure events—will provide important information about the development of components necessary for future vaccines. Because present results in people are inconclusive, further investigation into possible protective immunity is needed.

The ideal study to improve understanding of primary HCV infection and reinfection, with a specific focus on potential development of an HCV vaccine that would provide protection against viral persistence, would be designed in a specific way. Uninfected, high-risk individuals would be recruited and followed up, with tests for initial HCV infection every 1–3 months. All patients would ideally undergo HCV RNA testing to improve early detection; those infected for the first time would always have this test to characterise the course of primary HCV infection. Investigators would collect detailed information about HCV risk behaviour, including any longitudinal changes. Primary HCV infection cases with viral clearance would be followed up longitudinally for detection of HCV reinfection, with the same testing intervals as for initial detection. Individuals reinfected would be followed up for a long period to establish viraemia status and the incidence and course of further reinfection events. Blood samples would have to be taken during primary HCV infection and reinfection with standardised collection methods and stored for detailed immunological and virological studies. Finally, HCV reinfection would be confirmed through phylogenetic characterisation of initial and reinfection strains.

Without prospective studies appropriately designed to address whether HCV clearance provides protection against reinfection, pooling of information from existing cohorts with sufficient data is one way to move forward. The International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts (InC³) was established to create a merged multicohort project of pooled data from well characterised cohorts of IDUs with acute HCV, to enable new in-depth studies not possible from each individual study, and to bring together researchers across disciplines. InC³ has successfully pooled behavioural, clinical, and virological data from 539 participants with acute HCV infection from nine cohorts in Australia, Canada, Europe, and the USA.[84]

Conclusions

Data from chimpanzee and human studies of primary HCV infection, viral clearance, and HCV reinfection indicate that previous HCV infection is unlikely to provide substantial levels of acquired sterilising immunity. However, characterisation of the course of primary HCV infection and reinfection suggests that some protection against persistent HCV reinfection is developed through previous HCV infection.

Therefore, a vaccine that enhances spontaneous clearance of primary HCV could be more feasible than would a vaccine that prevents initial HCV infection.[20] The primary goal of such a vaccine would be to prevent the development of chronic HCV infection after repeat exposures. The prevention of chronic HCV infection would be a suitable endpoint, because chronic—not acute—HCV infection is associated with HCV-related morbidity and mortality.

Contributors
JG, MP, and GD developed the outline and concept for the Review, and finalised the first draft. All authors assisted in writing of the first draft according to their area of expertise and contributed to the final editing of the report.

Conflicts of interest
We declare that we have no conflicts of interest.

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