Unscrambling hepatitis C virus–host interactions

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The human suffering exacted by the hepatitis C virus is enormous. Hundreds of thousands of people die each year from liver failure and cancer caused by this infection. There is no vaccine, and the available antiviral drugs are toxic, expensive and only partly effective. Progress has been hindered by the absence of cell culture and small-animal models of the infection. Nonetheless, recent advances have yielded several promising new antiviral drugs and enhanced the prospects of developing a vaccine. The recent development of a robust *in vitro* hepatitis C virus infection system will aid this search.

The hepatitis C virus (HCV) is a noncytopathic hepatotropic member of the Flaviviridae that causes acute and chronic hepatitis, and hepatocellular carcinoma (HCC; Fig. 1)¹. The liver is its primary target organ, and the hepatocyte is its primary target cell. More than 170 million people are currently infected with HCV². Acute infection is usually asymptomatic, making early diagnosis difficult. A notable feature of HCV infection is its tendency towards chronicity: ~70% of acute infections become persistent, and chronic cases are often associated with serious liver disease¹ (Fig. 2). As a result, HCV infection is a leading killer worldwide and the commonest cause of liver failure in the United States (see review in this issue by Brown, page 973).

In common with hepatitis B and human immunodeficiency (HIV) viruses, HCV is primarily transmitted percutaneously³. Before the development of diagnostic tests, the infection was commonly passed on through blood and related products⁴, haemodialysis⁵ and organ transplantation⁶. Today, HCV primarily affects injecting drug users and their sexual partners⁶. It is a particular problem in correctional facilities, where 20–40% of inmates are infected, in contrast to ~2% of the general population⁷. It is opportunistic in HIV-infected individuals, ~25% of whom are co-infected with HCV (this figure rises to 50–90% among injecting drug users)⁸. Co-infection causes higher HCV titres and a more rapid progression to cirrhosis⁸.

The virus and its life cycle

HCV infects only humans and chimpanzees; there are no small-animal models. Moreover, until recently, cell culture systems were not available. Most of our knowledge of HCV has been derived from surrogate experimental systems that approximate true infection and often preclude definitive interpretation. Nonetheless, much has been learned in the 16 years since the HCV genome was first cloned by Houghton and colleagues⁹.

The HCV genome is a 9.6-kilobase uncapped linear single-stranded RNA (ssRNA) molecule with positive polarity. It contains 5' and 3' untranslated regions (UTRs) including control elements required for translation and replication¹⁰ (see review in this issue by Lindenbach and Rice, page 933). The UTRs flank an uninterrupted open reading frame encoding a single polypeptide of 3,010 or 3,011 amino acids, which is processed into structural (C, E1, E2 and p7) and nonstructural (NS2, NS3, NS4A, NS4B, NS5A and NS5B) subunits by host and viral proteases^{11,12}.

The HCV life cycle is entirely cytoplasmic. Replication occurs through

a minus-strand intermediate in a membrane-bounded compartment¹³, yielding double-stranded RNA (dsRNA) intermediates. The replicative intermediates are fully exposed to the cell dsRNA-sensing machinery^{14,15} and induce strong innate cellular responses following infection.

Although much of our understanding of HCV replication is based on subgenomic and genomic replicon systems developed by Bartenschlager and colleagues¹⁶, little is known about the mechanisms and host functions involved in viral entry, uncoating, trafficking, assembly and egress. However, three independent groups have recently reported the development of a robust HCV infection system *in vitro*^{17–19}, which should make these processes experimentally accessible.

This new system is based on a unique HCV genome (JFH1) derived from the blood of a Japanese patient with fulminant hepatitis²⁰ and has extraordinary replicative capacity *in vitro*²¹ (see review in this issue by Lindenbach and Rice, page 933). The ability to perform reverse-genetics experiments will facilitate identification of the unique elements required for infectivity *in vitro* and help understand their specific roles. Moreover, by making each step of the viral life cycle experimentally accessible, this system will assist the development of antiviral drugs and help analyse the neutralizing and curative potential of candidate vaccines.

The host–virus relationship

In common with other persistent viruses, HCV does not kill the cells it infects, but triggers an immune-mediated inflammatory response (hepatitis) that either rapidly clears the infection or slowly destroys the liver, causing the development of HCC (see review in this issue by Bowen and Walker, page 946). The outcome is largely determined by the efficiency of the antiviral immune response. Host–virus interactions are ideally investigated in cell culture and small-animal models; the former are only now becoming available. Nonetheless, many of the factors that determine the outcome of HCV infection are beginning to come into focus (see reviews in this issue by Gale and Foy, and Bowen and Walker, pages 939 and 946, respectively).

Host–outcome determinants

Innate immune response

HCV spreads rapidly in the liver after inoculation^{22,23}, and so the innate immune response might be expected to influence the outcome of infection. Indeed, prospective genomic analysis of the intrahepatic

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Figure 1 | Natural history of HCV infection. HCV is a noncytopathic virus that infects the liver and causes acute self-limited infection in 10–30% of patients with an associated inflammatory liver disease of variable severity that is mediated by the cellular immune response. In 70–90% of patients, however, HCV persists and causes chronic hepatitis with its life-threatening complications, including liver failure and hepatocellular carcinoma.

innate immune response in acutely infected chimpanzees suggests that HCV triggers a strong type-1 interferon (IFN- α/β) response as it spreads^{22,24}, but resists the effector functions of the downstream antiviral target genes that it induces. Importantly, the response is similar in animals that clear the infection and those that become persistently infected^{22,24}, implying that any influence on the outcome is indirect or obscure. Whatever its function, the innate intracellular immune response probably has a role in controlling HCV infection because the virus has developed several strategies to evade it (see review in this issue by Gale and Foy, page 939).

Adaptive immune response

The clearest determinants of the outcome of HCV infection are the magnitude, diversity and quality of the adaptive immune response. Viral clearance during self-limited infection is characterized by vigorous polyclonal CD4⁺ and CD8⁺ T-cell responses that are relatively weak and narrowly focused in chronically infected humans and chimpanzees. Moreover, the onset of viral clearance and liver disease coincide with that of the T-cell response and the entry of virus-specific T cells into the liver; primary failure to induce a T-cell response or functional exhaustion of an initially vigorous response predict viral persistence^{23,25}. However, the basis for variable immunological responsiveness to HCV has largely remained elusive. Indeed, we do not know whether the failure to respond vigorously in persistently infected subjects is caused by antigen overload during immunological priming, virus-induced defects in antigen presentation, hyperinduction of regulatory T cells, genetically determined restriction of the virus-specific T-cell repertoire or other causes (see review in this issue by Bowen and Walker, page 946). Therefore, whereas both primary and secondary immunological hyporesponsiveness to HCV seem to contribute to the establishment and maintenance of persistent infection, the reasons why they occur in selected subjects remain to be determined. Moreover, the virus can persist despite a multispecific CD4⁺ and CD8⁺ T-cell response^{23,25} by progressive mutational escape, which confirms the importance of the immune response in viral clearance and disease pathogenesis. An inbred mouse model of HCV infection would greatly facilitate these studies.

Viral outcome determinants

The viral factors influencing the outcome of HCV infection are beginning to come into focus.

HCV genotypes, replication and mutation rates

The six distinct genotypes of HCV show marked differences in geographic distribution, disease progression and response to therapy. However, the complex epidemiological differences in patient groups infected with each genotype make it difficult to ascribe variability in outcome to

the virus instead of the host (see review in this issue by Feld and Hoofnagle, page 967). The mutation rate of HCV is high (10^{-3} per nucleotide per generation), as is its replication rate ($\sim 10^{12}$ virions per day in humans)²⁶. This results in explosive expansion of the virus after inoculation and in the evolution of numerous viral quasispecies in each infected subject, which could influence the magnitude and efficacy of the antiviral immune response. Moreover, the virus produces a constant stream of escape variants that outrun the immune response and can eventually produce mutants with no corresponding receptors in the immunological repertoire^{23,25}. The influence of these parameters on the outcome of infection has been studied in a few acutely infected humans and chimpanzees and in many chronically infected individuals. The results show that B- and T-cell escape mutants are selected by the immune response during HCV infection and probably contribute to viral persistence (see review in this issue by Bowen and Walker, page 946).

Viral evasion strategies

The primary immune-evasion strategies fall into two distinct categories: subversion of the IFN response induced by the virus and mutational escape from the adaptive immune response.

According to the first strategy, when HCV infects the liver, it triggers the production of IFN and a range of antiviral genes that should control the infection — but do not^{22,27}. In fact, HCV seems to be resistant to these antiviral pathways, at least in the HCV replicon system, and several structural and nonstructural proteins have been shown to inhibit nonoverlapping functions of the innate immune response (see review in this issue by Gale and Foy, page 939). For example, the Gale group has shown that NS3/4A can block the phosphorylation and effector action of IFN regulatory factor 3 (IRF3)²⁸ by inactivating signalling by retinoic-acid-inducible gene I (RIG-I)²⁹, a cytoplasmic dsRNA-binding protein that activates cellular kinases that stimulate IRF3 (ref. 15). So, HCV seems to use several strategies to actively evade the immune responses that it induces. However, these evasion strategies were defined either biochemically or in transfected cell-culture systems, not in infected cells. It is premature to assume that they occur during natural infection until they have been validated *in vivo* or at least in the newly developed tissue-culture model of HCV infection.

Regarding the second strategy, mutational inactivation of B- and T-cell epitopes is common in HCV infection (see review in this issue by Bowen and Walker, page 946). B-cell epitopes are concentrated in the hypervariable region of the E2 protein³⁰, probably allowing the virus to persist in the presence of antibody that is neutralizing for its ancestors. The T-cell epitope mutations span the viral polyprotein³¹, often in residues that bind to major histocompatibility complex (MHC) molecules or are otherwise involved in antigen presentation. Mutations also occur in residues engaged by the T-cell receptor (TCR), making infected cells invisible to T cells expressing the corresponding TCR³². Although mutational escape probably contributes to the persistence of the virus, it is less clear whether it determines the outcome. Several groups have shown an association between certain human leukocyte antigen (HLA) alleles and the outcome of HCV infection²⁵. These differences might influence the breadth of the TCR repertoire and the ease with which the virus can escape. Confirmation of this hypothesis would be facilitated by an inbred mouse model of HCV infection.

What about treatment?

The standard treatment for chronic HCV infection is pegylated IFN- α plus ribavirin³³ (see review in this issue by Feld and Hoofnagle, page 967). Although the mechanism of action of these drugs is debated, with both antiviral and immunostimulatory mechanisms being implicated³⁴, the sustained response (cure) rates are far from ideal. Moreover, there is substantial associated toxicity³⁵, and the likelihood of success depends on viral and host factors that are often beyond the control of patients and physicians. Clearly, more effective and less toxic treatment regimens are needed.

Thanks to the HCV replicon system, much recent effort has been directed towards developing drugs that inhibit viral replication. Sev-

eral promising small-molecule inhibitors of the NS3/4A protease and the NS5B polymerase are in development (see review in this issue by De Francesco and Migliaccio, page 953). Early testing has uncovered strong antiviral activity both *in vitro* and in patients³⁶. As expected, escape variants have been rapidly selected by each drug, indicating that drug cocktails will probably be required to control this infection.

The hope is to target all aspects of the viral life cycle therapeutically, including those that are inaccessible using the replicon system. Indeed, this is becoming possible owing to the *in vitro* HCV infection model mentioned above^{17–19}.

De Francesco and Migliaccio also describe efforts to stimulate the innate immune response in chronically infected subjects. Furthermore, several groups are attempting to activate the adaptive immune response with therapeutic vaccines³⁷ (see reviews in this issue by Houghton and Abrignani, and Bowen and Walker, pages 961 and 946, respectively). Harnessing the immune response makes sense, as it is curative in subjects who spontaneously clear HCV infection and could potentially eliminate the drug-resistant viral clones selected by emerging antiviral drugs.

Prospects for a preventive vaccine

Developing a preventive vaccine is arguably the ultimate objective of this research. Traditional vaccine development based on the antibody response preventing incoming viruses from reaching their cellular targets has been challenging for mutation-prone HCV, which generates numerous antibody escape mutants³⁸.

Nonetheless, a vaccine might be within reach. Chimpanzees that are rechallenged after clearance of a primary HCV infection are protected against homologous and heterologous viral isolates³⁹. Moreover, those immunized with an adjuvanted recombinant HCV envelope vaccine that elicits E2 glycoprotein-specific antibodies and T-cell help are largely protected from chronic infection when challenged with a heterologous viral inoculum (see review in this issue by Houghton and Abrignani, page 961). This is a considerable advance, even though the immunized chimpanzees become acutely infected, because the morbidity of HCV is largely a consequence of chronic infection, from which they are protected.

As spontaneous viral clearance during acute HCV infection is characterized by a vigorous broadly reactive CD4⁺ and CD8⁺ T-cell response, much effort is being made to develop T-cell-based vaccines. Approaches include DNA-prime–protein boost, DNA-prime–poxvirus boost, recombinant adenovirus and Semliki Forest virus vectors, and recombinant α -viral particles (see review in this issue by Houghton and Abrignani, page 961). Despite their limitations, these techniques potentially offer enhanced immunogenicity, the ability to induce antibody as well as T-cell responses and the flexibility to stimulate cross-protective immunity.

Where do we go from here?

We arrive at the end of this article with more questions than answers as we try to understand the viral life cycle, the factors that determine the outcome of infection and how to develop a vaccine and more effective drugs for the prevention and treatment of HCV infection. We know that the virus infects the liver, but how many hepatocytes are infected, are other cells affected and does it disrupt luxury functions or have direct cytopathic effects? We know that HCV spreads and mutates rapidly and that a huge number of virions are produced every day. But we don't know the extent to which small differences in these parameters allow it to outrun the immune response and persist in certain patients. The virus survives despite a strong innate intracellular IFN response and has evolved the means to evade it, yet is the outcome determined by an undefined counter-regulatory gene or isoform expressed only in subjects who clear the infection? We know that certain HCV proteins can blunt the antiviral effects of IFN in transfected cells that overexpress them, but does this occur during natural infection *in vivo*? Viral clearance is associated with a sustained vigorous polyclonal adaptive immune response, yet why do some individuals mount such a response and others do not? Such questions must be answered to understand the outcome determinants, and prevent and terminate persistent HCV infection. New experimental systems are urgently needed if we wish to answer those difficult ques-

tions. The recent development of a cell-culture system supporting robust HCV infection^{17–19} is a huge step in the right direction. ■

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