Introduction

Following the discovery of hepatitis A and B viruses, it was almost certain that a third virus also attacked the liver. The main criteria for this thinking was the fact that, following blood transfusion, hepatitis with slightly different clinical features occurred in patients who were negative for both viruses, hence the disease was known as non-A, non-B post-transfusion hepatitis.

In 1989 the perpetrator, hepatitis C virus (HCV), was finally identified using an elegant molecular approach. Now, 15 years later, scientists have just begun to target key viral enzymes in an attempt to increase the limited treatment options currently available. Following a review of HCV, this article will also highlight some of the recent methodological breakthroughs that will potentially aid drug discovery.

HCV infection is a significant global problem, with approximately 3% of the world’s population infected and the rate of new cases increasing rapidly. The true prevalence is underestimated due to the asymptomatic nature of the acute infection. In Australia, around 225,000 people are infected and 15,000 new cases are reported each year. The majority (~60%) of all infections become persistent and lead to various clinical outcomes ranging from an asymptomatic carrier state to liver failure. Furthermore, patients with chronic HCV, in particular those with liver cirrhosis, are at high risk of developing hepatocellular carcinoma (HCC).

The virus itself has a positive sense 9.5kb RNA genome that replicates in the cytoplasm of the infected cell via minus-strand RNA intermediates. During infection, the genome acts as mRNA and is translated into a precursor polyprotein, which is cleaved into structural proteins by host cell peptidases and into non-structural (NS) proteins by two viral proteinases. As a consequence of the low fidelity of the polymerase, the viral genome is highly variable and is classified into six genotypes and over 80 subtypes. The predominant subtypes in patients in Australia are 1a, 1b and 3a.

Models of HCV for treatment development

No vaccine exists to prevent transmission of the disease, which has made HCV treatment a significant research focus in recent years. Combination therapy of pegylated interferon-α with ribavirin is currently the only real treatment option, although a significant (~40%) number of patients, especially those with genotype 1, remain infected after therapy. Discontinuation of treatment due to side effects is a major problem and, for many people, a liver transplant is the only cure.

Newer agents targeting the HCV replication enzymes such as the protease (NS3) and polymerase (NS5) may well be the answer, as seen in the treatment of human immunodeficiency virus. However, this is by no means a simple task as the virus is extremely difficult to study due to the lack of a reliable culture system or small animal model. Studies to date have relied upon the chimpanzee model, recombinant expression systems and, more recently, a subgenomic replicon system.

The HCV replicon is a RNA molecule that replicates in liver cell lines and contains only the non-structural genes of the virus. Until recently, this model only existed for genotype 1; however, a genotype 2a has recently been developed increasing the repertoire for drug screening models.

Recent research in Australia has shown that the HCV E1 and E2 glycoproteins were not retained in the ER as previously thought, but were in fact expressed on the cell surface. This finding has led to E1 and E2 being pseudotyped into retroviral particles, providing a model which has just begun to reveal how HCV enters cells. This model now provides the grounding to target another potential event in the viral lifecycle, namely cell entry, for the fight against this disease.

HCV polymerase

The HCV polymerase is a suitable target for antiviral therapy because of its vital role in the replication of HCV and because inhibitors of this virus-specific enzyme are less likely to have significant cytotoxicity due to the absence of an equivalent enzyme in humans.

Recent inhibitors have included dinucleotides, constrained peptides and monoclonal antibodies. Currently the majority of lead compounds are only tested on subtype 1b using in vitro and replicon systems. The net result of the current screening practices could mean that strategies to develop anti-HCV chemotherapies may identify inhibitors that only work on genotype 1.
Recent work in Australia has led to the development of the first in vitro system to study genotype 3a [Clancy et al. manuscript in preparation]. This model has enabled the characterisation of the 3a polymerase and, although the enzyme behaves in a similar fashion, both kinetically and biochemically, to genotype 1b enzymes, there are also some important differences in template utilisation. The model has also led to the development of novel ssDNA aptamers that bind with high affinity to the genotype 3a HCV polymerase [Jones et al. manuscript in preparation].

Conclusions

As the repertoire of testing systems increases, it is likely that future therapies for HCV will target viral enzymes and specific events in the HCV lifecycle. Combined therapies are likely to ensue, although we may have to wait some time for this. Further work is clearly needed to provide better models systems in which to study HCV replication and entry that are applicable to every genotype before an effective cure for HCV can be realised.

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References