Hepatitis C virus (HCV) — A review
Molecular biology of the virus, immunodiagnostics, genomic heterogeneity and the role of virus in hepatocellular carcinoma

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Hepatitis C virus (HCV), an RNA and a hepatotropic virus, is the leading cause of viral hepatitis worldwide. Infection with this virus causes a repertoire of liver diseases that include acute hepatitis, chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC), in addition to a number of extra-hepatic manifestations such as lichen planus, oral cancer, etc. Present, patients infected with this virus are treated with interferon either alone or in combination with ribavirin, a guanosine-like nucleoside analog. However, response to this treatment has been rather disappointing. For about a decade, lack of an alternative animal model other than chimpanzee, and an efficient cell culture system that could support long-term replication of the virus, hampered research on HCV. Despite this, a significant amount of information with regard to the molecular biology of the virus is available using bacterial cloning-expression systems, and based on computer predictions and analysis. Recent discovery of a cellular receptor to which the virus binds, identification of efficient cell culture/ cell-free systems, HCV replicons and the development of a chimeric mouse model, provide a platform to verify the existing knowledge about this virus in the coming years. Additionally these developments aid the researchers in identifying novel therapeutic agents, apart from allowing us to reassess the efficiency of the currently available therapeutics. Presented in this article are a review of existing information with regard to the molecular biology of the virus, immunodiagnostic assays, genomic heterogeneity and the role of the virus in hepatocellular carcinoma. Likely therapeutic strategies other than those currently available are also introduced.

Keywords: Genomic heterogeneity, Hepatitis C virus, Hepatocellular carcinoma, Immunodiagnostics, Molecular biology, Therapeutic strategies

As early as in 1974, it was apparent that a third virus other than hepatitis A virus (HAV) and hepatitis B virus (HBV) is responsible for a significant percentage of post-transfusion hepatitis (PTH) cases. However, it was only in 1989 that researchers at the Chiron Corporation, USA, could discover the virus responsible for the PTH cases. This virus was subsequently designated Hepatitis C virus (HCV). Discovery of HCV and its establishment as the leading cause of non-A, non-B hepatitis represents a major breakthrough in the fields of biomedical biotechnology, in general, and viral hepatitis, in particular. Current estimates put the prevalence of HCV infection at 1-2% of the World population. While illicit intravenous drug use and parenteral transmission remain the key routes of acquiring HCV, the mode of acquisition in almost 50% of the cases is still uncertain; such cases are labeled as “community acquired” and most likely result from cultural practices such as tattooing, body piercing, sharing razors, etc.

In a vast majority of the infected population, infection with this virus typically results in mild acute disease with apparent complete recovery in about 20% of cases, and about 80% of cases progress to asymptomatic chronic infection. Approximately 20% of such chronically infected patients develop cirrhosis after 22 years of infection on average. A number of such cases eventually progress to hepatocellular carcinoma (HCC). Nevertheless, documentary evidence shows that chronically infected cases can also progress directly to HCC without the intervening cirrhotic stage, and only a fraction progress to HCC via cirrhosis. In addition to this, HCV is now associated with a number of extra-hepatic manifestations such as lichen planus, oral cancer, membranoproliferative glomerulonephritis, etc.
Presently, diagnosis of the viral infection is done by a commercially available third generation ELISA, RT-PCR and strip-based diagnostics that include RIBA, LIA, etc., which are reviewed here. However, it might be worth mentioning at this stage that although the procedures mentioned here are sensitive and specific for detecting HCV infection, lack of an universally accepted standard procedure resulted in the development of a number of in-house protocols and assays, review of which is beyond the scope of this article.

Initial euphoria about the discovery of HCV died down when it was realized that this virus is a fastidious organism and no cell-culture system could support the long-term replication of the virus. Moreover, chimpanzee being the only animal model restricts studies on this virus to a very few labs the world over. Despite this, a significant amount of data are available with regard to the molecular biology of the virus using bacterial cloning and expression systems. A summary of this information is presented as Tables 1, 2, Figs 1, 2 and is reviewed here.

**Molecular biology of the virus**

A schematic representation of the HCV genome organization is shown as Fig. 1. Based on the comparative analysis of the genomes and the protein profiles of HCV isolates, the virus is now recognized as a separate genus—*Hepadnaviruses*—under the family *Flaviviridae*. Other genera of this family include the Flaviviruses and the Pestiviruses.

Hepatitis C virus (HCV) is a single-stranded positive sense RNA virus. The nucleic acid is approximately 9.4 kb in length and possesses a unique open reading frame (ORF) that encodes for a polyprotein precursor of approximately 3010 amino acids in length. Comprehensive analysis of HCV isolates revealed differences in length of the nucleic acid, and diversities of around 30% in their whole genome, leading to the definition of at least six genotypes and over 80 subtypes.

![Schematic representation of genomic organization of HCV. Polyprotein and cleavage sites on polyprotein.](image)

**Note:** Recently an alternate reading frame (ARF) and an alternative reading frame protein (ARFP) concept has been put forward and partial evidence for the same documented. Depicted in this schematic representation is the original ORF concept and the polyprotein encoded by the same. [For more details, see Table 2]
Flanking the ORF on either side are the 5' untranslated region (UTR) and the 3' UTR. The 5' UTR is about 341 nucleotides in length, well conserved in almost all the isolates and forms extensive secondary structures guiding the ribosomes in translation. This secondary structure is termed the internal ribosome entry site (IRES) and allows viral genome expression using a cap-independent mechanism. Despite being relatively well conserved, the 5' UTR shows type-specific variations that can be successfully exploited for genotyping of the virus isolates. On the other hand, the 3' UTR is highly divergent and varies in length depending on the isolate. This region is composed of three characteristic elements: the conventional 3' UTR, subsequent poly U stretch and the newly identified 3' terminal sequence named the 3' X tail. It is believed that the 3' UTR might function in initiation and regulation of genomic replication of HCV.

The nascent viral polyprotein is cleaved, by a combination of host cellular and viral proteinases, into at least ten functional proteins – Core, E1 and E2 glycoproteins (gp), p7, NS2, NS3, NS4a, NS4b, NS5a, NS5b, in that order. The cleavage sites on the polyprotein, their products and functions are summarized in Table 2 and Fig. 1. The core, E1 and E2 proteins are the putative structural proteins of the virus; the non-structural ones include p7, NS2, NS3, NS4a, NS4b, NS5a and NS5b. Functions of these cleavage products have been speculated by sequence comparisons with other known viruses, and evidence supporting these speculations was demonstrated in bacterial systems expressing these genes. Particular
noteworthy is the establishment of the function of the NS5b protein. The sequence of the NS5b protein, in general, and the amino acid motif G-D-D in particular, is totally conserved not only in HCV but also in Flaviviruses, Pestiviruses, Polioviruses, Tobacco mosaic virus and most of the RNA viruses. This motif is characteristic of RNA-dependent RNA-polymerases, and hence the function of NS5b in HCV has been speculated to be the viral polymerase. Behrens et al., demonstrated experimental evidence in support of this.

Many viruses are reported to have overlapping reading frames (OLRF). This might also be true in the case of HCV. Partial scientific evidence in this direction comes from the work of Walewski and colleagues at Mount Sinai School of Medicine, New York, wherein they have shown the presence of a computer predicted alternate reading frame (ARF). The authors demonstrated the presence of an alternate reading frame protein (ARFP) using synthetic peptides corresponding to the predicted ARFP sequence. Results from this and their subsequent study are interesting. The possibility of more number of ARFs or OLRFs in the genome of HCV cannot be ruled out. If found true with regard to all the genotypes and the numerous reported isolates, and coupled with the present concept of a single ORF, the ARF concept might provide an explanation for many of the questions left unanswered with the ORF concept that include persistence of the virus in human host despite a robust humoral and cellular immune response. Although speculative and needs to be verified, the virus might be switching, during its replication and survival, between the ORF and ARF probably without a loss in function of the proteins encoded by either of the reading frames.

Evolution of immunodiagnostic assays

Immunoscreening with the serum of a chronic NANBH patient of a cDNA library, constructed using random primers, of the viral nucleic acid extracted from the plasma of a chimpanzee infected with the infectious agent eventually led to the discovery of the virus. This clone designated 5-1-1, formed the basis of development of reagents and methodologies essential for diagnosis of the HCV infection. Furthermore, this clone also formed the starting point for characterization of HCV. Subsequently, an antigen designated c100-3, a 363 amino acid (a.a.) fusion polypeptide representing part of the now designated NS4 protein of HCV, constructed from three overlapping clones of 5-1-1, and expressed in yeast along with super oxide dismutase (SOD), formed the first immunodiagnostic procedure for detecting anti-HCV antibodies in patient sera thereby diagnosing the viral infection. This was the so-called first generation immunoassay to be made available. Soon it became apparent that there are a number of false positives associated with this assay that could probably be attributed to non-specific binding of IgG or immune complexes to the solid-phase. It was also reported that false positives were common in patients with autoimmune-chronic active hepatitis, hypergammaglobulinaemia and in stored frozen sera. Lack of sensitivity of this assay was also reported.

This situation eventually led to the development of the second-generation assay that included the c22-3 antigen along with the c100-3, representing a.a. 2-120 of HCV polyprotein, and the region amino acid 1192-1931 (includes NS3 and NS4 proteins), designated c200 or a smaller fragment of this termed c33c (only NS3). This assay was found to provide significant improvements in both sensitivity and specificity compared to the first generation assay. Presently a third generation assay is available in the market that includes an additional antigen, the NS5 protein product (a.a., 2054-2955). Together with this antigen, 60% of the total a.a. profile of HCV polyprotein is represented in the present day commercial assays.

One of the rather improperly documented aspects of HCV infection is the host humoral immune response, which in part could be attributed to the lack of a reliable assay system that can detect various antibodies elicited to different proteins of the virus. To overcome these impediments synthetic peptides or clones of the proteins of HCV are being used. Whereas the information thus generated does provide useful insights into the pathobiology of the virus, it needs to be confirmed and might not be accurate.

Initial reports on antibody responses involved the popular c100-3 antigen, which represents a portion of the NS4 protein. However, with the establishment of improper diagnosis using this antigen and with the availability of full-length sequences of HCV, a more precise analysis of humoral response is being achieved with the aid of synthetic peptides. The humoral epitopes that are identified so far are shown schematically as Fig. 2. Also summarized in the figure are the different regions used in various different diagnostics, etc.
The availability of full-length sequences of HCV isolates from different parts of the world also established the presence of variability in the sequences of the envelope gp and the NS5 protein. In enveloped viruses, antibody responses to the envelope proteins play an important role in the prevention of infection, particularly re-infection or reactivation of the virus. Especially in flaviviruses and pestiviruses, anti-envelope gp antibodies are a measure of recovery or immunity against the respective viral infection. Should a similar situation exist in HCV infection, detection of anti-HCV envelope gp antibodies would be of immense value. However, there is contradictory evidence available with regard to the protective ability of the envelope antibodies of HCV. Whereas Farci et al. reported a lack of protective immunity against reinfection with HCV, neutralizing ability of the envelope antibodies was reported by Rosa et al. Nevertheless, significant majority of the infected patients (60%-90%) develop antibodies against E2 gp of HCV, which is noteworthy. While the presence of anti-E2 gp antibodies in a majority of the infected individuals makes it an indispensable diagnostic marker, the exact role of these antibodies in the viral infection, in vivo, needs a thorough investigation. At present, not all commercially available diagnostic kits incorporate representative sequences from the envelope proteins of HCV. It is believed that majority of the anti-E2 gp antibody response is directed against assembled/discontinuous epitopes on the envelope gp that can be detected using E2 gp purified from mammalian cells, which was not available until recently. To overcome this impediment, Poduri et al. have used linear epitopes, from the immunodominant regions of the virus, without a compromise in either the sensitivity or the specificity of detection of the viral infection, as compared to the commercially available assays. A noteworthy finding from these studies is that over-a-period of time, almost all the patients infected with the virus were found to harbor antibodies to all the immunodominant epitopes shown in Fig. 2. This finding suggests that in long-term disease status, such as cirrhosis and HCC, the anti-HCV antibody response is no longer neutralizing the infection. In addition to this, it is now well established that antibodies to the hyper variable region (HVR1) of E2 gp (immunodominant epitope 383-409; epitope 8 in Fig. 2) are isolate specific and might not be able to protect the individuals from reinfection and in chronic infections. Taken together these results by far suggest that the antibody response against the immunodominant epitopes in HCV might be non-neutralizing. One probable explanation for this could lie in the observation that HCV is associated with low-density lipoproteins (LDL) that might be masking the epitopes thereby making them inaccessible for antibody binding and neutralization. This being the situation with regard to the anti-HCV antibodies and their protective ability, cellular immunity has been reported to be protective in the host. Particularly note worthy is the observation that there seems to be a vigorous response towards the core and the e2 proteins in patients who recover from the acute infection. Nonetheless, it appears that such an effective CTL response is only for a short period and persistence of the viral infection probably leads to a decline in the antiviral control. The vigorous response mounted during acute infection apparently has a negative impact on the liver in the sense that CTLs are documented to have a dual role in infections involving hepatotropic viruses such as HBV and HCV. Based on these observations it is tempting to speculate that a few of the epitopes on Hepatitis C viral proteins particularly the Core and E2 gp might be mimicking some of the cell surface proteins of hepatocytes and this makes sense given the fact that molecular mimicry by viruses is now a well documented aspect of virology. Moreover, Hijioka et al. have shown the presence of defective naked capsid particles in chronically infected patients, which might be a viral decoy for subverting the antiviral response. Collectively, these observations suggest that immune response against the capsid protein of HCV at least is not protective. Nevertheless, in addition to what has been stated earlier, it is essential to clarify the exact protective ability of anti-envelope gp antibodies, as immune serum globulins (ISG) or polyclonal immune globulin preparations offer a safe, low-cost and effective means for preventing HCV infection particularly in post-operative/pre-transfusion settings.

Availability of full-length sequence information of HCV from various parts of the world allowed researchers to design peptide assays that in most of the cases are far superior to the above three generations of immunoassays for HCV. History of the development of synthetic peptide-based immunoassays was parallel to the development of the above-described three-generation immunoassays. Of the earliest immunoassays developed, the first synthetic peptide-based enzyme immunoassays (PBEIA) used peptides from the
capsid/core protein of HCV\textsuperscript{75,76}. Subsequently, numerous reports using either oligopeptides or overlapping peptides appeared in various journals. Apart from permitting the formulation of immunosorbents with high signal/cut-off ratio for maximum sensitivity and specificity, peptide assays are cost-effective.

In addition to ELISA/antibody based diagnostics, circulating antigen in the patient sera is detected by scoring for the presence of HCV-RNA by RT-PCR. It should be borne in the mind of the reader that the discovery of the virus was via RT-PCR. A number of labs all over the world developed their own in-house primers for RT-PCR based diagnosis of HCV infection. While 5' UTR of HCV-RNA is used for diagnosis and RFLP typing of the virus\textsuperscript{67,77,78}, other regions such as the Core\textsuperscript{80} and NS5\textsuperscript{81} are amplified using either type specific primers or universal primers, essentially with an aim at genotyping of the virus once differential diagnosis has been done. RT-PCR based diagnostics, in addition to being sensitive and accurate, offers quantification of the pathogen that is essential once a patient is put on treatment. Variants of PCR based diagnosis such as bDNA assays, etc., are available in the market. Put simply, to get a complete picture of the viral infection before a patient is given treatment and for monitoring the viral dynamics during and after the treatment, it is prudent to take into account the results of both antibody and RT-PCR tests. In spite of this, RT-PCR diagnosis is not routinely implemented in third world countries owing to either economic reasons or lack of necessary facilities or qualified personnel.

Presently opinion is divided as to whether there is any need for further improvement of the currently available 3\textsuperscript{rd} generation immunoassays. While in countries such as USA, there might not be any necessity to improve the test, for reasons already mentioned, stored tropical samples continue to give false positive results. If a patient is also infected with malaria, a tropical disease, widespread in this part of the globe, the person's serum sample tends to give false positive results\textsuperscript{42}. Adding to this is the variations in the genome of the virus observed in a given region, makes the development of better-suited assays for detection of this viral infection, imperative. In this context RT-PCR based testing yields distinct results, which however as stated earlier is not a feasible

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{Schematic representation of major immunodominant epitopes identified on HCV polyprotein RT-PCR: reverse transcription-polymerase chain reaction; UTR: Untranslated region; \(\alpha\) – \(\kappa\): major immunodominant epitopes identified. Amino acid position of epitopes (\(\alpha\) – \(\kappa\)) on HCV polyprotein - \(\alpha\): 7-25\textsuperscript{63,65,67,68,69,70}; \(\beta\): 35-63\textsuperscript{64}; \(\gamma\): 222-248\textsuperscript{64,65}; \(\delta\): HVR1, 383-409\textsuperscript{62}; \(\varepsilon\): 413-436\textsuperscript{64,65,66}; \(\phi\): 440-464\textsuperscript{64,65}; \(\chi\): 837-880\textsuperscript{64,65}; \(\eta\): 1188-1465\textsuperscript{64,65}; \(\xi\): 1696-1739\textsuperscript{64,65}; \(\varphi\): 1916-1944\textsuperscript{64}; \(\kappa\): 2263-2318\textsuperscript{64,65}.}
\end{figure}
alternative in third world countries wherein the facilities, particularly in the rural areas, many a time are not available.

Despite the fact that only ~70-94% of repeatedly HCV antibody positive patient samples score positive for HCV-RNA by RT-PCR\(^\text{82,83}\), HCV-RNA detection is many-a-time used to confirm HCV-antibody screening tests. This is where strip-based tests such as INNO-LIA, RIBA, etc., offer a reliable alternative\(^64\). Apart from the antigens used in the 3\(^\text{rd}\) generation ELISA, representative sequences from the E2 gp (a.a. 383-410, HVR1; epitope \(\delta\), Fig. 2) are also included in these strip-based tests, thereby, in certain situations, making them a feasible alternative to RT-PCR, even in the third world countries.

**Prevalence and genetic heterogeneity of HCV (with specific reference to Indian Subcontinent)**

Once a diagnostic assay is available, it is imperative to estimate the extent and magnitude of the viral infection in the population. While current estimates put the number of affected individuals at 170 million people worldwide, prevalence statistics for the Indian subcontinent range from as low as 0.34%\(^66\) to 2.5%\(^65,67,68\) (Table 3). In addition to this, a vast number of cases go unreported and the insufficiency of the currently available diagnostic immunoassays in the Indian context has been clearly demonstrated\(^65-67,66,68\).

Hepatitis C Virus is an RNA virus and still evolving with an estimated frequency of 1.4-1.9\(\times\)10\(^{-3}\) mutations per nucleotide per year\(^88,89\). Because of this, the virus displays significant sequence diversities in the entire nucleic acid, in general, and in the amino terminus of the E2 gene, termed the Hyper variable region (HVR), in particular. This is due to the lack of proof reading activity of RNA-dependent RNA polymerase of RNA viruses leading to a high frequency of nucleotide substitutions during replication. This genetic heterogeneity of HCV has been classified under two headings - Quasispecies and Genotypes. While the genetic heterogeneity of HCV population observed within a single individual is referred to as Quasispecies\(^66,67,88,90\), the term Genotype is applied to the genome heterogeneity observed among different HCV isolates. Owing to accumulation of mutations during evolution of these viruses, HCV isolates can be divided into distinct groups\(^35,26,34,50,51\). To date, at least six genotypes and close to 80 subtypes have been identified and more are in the process of being characterized. Hence the present classification of HCV is incomplete and more types and subtypes are likely to be added. HCV types are now designated by Arabic numerical in their order of discovery (e.g., 1, 2, 3, etc.), while the subtypes are designated in lower case letters, also in their order of discovery (e.g., a, b, c, etc.)\(^91\). Overall there is no preponderance of genotypes geographically and more than one genotype is prevalent in almost all the countries from where data is available. However, the current pandemic of HCV is dominated by type 1, which is arguably the most severe form of the virus.

### Table 3 — Prevalence statistics for hepatitis C virus (HCV) infection in different categories in India

<table>
<thead>
<tr>
<th>Category</th>
<th>Prevalence range</th>
<th>Cumulative total of individuals analyzed in different studies</th>
<th>Actual prevalence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>General population(^*)</td>
<td>0.34-2.5%</td>
<td>82228</td>
<td>0.70%</td>
<td>66, 67, 84, 85, 140, 143, 146, 147, 149, 150, 151</td>
</tr>
<tr>
<td>Patients with different liver disorders(^\dagger)</td>
<td>2.35-42.10%</td>
<td>4530</td>
<td>3.42%</td>
<td>66, 67, 138-141, 144-146, 152</td>
</tr>
<tr>
<td>Hospital staff</td>
<td>0.0-9.09%</td>
<td>155</td>
<td>6.45%</td>
<td>144, 146, 151</td>
</tr>
<tr>
<td>Patients visiting STD clinics</td>
<td>0.64-21.10%</td>
<td>616</td>
<td>9.60%</td>
<td>142, 148</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>87529</td>
<td>0.90%</td>
<td></td>
</tr>
</tbody>
</table>

\(^*\)Apparently healthy voluntary blood donors taken as general population  
\(^\dagger\)Includes primarily patients with acute hepatitis, chronic hepatitis, cirrhosis, and hepatocellular carcinoma  
\(^\ddagger\)Sum of all the individuals examined in the references cited  
\(^\ddagger\)Assuming homogeneity in the samples

In addition to the above, a prevalence of 7.89% was reported from Lisu community of Arunachal Pradesh\(^147\).
Presence of numerous genotypes and their variants pose problems both in effective diagnosis and management of the viral infection. This situation necessitates development of reagents and methodologies taking into account all the prevalent types in a given country. Consequently, the diagnostics developed by Panigrahi et al.\textsuperscript{87} and Poduri et al.\textsuperscript{83-87} attain significance, while specific therapeutic regimen taking into account the prevalent types in the country as well as the variants of the virus as Quasispecies in a given individual is yet to evolve.

**Hepatitis C virus as a causative agent of hepatocellular carcinoma (HCC)**

Viruses represent a major risk factor in a significant portion of human cancers. Whereas most of the viruses that cause cancer have DNA as their genetic material or have a DNA intermediate in their replication, HCV is a typical RNA virus with no DNA intermediate\textsuperscript{8}. Hence the possibility of integration of the viral genome is probably an unlikely event. Regardless of this, it is now well established that HCV is strongly linked to the development of hepatocellular carcinoma (HCC) thereby representing a major complication in patients chronically infected with the virus\textsuperscript{8,9,97}.

Quite a significant number of reports have associated HCV with HCC\textsuperscript{8,10,11,98-100}. Contrary to the earlier report that HCV might not be an etiologic factor among Indian patients\textsuperscript{101}, Poduri et al.\textsuperscript{83,87} demonstrated that one majority (75\%) of the HCC patients had active HCV replication, while in the rest the virus appears to be sequestered. Moreover, all the patients with active viral replication have detectable antibodies to all of the identified immunodominant epitopes. In those patients where the virus appears to be sequestered, the antibody response seems to be directed mostly to the epitopes on the core, E2 gp and the NS3 proteins of the virus. While these results suggest a probable association of HCV with HCC in Indian patients, it is tempting to speculate that these three proteins, viz. E2 gp, Core & NS3, have a role to play in hepatocarcinogenesis.

Although these studies associate HCV with HCC, the exact role of the virus or which viral proteins are involved in this process remains to be clarified. There being controversial evidence with respect to the involvement of the p53 gene or its product\textsuperscript{102-104}, the core and the NS3 proteins are now speculated to play a role in hepatocarcinogenesis\textsuperscript{105-107}. It was put forward that these viral proteins might exert their influence in hepatocarcinogenesis in a manner similar to the E6 protein of Human papilloma viruses (HPV) or the E1 protein of adenoviruses, that degrade the p53 gene product leading to tumor formation. In addition to this, Kane et al.\textsuperscript{108} have demonstrated the presence of high levels of inducible nitric oxide synthetase (iNOS) and nitric oxide in patients who are chronically infected with HCV. Kane et al. hypothesized that genomic instability results from prolonged exposure of hepatocytes to nitric oxide, a well-known mutagen. Subsequently, subversion of cellular repair mechanisms and apoptotic strategies by the viral proteins, such as the core and the NS3, leads to a malignant transformation eventually progressing to HCC. These hypotheses are put together as one possible disposition of HCV in inducing hepatocellular carcinoma in Fig. 3. Although researchers all over the world might turn their attention towards the ARF concept to clarify the role of the virus in HCC eventually, it would be premature at present to contemplate on the possible role of ARF/ARFPs in hepatocarcinogenesis.

**Future perspectives**

While a satisfactory prophylaxis is still not available, interferon either alone or in combination with ribavirin are the currently used drugs of choice. However, only about 11-30\% of the patients put on the treatment respond\textsuperscript{108}, and approximately 50-60\%...
of patients respond to combination therapy\textsuperscript{34,109} making search for alternative strategies imperative. A plausible direction in this regard is given by the observation that there is improper vertical transmission of the virus and the lack of an efficient cell culture system. It is now documented that there is an apparent suppression of liver damage during gestation\textsuperscript{110}. While this observation supports the concept that liver damage in hepatitis C infection is related to immune response\textsuperscript{111}, elevated levels of lactoferrin, a broad-spectrum immunomodulator, in gestating and lactating women\textsuperscript{112} might be the actual reason for the improper vertical transmission of the virus. Supporting this statement is the documentation that lactoferrin binds HCV\textsuperscript{113} and markedly inhibits the viral replication in cultured cells\textsuperscript{114,115}. Ongoing trials for treating HCV infected patients with lactoferrin are showing promising results\textsuperscript{116-118}. The small amount of lactoferrin present in fetal bovine serum (FBS) used, might in fact be inhibiting the viral replication in cell cultures. In this context, HCV replicons\textsuperscript{119} and the cell free systems\textsuperscript{120,121} are bound to make an impact. It was recently demonstrated that the success achieved with regard to HCV replicons and identification of mutations that permit HCV replication\textsuperscript{122} in cell cultures need not necessarily apply to the \textit{in vivo} infectivity in chimpanzees\textsuperscript{123}. A probable explanation for this could lie in the observation that the HCV replicons are exceedingly artificial. HCV replicons were designed to be bicistronic resulting in translation of the first cistron (neomycin phosphotransferase) being directed by HCV-IRES and translation of the second cistron by encephalomyocarditis virus (EMCV) IRES. Additionally, these molecules were derived from an HCV consensus genome. Nevertheless, this system is greatly suitable for drug screening as all the known HCV enzymes are encoded by this system.

An alternative approach in treating HCV induced HCC lies in the observation that mice immunized with viral peptides complexed to heat shock protein (HSP-70) resulted in protective immunity and specific cytotoxic T-lymphocytes (CTL)\textsuperscript{124,125}. Consistent with these observations in mice, it is reported that cancer patients immunized with autologous cancer derived heat shock protein preparations develop anti-tumor immunity\textsuperscript{126}. Moreover, HSP-peptide complexes can be reconstituted \textit{in vitro} to elicit peptide-specific CTL response as well as anti-tumor immunity\textsuperscript{127}. Taken together these findings clearly suggest that preparations of HSP-peptide complexes is an alternative and viable option for combating viral infections where patients do not respond to the conventional strategies that are being currently used.

While the above two strategies take advantage of the host defense mechanisms, active research targeting viral proteins or stages in the viral replication is on-going around the world. Significant among these include ribonucleoside analogs\textsuperscript{128}, ribozymes\textsuperscript{129}, antisense RNA\textsuperscript{130}, protease inhibitors\textsuperscript{131}. Among those that are in the pipeline, include Glycyrrhizin, a triterpene glycoside and extract of licorice root, in combination with interferon\textsuperscript{132}. This herbal preparation is known to have antiviral activity. It is used routinely in the traditional medicine in the eastern parts of the world to treat diabetes and nephritis. In addition to these, research on the protective ability of immune serum globulin (ISG) or polyclonal immune globulin preparations offer a viable substitute particularly in post-operative/pre-transfusion settings. Whereas the discovery of CD81 as a possible receptor for entry of the virus into cells\textsuperscript{133} does definitely provide an explanation to the extra-hepatic manifestations observed, identification of the actual receptor responsible for hepatotropism should expedite the drug discovery process with regard to this viral infection, apart from enabling researchers in reassessing the efficiency of the currently available therapeutic regimen. Identification of cell culture systems that support HCV replication\textsuperscript{119,120,121,122,134,135} and development of the chimeric mouse-model\textsuperscript{136} should also aid in this process.

**Conclusion**

With the current pandemic of HCV affecting about 170 million people worldwide, emphasis should be mainly on evaluating the actual role of anti-E2 gp antibodies, as these form an indispensable diagnostic marker, probably might also provide an insight to the disease progression. Additionally, it can be predicted that a significant proportion of those affected eventually develop HCC. Hence elucidating whether HCV exerts its influence directly or indirectly in the development of HCC becomes imperative.

Recent noteworthy developments such as identification of CD81 as the possible receptor for entry of the virus into the cells\textsuperscript{133}, identification of cell culture systems\textsuperscript{119,122,134}, the ARF concept\textsuperscript{17,38} and the development of a mouse model system\textsuperscript{36} should aid in delineating a number of hypotheses that are in circulation in the literature. Coming years will witness development of novel and much better therapeutics that might include polyherbal preparations.
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References


