Hepatitis C virus: The Indian scenario

M. N. Khaja*, S. K. Munpally†, M. M. Hussain* and C. M. Habeebullah*,#

*Centre for Liver Research and Diagnostics, Deccan College of Medical Sciences and Allied Hospitals, Kanchanbagh, Hyderabad 500 058, India
†Centre for Plant Molecular Biology, Osmania University, Hyderabad 500 007, India

Hepatitis C virus (HCV), an RNA virus, is now well established to be heterogeneous in nature, showing multiple genotypes and subtypes, with the basic structure and genome organization being conserved. Transmitted by parenteral and nosocomial routes, infection with this virus is the leading cause of chronic liver disease. There being no vaccine and the current treatments successful only up to 11–30%, hepatitis C is rarely diagnosed until its chronic stages, when it can cause severe liver damage. In the present article we review the currently available information with regard to molecular biology, pathobiology and epidemiology of HCV infection. Also included in the review are currently approved therapeutic interventions, and strategies for the control of the viral infection. In addition, we also review the work done on this virus, particularly with reference to diagnosis and genotyping of the virus in India. Finally, we explore the possible approaches towards developing an effective vaccine against HCV.

VIRAL hepatitis, caused by any of the six hepatotropic viruses, viz. hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV) and hepatitis G virus (HGV), represents a major health problem worldwide. Among these, HCV is now established to be the major causative agent of post-transfusional Non-A, Non-B hepatitis (PT-NANBH)[1]. With approximately 170 million people worldwide estimated to be infected with HCV, a figure that is four times the HIV infection status, HCV has the potential to be the next pandemic.

HCV, now documented to be heterogeneous in nature, belongs to the genus *Hepaciviruses* and family Flaviviridae[2,3]. Despite significant progress in the field of biotechnology, reliable diagnostic procedures, an alternative animal model other than the chimpanzee, an efficient cell culture that can support long-term replication of the virus and effective therapeutic strategies are still lacking. In spite of this, a significant amount of information with regard to the molecular biology of the virus is available using bacterial cloning and expression systems, which is reviewed here. Also included in this review are the pathobiology, diagnosis, treatment and strategies for control of the viral infection.

#For correspondence. (e-mail: cmhabib@rediffmail.com)

Molecular biology

The genome of HCV comprises a single-stranded positive-sense RNA of approximately 9.6 kb in length and contains a single open reading frame (ORF) that encodes for a non-functional polyprotein of approximately 3000 amino acids in length[4]. This non-functional polyprotein is cleaved co- and post-translationally by cellular and viral proteases to yield at least ten different functional protein products. Structural proteins are the major components of the mature virion, which are coded in the 5′ quarter of the ORF and arranged as C–E1–E2 and p7, while the non-structural proteins are encoded in the 3′ three-quarters of the ORF in the order NS2–NS3–NS4A/B–NS5A/B (Figure 1). These are involved in polyprotein processing and replicative functions of the virus[5,6] (Table 1). The envelope glycoprotein E2 exhibits a great degree of heterogeneity, both in the nucleic acid and protein sequence[7,8]. This region is termed the hyper-variable region-1 (HVR-1) of HCV. In infected individuals, quasi-species arise mainly by accumulating mutations in the HVR-1 induced by strong immune pressure[9]. Despite this genetic heterogeneity, the structure of the glycoprotein is preserved indicating the importance of this region for virus survival[10,11]. However, recently it was demonstrated that deletion of the HVR-1, although did not restrict the outcome of infection, nevertheless, affected virus replication as observed by low levels of virus especially in the early weeks of infection[12].

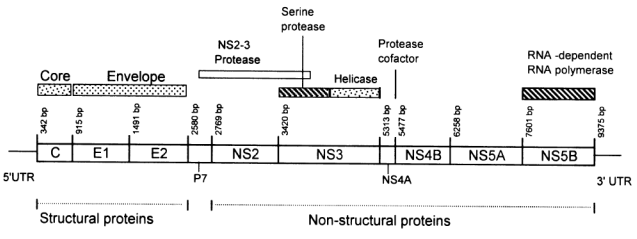

Figure 1. Schematic of HCV genome organization showing the location of HCV genes and proposed functions of gene products. 5′ and 3′ non-coding regions (NCR) are indicated. Numbering refers to nucleotide positions of genes, based on the sequence of HCV genotype.

The 5′ UTR (untranslated region) of HCV-RNA is highly conserved and forms a secondary structure that guides the ribosomes for translation. This secondary structure is termed the internal ribosome entry site (IRES). Although the 5′ UTR is highly conserved, it shows variations which are type-specific and can be exploited for typing of the virus.

HCV being an RNA virus exhibits a remarkable degree of heterogeneity throughout the length of its nucleic acid[13]. Traditionally, this heterogeneity has been classified as (i) quasi-species and (ii) genotypes[14]. The term quasi-species is applied to the heterogeneity in the nucleic acid sequences of the virus isolated from a single patient, while the term genotypes refers to the differences in the sequences that are observed among isolates from different patients. This heterogeneity arises as a consequence of lack of proof-reading activity of RNA-dependent RNA polymerase during replication of the virus. Over a period of time, these mutations get accumulated at specific regions, leading to what are known as the genotypes. To date at least six genotypes and more than 120 subtypes of the virus have been identified, and more are in the process of being characterized. Hence the present classification of HCV is incomplete, as information from a number of isolates is still awaited.

Identification of these genotypes is done by at least six methods, discussed later in the article, while the clinical significance of the genotypes and quasi-species with respect to treatment are also discussed. Geographic distribution of various genotypes is given in Table 2, while data on genotyping studies from India are summarized in Table 3. From Table 3 it can be observed that numerous subtypes of genotype 3 are found in India, a pattern reflective of long-standing endemic infection. Contrary to this, although one or more types are present in other parts of the world, each is represented by few subtypes, suggestive of recent transmissions.

Table 1. Function and size of the cleavage products of HCV polyprotein

Cleavage product	Size (kDa)	Function
Core	21	Structural, forms the viral nucleocapsid; Oncoprotein (?)
E1	31	Structural, forms heterodimer with E2 gp; forms envelope of the virus
E2	70	Structural, forms heterodimer with E1 gp; forms envelope of the virus; mutations in this region determine the response to IFN
P7	7	Unknown
NS2	23	Forms autoprotease with NS3
NS3	70	Serine protease, Oncoprotein (?)
NS4a	8	Protease cofactor
NS4b	27	Unknown
NS5a	56	IFN sensitivity-determining region
NS5b	68	RNA-dependent RNA polymerase

gp, Glycoprotein; IFN, Interferon. Other abbreviations carry their usual significance.

Pathobiology of the virus

Natural history, clinical profile and hepatic manifestation

Natural history of hepatitis C and related data are limited, as the onset of disease is indolent and protected in many individuals (Figure 2). The natural history of this disease appears to differ according to geography and alcohol use. HCV-RNA is detected in the blood after 1–3 weeks by polymerase chain reaction (PCR). Virtually all patients develop liver cell injury as shown by raised alanine amino transaminase (ALT) levels. A majority of patients are asymptomatic and anicteric. Only 25–40% of the patients develop malaise, weakness and anorexia, and some become icteric[15]. Antibodies to HCV almost invariably become detectable during the course of illness. Anti-HCV can be detected in 50–70% of the patients at the onset of disease symptoms and in approximately 90% of the patients three months after the onset of infection. HCV is self-limited in only 15% of the patients. Recovery is characterized by disappearance of HCV-RNA and

Table 2. HCV genotype distribution in different parts of the world

Genotype (and its variants)*	Geographic region
1	North and South America; Europe
2	North and South America
3	Nepal, Bangladesh, Pakistan and South Asia
4	Egypt
5	Central and South America
6	China, Japan, and Southeast Asia

*Nomenclature of genotypes, as proposed by Simmonds *et al.*[61]. For genotypes prevalent in India, see Table 3. In addition to the genotypes mentioned above, a number of novel isolates have been reported from various parts of the world, that are in the process of being characterized and hence are not mentioned in the table.

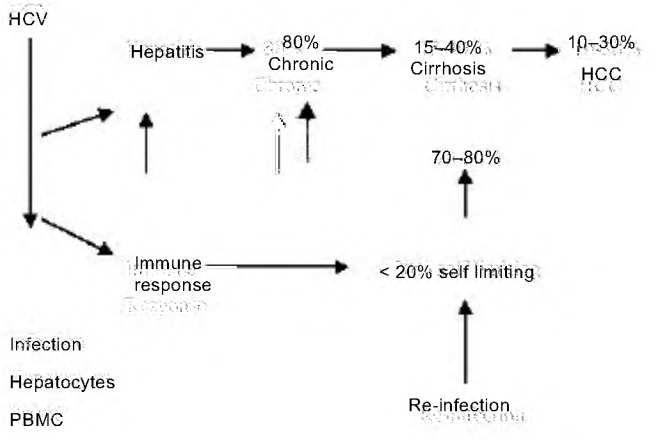

Figure 2. Natural history of hepatitis C virus infection.

normalization of ALT. About 85% of the HCV-infected individuals fail to clear the virus by six months, and develop chronic hepatitis with persistent and sometimes intermittent viremia[16,17]. A majority of patients have elevated ALT levels that can fluctuate widely. However, antibodies to HCV or circulatory HCV-RNA can be demonstrated in virtually all patients. In chronic hepatitis C, inflammatory cells infiltrate the portal tracts and parenchyma cells. The margin of the parenchyma and portal tracts may become inflamed, leading to liver cell necrosis. When the disease progresses, the inflammation and liver cell death may lead to fibrosis; such fibrosis can progress to cirrhosis in 20% of the patients within two decades of the onset of infection. Once cirrhosis is established, complications can ensue that are secondary to liver failure such as jaundice, ascites, variceal haemorrhage and encephalopathy.

Extra-hepatic manifestations

Many extra-hepatic manifestations, such as lichen planus, oral cancer, porphyria cutanea tarda, membrano-glomerulonephritis, etc. have been documented to be associated with the viral infection. In addition, autoimmune diseases, viz. autoimmune thyroiditis and mixed cryoglobulinemia are also found to be associated with HCV infection[18–21]. Although HCV is primarily a hepatotropic virus, its association with extra-hepatic manifestations and autoimmune diseases is still not clear. A probable explanation might lie in the observation that CD81 is now documented to be a receptor for entry of this virus into the cells[22]. It may be noted at this stage that CD81 is a molecule that is expressed by many of the cells, resulting in chance, non-specific binding of the virus to different cells. Once inside the cells, availability of the necessary components aids in effective replication of HCV, which probably results in the manifestations observed.

While autoantibody response in patients infected with HCV has been documented, this aspect of HCV pathobiology is still the grey area and requires more studies in this direction. Despite this, it is tempting to speculate that the virus might be mimicking host cellular proteins, resulting in the development of autoantibody response, thereby manifesting in autoimmune disorders mentioned above.

Whatever might be the reason for the extra-hepatic manifestations, further work would be essential to delineate whether the virus exerts its influence directly or indirectly in these disorders.

Epidemiology, mode of transmission and prevalence

HCV is transmitted primarily through the parenteral route and sources of infection include injection, drug abuse, needle-stick accidents and transfusion of blood and blood products, with transmission by sexual routes being debatable. Several studies suggest that HCV transmission by sexual contact is 10–20% in the US. However, Alter[23] suggests that this might be a predominant route as 80% of the population is heterosexual, which makes sense, as a large number of people are chronically infected. Dentists practising oral surgery, practitioners of folk medicine and those involved in hairdressing, ear-piercing and tattooing are also at higher risk for HCV. We have evaluated the risk factors associated with HCV infection among 161 patients who had reported at our centre. Among the risk factors evaluated, renal transplant and/or haemodialysis, and surgery six months prior to infection were predominant, with blood transfusion and multiple sexual contacts being additional factors.

HCV is the major cause of post-transfusion NANB hepatitis. However, patients with chronic renal failure (CRF), haemophilia and thalassaemia are at risk of developing transfusion-related NANB virus because of

Table 3. Region-wise distribution of HCV genotypes in India

Place	No. of samples analysed	Method	Genotype reported/found*	Reference
Delhi	11	Direct sequencing	1b, 3a, 3b, 3g	62
Mumbai	5	PCR-based typing	3, 4 and 5	63
Mumbai	61	Direct sequencing	1a, 1b, 2a, 2b, 2c, 3a, 3b, 3c, 3d, 3f, 3g	64
Hyderabad	45	Type-specific PCR amplification of *NS5* gene of HCV-RNA	1a, 1b, 3b	Ongoing study
Chennai	32	Direct sequencing	1b, 3b and novel isolates	65
Thiruvananthapuram	11	PCR–RFLP and direct sequencing of 5′ UTR	3b	Poduri and Das 2001 (unpublished data; pers. commun.)

*Nomenclature of genotypes, as proposed by Simmonds *et al.*[61].
Although limited, the above table clearly shows that in addition to novel types, almost all the reported genotypes of HCV are present in India. Thus monitoring of HCV genotypes in India on a regular basis becomes imperative. Furthermore, data from other parts of the country are awaited.
Note that numerous variants of type 3 are found in India, representative of endemic infection over a long period.
In addition to the data presented in the table, *full-length sequence* from a single isolate of HCV from an infected Indian patient has been deposited in the Genebank which bears the *accession number AY051292*.

repeated blood transfusions and multiple medical interventions. HCV infection is detected in more than 90% of the patients with post-transfusion NANB hepatitis, 60–80% of haemophilic patients receiving replacement therapy and 60–70% chronic liver disease patients with history of blood transfusions[24].

HCV infection in voluntary blood donors ranges between 1.85% (New Delhi)[25] and 2.5% (Hyderabad, unpublished data). The prevalence of HCV around the world ranges from 0.2 to 2%. Infectivity rates vary widely between 0.3 and 24.5%. There is a relatively low prevalence of HCV antibodies in blood donors from the US and northern Europe, including the UK, France and Germany[26–29]. Higher prevalence rates have been found in South-east Asian countries, such as Thailand, Malaysia and India[30–32]. Even higher prevalence rates between 1.4 and 2.1% have been observed in northern African and Arabian countries, such as Libya, Yemen, Saudi Arabia and Ethiopia[33–36]. The highest prevalence has been reported in Ukraine and in the Central African countries of Gabon and Cameroon, as well as in Egypt[37,38]. In Egypt the prevalence of HCV is highest in various regions ranging from 6 to 28% because the use of parenteral antihistosomal therapy[39].

Diagnosis

As with any disease, an accurate diagnosis of HCV infection is essential before patients are counselled and treatment is initiated. Since the identification and molecular characterization of HCV in 1989 by Choo and colleagues[1], a variety of diagnostic tests based on the detection of either the anti-HCV antibodies or HCV-RNA in patient sera have been developed.

Presently, a third-generation ELISA that incorporates antigens from the core, NS3, NS4 and the NS5 proteins of HCV, representing about 60% of the total amino acid profile of HCV polyprotein, is available in the market[40]. Although this ELISA is significantly sensitive, a major drawback of this assay is that it fails to differentiate between active and post-infection cases. In addition to this, it is now well documented that the commercially available third-generation ELISA cannot be used to detect the viral infection in Indian patients owing to genotype variations[41–44]. It may be noted at this stage that the commercial third-generation ELISA is based on genotype 1 and that the genotype-specific antibody response in this virus is now documented[45]. To overcome this problem, sensitive diagnostic peptide-based enzyme immunoassays that are cost-effective and designed to detect HCV infection in Indian patients have been developed[43,44,46]. Presently, an indigenous peptide-based HCV EIA kit is available (Xcyton™, Bangalore, India). Significance of detection of HCV-RNA in patient sera by RT–PCR lies in the observation that a positive result in RT–PCR is indicative of active viral replication. A significant contribution in this direction is the sensitive RT–PCR method developed by Das *et al.*[41] taking into account the sequence variations of the prevalent strains of the virus in India. Despite this, ELISA-based testing is regularly adopted for its relative ease of implementation, automation and cost-effectiveness.

In addition to these two assays, a recombinant immunoblot assay (RIBA) is available and is also designed to detect antibodies in the patient sera. Antigens from all the reported proteins of the virus are included in this assay. This test is essentially designed to confirm the results obtained in the third-generation ELISA.

Once a patient is found to be positive for HCV-RNA by RT–PCR, genotyping of the virus comes into play. Genotyping of the virus is essential as some types of the virus, particularly type 1b, have been clearly demonstrated to be resistant to the currently available therapeutic interventions. Genotyping of the virus is currently done by at least six methods, apart from direct sequencing of the viral nucleic acid. These include: (1) RFLP typing of the 5′ UTR; (2) Type-specific PCR amplification of the core gene; (3) Type-specific PCR amplification of the *NS5* gene; (4) Serotyping using type-specific peptides from the NS4 region of HCV polyprotein; (5) InnoLiPA (Line Probe Assay) using type-specific probes, and (6) Direct sequencing of the *NS5B* gene.

Details of the prevalent genotypes reported from India along with the method adopted are given in Table 3. Although limited, Table 3 clearly shows that in addition to novel types, almost all the reported genotypes of HCV are present in India. Thus monitoring of HCV genotypes in India on a regular basis becomes imperative. Furthermore, data from other parts of the country are awaited. Note from Table 3 that numerous variants of type 3 are found in India, indicative of a long-standing endemic infection with this genotype in the country. In addition to the data presented in Table 3, full-length sequence from a single isolate of HCV from an infected Indian patient has been deposited in the GenBank with accession number AY051292, which shows significant homology with genotype 1 of HCV.

Treatment and strategy for control

Although a decade has elapsed since its discovery, no satisfactory therapy for management of HCV infection is available. However, patients infected with this virus are now being treated with either interferon-2α (IFN-2α) alone or in combination with Ribavirin[47], and these are the only approved drugs of choice. However, only 11–30% of the patients respond to this treatment[48].

The standard dosage regimen with the above-mentioned combination of drugs includes 0.5–3.0 mg IFN-2α/kg body weight thrice a week plus 800 mg Ribavirin daily, for at least 45–52 weeks.

Modified forms of IFN, such as Pegylated IFN, etc. are available and have been shown to achieve a more sustained virologic response in chronic hepatitis C patients, particularly in those infected with genotype 1 (ref. 49). Addition of polyethylene glycol (PEG) to IFN produces a product that has significantly longer half-life and favourable pharmacokinetics. This pegylation of IFN reduces the dosage to once a week instead of thrice a week as with normal IFN. Similar modifications of IFN are being tested both in India and the rest of the world.

The poor response towards IFN either alone or in combination with Ribavirin raises questions as to what could be the probable reasons for the failure. It was found that the *E2* and *NS5A* gene products as well as mutations in the corresponding regions of the HCV-RNA might be the determining factors for response to IFN. More mutations at the *E2* gene are associated with a poor response to IFN. Conversely, more mutations at the *NS5* gene are associated with a better response to IFN[50–54]. It is believed that the products of these two genes interact with IFN-inducible PKR protein kinase and repress its activity, thereby subverting the downstream process of IFN action[51,54].

Until effective vaccine and therapeutic strategies are developed, it would be prudent to prevent the spread of this viral infection. As this virus is transmitted primarily by parenteral and nosocomial routes, stringent screening of blood and related products decreases the risk of PT-NANBH. Adopting universal precautions in hospital and health care settings prevents the nosocomial spread of the virus. Implementing the above-mentioned procedures significantly decreased nosocomial infections in developed countries. However, as stated earlier, the mode of acquisition in approximately 50% of the cases is still not known. Probably these can be attributed to cultural practices that involve body-piercing as in tattooing, ear-piercing, etc. If this were found to be true, then educating and creating awareness among the masses about the consequences of such practices may partly control the spread.

Future perspectives

Infection with HCV is unique and represents an enigma to the clinician for the simple reason that majority of the patients remain asymptomatic, with fluctuating liver enzyme levels. Thus, rather than developing novel therapeutic strategies, development of a vaccine that can prevent the viral infection should be of paramount importance. A major impediment in this direction is the variation in the genome of HCV observed either as quasi-species or as genotypes. While the discovery of CD81 as a possible receptor for HCV is noteworthy, identification of the actual receptor for entry of the virus into hepatocytes, which is responsible for the hepatotropism of HCV, would partly help in overcoming the above-mentioned obstacle. However, many of the viruses have multiple binding sites both on the target cells as well on the viral envelope. If this were also found to be true in the case of HCV, and coupled with the observation that HCV has a high mutation rate, development of an effective vaccine represents a challenge to human kind. It may be noted at this stage that binding of the virus to the cellular receptor requires glycosylation of the viral proteins and involves a carbohydrate moiety during this process. Thus targeting potential N-linked glycosylation sites on the viral structural proteins, particularly the envelope proteins, makes for an effective strategy against the virus[55]. In addition to this, many members of the Flaviviridae family, to which HCV also belongs, may enter the cell by binding to low-density lipoprotein (LDL) receptors. Recently, Agnello *et al.*[56] have demonstrated a direct correlation between the level of cell surface-expressed LDL receptor and the number of HCV-RNA positive cells. Furthermore, HCV particles are observed to be associated with beta-lipoproteins[57]. Taken together, these results also suggest LDL-receptors as possible sites of entry of the virus into the cells. However, it remains to be seen whether interaction of HCV with LDL-receptor or CD81 leads to a productive infection.

Recently, a cell culture model for HCV replication has been reported[58,59]. This should provide vital insights into understanding HCV-RNA replication and the role of various encoded proteins, apart from serving as an antiviral drug-screening system. A major breakthrough was achieved in August 2001 by David *et al.*[60] with the development of a mouse model that helps in studying HCV *in vivo* and also in the development of therapeutics and possibly a vaccine. Researchers should exploit these two models in exploring the potential of anti-HCV activity of preparations used in the Indian systems of medicine, particularly with specific reference to phyllanthus extracts which have shown promise with regard to HBV. Furthermore, the above-mentioned models should also help in reassessing the viral factors that are responsible for poor response to the currently available therapeutic intreventions.

Conclusions

Compared to the progress made in Western countries, research in the Indian subcontinent is going on at a slow pace. In spite of the fact that HCV continues to be a major threat to the Indian population, mandatory blood screening is not rigorously implemented. Till recently, complete cloning and sequencing of even a single Indian isolate of HCV has not been done. Guntaka *et al.*[66] have recently completed the sequencing of one isolate of HCV from an infected Indian patient. Complete coding sequence can be retrieved from GenBank using Accn No. AY051292. This information should make it possible to

speed up research on Indian species and prevailing quasi-species of HCV.

1. Choo, Q. L. *et al.*, *Science*, 1989, **244**, 359–362.
2. Rice, C. M., *Flaviviridae: The Virus and their Replication in Virology* (eds Fields, B. N. *et al.*), Lippinocott-Raven, Philadelphia, 1996, p. 931.
3. Mayo, M. A. and Pringle, C. R., *J. Gen. Virol.*, 1998, **79**, 649–657.
4. Major, M. E., Feinstone, S. M., Contal, P., Janot, C. and Treiff, F., *Pathol.-Biol. Paris*, 1993, **41**, 149–153.
5. Thomson, M. and Jake Liang, T., in *Biomedical Research Reports: Hepatitis C* (eds Jake Liang, T. and Hoofnagle, J. H.), Academic Press, 2000, pp. 1–24.
6. Reed, K. E. and Rice, C. M., *Curr. Top. Microbiol. Immunol.*, 2000, **242**, 55–84.
7. Simmonds, P., *J. Hepatol.*, 1999, **31**, 54–60.
8. Farci, P. and Purcell, R. H., *Liver Dis.*, 2000, **20**,103–126.
9. Forns, X., Purcell, R. H. and Bukh, J., *Trends Microbiol.*, 1999, **7**, 402–410.
10. McAllister, J. *et al.*, *J. Virol.*, 1998, **72**, 4893–4905.
11. Smith, D, B., *J. Viral Hepatol.*, 1999, **6**, 41–46.
12. Forns, X., Thimme, R., Govindarajan, S., Emerson, S. U., Purcell, R, H, Chisari, F. V. and Bukh, J., *Proc. Natl. Acad. Sci. USA*, 2000, **21**, 13318–13323.
13. Okamoto, H., Kojima, M., Okada, S., Yoshizama, H., Iizuka, H. and Tanaka, T., *Virology*, 1992, **190**, 894–899.
14. Martell, M. *et al.*, *J. Virol.*, 1992, **66**, 3225–3229.
15. National Institute of Health Consensus Development Conference Panel Statement, *Hepatology*, (Suppl. 1), 1997, **26**, 23–103.
16. Alter, M. J. *et al.*, *N. Engl. J. Med.*, 1999, **341**, 556–562.
17. Conry-Cantilena, C. *et al.*, *ibid*, 1996, **334**, 1691–1696.
18. Monti, G. *et al.*, *Q. J. Med.*, 1995, **88**, 115–125.
19. Monteverde, A., Ballare, M. and Pileri, S., *Springer Sem. Immunopathol.*, 1997, **19**, 99–110.
20. Johnson, R. J. *et al.*, *Kidney Int.*, 1994, **46**, 1700–1704.
21. Stehamn-Breen, C., Alpers, C. E., Fleet, W. P. and Johnson, R. J., *Nephron*, 1999, **81**, 37–49.
22. Pileri, P. *et al.*, *Science*, 1998, **282**, 938–941.
23. Alter, M, J., Cited from the John Ohen – *The Scientific Challenge of Hepatitis C Virus*, 1999, **285**, 26–30.
24. Zuckerman, A. J., *Br. Med. J.*, 1984, **299**, 871–873.
25. Panigrahi, A. K., Panda, S. K., Dixit, R. K., Rao, K. V. S., Acharya, S. K., Dasarathy, S. and Nanu, A., *J. Med. Virol.*, 1997, **51**, 167–174.
26. Aymard, J. P., Botte, C., Contal, P., Janot, C. and Treiff, F., *Pathol.-Biol. Paris*, 1993, **41**, 149–153.
27. Murphy, E. L. *et al.*, *JAMA*, 1996, **275**, 995–1000.
28. Mutimer, D. J. *et al.*, *J. Viral Hepatol.*, 1995, **2**, 47–53.
29. Shakil, A. O. *et al.*, *Ann. Intern. Med.*, 1995, **123**, 330–337.
30. Suwanagool, S., Tieangrim, S., Ratasanuwan, W., Mekanantangosol, S., Leungrojankul, P. and Kunasol, P., *J. Med. Assoc. Thailand*, 1195, **78**, 611–617.
31. Irshad, M., Archarya, S. K. and Joshi, Y. K., *Indian J. Med. Res.*, 1995, **102**, 162–164.
32. Duraisamy, G., Zuridah, H. and Ariffin, M. Y., *Med. J. Malays.*, 1993, **48**, 313–316.
33. Al-Faleh, F. Z. *et al.*, *Ann. Trop. Med. Parasitol.*, 1995, **89**, 431–437.
34. El Guneid, A. M., Gunaid, A. A., O'Neill, A. M., Zureikat, N. I., Coleman, J. C. and Murray-Lyon, I. M., *J. Med. Virol.*, 1993, **40**, 330–334.
35. Frommel, D., Tekle-Haimanot, R., Berhe, B., Verdier, M., Preux, P. M. and Denis, F. A., *Am. J. Trop. Med. Hyg.*, 1993, **49**, 435–439.
36. Saleh, M. G., Pereira, L. M., Tibbs, C. J., Ziu, M., Al-Fituri, M. O., Williams, R. and McFarlane, I. G., *Med. Hyg.*, 1994, **88**, 292–294.
37. Delaporte, E. *et al.*, *Trans. R. Soc. Trop. Med. Hyg.*, 1993, **87**, 636–637.
38. Kowo, M. P., Goubau, P., Ndam, E. C, Njoya, O., Sasaki, S., Seghers, V. and Kesteloot, H., *ibid*, 1995, **89**, 484–486.
39. Franc, C. *et al.*, *Lancet*, 2000, **91**, 355–388.
40. Hiroshi Aoki, Toshiki Yamamoto, Kazunori Kajino, Yasuyuki Arakawa and Okio Hino, *Intervirology*, 1996, **15**, 19–25.
41. Das, M. R. *et al.*, *Curr. Sci.*, 1993, **65**, 477–483.
42. Panigrahi, A. K., Nanda, S. K., Dixit, R. K., Acharya, S. K., Zuckerman, A. J. and Panda, S. K.,. *J. Med. Virol.*, 1994, **44**, 176–179.
43. Poduri, C. D., Khanna, A., Sugunan, V. S. and Das, M. R., *Immunologist*, (Suppl. 1), 1998, **6**, 429.
44. Poduri, C. D. *et al.*, *Acta Virol.*, 2001, **45**, 1–6.
45. Bhattacherjee, V. *et al.*, *J. Gen. Virol.*, 1995, **76**, 1737–1748.
46. Panigrahi, A. K., Nayak, B., Dixit, R., Acharya, S. K. and Panda, S. K., *Trop. Gastroenterol.*, 1998, **19**, 105–107.
47. Hoofnagle, J. H., *Digestion*, 1998, **59**, 563–578.
48. Neumann, A. *et al.*, *Science*, 1998, **282**, 103–107.
49. Manns, M. P. *et al.*, *Lancet*, 2001, **358**, 958–965.
50. Enomoto, N. *et al.*, 1996, *N. Engl. J. Med.*, **334**, 77–81.
51. Gale, M. J. Jr. *et al.*, *Virology*, 1997, **230**, 217–227.
52. Pawlotsky Jean-Michel, Germanidis Georgios, Neumann Avidan, U., Pellerin Muriel, Frainais Pierre-Olivier and Dhumeaux Daniel, *J. Virol.*, 1998, **72**, 2795–2805.
53. Polyak, S. J. *et al.*, *ibid*, 1998. **72**, 4288–4296.
54. Taylor, D. R., Shi, S. T., Romano, P. R., Barber, G. N. and Lai, M. M., *Science*, 1999, **285**, 107.
55. Rumenapf, T., Unger, G, Strauss, J. H. and Thiel, H. J., *J. Virol.*, 1993, **67**, 3288–3294.
56. Agnello, V., Abel, G., Elfahal, M., Knight, G. B. and Zhang, Q. X., *Proc. Natl. Acad. Sci. USA*, 1992, **96**, 12766–12771.
57. Thomssen, R., Bonk, S., Profe, C., Heermann, K. H., Kochel, H. G. and Uy, A., *Med. Microbiol. Immunol.*, 1992, **181**, 293–300.
58. Lohamn, V., Korner, F., Koch, J. O., Herian, V., Theilmann, L. and Bartenschlager, *Science*, 1999, **285**, 110–113.
59. Blight, K. J., Alexander, A. K. and Rice, C. M., *Science*, 2000, **290**, 1972–1974.
60. David, F. M. *et al.*, *Nature Med.*, 2001, **7**, 927–933.
61. Simmonds, P. *et al.*, *Hepatology*, 1994, **19**, 1321–1324.
62. Panigrahi, A. K. *et al.*, *J. Med. Virol.*, 1996, **48**, 191–198.
63. Sawant *et al.*, *J. Assoc. Physicians India*, 1999, **47**, 580–583.
64. Amarapurkar, D. N. *et al.*, *ibid*, 2001, **49**, 983–985.
65. Valliammai, T. *et al.*, *J. Gen. Virol.*, 1995, **76**, 711–716.
66. Guntaka, R. V. *et al.*, Nucleotide sequence of Indian strain of HCV, Acc. No. AY051292.

ACKNOWLEDGEMENTS. We acknowledge the critical suggestions and inputs from C. D. Poduri.

Received 11 May 2001; revised accepted 27 June 2002