Variability or conservation of hepatitis C virus hypervariable region 1? Implications for immune responses

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The hypervariable region 1 (HVR1) of the E2 protein of hepatitis C virus (HCV) is highly heterogeneous in its primary sequence and is responsible for significant inter- and intra-individual variation of the infecting virus, which may represent an important pathogenetic mechanism leading to immune escape and persistent infection. A binding site for neutralizing antibodies (Ab) has also been allegedly identified in this region. Prospective studies of serological responses to synthetic oligopeptides derived from naturally-occurring HVR1 sequences showed promiscuous recognition of HVR1 variants in most patients via binding to C-terminal amino acid residues with conserved physicochemical properties. Monoclonal antibodies generated by immunization of mice with peptides derived from natural HVR1 sequences were shown to recognize several HVR1 variants in line with evidence gathered from studies using human sera. In addition, selected mAbs were able to bind HVR1 in the context of a complete soluble form of the E2 glycoprotein, indicating recognition of correctly folded sequences, and were shown to specifically capture circulating and recombinant HCV particles, suggesting that HVR1 is expressed on intact virus particles and therefore potentially able to interact with cellular receptor(s). These findings suggest that it is possible to induce a broadly reactive clonal immune response to multiple HCV variants and that this mechanism could be used in principle to induce protective immunity for a large repertoire of HCV variants.

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1. Introduction

Infection with hepatitis C virus (HCV) is a leading cause of chronic liver disease and it is now known to infect approximately 3% of the world's population. The HCV genome is translated as a single polyprotein which undergoes processing by host and viral proteases to produce structural and nonstructural proteins, respectively (Lindebach and Rice 2001). The virus displays a high mutation rate, and at least 6 major genotypes have been recognized based on nucleotide sequencing of conserved and non conserved regions (Simmonds 1999). The HCV-specific immune response is generally unable to clear the virus and, therefore, spontaneous resolution is extremely rare, while over 70% of acute infections eventually become persistent. Most infections remain asymptomatic for several years resulting in late recognition of the disease (Everhart *et al* 1997). The high proportion of chronic HCV infections may be due to active escape mechanisms which eludes the host immune response during the acute infection

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Abbreviations used: Ab, Antibodies; HCV, hapatitis C virus; HVR1, hypervariable region 1; mAb, monoclonal antibodies; SRBI, scavenger receptor class B type I.

through early selection of philogenetically divergent mutants (Farci *et al* 2000) most likely generated by an inappropriate selective pressure exerted by the host immune response, or to the inability of HCV to induce an efficient protective immunity. Interestingly, a dramatic reduction in genetic diversity leading to an increasingly homogeneous viral population has been recently shown to be a consistent feature associated with viral clearance in sustained responders to interferon treatment, independently of viral genotype (Farci *et al* 2002). While most HCV infections do elicit cellular and humoral immune responses (Missale *et al* 2001), there is little conclusive evidence in support of the existence of protective immunity. A vaccine containing proteins of the viral envelope will soon be evaluated in phase I clinical trials.

2. What role for hypervariable region 1?

In contrast with hepatitis B virus infection in which envelope-specific neutralizing antibodies (Ab) closely correlate with clinical recovery, patients chronically infected with HCV invariably have envelope specific Ab detectable in their serum, indicating an ongoing B cell response (Cerino *et al* 1997). The significance and utility of such circulating Ab are currently uncertain, since re-challenge of experimentally infected chimpanzees with high levels of circulating anti-HCV immunoglobulin still results in the reappearance of viremia (Farci *et al* 1992). Our current understanding of the possible functions of B-cell responses in HCV infection is depicted in figure 1.

Passive immunization studies and *in vitro* neutralization of HCV isolates with hyperimmune serum-specific for the hypervariable region 1 (HVR1) would suggest a protective role for anti-HVR1 Ab (Farci *et al* 1994, 1996). It is known, however, that such Ab appear to co-exist with the HVR1 variants they recognize and are frequently cross-reactive with unrelated HVR1 sequences isolated from different patients (Lesniewsky *et al* 1993; da Silva-Cardoso *et al* 1995; Scarselli *et al* 1995; Zibert *et al* 1995, 1997; Cerino *et al* 1997; Jackson *et al* 1997; Yoshioka *et al* 1997; Hattori *et al* 1998; Mondelli *et al* 1999; Shang *et al* 1999). Thus, the significance of HVR1-specific humoral immune responses, and their relationship to HVR1 sequence variation, are still incompletely defined.

Available evidence would suggest that HVR1 variation has an adaptive significance and results from a conti-

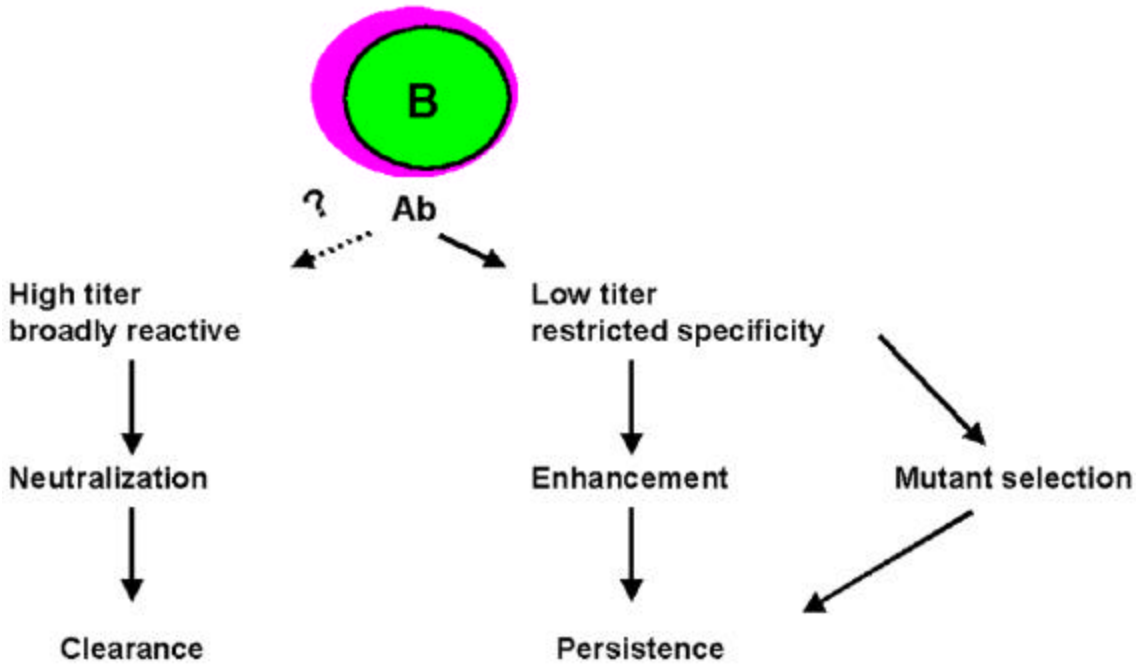

Figure 1. Proposed functions of B-cell responses in HCV infection. Production of highly efficient neutralizing antibodies to HCV is currently uncertain, although it is thought to occur only in certain circumstances. Such antibodies should be produced at high concentration and possibly have broad specificity, though this is currently unknown. However, in most circumstances neutralizing antibodies would be produced at low concentrations and with restricted specificity; this would on the one hand favour selection of viral mutants and on the other hand it could generate an enhancement mechanism which would increase the spread of the infection to other susceptible cells. These phenomena may have a deleterious effect on the evolution of HCV infection.

nuous selection process which is likely controlled by humoral immune responses, as suggested by a minimal or absent sequence mutation rate in subjects with congenital immunoglobulin defects (Kumar *et al* 1994; Booth *et al* 1998). Moreover, appearance of circulating anti-HVR1 Ab in chimpanzees inoculated with an identical HCV strain was associated with HVR1 sequence variation, whereas no sequence mutations were observed in the absence of detectable HVR1-specific humoral immune responses (van Doorn *et al* 1995). Evidence in support of Ab-driven HVR1-variant selection has also been provided by others. For instance, Ray *et al* (1999) showed that persistent viremia was associated with higher inter-sample antonymous vs synonymous substitutions (*Ka/Ks*), suggesting that HVR1 can function as an immunological decoy, stimulating a strong, immune response which would be responsible for variant selection, but would be ineffective to clear HCV. Recent additional data obtained from a prospective study of viral evolution during perinatal infection argues in favour of a dominant role of positive selection for amino acid changes in driving the pattern of HCV genetic diversification (Manzin *et al* 2000).

In contrast with these studies, others failed to find evidence of strong selective pressure driving the emergence of viral variants. Immune responses to HVR1 were generally weak and not correlated with nucleotide or amino acid substitutions (Bassett *et al* 1999; Major *et al* 1999). However, it is important to emphasize that those data were generated in chimpanzees, the only available model of HCV infection, although largely inadequate for pathogenetic studies on HCV infection, because of distinct disease features which differentiate human from primate infection. The role of the host immune response in selecting HVR1 variants has also been recently questioned by Allain *et al* (2000) who failed to detect a correlation between the evolutionary rate or the heterogeneity of the viral quasispecies in the patients studied and the strength of immune responses to HVR1 epitopes, suggesting that genetic drift is independent on the host immune pressure. However, in view of the overwhelming evidence in support of an active selection process, mechanisms of HCV variation, such as random genetic drift, are unlikely to be operative in this setting.

In a previous study we prospectively followed serological responses to synthetic oligopeptides derived from HVR1 sequences of patients with acute and chronic HCV infection obtained at baseline and after a defined follow-up period (Mondelli *et al* 1999). Extensive serological cross-reactivity for unrelated HVR1 peptides was observed in the majority of the patients. Ab responses were restricted to the IgG1 subclass and were focused on the carboxy-terminal end of the HVR1 region. Cross-reactive Ab could also be readily elicited following immunization of mice with multiple antigenic peptides carrying HVR1 sequences derived from our patients. Consistent with the inter-isolate Ab cross-reactivity is the recently reported HLA class II-restricted CD4+ T-cell response to conserved motifs located at the C terminus of the HVR1 sequence (Shirai *et al* 1999). Indeed, the predominant T-cell response to the HVR1 region was directed against the C terminus, similarly to findings obtained by others who also showed stronger HVR1-specific CD4 T-cell responses in patients who cleared the infection after successful antiviral therapy (Del Porto *et al* 2000).

3. HVR1 is conformationally conserved and is expressed on HCV particles

To investigate further the molecular basis for Ab cross-reactivity for unrelated HVR1 sequences, we generated a panel of murine monoclonal antibodies (mAb) from mice immunized with HVR1 surrogate peptides (mimotopes), affinity-selected with sera from HCV-infected patients from a phage-display library (Puntoriero *et al* 1998). A significant number of antigen-specific hybridomas was obtained after immunization with a pool of nine mimotopes. The mAbs were shown to recognize a number of 16-mer and 27-mer peptides derived from natural HVR1 sequences isolated from patients with acute and chronic HCV infection, and a major binding site was mapped at amino acid position 390–405, akin to our previous findings using human sera (Cerino *et al* 2001). HVR1 mimotope-specific mAb were also able to efficiently compete with sera from HCV-infected patients for binding to peptides derived from natural HVR1 sequences, thus confirming previous data obtained with polyclonal Ab, showing that HVR1 peptide mimotopes are efficient antigenic and immunogenic mimics of naturally occurring HCV variants.

A plausible explanation accounting for the promiscuous binding characteristics of HVR1-specific antibodies comes from a recent theoretical study on the structural conformation of HVR1 which showed either a broad amino acid repertoire at each position despite a remarkable residue conservation in specific sites or replacements with amino acids with similar physicochemical properties, usually positively charged basic residues, in the most variable segments (Penin *et al* 2001). The very similar hydropathy and antigenicity profiles of HVR1 variants revealed a substantial conformational conservation, thus providing a plausible explanation for the extensive cross-reactivity demonstrated by Ab and confirming the existence of an active selection process. Interestingly, two sites with a high antigenicity score were identified at positions 1–13 and 19–24, a pattern which is predicted also with philogenetically distant variants (Penin *et al* 2001). The latter

site contains the immunodominant B-cell epitope(s) described previously (Scarselli *et al* 1995; Mondelli *et al* 1999) and is compatible with B-cell epitope mapping data (Zibert *et al* 1995, 1997; Mondelli *et al* 1999).

The possibility to generate broadly reactive antibodies may represent a useful approach to overcome the natural diversity of a virus such as HCV, suggesting that mimotope-based vaccines can be used as potentially effective HCV immunogens. This assumption is based on evidence indicating that antibodies to HVR1 can prevent HCV infection in the chimpanzee model by both *in vitro* and *in vivo* neutralization of a pedigreed virus inoculum of HCV strain H (Farci *et al* 1994, 1996). However, exposure of HVR1 on complete viral particles has not been formally proven and, in principle, the role of this sequence in binding neutralizing antibodies is far from being established. A recent preliminary study suggested that mAbs obtained by immunization with peptides derived from natural HVR1 isolates were able to capture *bona fide* viral particles only from homologous HCV isolates and could also prevent infection of an allegedly susceptible cell line *in vitro* (Zhou *et al* 2000). These findings are in partial agreement with our data in that one of our mAbs was also able to capture *bona fide* and recombinant viral particles, although there was no apparent genotype or isolate-specific recognition (Cerino *et al* 2001). Similar findings were reported by Li *et al* (2001), who were able to show that mAbs generated by immunization with HVR1 peptides, specific for conserved motifs, could capture HCV RNA from plasmas of patients and were also able to block HCV binding to Molt-4 cells which, in previous experiments (Hamaia *et al* 2001), were shown to efficiently bind HCV. These observations fit with the idea of a significant structural conservation of HVR1, as discussed above.

It may be argued that mAbs raised against HVR1 peptides are unable to recognize the same sequence when expressed in the context of a correctly-folded complete E2 glycoprotein which included HVR1. However, our mAbs were shown to recognize correctly-folded E2 polypeptides expressing the same HVR1 sequences synthesized as linear peptide, providing additional evidence supporting the existence of immunodominant, conformation-independent epitope(s) in the C-terminal sub-region which, under certain circumstances, may be exposed on integral viral particles.

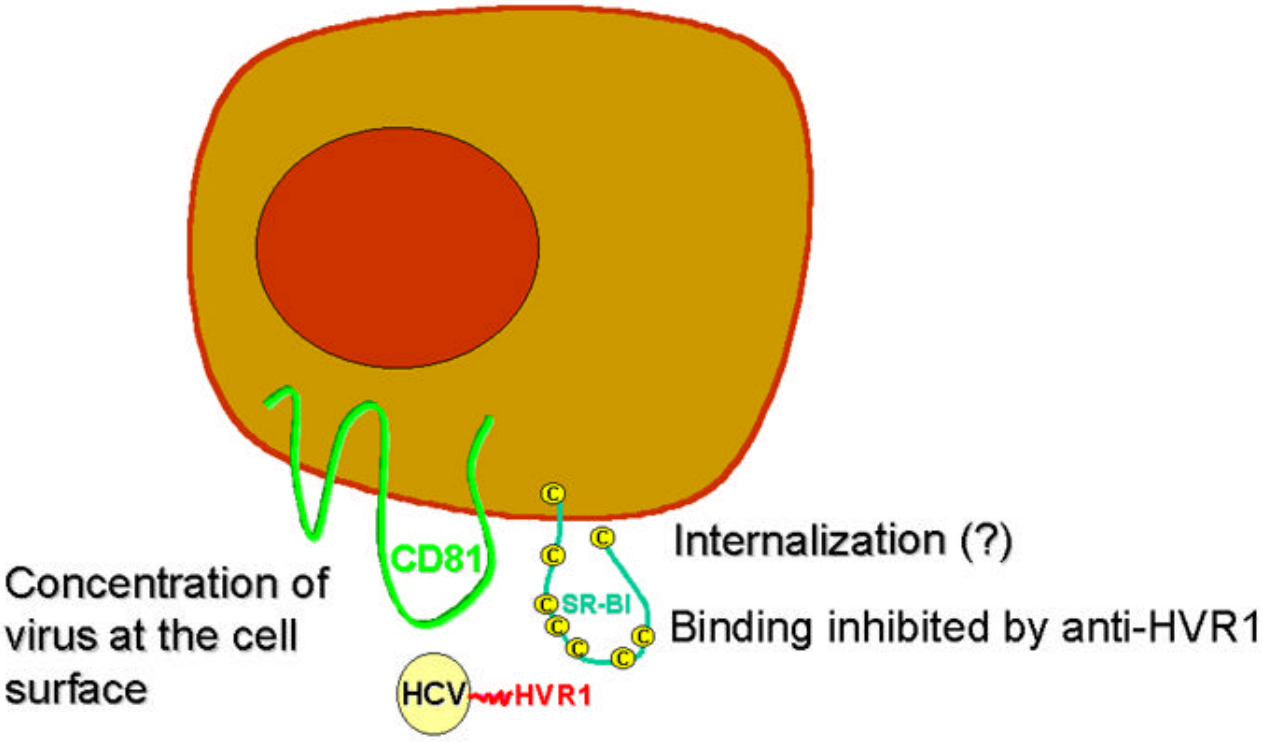

Figure 2. Possible mechanisms of HCV-receptor(s) interaction on the hepatocyte. CD81, a tetraspanin expressed on hepatocytes and B cells (among many other cells), binds HCV via HVR1-independent ligand(s) and concentrates viral particles on the cell surface. However, the recently identified HCV co-receptor, the scavenger receptor class B type I (SRBI), would bind to viral particles which would then be internalized. The role of lipoproteins, which normally coat circulating viral particles, in the viral uptake process is still undefined, although the LDL receptor has been proposed as an additional candidate co-receptor molecule for HCV (Agnello *et al* 1999).

4. Conclusions and outlook

The role, if any, played by HVR1 in host-virus interactions at the protein level is still unknown. Current evidence suggests that mammalian cell-derived E2 glycoproteins (Petracca *et al* 2000) and, to a much lesser extent, HCV-LP 1a (M Triyatni and T J Liang, unpublished observations) can bind human CD81, a candidate receptor molecule for HCV. Yet HCV-LP can penetrate into HepG2 cells via alternative receptor(s) which are constitutively expressed on hepatocytes (M Triyatni and T J Liang, unpublished observations), supporting the hypothesis that HCV requires a second receptor molecule for internalization (Petracca *et al* 2000) (figure 2). Preliminary evidence suggests that HVR1 is not involved in binding to CD81 (Flint *et al* 1999; Patel *et al* 2000). Recently, a novel HCV candidate receptor has been identified on human hepatocellular carcinoma cell lines. Such a putative receptor, the scavenger receptor class B type I (SRBI), binds E2 via HVR1 and is species-specific (Scarselli *et al* 2002). Thus, high-affinity anti-HVR1 Ab elicited by immunization could modulate HCV infection by inhibiting binding of viral particles to SRBI. This approach may have important implications for immunotherapy or prophylaxis of HCV infection.

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References

Agnello V, Abel G, Elfahal M, Knight G B and Zhang Q X 1999 Hepatitis C virus and other Flaviviridae viruses enter cells via low density lipoprotein receptor; *Proc. Natl. Acad. Sci. USA* **96** 12766–12771

Allain J-P, Dong Y, Vandamme A-M, Moulton V and Salemi M 2000 Evolutionary rate and genetic drift of hepatitis C virus are not correlated with the host immune response: studies of infected donor-recipient clusters; *J. Virol.* **74** 2541–2549

Bassett S E, Thomas D L, Brasky K M and Lanford R E 1999 Viral persistence antibody to E1 and E2, and hypervariable region 1 sequence stability in hepatitis C virus-inoculated chimpanzees; *J. Virol.* **73** 1118–1126

Booth J C L, Kumar U, Webster D, Monjardino J and Thomas H C 1998 Comparison of the rate sequence variation in the hypervariable region of E2/NS1 region of hepatitis C virus in normal and hypogammaglobulinemic patients; *Hepatology* **27** 223–227

Cerino A, Bissolati M, Cividini A, Nicosia A, Esumi M, Hayashi N, Mizuno K, Slobbe R, Oudshoorn P, Silini E, Asti M and Mondelli M U 1997 Antibody responses to the hepatitis C virus (HCV) E2 protein: relationship to viraemia and prevalence in anti-HCV seronegative subjects; *J. Med. Virol.* **51** 1–5

Cerino A, Meola A, Segagni L, Furione M, Marciano S, Triyatni M, Liang T J, Nicosia A and Mondelli M U 2001 Monoclonal antibodies with broad specificity for hepatitis C virus hypervariable region 1 variants can recognize viral particles; *J. Immunol.* **167** 3878–3886

da Silva-Cardoso M, Simoneit K, Nemecek V, Epple S, Koerner K and Kubanek B 1995 The serology of hepatitis C virus infection: antibody cross-reaction in the hypervariable region 1; *Arch. Virol.* **140** 1705–1713

Del Porto P, Puntoriero G, Scottà C, Nicosia A and Piccolella E 2000 High prevalence of hypervariable region 1-specific and cross–reactive CD4(+) T cells in HCV-infected individuals responsive to IFN-alpha treatment; *Virology* **269** 313–324

Everhart J E, Stolar M and Hoofnagle J H 1997 Management of hepatitis C: a national survey of gastroenterologists and hepatologists; *Hepatology* **26** 78S–82S

Farci P, Alter H J, Govindarajan S, Wang D C, Engle R, Lesniewski R R, Mushahwar I K, Desai S M, Miller R H, Ogata N and Purcell R H 1992 Lack of protective immunity against reinfection with hepatitis C virus; *Science* **258** 135–140

Farci P, Alter H J, Wong D C, Miller R H, Govindarajan S, Engle R, Shapiro M and Purcell R H 1994 Prevention of hepatitis C virus infection in chimpanzees after antibody-mediated in vitro neutralization; *Proc. Natl. Acad. Sci. USA* **91** 7792–7796

Farci P, Shimoda A, Coiana A, Diaz G, Peddis G, Melpoder J C, Strazzera A, Chien D Y, Munoz S J, Balestrieri A, Purcell R H and Alter H J 2000 The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies; *Science* **288** 339–344

Farci P, Shimoda A, Wong D, Cabezon T, De Gioannis D, Strazzera A, Shimizu Y, Shapiro M, Alter H J and Purcell R H 1996 Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein; *Proc. Natl. Acad. Sci. USA* **93** 15394–15399

Farci P, Strazzera R, Alter H J, Farci S, Degioannis D, Coiana A, Peddis G, Usai F, Serra G, Chessa L, Diaz G, Balestrieri A and Purcell R H 2002 Early changes in hepatitis C viral quasispecies during interferon therapy predict the therapeutic outcome; *Proc. Natl. Acad. Sci. USA* **99** 3081–3086

Flint M, Maidens C L, Loomis-Pric D, Shotton C, Dubuisson J, Monk P, Higginbottom A, Levy S and McKeating J A 1999 Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81; *J. Virol.* **73** 6235–6244

Hamaia S, Li C and Allain J-P 2001 The dynamics of hepatitis C virus binding to platelets and 2 mononuclear cell lines; *Blood* **98** 2293–2300

Hattori M, Yoshioka K, Aiyama T, Iwata K, Terazawa Y, Ishigami M, Yano M and Kakumu S 1998 Broadly reactive anti-

bodies to hypervariable region 1 in hepatitis C virus-infected sera: relation to viral loads and response to interferon; *Hepatology* **27** 1703–1710

Jackson P, Petrik J, Alexander G J M, Pearson G and Allain J P 1997 Reactivity of synthetic peptides representing selected sections of hepatitis C virus core and envelope proteins with a panel of hepatitis C virus-seropositive humam plasma; *J. Med. Virol*. **51** 67–79

Kumar U, Monjardino J and Thomas H C 1994 Hypervariable region of hepatitis C virus envelope glycoprotein (E2/NS1) in an agammaglobulinemic patient; *Gastroenterology* **10** 1072–1075

Lesniewsky R R, Boardway K M, Casey J M, Desai S M, Devare S G and Leung T K 1993 Hypervariable region 5′-terminus of hepatitis C virus E2/NS1 encodes antigenically distinct variants; *J. Med. Virol*. **40** 150–156

Li C, Candotti D and Allain J-P 2001 Production and characterization of monoclonal antibodies specific for a conserved epitope within hepatitis C virus hypervariable region 1; *J. Virol*. **75** 12412–12420

Lindebach B D and Rice C M 2001 Flaviviride: the viruses and their replication; in *Fields virology* (eds) D M Knipe and P M Howley (Philadelphia: Lippincott Williams and Wilkins) pp 991–1041

Manzin A, Solforosi L, Debiaggi M, Zara F, Tanzi E, Romano L, Zanetti A R and Clementi M 2000 Dominant role of host selective pressure in driving hepatitis C virus evolution in perinatal infection; *J. Virol*. **74** 4327–4334

Major M E, Mihalik K, Fernandez J, Seidman J, Kleiner D, Kolyhalov A A, Rice C M and Feinstone S M 1999 Long-term follow-up of chimpanzees inoculated with the first infectious clone for hepatitis C virus; *J. Virol*. **73** 3317–3325

Missale G, Penna A, Boni C, Malpeli M, Zerbini A, Pilli M, Urbani S and Ferrari C 2001 Comparative pathogenesis of HBV and HCV; *Virus Res*. **82** 19–23

Mondelli M U, Cerino A, Lisa A, Brambilla S, Segagni L, Cividini A, Bissolati M, Missale G, Bellati G, Meola A, Bruniercole B, Nicosia A, Galfré G and Silini E 1999 Antibody responses to hepatitis C virus hypervariable region 1: evidence for cross-reactivity and immune-mediated sequence variation; *Hepatology* **30** 537–545

Patel A, Wood J, Penin F, Dubuisson J and McKeating J 2000 Construction and characterization of chimeric E2 glycoprotein of hepatitis C virus: analysis of E2 regions interacting with CD81; *J. Gen. Virol*. **81** 2873–2883

Penin F, Combet C, Germanidis G, Frainais P-O, Deléage G and Pawlotsky J-M 2001 Conservation of the conformation and positive charges of hepatitis C virus E2 glycoprotein hypervariable region 1 points to a role in cell attachment; *J. Virol*. **75** 5703–5710

Petracca R, Falugi F, Galli G, Norais N, Rosa D, Campagnoli S, Burgio V, di Stasio E, Giardina B, Houghton M, Abrignani S and Grandi G 2000 Structure-function analysis of hepatitis C virus envelope-CD81 binding; *J. Virol*. **74** 4824–4830

Puntoriero G, Meola A, Lahm A, Zucchelli S, Bruno B, Tafi R, Pezzanera M, Mondelli M U, Cortese R, Tramontano A, Galfrè G and Nicosia A 1998 Towards a solution for hepatitis C virus hypervariability: mimotopes of the hypervariable region 1 can induce antibodies cross–reacting with a large number of viral variants; *EMBO J*. **17** 3521–3533

Ray C S, Wang Y-M, Laeyendecker O, Ticehurst J R, Villano S A and Thomas D L 1999 Acute hepatitis C virus structural gene sequences as predictors of persistent viremia: hypervariable region 1 as a decoy; *J. Virol*. **73** 2398–2346

Scarselli E, Ansuini H, Cerino R, Roccasecca R M, Acali S, Filocamo G, Traboni C, Nicosia A, Cortese R and Vitelli A 2002 The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus; *EMBO J*. **21** 5017–5025

Scarselli E, Cerino A, Esposito G, Silini E, Mondelli M U and Traboni C 1995 Occurence of antibodies reactive with more than one variant of the putative envelope glycoprotein (E2; gp70) hypervariable region 1 in hepatitis C viremic patients; *J. Virol*. **69** 4407–4412

Shang D, Zhai W and Allain J-P 1999 Broadly cross-reactive, high-affinity antibody to hypervariable region 1 of the hepatitis C virus in rabbits; *Virology* **258** 396–405

Shirai M, Arichi T, Chen M, Nishioka M, Ikeda K, Takahashi H, Enomoto N, Saito T, Major M E, Nakazawa T, Akatsuka T, Feinstone S M and Berzofsky J A 1999 T cell recognition of hypervariable region-1 from hepatitis C virus envelope protein with multiple class II MHC molecules in mice and humans: preferential help for induction of antibodies to the hypervariable region; *J. Immunol*. **162** 568–576

Simmonds P 1999 Viral heterogeneity of the hepatitis C virus; *J. Hepatol*. **31S** 54–60

van Doorn L J, Capriles I, Maertens G, DeLeys R, Murray K, Kos T, Schellekens H and Quint W 1995 Sequence evolution of the hypervariable region in the putative envelope region E2/NS1 of hepatitis C virus is correlated with specific humoral immune responses; *J. Virol*. **69** 773–778

Yoshioka K, Aiyama T, Okumura A, Takayanagi M, Iwata K, Nagai Y and Kakumu S 1997 Humoral immune response to the hypervariable region of hepatitis C virus differs between genotypes 1b and 2a; *J. Infect. Dis*. **175** 505–510

Zibert A, Kraas W, Meisel H, Jung G and Roggendorf M 1997 Epitope mapping of antibodies directed against hypervariable region 1 in acute self-limiting and chronic infections due to hepatitis C virus; *J. Virol*. **71** 4123–4127

Zibert A, Schreier E and Roggendorf M 1995 Antibodies in human sera specific to hypervariable region 1 of hepatitis C can block viral attachment; *Virology* **208** 653–661

Zhou Y H, Shimizu Y K and Esumi M 2000 Monoclonal antibodies to the hypervariable region 1 of hepatitis C virus capture virus and inhibit virus adsorption to susceptible cells in vitro; *Virology* **269** 276–283