

# Hypervariable region 1 of hepatitis C virus: immunological decoy or biologically relevant domain?

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## Abstract

The hypervariable region 1 (HVR1) of the E2 protein of hepatitis C virus (HCV) is highly heterogeneous and is responsible for significant inter- and intra-individual variation of the infecting virus, which may represent an important pathogenetic mechanism leading to escape and persistent infection. Moreover, a binding site for neutralizing antibodies (Ab) has been allegedly identified in this region. Prospective studies of serological responses to synthetic oligopeptides derived from HVR1 sequences of patients with acute and chronic HCV infection showed extensive serological cross-reactivity for unrelated HVR1 peptides in the majority of the patients. A significant correlation was found between HVR1 sequence variation, and intensity, and cross-reactivity of humoral immune responses providing strong evidence in support of the contention that HCV variant selection is driven by the host immune pressure. Monoclonal Ab (mAb) generated following immunization of mice with peptides derived from natural HVR1 sequences also showed cross-reactivity for several HVR1 sequences attesting to the existence of conserved amino acid motifs among different variants. These findings suggest that it is possible to induce a broadly cross-reactive immune response to HVR1 and that this mechanism can be used to generate protective immunity for a large repertoire of HCV variants. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Hepatitis C virus; Hypervariable region 1; Immune response; Monoclonal antibodies

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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  $\approx 3\%$  of the world's population

(World Health Organization, 1997). The HCV genome is translated as a single polyprotein which undergoes processing by host and viral proteases to produce structural and non structural proteins, respectively (Houghton, 1996). The virus displays a high mutation rate and at least six major genotypes have been recognized based on nucleotide sequencing of conserved and non conserved regions (Simmonds, 1999). The HCV specific im-

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immune response is generally unable to clear the virus and, therefore, spontaneous resolution is extremely rare, while over 80% 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 eluding the host immune response or to the inability of HCV to induce an efficient protective immunity. While most HCV infections do elicit immune responses, there is little conclusive evidence in support of the existence of protective immunity. A vaccine containing proteins of the viral envelope is currently being evaluated in phase I clinical trials.

## **2. Hypervariable region 1: immunological decoy or binding site for neutralizing antibodies?**

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 rechallenge of experimentally infected chimpanzees with high levels of circulating anti-HCV immunoglobulin still results in the reappearance of viremia (Farci et al., 1992). However, passive immunization studies and *in vitro* neutralization of HCV isolates with hyperimmune serum specific for the hypervariable region 1 (HVR1) would argue in favour of a protective role of anti-HVR1 Ab. It is well 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 (Cerino et al., 1997; Zibert et al., 1997; Scarselli et al., 1995; da Silva-Cardoso et al., 1995; Jackson et al., 1997; Zibert et al., 1995; Hattori et al., 1998; Yoshioka et al., 1997; Lesniewsky et al., 1993; Mondelli et al., 1999). Thus, the significance of HVR1-specific humoral immune responses and their relationship to HVR1 sequence variation are largely undefined.

Available evidence would suggest that HVR1 variation has an adaptive significance and results from a continuous 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). However, formal and definitive evidence in support of Ab-driven HVR1 variant selection is lacking and, in principle, other mechanisms of variation, such as random drift, could be envisioned.

In a recent 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 carboxyterminal end of the HVR1 region. Cross-reactive Ab could be readily elicited following immunization of mice with multiple antigenic peptides carrying HVR1 sequences derived from our patients. The finding of a statistically significant correlation between HVR1 sequence variation, and intensity, and cross-reactivity of humoral immune responses provided evidence in support of the contention that HCV variant selection is driven by the host's immune pressure. Consistent with the interisolate 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, possibly restricted by DR4 and DR1 which have binding sites for HVR1. T-cell mediated help for HVR1-specific Ab production is likely to be regulated by a phenomenon called 'T-B reciprocity' in which

Ab on the surface of B cells bind Ag and are internalized with the Ag receptor-mediated endocytosis into intracellular compartments for processing and loading of MHC class II molecules. Thus, Ab may influence the susceptibility of the antigenic protein to proteolytic processing, by determining which peptides are subsequently presented to class II-restricted CD4 T cells and eventually identify which helper T cells can help that B cell (Berzofsky, 1983). The outcome of such mechanism would be that Ag-specific B cells preferentially present Ag to CD4 cells specific for certain epitopes and CD4 cells of different specificity would preferentially provide help for B cells specific for certain epitopes more than others on the same protein. This recently described attractive hypothesis awaits a direct demonstration in a suitable model system.

Another interesting finding which may be relevant to the pathogenesis of chronic HCV infection was that the extent and the quality of cross-reactive responses to this region may influence the outcome of HCV-induced liver disease. It is noteworthy that in a recent report, we documented a significantly higher HVR1 sequence diversification as a function of time in patients infected with type 2 compared with those infected with other types (predominantly type 1) (Brambilla et al., 1998). As HVR1 variation has an adaptive significance, it is conceivable that higher variant selection would result from stronger immune pressure targeted on HVR1 in this patient subgroup, as also suggested by others (Yoshioka et al., 1997). Since maximal time-related HVR1 sequence diversification is observed in type 2-infected patients (Brambilla et al., 1998), our findings provide additional corroborative evidence that HVR1 sequence variation is driven by Ab. It is of interest that type 2-infected patients generally show milder histological lesions and lower serum levels of enzymes associated with hepatocellular necrosis (Silini et al., 1995; Puoti et al., 1997), and it is therefore possible that more vigorous and heterogeneous cross-reactive responses to HVR1 are associated with a more benign prognosis and possibly slower disease progression in this set-

ting. Consistent with a central role for humoral immune responses in the control of HCV infection is also the observation that HCV RNA positive individuals with congenital immunoglobulin defects are characterized by a more severe liver disease, compared with those negative for HCV RNA (Bjørø et al., 1994).

Evidence in support of Ab-driven HVR1 variant selection has also been provided by others. For instance Ray et al. (1999) showed that persistence viremia was associated with higher intersample 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 argue in favor 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 reports 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. The reasons for these apparent discrepancies should be further examined in the early phase of acute HCV infection when immune responses to HVR1 are usually absent or highly focused on a single or

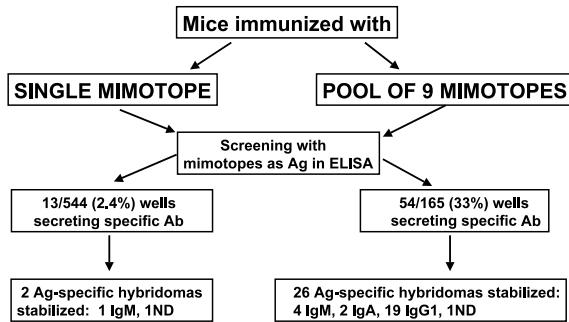


Fig. 1. Experimental procedure employed for generation of anti-HVR1 mAb.

limited number of HVR1 variants (Mondelli et al., 1999). The biological implications of the evolutionary rate of HVR1 in acute hepatitis have been recently addressed by Farci et al. (2000) who found that early diversification of the HCV quasispecies correlated with persistent infection, whereas acute self-limited infection was characterized by evolutionary stasis. Consistent with the hypothesis of selective pressure exerted by the host immune response, the sequence changes occurred exclusively within HVR1 and correlated with Ab responses.

### 3. Hypervariable region 1-specific Ab cross-reactivity can be demonstrated at the clonal level and is focused on conserved motifs

To investigate further the molecular basis for Ab cross-reactivity for unrelated HVR1 sequences, we generated a panel of murine monoclonal Ab 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 significantly higher number of antigen-specific clones was obtained after immunization with a pool of nine mimotopes compared with immunization with only one mimotope (21% of specific hybridomas vs. 0.7%, respectively), and in the latter conditions only IgM mAb were generated, whereas also IgA- and IgG1-secreting mAb were obtained using the former experimental approach (Fig. 1). HVR1 mimotope-specific mAb were also shown to recognize a number of 16-mer and 27-mer peptides (Fig. 2) derived from natural HVR1 sequences isolated from patients with acute and chronic HCV infection and a major binding site could be mapped at amino acid position 390–405, akin to our previous findings using hu-

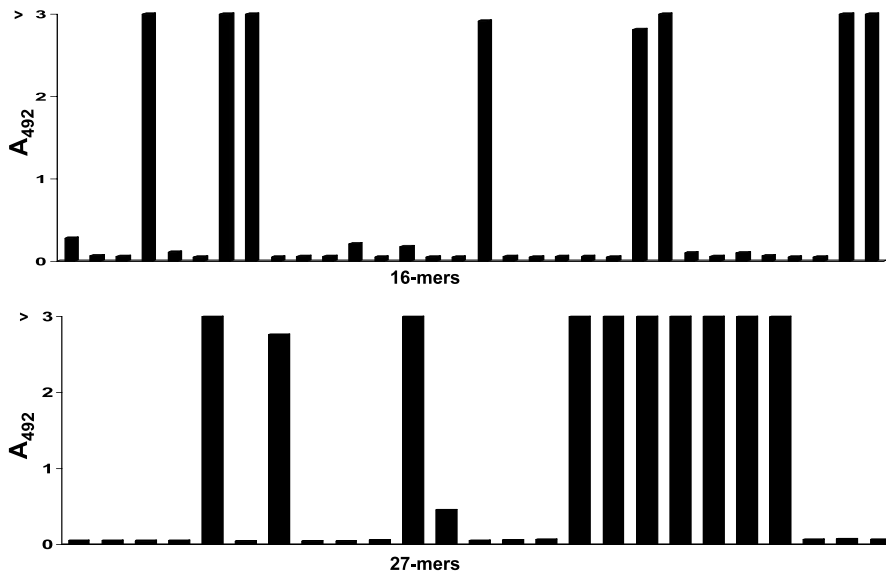


Fig. 2. Reactivity of a representative murine mAb (IgG1) with peptides derived from natural HVR1 sequences (immunogen: pool of nine mimotopes).

man sera. 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. Moreover, the majority of mAb were able to recognize HVR1 in the context of C-terminal-truncated, soluble E2 glycoprotein, indicating that conformational modifications are not critical for Ab binding to this region. These findings demonstrate that it is possible to induce a broadly cross-reactive antibody response at the clonal level as shown by others (Zhou et al., 1999) and that the efficiency of this process can be greatly enhanced following immunization with multiple HVR1 surrogate peptides. Such mechanism could be exploited for the development of an effective HCV vaccine inducing broad specificity for a large repertoire of viral variants.

#### 4. Conclusions

The assessment of neutralizing Ab responses *in vitro*, which is the first correlate of immunity examined, is usually a problem for HCV because the virus does not grow efficiently in cell culture. There is no obvious solution to this problem. Although E2 carries a biologically relevant binding site for CD81 (Pileri et al., 1998), a high affinity receptor for HCV, this molecule is probably much less efficient in internalizing the virus. Moreover, the binding site for CD81 is reportedly not located within the HVR1 region, but probably in the HVR2 region downstream from HVR1 as predicted from structural modelling of the E2 protein (Yagnik et al., 2000). What role then could be envisioned for HVR1 beside from being a target for immune selection? There is increasing evidence suggesting that an additional receptor is required for efficient internalization of HCV. If HVR1 is a critical region for binding to this additional receptor, an immune response to it would be able to inhibit HCV penetration into susceptible cells. The demonstration that an anti-

body raised to a HVR1 peptide is capable of preventing HCV infection in chimpanzees (Farci et al., 1996) lends further support to the concept that HVR1 is not simply an immunological decoy.

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