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Virology 332 (2005) 538–549

VIROLOGY

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Immunoglobulin mimicry by Hepatitis C Virus envelope protein E2

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Received 28 September 2004; accepted 19 November 2004
Available online 12 January 2005

Abstract

Hepatitis C virus (HCV) establishes persistent infection in the majority of infected individuals. The currently accepted hypothesis of immune evasion by antigenic variation in hypervariable region 1 (HVR1) of glycoprotein E2 does not however, explain the lack of subsequent immune recognition. Here, we show that the N-terminal region of E2 is antigenically and structurally similar to human immunoglobulin (Ig) variable domains. E2 is recognized by anti-human IgG antibodies and also possesses common amino acid (aa) sequence features of the conserved v-gene framework regions of human Ig light chains in particular but also heavy chains and T cell receptors. Using a position specific scoring system, the degree of similarity of HVR1 to Ig types correlated with immune escape and persistence in humans and experimentally infected chimpanzees. We propose a unique role for threshold levels of Ig molecular mimicry in HCV biology that not only advances our concept of viral immune escape and persistent infection but also provides insight into host-dependent disease patterns.

Keywords: Immunoglobulin mimicry; Hepatitis C; Glycoprotein

Introduction

Hepatitis C virus (HCV) currently infects 170 million people constituting a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Alter and Seeff, 2004; Major et al., 2004). HCV is the only RNA virus infecting humans (excepting retroviruses) that persists in the majority of infected individuals. Viruses, especially those with RNA genomes, can undergo mutation at high frequencies, and under novel selective pressures, rapidly generate populations of viral variants. Such variability can provide a means of evading clearance by both T- and B-cell immunity. Accumulated data suggest that hypervariable region 1 (HVR1), located in a stretch of 27–31 residues at the amino terminus of the second envelope glycoprotein (E2) is the main target of the anti-HCV neutralizing response and therefore plays a significant role in the establishment of viral persistence (Farci et al., 2000; Kato, 2001). During HCV infection, amino acid substitutions in HVR1 generates populations of genetically related variants, termed quasispecies (Domingo et al., 1997; Ducoulombier et al., 2004; Hijikata et al., 1991), some of which are antibody escape mutants that are not recognized by the immune response and persist after seroconversion (Kato, 2001; Pavio and Lai, 2003). Although this is the most accepted hypothesis, the important question as to why the virus epitopes within HVR1 cannot be subsequently recognized by the immune system is unknown. The role of HVR1 in persistence has become controversial because recombinant HCV lacking HVR1, can persist in chimpanzees, but the resultant virus was not able to establish

Abbreviations: aa, amino acid(s); BLAST, basic local alignment search tool; CDR, complementarity determining region; E2, envelope protein 2; eIF2α, eukaryotic initiation factor 2α; FR, framework region; HCV, hepatitis C virus; Ig, immunoglobulin; HVR1, hypervariable region 1; IFN, interferon; IMGT, Immunogenetics database; pi, post infection; pd, post-diagnosis; PKR, protein kinase R; TCR, T cell receptor.

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doi:10.1016/j.virol.2004.11.041

persistent infection on subsequent transmission suggesting a decreased ability in this regard (Forns et al., 2000).

Molecular mimicry, where viruses express proteins that are structurally similar to host defense proteins and immunomodulators, is an important immune-evasion strategy used to promote survival and persistence, especially in DNA viruses (Ploegh, 1998; Seet et al., 2003; Vossen et al., 2002). HCV is also known to employ molecular mimicry to resist type I IFN (Taylor et al., 2000). The virally induced antiviral cytokine, type I interferon (IFN), acts in part through the dsRNA-dependent protein kinase (PKR) to inhibit protein synthesis through phosphorylation of eukaryotic initiation factor 2α, (eIF2α). The HCV envelope protein E2 contains a 12 aa sequence identical to phosphorylation domains of both eIF2α and the PKR kinase (Taylor et al., 1999). This domain operates to prevent PKR-dependent phosphorylation of eIF2α and inhibition of protein synthesis. The extent of PKR-eIF2α homology of this domain correlates with the ability of HCV to resist type I IFN treatment.

We reasoned that other instances of molecular mimicry, targeted to other human proteins, could also be contributing to HCV persistent infection. We found a second instance of molecular mimicry, where HCV encodes a sequence in E2 that is homologous to human immunoglobulins (Ig), and specifically possesses typical structural features of the variable region of Ig such that it cross-reacts with anti-human-IgG. Using a bioinformatic and evolutionary approach, sequence analysis of HCV variants that arise in the course of primary infection in humans and chimpanzees showed that the degree of similarity of HVR1 or its epitopes with Ig types was directly related to viral escape and persistence in a host specific manner, indicating a significant role for viral molecular mimicry.

Results

E2 is structurally similar to IgG

To elucidate the mechanism of immune escape by HCV, an investigation of E2 mimicry was performed. Surprisingly, full-length recombinant HCV genotype 3a E2 proteins expressed in *Escherichia coli* (*E. coli*) were repeatedly seen to bind antibody to human IgG Fab fragment (Fig. 1A, lanes 1 and 2), suggesting structural similarity between E2 and human Ig molecules. This binding was also obtained when using the first 113 or 123 aa of E2 (encompassing hypervariable regions 1 and 2 (HVR1, HVR2)) from different HCV variants of genotype 1a whether synthesized

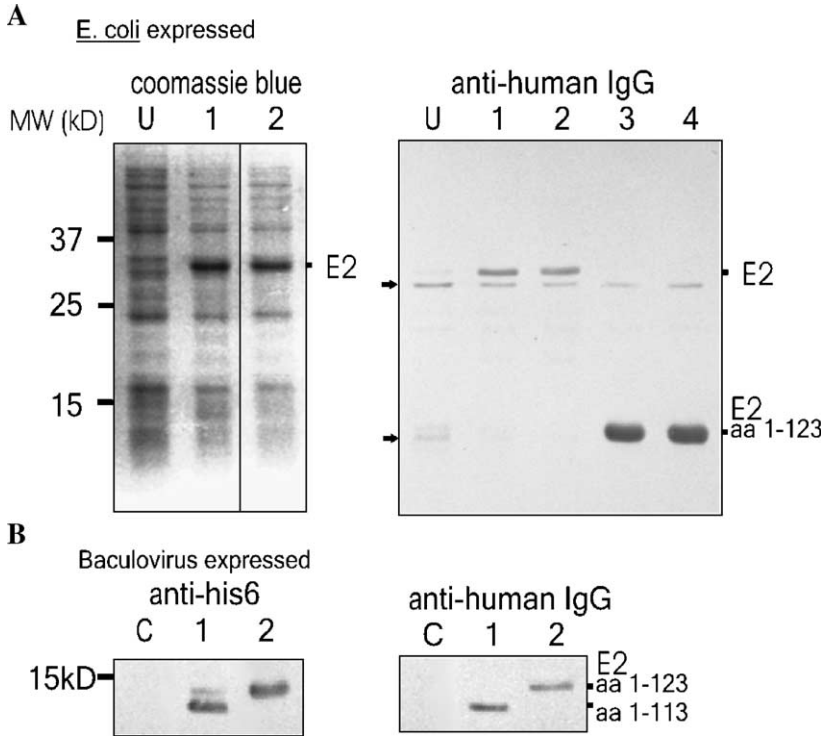

Fig. 1. Anti-human-IgG Binds HCV E2. (A) Full length HCV 3a E2 (aa1–322) or amino terminal regions from aa1–123 of HCV 1a proteins were expressed in *E. coli.* The left panel shows total protein staining as a sample loading control (Coomassie blue) and the right panel shows anti human-IgG Fab fragment binding. Samples: U-uninduced HCV 3a clone B-d8-1 (aa1–322), 1—HCV 3a B-1 (aa1–322), 2—HCV 3a B-2 (aa1–322); 3—HCV 1a A-5 (aa1–123); 4—HCV 1a A-9 (aa1–123). In addition to the specific E2 proteins, two *E. coli* protein bands were observed to bind anti-human IgG (open arrows). (B) Amino terminal fragments of HCV1a were cloned and expressed in baculovirus infected insect cells. The left panel shows the loading controls that were detected by binding to anti-his6 antibody and the right panel is stained with anti-human-IgG Fab fragment. Sample C—cell lysate from empty baculovirus vector; 1—HCV 1a A19 (aa1–113); 2—HCV 1a A-5 (aa1–123).