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Development and characterization of a human monoclonal antibody targeting the N-terminal region of hepatitis C virus envelope glycoprotein E1

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ABSTRACT

Monoclonal antibodies (mAbs) targeting the hepatitis C virus (HCV) envelope have been raised mainly against envelope protein 2 (E2), while the antigenic epitopes of envelope protein 1 (E1) are not fully identified. Here we describe the detailed characterization of a human mAb, designated A6, generated from an HCV genotype 1b infected patient. ELISA results showed reactivity of mAb A6 to full-length HCV E1E2 of genotypes 1a, 1b and 2a. Epitope mapping identified a region spanning amino acids 230–239 within the N-terminal region of E1 as critical for binding. Antibody binding to this epitope was not conformation dependent. Neutralization assays showed that mAb A6 lacks neutralizing capacity and does not interfere with the activity of known neutralizing antibodies. In summary, mAb A6 is an important tool to study the structure and function of E1 within the viral envelope, a crucial step in the development of an effective prophylactic HCV vaccine.

1. Introduction

Hepatitis C virus (HCV) is a member of the *Hepacivirus* genus within the *Flaviviridae* family. Based on the 9.6 kb-long RNA genome sequence, HCV is classified into 7 genotypes (1 − 7) and multiple subtypes (a, b, c, etc.) (Bukh, 2016). More than 170 million people worldwide are estimated to be infected with HCV (Petruzziello et al., 2016). More than 70% of individuals with acute HCV infection will become chronically infected, which after several decades may lead to liver cirrhosis and hepatocellular carcinoma. Despite the remarkable improvement of HCV treatment regimens using direct-acting antivirals (DAAs), mutations occur that bring about resistance and negatively impact treatment outcome (Li and De Clercq, 2017; Pawlotsky, 2016). The risk of acquiring HCV infection is high among injection drug users due to the high prevalence of HCV in this population. The high mutation rate of HCV, leading to continuous release of closely related viral variants that escape the host's adaptive immune response, represents a major hurdle for the development of a vaccine. Identification of conserved epitopes that induce protective immunity would strongly promote vaccine design.

Inside the host cell, viral RNA is translated into a single polyprotein precursor that is cleaved into structural (core, envelope E1 and E2) and non-structural (P7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) proteins (Bartenschlager et al., 2011). Structural proteins are responsible for the formation of the HCV virus particle, while the non-structural proteins are essential for viral replication, translation and assembly. Our insight

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in the HCV life cycle was hampered for a long time but the development of the HCV pseudoparticle (HCVpp) and HCV cell culture (HCVcc) systems paved the way for a more comprehensive exploration of virus infectivity (Hsu et al., 2003; Wakita et al., 2005). Both systems are now widely used for studying HCV entry and screening for viral inhibitors (Catanese and Dorner, 2015). The envelope proteins E1 and E2 are highly glycosylated membrane-associated proteins consisting of an N-terminal ectodomain and a C-terminal transmembrane domain. The E1 protein is 192 amino acids (AA) long (from AA 192 to 383) while the E2 glycoprotein is 363 residues (AA 384–746) in length according to the reference strain H77 (accession no. AF011751). E2 functions as mediator for viral entry through interaction with host attachment factors and receptors (Dubuisson and Cosset, 2014). It is also the main target for the host's adaptive immune system. Recently the E1 protein was also shown to be involved in viral entry and virion assembly (Haddad et al., 2017).

The essential role of cell-mediated immunity in HCV clearance has been extensively reported (Abdelwahab, 2016). The early generation of neutralizing antibodies has been associated with resistance to infection in individuals at high-risk of exposure, spontaneous clearance during acute infection and sustained virologic response after therapy (Ndongo et al., 2010; Osburn et al., 2014; Swann et al., 2016). Although the envelope of HCV contains multiple immunogenic epitopes, the majority of monoclonal antibodies (mAbs) isolated from infected patients or vaccinated animals have been identified as E2-specific (Tabll et al., 2015). The biological activity of these antibodies is diverse and varies from neutralizing to non-neutralizing or even interfering (Wang et al., 2011a). Polyclonal antibodies from HCV-infected patients were able to protect animal models like humanized mice and chimpanzees from HCV challenge (Bukh et al., 2015; Meuleman et al., 2011; Vanwolleghem et al., 2008). We and others previously reported that neutralizing antibodies targeting the E2 protein could protect from HCV infection in vitro and in vivo (Desombere et al., 2016; Keck et al., 2016; Mesalam et al., 2016). In addition, administration of mAb MBL-HCV1, targeting E2, delayed viral rebound following liver transplantation, while complete protection was reported when combined with the polymerase inhibitor sofosbuvir (Chung et al., 2013; Smith et al., 2017). These observations point towards the importance of humoral immunity and neutralizing antibodies in HCV clearance.

Despite the availability of multiple in vitro systems as well as experimental animal models, little is still known about the structure and actual function of the E1 glycoprotein (Douam et al., 2014; Haddad et al., 2017; Lavillette et al., 2007; Wahid et al., 2013). Evidence for the immunogenicity and the induction of neutralizing antibodies by E1 has been reported. However, only few mAbs have been raised against this protein compared to the numerous anti-E2 mAbs described in the literature. This may be related to the difficulty to express the E1 protein as a correctly folded monomer (Op De Beeck et al., 2001). Polyclonal antibodies from mice vaccinated with E1-HCVpp or recombinant E1 protein were able to neutralize HCVcc (Dreux et al., 2006; Pietschmann et al., 2006). Also, synthetic peptides covering the C terminal region of E1 were able to react with 32% of sera from infected patients in one study (Siemoneit et al., 1995) while 92% reactivity was reported in another study (Ray et al., 1994). In addition, immune sera of rabbits vaccinated with a synthetic peptide encompassing AA 315–323 prevented the binding and entry of HCV particles into HepG2 cells (El-Awady et al., 2006). However, this finding has not been confirmed using the HCVpp or HCVcc systems.

Two main regions have been identified as reactive domains for anti-E1 antibodies. The first region is the N- terminal region identified by the human mAb H-111 (AA 192–202) (Keck et al., 2004b) and the murine mAb A4 (AA 197–207) (Dubuisson et al., 1994). While these antibodies recognize E1 presented on the viral envelope, mAb H-111 is only weakly neutralizing while no neutralization has been reported for mAb A4. The second region encompasses AA 313–327, which is located at the C terminus and is identified mainly by mAbs IGH505 and IGH526 (Kong et al., 2015; Meunier et al., 2008). We isolated a monoclonal antibody, designated A6, from a HCV genotype 1b infected patient. Epitope mapping identified AA 230–239 within the N-terminal region of E1 as the critical site for binding. This mAb showed affinity towards a panel of HCV envelope proteins of genotypes 1a, 1b and 2a, but lacked any neutralizing or interfering activity.

2. Materials and methods

2.1. Human sera, cell lines and antibodies

Blood samples were collected from HCV infected patients who were followed at the Ghent University Hospital. The study was approved by the local ethical committee and all patients involved gave informed consent. Human embryonic kidney cells (293T) and human hepatoma cell lines (Huh-7.5RFP-NLS-IPS and Hep3B) were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum, 1% non-essential amino acids, 1% L-glutamine and antibiotics. The human anti-E1 mAb IGH526, the murine anti-E1 mAb A4, the murine anti-E2 mAb AP33 and the human anti-E2 mAbs MRCT10, 1:7, HC84.26, CBH-7 and HC-1AM were previously described (Allander et al., 2000; Dubuisson et al., 1994; Keck et al., 2004a, 2012; Kong et al., 2015; Owsianka et al., 2001; Pantua et al., 2013; Wang et al., 2011b).

2.2. Amplification and expression of HCV envelope

RNA was extracted from the plasma of infected patients using ZR Viral RNA kit (Zymo Research) followed by cDNA synthesis using superscript III reverse transcriptase (Invitrogen) and random primers. The full-length E1E2 sequence was amplified by nested PCR. The first PCR was performed using LongAmp DNA polymerase (NEB) and the following primers: (F) 5`-CGT AGG TCG CGT AAC TTG GGT AA-3′ and (R) 5`-GTG CGC CTC GGC CCT GGT GAT AAA-3`. The second-round PCR was performed using Pfu DNA polymerase (Promega) and primers: (F) 5'-TAT AGA TAT CAT GGG GTA CAT TCC GCT CGT C-3' and (R) 5'-ATA TGA TAT CTT ACT CAG CCT GAG CTA TCA G-3'. PCR products were analyzed using 1% agarose gel electrophoresis and the bands corresponding to E1E2 were eluted and cloned into the pCDNA3.1/Hygro expression vector (Invitrogen). The inserts were sequenced (Center for Medical Genetics, Ghent University, Belgium), multiple aligned and analyzed using BioEdit version 7.2.0, Clone manager 9 professional and CLC main workbench version 7.6.4 (QIAGEN). HCV E1E2 sequences covering all 7 genotypes (1a, 1b, 2a, 2b, 3a, 4a, 5a, 6a and 7a) were retrieved from the European HCV database (https://euhcvdb.ibcp.fr/euHCVdb/) and the NCBI (https://www.ncbi.nlm.nih.gov/nucleotide/) and used for alignment with Belgian isolates. Based on amino acid sequences of the whole E1E2, neighbor joining phylogenetic tree was constructed using 1000 replicates bootstrapping analysis of CLC main workbench version 7.6.4. For expression of E1E2 in mammalian cells, constructs encoding the E1E2 region of genotypes 1–6 were used for transfection of 293T cells using ProFection mammalian transfection kit (Promega). After 48 h, lysis buffer (Promega) and protease inhibitors (Roche) were added and the cell lysate was centrifuged at 13,000 rpm and 4 °C. The supernatant was collected and stored at –80 °C until use. Constructs containing the following viral strains were used: H77, UKN1A20.8, UKN1A14.38, J4, UKN1B5.23, UKN1B12.16, P5VD, P5VE, P5VF, JFH1, UKN2A1.2, UKN2B2.8, S52, UKN4.11.1, UKN5.14.4 and UKN6.5.8 in addition to 28 Belgian isolates (Desombere et al., 2016; Fafi-Kremer et al., 2010; Lavillette et al., 2005; Owsianka et al., 2005).

2.3. Generation of anti-HCV mAb

The hybridoma cells were generated from the peripheral blood mononuclear cells (PBMCs) of a genotype 1b HCV infected patient as described previously (Depraetere et al., 2001). Clones that secreted E1-specific antibodies were identified using a prototype version of the