BASIC—LIVER, PANCREAS, AND BILIARY TRACT

Monoclonal Anti-Claudin 1 Antibodies Prevent Hepatitis C Virus Infection of Primary Human Hepatocytes

ISABEL FOFANA,[*,‡] SOPHIE E. KRIEGER,[*,‡] FRITZ GRUNERT,[§] SANDRA GLAUBEN,[§] FEI XIAO,[*,‡] SAMIRA FAFI–KREMER,[*,‡] ERIC SOULIER,[*,‡] CATHY ROYER,[*,‡] CHRISTINE THUMANN,[*,‡] CHRISTOPHER J. MEE,[‖] JANE A. MCKEATING,[‖] TATJANA DRAGIC,[¶] PATRICK PESSAUX,[#] FRANCOISE STOLL–KELLER,[*,‡] CATHERINE SCHUSTER,[*,‡] JOHN THOMPSON,[§] and THOMAS F. BAUMERT[*,‡,**]

[*]Inserm, U748, Strasbourg, France; [‡]University of Strasbourg, Strasbourg, France; [§]Genovac GmbH, Freiburg, Germany; [‖]Hepatitis C Research Group, Institute for Biomedical Research, University of Birmingham, Birmingham, United Kingdom; [¶]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York; [#]Pôle des Pathologies Digestives, Hépatiques et Transplantation, and [**]Pôle Hépato-digestif, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

BACKGROUND & AIMS: Hepatitis C virus (HCV) infection is a challenge to prevent and treat because of the rapid development of drug resistance and escape. Viral entry is required for initiation, spread, and maintenance of infection, making it an attractive target for antiviral strategies. The tight junction protein claudin-1 (CLDN1) has been shown to be required for entry of HCV into the cell. **METHODS:** Using genetic immunization, we produced 6 monoclonal antibodies against the host entry factor CLDN1. The effects of antibodies on HCV infection were analyzed in human cell lines and primary human hepatocytes. **RESULTS:** Competition and binding studies demonstrated that antibodies interacted with conformational epitopes of the first extracellular loop of CLDN1; binding of these antibodies required the motif W(30)-GLW(51)-C(54)-C(64) and residues in the N-terminal third of CLDN1. The monoclonal antibodies against CLDN1 efficiently inhibited infection by HCV of all major genotypes as well as highly variable HCV quasispecies isolated from individual patients. Furthermore, antibodies efficiently blocked cell entry of highly infectious escape variants of HCV that were resistant to neutralizing antibodies. **CONCLUSIONS: Monoclonal antibodies against the HCV entry factor CLDN1 might be used to prevent HCV infection, such as after liver transplantation, and might also restrain virus spread in chronically infected patients.**

Keywords: Antiviral; Genetic Barrier; Host Factor; Receptor; Treatment.

Hepatitis C virus (HCV) is a major cause of cirrhosis and hepatocellular carcinoma worldwide.[1] Current antiviral treatment consisting of pegylated interferon-α (IFN-α) and ribavirin is limited by resistance, adverse effects, and high costs.[2] Although the clinical development of novel antivirals targeting HCV protein processing has been shown to improve sustained virological response, toxicity of the individual compounds and development of viral resistance remain major challenges.[3]

The absence of strategies for prevention of HCV infection is a major problem for patients undergoing liver transplantation for HCV-related end-stage liver disease.[1] Because of viral evasion from host immune responses and immunosuppressive therapy, reinfection of the graft is universal and characterized by accelerated progression of liver disease.[1,4] Recurrent HCV liver disease in the graft with poor outcome has become an increasing problem facing hepatologists and transplant surgeons. Thus, novel antiviral preventive and therapeutic strategies are urgently needed.[5]

HCV entry into target cells is a promising target for antiviral preventive and therapeutic strategies[6,7] because it is essential for initiation, spread, and maintenance of infection.[6,7] Furthermore, cross-neutralizing antibodies inhibiting HCV entry have been shown to be associated with control of HCV infection and prevention of HCV reinfection in cohorts with self-limited acute infection.[8,9]

HCV entry is a multistep process involving several host factors including heparan sulfate,[10] CD81,[11] scavenger receptor B1,[12] claudin-1 (CLDN1),[13] and occludin.[14] Among the host cell entry factors, tight junction (TJ) protein CLDN1 is a promising antiviral target because it is essential for HCV entry, and, to date, there is no evidence for CLDN1-independent HCV entry.[13,15] Fur-

Abbreviations used in this paper: CLDN, claudin; CMFDA, 5-chloromethylfluorescein di-acetate; CTRL, control; EL1, extracellular loop 1; HA-tag, hemaglutinin-tag; HCV, hepatitis C virus; HCVcc, cell culture-derived HCV; HCVpp, HCV pseudoparticles; IC_{50}, 50% inhibitory concentration; IgG, immunoglobulin G; K_d, dissociation constant; mAb, monoclonal antibody; MFI, mean fluorescence intensity; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PHH, primary human hepatocytes; TJ, tight junction.

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thermore, CLDN1 has been suggested to play an important role in cell-cell transmission.[16] In contrast to other HCV entry factors such as CD81 or scavenger receptor BI, CLDN1 is predominantly expressed in the liver.[17] CLDN1 is also expressed in the kidney.[17]

To date, the exploration of CLDN1 as an antiviral target has been hampered by the lack of antibodies targeting surface expressed epitopes.[13] In this study, we demonstrate for the first time successful production of anti-CLDN1 monoclonal antibodies (mAbs) that inhibit HCV infection. These results suggest that targeting CLDN1 with specific mAbs may constitute a novel antiviral approach to prevent primary HCV infection, such as after liver transplantation, and might also restrain virus spread in chronically infected patients.

Materials and Methods

Primary Hepatocytes and Cell Lines

Culture of primary human hepatocytes (PHH),[18] human hepatoma cell lines (Huh7.5.1),[19] and HepG2,[20] human embryonic kidney cell lines (HEK293T),[9] and BOSC23, and Chinese hamster ovary (CHO) cells[18] has been described.

Production and Screening of anti-CLDN1 mAbs

Anti-CLDN1 mAbs were raised by genetic immunization of Wistar rats using a eukaryotic expression vector encoding the full-length human CLDN1 complementary DNA according to proprietary GENOVAC technology (GENOVAC GmbH, Freiburg, Germany). Following completion of immunization, antibodies were selected by flow cytometry for their ability to bind to human CLDN1 expressed on the cell surface of nonpermeabilized HEK293T and BOSC23 cells and CHO cells, which had been transfected with pCMV-SPORT6/CLDN1. For imaging studies, Huh7.5.1 cells were stained with rat isotype control or anti-CLDN1 mAb OM-4A4-D4 (10 μg/mL) and analyzed as described.[18]

Epitope Mapping

Competition between anti-CLDN1 mAbs for cellular binding was measured by a cell-based enzyme-linked immunosorbent assay and labeled antibodies: Huh7.5.1 cells were incubated for 60 minutes with 0.1 μg/mL biotinylated anti-CLDN1 mAb (Sulfo-NHS-LC-Biotin; Thermo Scientific, Illkirch, France) together with increasing concentrations of unlabeled anti-CLDN1 mAbs as competitors. Following washing with phosphate-buffered saline, binding of biotinylated antibody was detected by incubation with streptavidin labeled with horseradish peroxidase. Curves determined by measurement of binding in the presence of an isotype-matched control were compared with those determined in the presence of the competing antibody. Epitope mapping was performed using plasmids encoding for CLDN1 containing defined mutations and a cytoplasmic N-terminal hemaglutinin-tag (HA-tag).[21] To study binding of anti-CLDN1 mAbs to mutant CLDN1, binding of mAb OM-7D3-B3 to BOSC23 transfected with CLDN1 expression constructs was determined by flow cytometry. Flow cytometric quantitation of HA-tag expression using an anti-HA antibody (Covance, Münster, Germany) served as internal control. The transfected cells were either permeabilized with Cytoperm/Cytofix (BD Biosciences, Heidelberg, Germany) for analysis of cytoplasmic HA-tag expression or untreated for analysis of anti-CLDN1-mutant CLDN1 interactions. For fluorescence-activated cell sorter analysis, transfected cells were incubated with 20 μg/mL anti-CLDN1 mAb or anti-HA for 30 minutes followed by incubation with 10 μg/mL anti-rat immunoglobulin G (IgG) mAb (for anti-CLDN1) or 10 μg/mL anti-mouse IgG mAb labeled with phycoerythrin (for anti-HA). The half-saturating concentrations (apparent K_d [antibody disassociation constant]) were determined as described.[22]

Cell Culture-Derived HCV and HCV Pseudoparticles Production and Infection

Cell culture-derived HCV (HCVcc) (Luc-Jc1, Luc-Con1),[23] HCV pseudoparticles (HCVpp) (strains H77, HCV-J, JFH1, UKN3A1.28, UKN4.21.16, UKN5.14.4, UKN6.5.340, P01VL, P02VH, P02VI, P02VJ, P03VC, P04VC, P04VD, P04VE, P05VD, P05VE, P05VF, P06VG, P06VH, P06VI)[24] and vesicular stomatitis virus pseudoparticles (VSVpp) were produced as described.[24] Patient-derived HCVpp were produced from 6 patients (P01–P06) undergoing liver transplantation using full-length E1E2 expression constructs generated from circulating HCV as described.[9,24] Huh7.5.1, Huh7 cells, or PHH were preincubated with antibodies for 1 hour and incubated for 4 hours at 37°C with HCVcc or HCVpp. Viral infection was analyzed as described.[18,23] For antibody-mediated neutralization, HCVpp were preincubated with autologous anti-HCV serum,[24] anti-E2 mAb (IGH461; Innogenetics, Gent, Belgium),[25] and anti-HCV IgG purified from a chronically infected patient as described.[25,26]

Toxicity Assays

Cytotoxic effects on cells were assessed by analyzing the ability to metabolize 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as described previously.[27] Huh7.5.1 cells and PHH from 3 different donors were preincubated with isotype control, anti-CLDN1 OM-7D3-B3 mAbs, anti-Fas (Beckman Coulter, Marseille, France), flavopiridol (Sigma, Taufkirchen, Germany), or compound C (Sigma). TJ integrity was analyzed as described.[20]

Statistical Analysis

Results are expressed as means ± standard deviation (SD). Statistical analyses were performed using Student t test, with a P value of $<.05$ being considered statistically significant.