Antiviral Research 101 (2014) 37–44

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Impact of single nucleotide polymorphisms in the essential HCV entry factor CD81 on HCV infectivity and neutralization

Maximilian Deest [a,b], Sandra Westhaus [a,b,d], Eike Steinmann [c], Michael P. Manns [a,b], Thomas von Hahn [a,b,d], Sandra Ciesek [a,b,*]

[a] Department of Gastroenterology, Hepatology and Endocrinology, Medical School Hannover, Germany
[b] German Centre for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Germany
[c] Division of Experimental Virology, TWINCORE, Centre for Experimental and Clinical Infection Research, A Joint Venture Between the Medical School Hannover (MHH) and the Helmholtz Centre for Infection Research (HZI), Hannover, Germany
[d] Institute for Molecular Biology, Medical School Hannover, Hannover, Germany

ARTICLE INFO

Article history:
Received 23 July 2013
Revised 22 October 2013
Accepted 30 October 2013
Available online 7 November 2013

Keywords:
Hepatitis C virus
CD81 antibodies
Single nucleotide polymorphism
Entry

ABSTRACT

End stage liver disease caused by chronic infection with the hepatitis C virus (HCV) is a leading indication for liver transplantation, yet outcomes are poor since the liver graft is rapidly re-infected by HCV. Antibodies against the essential HCV receptor CD81 have been shown to inhibit HCV cell entry *in vitro* and *in vivo* and may represent an attractive treatment option. However, several CD81 variants exist at low levels in human populations.

We aimed to investigate to what extent these variants function as HCV receptors and would be amenable to therapeutic interventions with CD81 antibodies. We used lentiviral expression to introduce wildtype or variant CD81 in the $CD81^{low}$ Lunet N4 cell line. HCV replication cycle steps and neutralization by CD81 antibodies were then investigated using full length HCV reporter viruses (HCVcc) as well as HCV pseudoparticles (HCVpp).

We found that all tested CD81 variants support cell entry by HCVpp and HCVcc with an efficiency similar to wildtype CD81. Other replication cycle steps, namely intracellular RNA replication and release of new particles, were also unaffected by the presence of CD81 variants. Importantly, four neutralizing antibodies directed against the CD81 LEL (5A6, JS81, 1D6 and 1.3.3.22) retained their ability to inhibit HCV infection when wildtype CD81 on target cells was replaced with any of the CD81 variants.

These data indicate that CD81 variants that exist in the human population are fully functional as HCV receptors and their presence would not diminish the efficacy of therapeutic regimens that include CD81-antibodies.

1. Introduction

About 160 million individuals worldwide are currently chronically infected with the hepatitis C virus (HCV) and thus at risk for the development of cirrhosis, end-stage liver disease and hepatocellular carcinoma (Lavanchy, 2011). Individuals undergoing orthotopic liver transplantation (OLT) for complications of HCV infection pose a particular clinical problem: graft re-infection with HCV occurs in nearly all cases and long-term outcomes are unsatisfactory (Ciesek and Wedemeyer, 2012). Prevention of graft re-infection, as it is routinely achieved in the case of hepatitis B, is a major clinical goal, but will likely require efficient pharmacological means of preventing viral entry into hepatocytes. However, as yet such compounds are not available or even in advanced development.

HCV has a narrow host range and primarily infects and replicates in human hepatocytes (Burgel et al., 2010). One reason for this is that all stages of HCV's replication cycle depend on various cellular host factors. For example, HCV cell entry requires at least four host factors on the hepatocyte surface: the tetraspanin CD81, scavenger receptor class B type I (SR-BI) and the tight junction components claudin 1 (CLDN1) and occludin (OCLN) (von Hahn et al., 2010). Additional cellular factors are also involved, but seem to be ubiquitously present since it appears that the presence of the above four determines whether a cell allows HCV entry or not (Ploss et al., 2009).

The essential HCV entry factor human CD81 (hCD81) is expressed in all nucleated cells and belongs to the tetraspanin super

* Corresponding author at: Department of Gastroenterology, Hepatology and Endocrinology, Medical School Hannover, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany. Tel.: +49 511 532 4585; fax: +49 511 532 4283.
E-mail address: ciesek.sandra@mh-hannover.de (S. Ciesek).

0166-3542/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.antiviral.2013.10.018

family (Bitzegeio et al., 2010). It carries six structural domains: Four are transmembrane domains and two are extracellular loops containing a small (SEL) and a large (LEL) extracellular loop. CD81 plays a vaguely defined role in many cell processes including cell morphology, signal transduction, adhesion and proliferation and differentiation of cells of the immune system (Levy et al., 1998). It has been shown previously that HCV infection is sensitive to neutralization with anti-CD81 antibodies *in vitro* and *in vivo* (Meuleman et al., 2008). Therefore CD81 is considered to be an attractive therapeutic target as it offers a prospect of preventing HCV infection after OLT by blocking cell entry.

The clinical course of HCV infection is highly variable between individual hosts: up to 80% of acutely HCV infected patients develop a chronic infection while 20% clear infection spontaneously. Among those who remain chronically infected some remain asymptomatic for life while others require liver transplantation or die from the complications of chronic infection (Di Bisceglie, 2000). Two genetic variations in the interleukin-28B gene are associated with spontaneous or treatment-induced resolution of infection (Ge et al., 2009; Tanaka et al., 2009). Very recently, it was shown that a different nearby deletion variant (ss469415590) that creates a novel gene (interferon lambda 4) may be even more strongly predictive and in fact mechanistically responsible for the observed phenotype (Prokunina-Olsson et al., 2013). Additionally, we recently identified three coding non-synonymous SNP's in PPIA, the gene encoding for the essential HCV replication factor cyclophilin A, which are associated with resistance to HCV infection *in vitro* (von Hahn et al., 2012). However, additional genetic factors must clearly be involved in determining the course of hepatitis C.

Several genetic variants in the CD81 gene have been described in humans, but it is unknown if all of these CD81 variants facilitate HCV entry. Even more important, it has never been investigated if anti-CD81 antibodies can neutralize HCV infection if one of the CD81 variants is present.

The aim of this study was to investigate the impact of intraspecies genetic variation in the essential HCV entry factor CD81. By using the Lunet N4 cell line, which has very low endogenous CD81 and re-introduction of CD81 variants by lentiviral transduction we could show that all seven known coding non-synonymous SNP's were able to support HCV entry *in vitro*. Importantly, SNP's in the coding region of the CD81 gene did not appear to have an effect on neutralization by several CD81 antibodies or cell-to-cell-transmission of HCV. These data suggest that it is not necessary to test patients for CD81 SNP's before potential treatment with CD81 antibodies.

2. Materials and methods

2.1. DNA constructs

Variants were created by standard polymerase chain reaction mutagenesis into the wildtype hCD81 open reading frame contained in the lentiviral pWPI vector (pWPI-BLA) (von Hahn et al., 2012). Restriction sites for *Pme*I and *Spe*I were used. Detailed cloning strategies are available upon request. All modified hCD81 sequences were confirmed by direct sequencing (Eurofins MWG Operon, Ebersberg, Germany). The constructs encoding the HCV E1/E2 proteins for pseudotypes of H77 (genotype 1a) or J6 (genotype 2a) and the full length reporter virus genome Luc-Jc1 have been described previously (Ciesek et al., 2011b).

2.2. Cell culture and cell lines

Lunet N4 subclones and Huh-7.5 cells were maintained in Dulbeccos modified eagle medium (DMEM, Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal bovine serum, L-glutamine, non-essential amino acids, penicillin and streptomycin. Cells harboring the hCD81 variants were kept in the presence of 5 μg/ml Blasticidin.

2.3. Pseudotyping of lentiviral particles and transduction of target cells

Pseudoparticles were generated as previously described (Ciesek et al., 2011b). Briefly three plasmids were cotransfected into 293T cells. These encoded (I) a gutted lentiviral genome containing either a firefly luciferase reporter (CSFlucW2) or any of the CD81 variants described above, (II) HIV gag-pol and (III) either the HCV glycoproteins E1 and E2 of strain H77 or J6 or the G protein of Vesicular Stomatitis Virus (VSV-G). Supernatants were collected at 48 h post transfection, passed through a 0.45 μm-pore-size filter and added to the target cells for 6 h. Luciferase assay was performed 72 h post transduction.

2.4. Cell culture grown HCV (HCVcc) infection

Huh-7.5 cells were electroporated with an *in vitro* generated RNA transcript of the HCV genome F-Luc Jc1 containing a firefly luciferase reporter gene as previously described (Ciesek et al., 2011a). For infection experiments supernatant at 48 h was passed through a 0.45 μm pore size filter and used to infect target cells. 500 μl containing virus supernatant with a viral titer of 1×10^4 in $TCID_{50}$ (MOI 0.05–0.1) was used to inoculate target cells for 5 h. Quantification of HCV infection was performed by measuring luciferase activity. To assess RNA replication separate from cell entry, luciferase activity was measured in electroporated instead of infected cells.

2.5. Luciferase assay

Infected or transfected cells were washed twice with phosphate-buffered saline and lysed in 1000 μl/well (6-well plate) or 350 μl/well (12-well plate) luciferase lysis buffer (1% Triton X-100, 25 mM glycylglycine, 15 mM $MgSO_4$, 4 mM EGTA and 1 mM DTT, pH 7.8). Firefly luciferase activity was measured as described previously using a luminometer (Lumat LB9508; Berthold, Freiburg, Germany) (Ciesek et al., 2011a).

2.6. Antibodies

For neutralisation studies we used the following CD81 antibodies: 1D6 (AbD serotec, Düsseldorf, Germany), 5A6 (Santa Cruz Biotechnology, Santa Cruz, USA), 1.3.3.22 (Ancell, Bayport, USA) and JS81 (Becton Dickinson, Heidelberg, Germany).

2.7. Flow cytometery

Lunet N4 and Huh-7.5 cells were stained with an anti-CD81 antibody (clone JS-81, Becton Dickinson, Heidelberg, Germany) and secondary anti-mouse antibody labeled with FITC (Invitrogen, Paislay, UK). For measurement BD FACS Canto flow cytometer (Becton Dickinson, Heidelberg, Germany) was used. FlowJo software (Tree Star, Ashland, OR) was used for data analysis.

2.8. Detection of cell-to-cell transmission

To investigate cell-to-cell transmission of HCV in adjacent cells, we performed an agarose overlay assay where transmission from HCV positive donor cells to HCV negative target cells in co-culture was assessed (Ciesek et al., 2011b). Target cells with CD81 variants were transduced with the pTRIP-tagRFP-NLS-IPS1 reporter construct (Jones et al., 2010). This construct contains a fusion protein

of the red fluorescent protein tagRFP, an SV40 nuclear localization sequence (NLS) and the C-terminal part of the interferon beta promoter stimulator protein 1 (IPS1). IPS1 contains a recognition site for the HCV NS3/4 protease and a transmembrane segment that is lodged in the outer mitochondrial membrane. In uninfected cells the tagRFP reporter is tethered to the mitochondrial membrane resulting in a cytosolic distribution of fluorescence. If cells are infected with HCV, NS3/4A cleaves the tagRFP-NLS fusion from its mitochondrial anchor and the reporter relocates to the nucleus. Donor and target cells were mixed 1:1 and seeded at 4×10^4 cells per well. The cell mixture was immediately overlaid with medium containing 1% agarose in order to prevent virus spread through the cell-free route. Thus all infection events observed in target cells are assumed to be due to cell-to-cell spread. Cell-to-cell-spread to target cells indicated by nuclear localization of tagRFP was monitored through fluorescent microscopy. The percentage of HCV positive target cells was determined after 96 h of co-culture.

2.9. Statistical analysis

The *in vitro* experiments were repeated on separate occasions. Each repetition was performed in multiple replicates. Unless stated otherwise the mean ± standard deviation of the replicates from one representative experiment is shown with the number of replicates indicated. Numerical data was analyzed using Excel (Microsoft, Redmond, WA, USA).

3. Results

3.1. Coding non-synonymous SNP's in the CD81 gene are present in human populations

Seven coding non-synonymous SNP's in the CD81 gene causing amino acid changes have been reported in the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP) (Fig. 1). Six out of these are located in the CD81 LEL that also contains the HCV E2 binding site. Only the V211M variant is located in the fourth transmembrane domain. There is incomplete information on the frequency of CD81 SNP's in the human population. In the 1000 genomes project (http://www.1000genomes.org/) the rs149336067, rs184960624 and rs148404749 variants have each been detected in one out of 2000 alleles in Tuscan, Utah, Yoruba residents, respectively. The other variants were not detected in the 1000 genomes panel.

A

rs number	aa exchange	location	MAF in 1000Genomes	Population with SNP
rs181171080	V135M	LEL	0.0005	Tuscan residents
rs149336067	N173S	LEL	n/a	
rs138631483	N173D	LEL	n/a	
rs184960624	D195G	LEL	0.0005	Utah residents
rs144435973	D195N	LEL	n/a	
rs148404749	D196E	LEL	0.0005	Yoruba residents
rs139884987	V211M	TM4	n/a	

MAF = minor allele frequence; LEL= large extracellular loop, n/a = not applicable

B

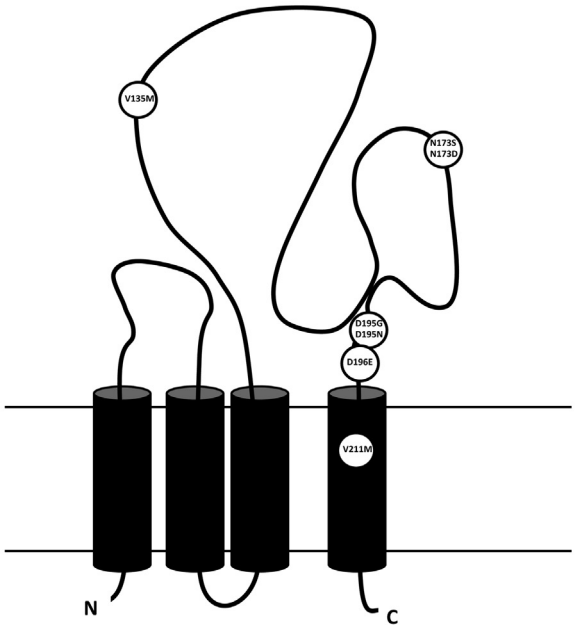

Fig. 1. Non-synonymous SNP's in CD81. (A) Schematic representation of CD81 with the position of seven amino acid changes caused by known non-synonymous SNP's in the coding region. (B) Topological overview of seven SNP's in human CD81 investigated in this study.

3.2. Generation and characterization of Lunet N cell lines with CD81 variants

To study the impact of variations in CD81 on HCVcc and HCVpp we used a cell line with low endogenous CD81 called Lunet N4 (Bitzegeio et al., 2010; Witteveldt et al., 2009). This cell line expresses very low levels of CD81 and thus is not susceptible to infection with HCVcc or HCVpp. We introduced the seven SNP's into lentiviral CD81 expression constructs and used these to transduce Lunet N4 cells. Transduction efficiency was assessed by flow cytometry and more than 95% transduction rates were confirmed in all cases (Fig. 2). Moreover, each of the cell lines expressed CD81 in comparable quantity on their surface. The cell line expressing wildtype human CD81 served as a control. To ensure, that surface expression is similar to naïve cells, we additionally analyzed CD81 surface expression on naïve Huh7.5 cells and could not detect any differences to the transduced Lunet cells.

3.3. Impact of human CD81 variants on HCVcc and HCVpp entry in vitro

To evaluate the effects of CD81 SNP's on HCV entry, we used HCV pseudoparticles (HCVpp) and cell culture derived Luc-Jc1 virus (HCVcc). To determine the impact of SNP's in the CD81 gene

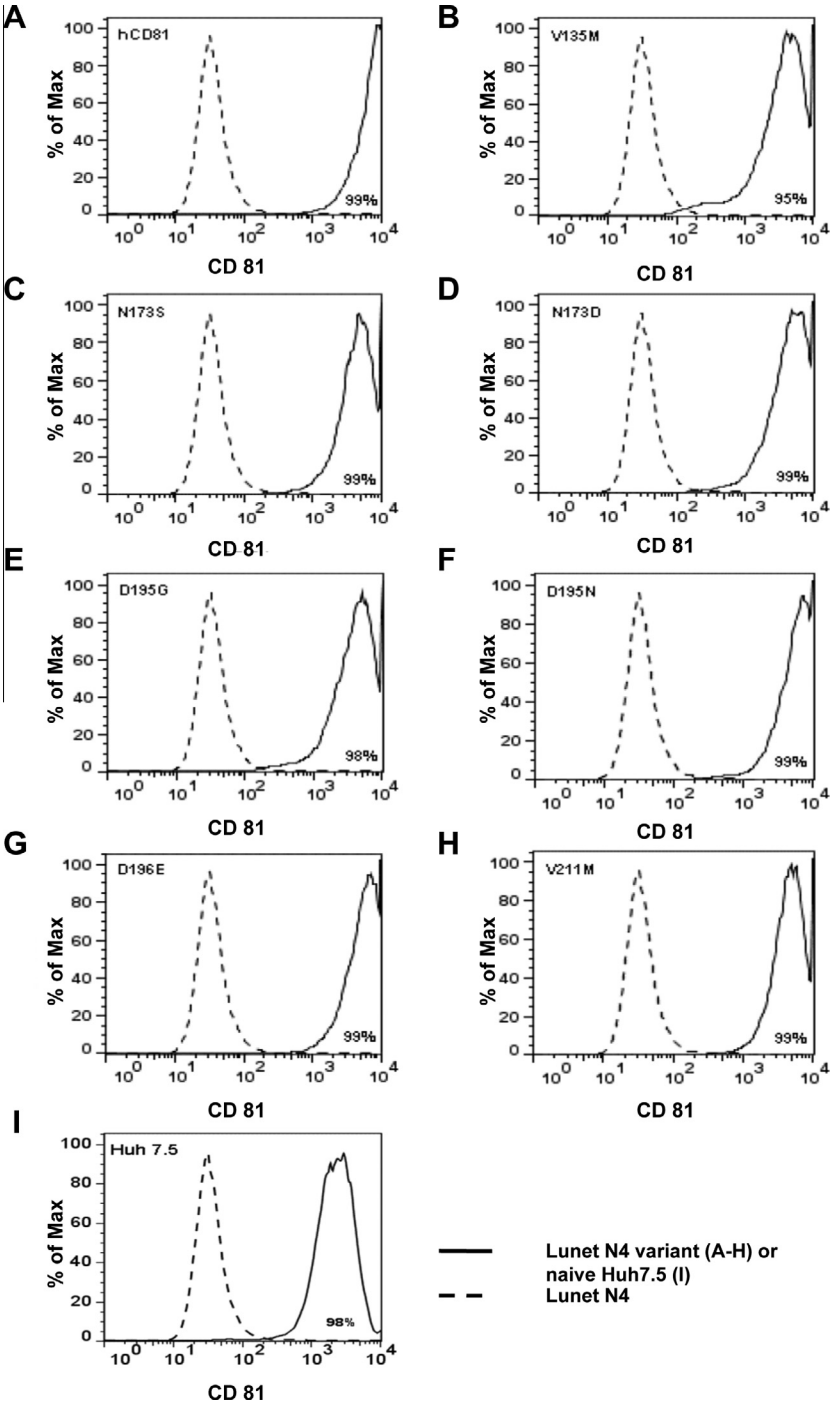

Fig. 2. Characterization of novel Lunet N4 cell lines with CD81 variants. (A–I) Surface expression of CD81 in different cell lines was determined by FACS analysis. All generated cell lines express the entry factor CD81 while Lunet N4 have a very low surface expression of CD81. As a control we used naïve Huh-7.5 cells.

on HCVcc entry, the different cell lines were infected with HCVcc for 5 h and after 48 h luciferase activity was quantified as a measure of RNA replication. As shown in Fig. 3 all CD81 SNP's facilitate HCVcc entry similar to wildtype CD81 (Fig. 3A). To investigate the effects of these CD81 SNP's on HCV entry alone we next used HCV pseudoparticles. HCVpp only test the entry step in the HCV life cycle without any interference from effects on HCV replication. Here we did not observe any inhibitory or stimulatory effect of the CD81 variants compared to wildtype CD81 on HCVpp bearing the HCV-glycoproteins E1E2 of genotype 1 (H77) or genotype 2 (J6, Fig. 3B). Thus, coding CD81 SNP's are unlikely to also have an effect of HCV entry *in vivo*.

3.4. No impact of CD81 SNP's on HCV replication and release of new viral particles

To investigate if CD81 SNP's have an impact on later steps in the HCV replication cycle, e.g. RNA replication and release of new viral particles we transfected the cell lines expressing the different CD81 variants with HCVcc and measured HCV replication by luciferase activity after 4, 24, 48 and 72 h post transfection. To investigate the release of new infectious particles the supernatant of electroporated cells was taken after 48 h, filtered through a 0,45 μm pore size filter and passed onto naïve Huh-7.5 cells. Medium was changed after 5 h, cells were lysed after 48 h and luciferase assay was performed. Even if there were slightly differences in HCV RNA replication or release of new particles *in vitro* all CD81 variants allow HCV RNA replication and particle release. Thus, genetic polymorphisms in the CD81 gene do not have any strong impact on virus replication (Fig. 4A) or *de novo* production of viral progeny (Fig. 4B).

3.5. Neutralisation with CD81 antibodies is not altered by coding non-synonymous SNP's in CD81

CD81 is thought to be an attractive target for HCV therapy, since cellular targets are likely to have higher barrier to resistance and drugs targeting cellular targets are often effective in all HCV genotypes. On the other hand it is possible, that genetic variations in the host protein might influence the efficiency of antibodies directed against CD81. Until now, it is unclear if SNP's in CD81 would influence the antiviral efficacy of CD81 antibodies. Several antibodies are able to block HCV infection *in vitro* and *in vivo* by binding to the LEL of CD81. We here investigated if the CD81 antibodies JS81, 1.3.3.22, 1D6 and 5A6 are able to inhibit HCVpp and HCVcc entry when one of the seven CD81 variants were present instead of wildtype CD81. Cell lines were inoculated with increasing concentrations of CD81 antibodies (0.05–5 μg/ml) 1 h before infection with HCV. After 1 h, cells were infected with HCVcc again in the presence of the different CD81 antibodies for 5 h. Cells were lysed after 48 h and luciferase assay was performed. Neutralization of HCV infection in a concentration dependent manner was observed for all cell lines. While treatment with CD81 antibodies JS81 or 1.3.3.22 led to complete neutralization at a concentration of 5 μg/ml (Fig. 5), CD81 antibodies 1D6 and 5A6 were only able to

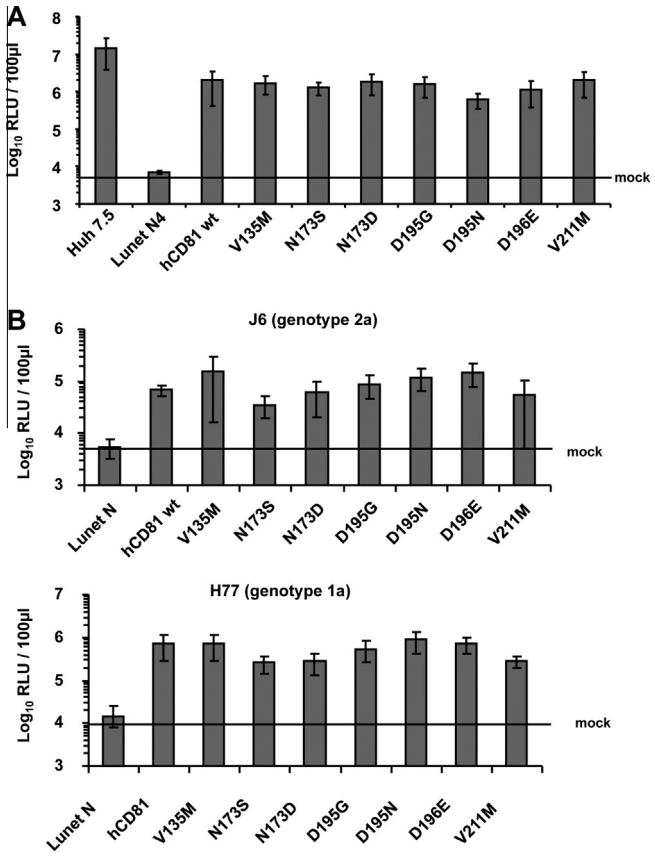

Fig. 3. Effect of different CD81 SNP's on the HCV entry. (A) Lunet N4 cells expressing human CD81 variants were inoculated with supernatant containing Jc1-Luc reporter virus. Luciferase activity was assayed 48 h post infection. Mean values and the standard deviation of three independent experiments are shown. (B) HCV pseudotypes bearing the J6 (upper part) or H77 (lower part) glycoproteins were used to infect Lunet N4 expressing different CD81 SNP's. Pseudotypes with the glycoprotein of the vesicular stomatitis virus (VSV-G) served as control. One of three independent experiments is shown. Mean values of quadruplicate measurement including the standard deviation are given.

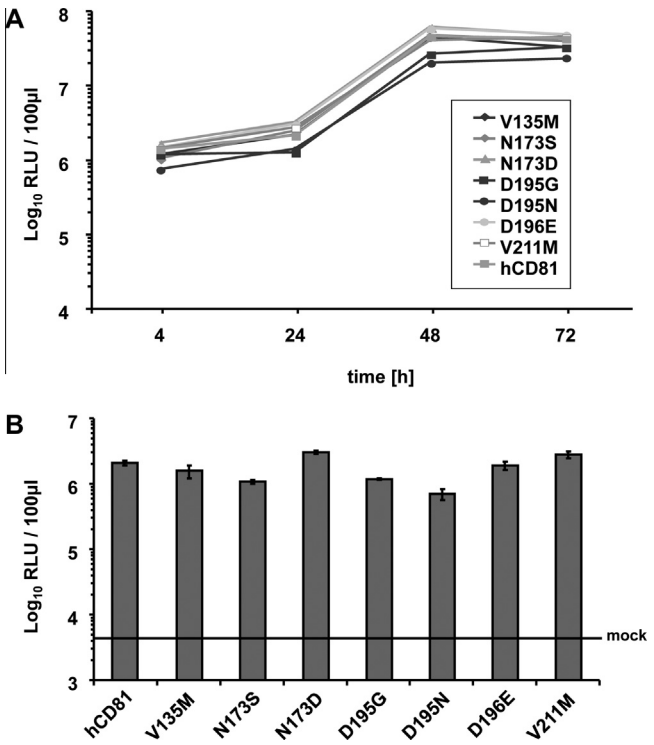

Fig. 4. Effects of CD81 SNP's on HCV RNA replication and release of new viral particles. (A) Lunet N4 cells expressing different CD81 variants were electroporated with Jc1-Luc RNA transcripts. HCV RNA replication was quantified by measuring luciferase activity after 4, 24, 48 and 72 h. Mean values of three independent experiments are shown. (B) To asses efficiency of viral particle production the different cells were transfected with Jc1 wildtype RNA transcripts and after 48 h supernatant containing viral particles were filtered and transferred to naïve Huh7.5 cells. Luciferase activity was measured after 48 h.

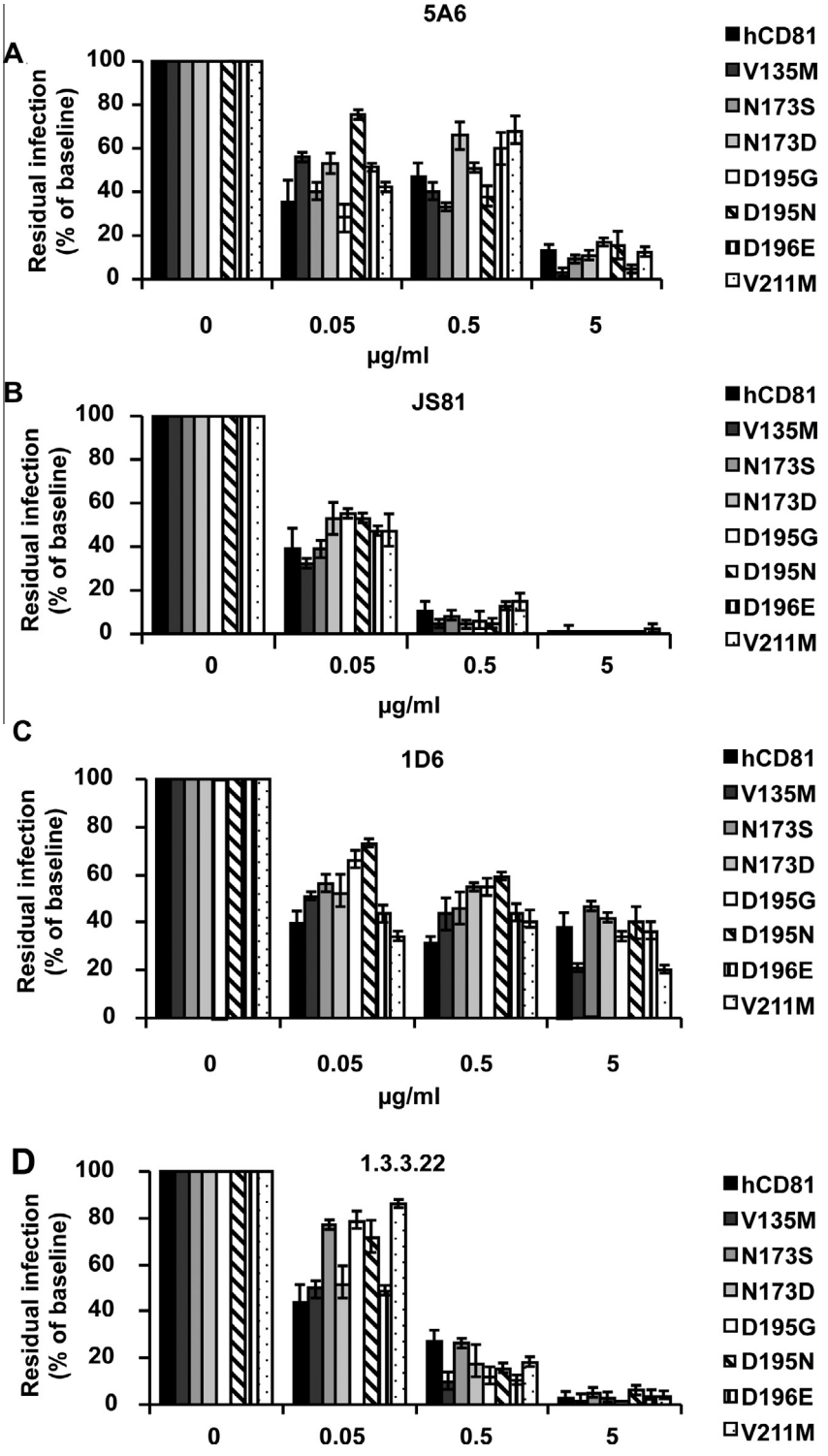

Fig. 5. Effect of CD81 SNP's on HCV neutralization with CD81 antibodies. (A–D) For neutralisation experiments cells were pretreated with CD81 antibodies. After 30 min target cells were washed three times with PBS and exposed to 500 µl supernatant containing Jc1-Luc reporter virus and the CD81 antibody in the same concentration as during pretreatment. After 5 h cells were washed and then kept in medium without antibody. Luciferase activity was measured after 48 h.

block 60–70% of infection. However, we could not detect any difference in sensitivity to any specific antibody between any of the CD81 variants. Thus in the presence of any of the CD81 SNP's HCV infection remains sensitive to neutralization with the tested anti-CD81 antibodies JSS81, 1.3.3.22, 1D6 and 5A6. Thus SNP's in the coding region of CD81 can be neglected when patients are treated with regimens containing these CD81 antibodies.

3.6. Variants in the essential HCV entry factor CD81 have no effect on cell-to-cell transmission

It has been shown, that *in vitro* HCV can be transmitted either as cell free virions or in a direct cell-to-cell manner. To investigate if any of the CD81 variants has an influence on cell-to-cell spread of HCV, we performed an agarose overlay assay. As "donor cells" we

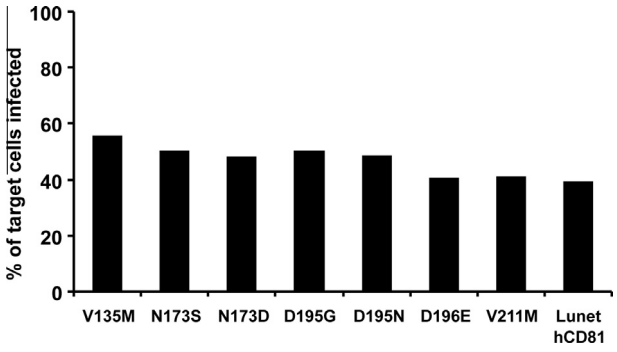

Fig. 6. All CD81 variants allow cell-to-cell spread of HCV. To investigate cell-to-cell-transmission a fully H77/JFH1 infected population of non-fluorescent Huh7.5 cells was put in co-culture with uninfected target cells (Lunet cells expressing CD81 variants as well as a tagRFP-NLS-IPS reporter (see methods section). Percentage of infected target cells as indicated by nuclear localization of tagRFP per well at 96 h of co-culture is shown.

used Huh7.5 cells infected with HCVcc (H77/JFH chimera) at a high multiplicity of infection. It was confirmed that >99% of donor cells were HCV positive by HCV NS5A immunofluorescence staining. Donor cells were co-cultured with Lunet "target" cells expressing the different CD81 variants and a reporter construct where HCV infection can be detected as the single cell level by relocation of the monomeric fluorescent protein tagRFP from a mitochondrial (uninfected) to a nuclear (infected) localization (Jones et al., 2010). As shown in Fig. 6, all Lunet cells expressing CD81 variants allowed cell-to cell spread to a comparable extent. This indicates, that CD81 SNP's do not affect efficiency of HCV cell-to-cell transmission.

4. Discussion

This study is the first to describe how HCV infection could be affected by genetic variation in the essential HCV entry factor CD81 *in vitro*. To enable us and others to address this topic we used the Lunet N4 cell line, which has a very low level of endogenous CD81. Upon reintroduction of the wildtype variant of CD81 this cell line becomes again permissive to all stages of the HCV replication cycle. All seven CD81 SNP's in the coding region of CD81 are able to facilitate HCVcc and HCVpp entry *in vitro* independent of HCV genotype. Moreover, we show that these SNP's have no influence on antiviral treatment with CD81 antibodies *in vitro*.

HCV entry is a complex multi-step process and multiple host factors are involved (Sandmann and Ploss, 2013; von Hahn et al., 2010). Nowadays, numerous host factors essential for HCV entry have been detected yet our understanding what each factor contributes, how they interact with the virus and with one another and how these interactions are orchestrated is still limited. Variation within a host species is thought to account for variation in the clinical course of hepatitis C. However, we here show that all tested CD81 variants allow HCV entry and thus likely play no or only a minor role in the course of disease.

Furthermore, understanding the impact of variants of the human species is key to predicting treatment response and may in the future be used to individualize treatment strategies. One famous example for variations in host genes with impact on the treatment of HCV infection is IL-28B/IFNL4: recently it has been shown by different groups that variants of the IL-28B/IFNL4 gene are strongly associated with the course of an acute HCV infection in addition to the response to antiviral interferon-based treatment (Ge et al., 2009; Prokunina-Olsson et al., 2013; Suppiah et al., 2009; Tanaka et al., 2009; Thomas et al., 2009).

The essential HCV entry factor CD81 is characterized by high variability with a high number of genetic variants (http://www.ncbi.nlm.nih.gov/snp). We have assessed the frequency of the seven known coding non-synonymous SNP's and found that all of them facilitate HCV entry *in vitro*. All of these SNP's are rare (allele frequency <1%) and we believe that this makes a major clinical impact of these variants unlikely. However, further studies with larger cohorts would be necessary to determine the correct frequency of CD81 SNP's *in vivo* or to rule out any clinical impact that these variants might have that is not mirrored in the available *in vitro* systems.

Today, CD81 may be a very promising target for antiviral therapy – especially to block HCV entry and thus reinfection after OLT, since it is expected, that targeting cellular host factors works for all HCV genotypes and has a higher barrier to drug resistance in comparison to drugs targeting directly the virus and its enzymes (Lupberger et al., 2011; von Hahn et al., 2011). Until now it was unknown if these CD81 antibodies are effective in humans with a SNP in the coding region of CD81. In this study we were able to show that HCV infection of all cell lines expressing seven different CD81 SNP's remains sensitive to treatment with four commercially available CD81 antibodies. While CD81 antibodies JS81 and 1.3.3.22 completely neutralize all CD81 variants, treatment with CD81 antibodies 1D6 and 5A6 only led to partial neutralization of HCV infection. However, we believe that these SNP's can be neglected when an antiviral therapy including these CD81 antibodies is planned.

In conclusion, we here reported on seven CD81 SNP's present in human populations that all facilitate HCV entry *in vitro*. Importantly, from a pharmacogenomic perspective, the reported SNP's are not expected to result in altered sensitivity of HCV to neutralization with any of four different CD81 antibodies.

Conflict of interest

All authors: none to declare.

Acknowledgements

We are grateful to Takaji Wakita and Jens Bukh for JFH1 and J6CF isolates, respectively, to Dirk Lindemann for pczCFG5-IZ and the pczVSV-G construct, to Charles Rice for Huh-7.5 cells and to Thomas Pietschmann for Lunet N4 cells. This study was supported by the German Center of Infection Research (DZIF).

References

Bitzegeio, J., Bankwitz, D., Hueging, K., Haid, S., Brohm, C., Zeisel, M.B., Herrmann, E., Iken, M., Ott, M., Baumert, T.F., Pietschmann, T., 2010. Adaptation of hepatitis C virus to mouse CD81 permits infection of mouse cells in the absence of human entry factors. PLoS Pathog. 6, e1000978.

Burgel, B., Friesland, M., Koch, A., Manns, M.P., Wedemeyer, H., Weissenborn, K., Schulz-Schaeffer, W.J., Pietschmann, T., Steinmann, E., Ciesek, S., 2010. Hepatitis C virus enters human peripheral neuroblastoma cells - evidence for extrahepatic cells sustaining hepatitis C virus penetration. J Viral Hepat 18, 562–570.

Ciesek, S., von Hahn, T., Colpitts, C.C., Schang, L.M., Friesland, M., Steinmann, J., Manns, M.P., Ott, M., Wedemeyer, H., Meuleman, P., Pietschmann, T., Steinmann, E., 2011a. The green tea polyphenol, epigallocatechin-3-gallate, inhibits hepatitis C virus entry. Hepatology 54, 1947–1955.

Ciesek, S., Wedemeyer, H., 2012. Immunosuppression, liver injury and post-transplant HCV recurrence. J. Viral Hepat. 19, 1–8.

Ciesek, S., Westhaus, S., Wicht, M., Wappler, I., Henschen, S., Sarrazin, C., Hamdi, N., Abdelaziz, A.I., Strassburg, C.P., Wedemeyer, H., Manns, M.P., Pietschmann, T., von Hahn, T., 2011b. Impact of intra- and interspecies variation of occludin on its function as coreceptor for authentic hepatitis C virus particles. J. Virol. 85, 7613–7621.

Di Bisceglie, A.M., 2000. Natural history of hepatitis C: its impact on clinical management. Hepatology 31, 1014–1018.

Ge, D., Fellay, J., Thompson, A.J., Simon, J.S., Shianna, K.V., Urban, T.J., Heinzen, E.L., Qiu, P., Bertelsen, A.H., Muir, A.J., Sulkowski, M., McHutchison, J.G., Goldstein,

D.B., 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461, 399–401.
Jones, C.T., Catanese, M.T., Law, L.M., Khetani, S.R., Syder, A.J., Ploss, A., Oh, T.S., Schoggins, J.W., MacDonald, M.R., Bhatia, S.N., Rice, C.M., 2010. Real-time imaging of hepatitis C virus infection using a fluorescent cell-based reporter system. Nat. Biotechnol. 28, 167–171.
Lavanchy, D., 2011. Evolving epidemiology of hepatitis C virus. Clin. Microbiol. Infect. 17, 107–115.
Levy, S., Todd, S.C., Maecker, H.T., 1998. CD81 (TAPA-1): a molecule involved in signal transduction and cell adhesion in the immune system. Annu. Rev. Immunol. 16, 89–109.
Lupberger, J., Zeisel, M.B., Xiao, F., Thumann, C., Fofana, I., Zona, L., Davis, C., Mee, C.J., Turek, M., Gorke, S., Royer, C., Fischer, B., Zahid, M.N., Lavillette, D., Fresquet, J., Cosset, F.L., Rothenberg, S.M., Pietschmann, T., Patel, A.H., Pessaux, P., Doffoel, M., Raffelsberger, W., Poch, O., McKeating, J.A., Brino, L., Baumert, T.F., 2011. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. Nat. Med. 17, 589–595.
Meuleman, P., Hesselgesser, J., Paulson, M., Vanwolleghem, T., Desombere, I., Reiser, H., Leroux-Roels, G., 2008. Anti-CD81 antibodies can prevent a hepatitis C virus infection in vivo. Hepatology 48, 1761–1768.
Ploss, A., Evans, M.J., Gaysinskaya, V.A., Panis, M., You, H., de Jong, Y.P., Rice, C.M., 2009. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. Nature 457, 882–886.
Prokunina-Olsson, L., Muchmore, B., Tang, W., Pfeiffer, R.M., Park, H., Dickensheets, H., Hergott, D., Porter-Gill, P., Mumy, A., Kohaar, I., Chen, S., Brand, N., Tarway, M., Liu, L., Sheikh, F., Astemborski, J., Bonkovsky, H.L., Edlin, B.R., Howell, C.D., Morgan, T.R., Thomas, D.L., Rehermann, B., Donnelly, R.P., O'Brien, T.R., 2013. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat. Genet. 45, 164–171.
Sandmann, L., Ploss, A., 2013. Barriers of hepatitis C virus interspecies transmission. Virology 435, 70–80.
Suppiah, V., Moldovan, M., Ahlenstiel, G., Berg, T., Weltman, M., Abate, M.L., Bassendine, M., Spengler, U., Dore, G.J., Powell, E., Riordan, S., Sheridan, D., Smedile, A., Fragomeli, V., Muller, T., Bahlo, M., Stewart, G.J., Booth, D.R., George, J., 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat. Genet. 41, 1100–1104.
Tanaka, Y., Nishida, N., Sugiyama, M., Kurosaki, M., Matsuura, K., Sakamoto, N., Nakagawa, M., Korenaga, M., Hino, K., Hige, S., Ito, Y., Mita, E., Tanaka, E., Mochida, S., Murawaki, Y., Honda, M., Sakai, A., Hiasa, Y., Nishiguchi, S., Koike, A., Sakaida, I., Imamura, M., Ito, K., Yano, K., Masaki, N., Sugauchi, F., Izumi, N., Tokunaga, K., Mizokami, M., 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat. Genet. 41, 1105–1109.
Thomas, D.L., Thio, C.L., Martin, M.P., Qi, Y., Ge, D., O'Huigin, C., Kidd, J., Kidd, K., Khakoo, S.I., Alexander, G., Goedert, J.J., Kirk, G.D., Donfield, S.M., Rosen, H.R., Tobler, L.H., Busch, M.P., McHutchison, J.G., Goldstein, D.B., Carrington, M., 2009. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461, 798–801.
von Hahn, T., Ciesek, S., Manns, M.P., 2011. Arrest all accessories-inhibition of hepatitis C virus by compounds that target host factors. Discov. Med. 12, 237–244.
von Hahn, T., Schiene-Fischer, C., Van, N.D., Pfaender, S., Karavul, B., Steinmann, E., Potthoff, A., Strassburg, C., Hamdi, N., Abdelaziz, A.I., Sarrazin, C., Muller, T., Berg, T., Trepo, E., Wedemeyer, H., Manns, M.P., Pietschmann, T., Ciesek, S., 2012. Hepatocytes that express variants of cyclophilin a are resistant to HCV infection and replication. Gastroenterology 143 (439–447), e431.
von Hahn, T., Steinmann, E., Ciesek, S., Pietschmann, T., 2010. Know your enemy: translating insights about the molecular biology of hepatitis C virus into novel therapeutic approaches. Expert Rev. Gastroenterol. Hepatol. 4, 63–79.
Witteveldt, J., Evans, M.J., Bitzegeio, J., Koutsoudakis, G., Owsianka, A.M., Angus, A.G., Keck, Z.Y., Foung, S.K., Pietschmann, T., Rice, C.M., Patel, A.H., 2009. CD81 is dispensable for hepatitis C virus cell-to-cell transmission in hepatoma cells. J. Gen. Virol. 90, 48–58.