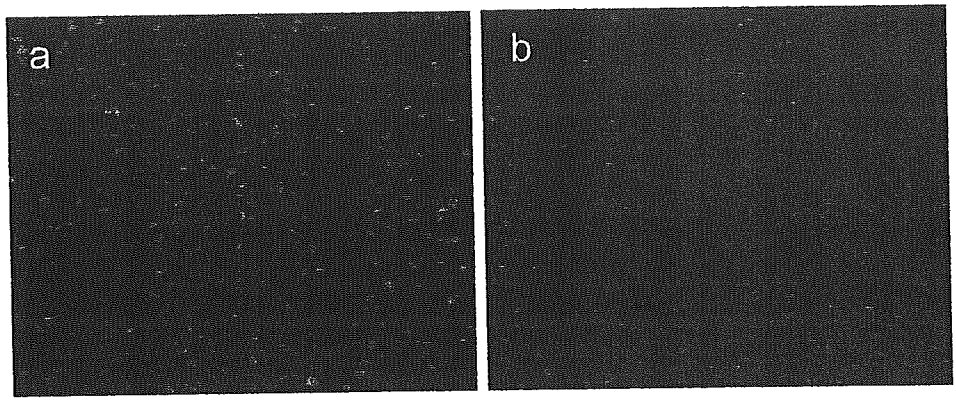
A


1 2 3 4 5 6 7
5'UTR

B


a
b

C

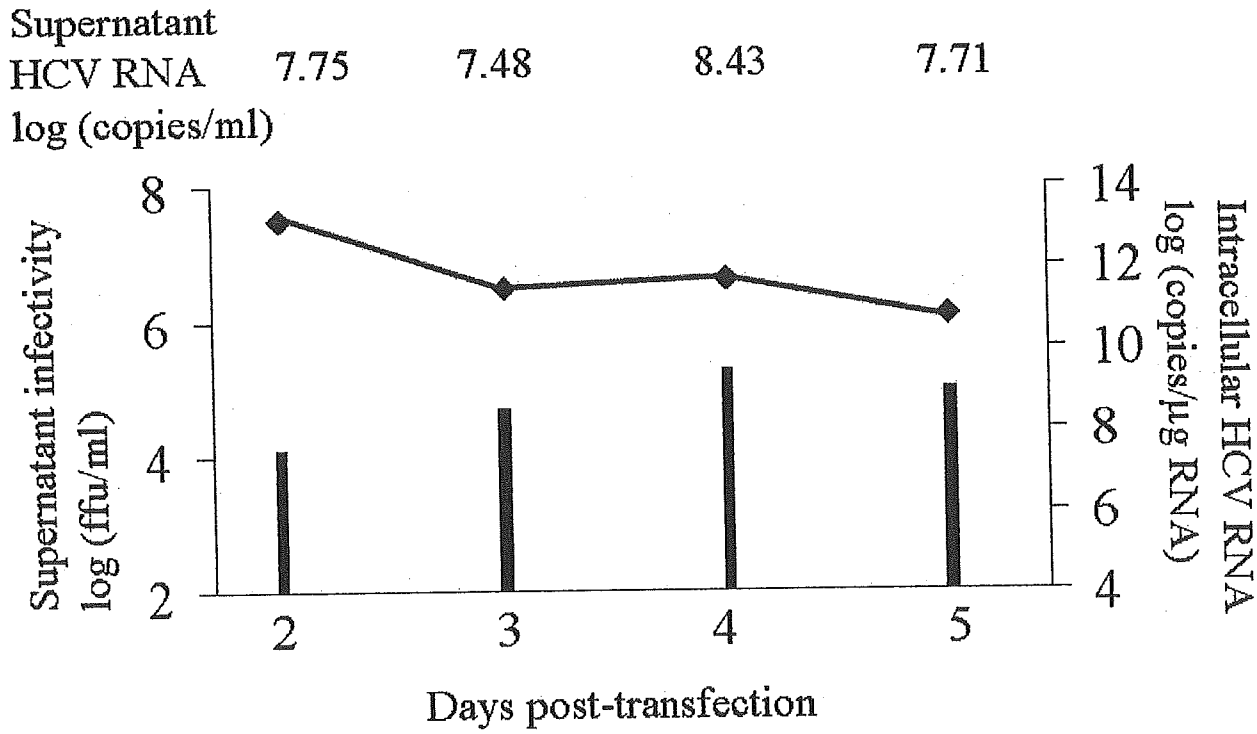

Supernatant
HCV RNA
log (copies/ml)
7.75
7.48
8.43
7.71
Supernatant infectivity
log (ffu/ml)
8
6
4
2
14
12
10
8
6
4
Intracellular HCV RNA
log (copies/μg RNA)
2
3
4
5
Days post-transfection

Fig. 4

D

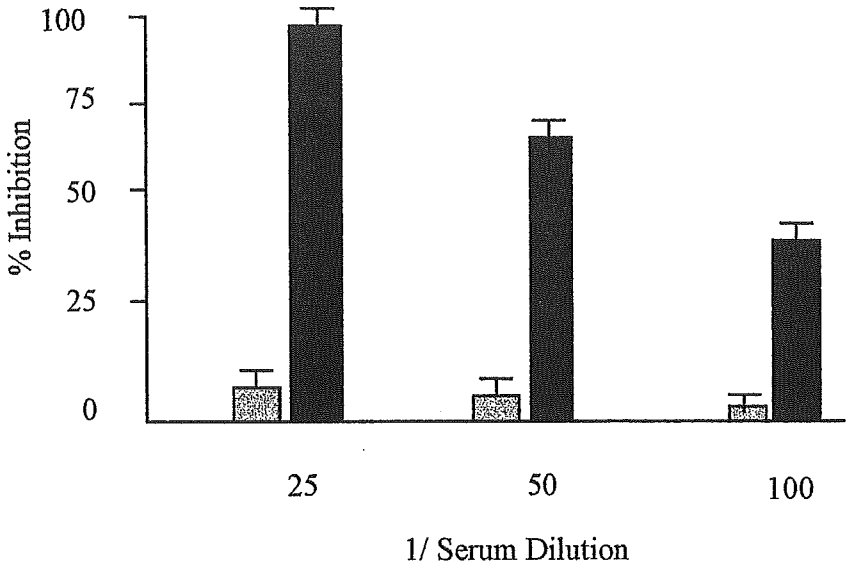


Fig. 4

Characterization of the replication sensitivity to cyclosporin A among strains of hepatitis C virus

Naoto Ishii[1+], Koichi Watashi[1+], Takayuki Hishiki[1], Kaku Goto[1], Daisuke Inoue[1], Makoto Hijikata[1], Takaji Wakita[2], Nobuyuki Kato[3], and Kunitada Shimotohno[1]*

Laboratory of Human Tumor Viruses, Department of Viral Oncology, Institute for Virus Research, Kyoto University, Kyoto,[1] Department of Microbiology, Tokyo Metropolitan Institute for Neuroscience, Tokyo,[2] and Department of Molecular Biology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama,[3] Japan

Running title: sensitivity of HCV replication to cyclosporin A

[+] N.I. and K.W. contributed equally to this work.

***Correspondence to;** Kunitada Shimotohno, Ph.D.

Laboratory of Human Tumor Viruses, Department of Viral Oncology, Institute for Virus Research, Kyoto University

53 Kawaharacho, Shogoin, Sakyo-ku, Kyoto, 606-8507, Japan

Phone: 81-75-751-4000, Fax: 81-75-751-3998

E-mail: kshimoto@virus.kyoto-u.ac.jp

Abstract

Recently, a production system for infectious particles of hepatitis C virus (HCV) utilizing the genotype 2a JFH1 strain has been developed. This strain has high capacity to replicate in the cells. Cyclosporin A (CsA) has a suppressive effect on HCV replication. In this report, we characterize the anti-HCV effect of CsA. We observe that the presence of viral structural proteins does not influence the anti-HCV activity of CsA. Among HCV strains, the replication of genotype 1b replicons was strongly suppressed by treatment with CsA. In contrast, JFH1 replication was less sensitive to CsA and its analog, NIM811. Replication of JFH1 did not require the cellular replication cofactor, cyclophilin (CyP)B. CyPB stimulated the RNA binding activity of NS5B in the genotype 1b replicon, but not the genotype 2a JFH1 strain. These findings provide an insight into the mechanisms of diversity governing virus-cell interactions and in sensitivity of these strains to anti-viral.

Introduction

Hepatitis C virus (HCV), a member of the flaviviridae family, has a positive-strand RNA genome (1, 26). The genome encodes a large precursor polyprotein, which is cleaved by host and viral proteases to generate at least ten functional viral proteins: core, envelope (E)1, E2, p7, nonstructural protein (NS)2, NS3, NS4A, NS4B, NS5A, and NS5B (6, 8). NS5B is an RNA-dependent RNA polymerase (RdRp) that is crucial for viral genome replication (1, 26). There is genetic heterogeneity within the HCV genome. Currently, these differences are classified into six genotypes that are further segregated into a series of subtypes (4, 23). In Japan, genotype 1b is predominant; roughly 65% of cases of HCV-related chronic hepatitis involve genotype 1b. By comparison, genotype 2a is present 17% of these patients (13, 23).

Sustained infection of HCV is the major cause of chronic liver diseases such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (16). Rarely, HCV causes fulminant hepatitis (13). The predominant treatment for HCV-infected patients is interferon (IFN)/peg-IFN alone or in combination with ribavirin (19, 20). However, alternative anti-HCV therapies are needed because virus is not eliminated in about a half of the treated patients (19, 20). Lohmann et al. have developed the HCV subgenomic replicon system, in which an HCV subgenomic replicon autonomously replicates in Huh-7 cells (HCV replicon cells) (18). This replicon comprises the HCV 5'-untranslated region (UTR) containing an internal ribosomal entry site (IRES), the neomycin phosphotransferase gene, the encephalomyocarditis virus (EMCV) IRES, the coding region for HCV NS3 through NS5B, and the HCV 3'-UTR (subgenomic replicon), but lacks coding region for the core and envelope proteins as well as p7 and NS2 (Fig. 1). Subsequently, a genome-length (fullgenome) replicon has been developed. This construct

contains a full-genome length of HCV including the coding regions for core through NS2 (Fig. 1) (5, 10). We can evaluate HCV replication using these subgenomic or genome-length replicon systems. Previously, we have established an HCV subgenomic replicon cells carrying HCV genotype 1b NN strain (15, 29). We demonstrated that an immunosuppressant, cyclosporin A (CsA), has an anti-HCV activity in these cells (29). In addition, we have determined the molecular mechanism of anti-HCV effect of CsA on this replicon; Cyclophilin (CyP)B, one of the cellular targets of CsA, is a cellular replication cofactor of HCV genome (31). CyPB interacts with NS5B to promote its RNA binding activity (The detailed description can be obtained in (31)). CsA is suggested to suppress HCV genome replication by inhibiting the functional association of CyPB with NS5B. Another group also reported anti-HCV function of CsA using a subgenomic replicon of other genotype 1b strain, HCV-N (22). In this study, we demonstrate that CsA also has a strong anti-HCV activity in other available genotype 1b replicons, carrying the Con1 and O strains (12, 18).

Recently, Wakita et al. have reported that a replicon of HCV genotype 2a JFH-1 strain, which was isolated from a type-C fulminant hepatitis, has a much stronger replication activity than genotype 1b replicons in Huh-7 cells (13, 27). A production system of infectious viral particles has been recently established with this high replication-competent strain (17, 27, 34). This viral strain may acquire a growth advantage compared with many other strains, although the underlying mechanism is unknown. In this study, we described a characteristic difference of the replication of JFH1 compared to genotype 1b replicons.

Here, we report that the replication of JFH1 is less sensitive to CsA than genotype 1b strains although the interaction of CyPB with NS5B is observed with this replicon. However, genome

replication and RNA binding activity of NS5B is independent of CyPB. We have exploited a chemical compound to demonstrate how strain diversity can be generated by underlying differences in the mechanisms of the virus-cell interaction. These findings provide important insight into the mechanisms that mediate the efficacy of anti-viral agents.

Materials and Methods

Cell culture

Huh-7 cells were cultured in Dulbecco's Modified Eagle Medium (Invitrogen) with 10% fetal bovine serum, non-essential amino acids (Invitrogen), and L-glutamine (Invitrogen). MH-14, #50-1, MH14#W31, SN1, sO (formerly named as 1B2R1), JFH1#4-1, and JFH#2-3 cells (12, 13, 15, 18, 29), carrying subgenomic replicons, and NNC#2, SN1A#2, and SNC#7 cells, carrying full-genome replicons, were cultured in the above medium supplemented with 300-500 μg/ml G418 (Invitrogen). In the assay measuring the response to CsA, NIM811, or PSC833 (Fig. 2, 3, and 4), we seeded small numbers of each replicon cells (7-15 $x10^3$ cells/12 well plate) and treated with each drug. Culture medium was changed every three days (CsA, NIM811, or PSC833 was supplemented in the fresh medium for the treatment groups). We did not perform any passages in the assay period. At day 7, the cells were 70-90 % confluent. A schematic representation of the constructs of HCV replicon RNAs, the name of HCV strains from which the replicon RNA sequences are derived, and the name of replicon cell clones used in this study is summarized in Fig. 1. Since many replicon clones were used in this study, we showed "strain/genotype/length of the replicon construct" in parenthesis after the names of each cell

clone in the result section and figure legends of this manuscript to avoid confusion of the names. For example, MH-14 (NN/1b/SG), JFH1#4-1 (JFH1/2a/SG), and SN1A#2 cells (Con1/1b/FL). SG and FL indicate subgenomic and fullgenome replicon, respectively.

Establishment of replicon cells

MH-14, #50-1, sO, JFH1#4-1, and JFH1#2-3 cells were described previously (12, 13, 15, 29). The replicon RNAs were produced using MEGAscript T7 kit (Ambion) from pMH14, pSN1, pNNC, pSN1A, and pSNC plasmids for the establishment of the replicon cells MH14#W31, SN1, NNC#2, SN1A#2, and SNC#7 cells, respectively. For the establishment of MH14#W31, we transfected RNA into the Huh-7 cell strain which was identical to the parental cells of JFH1#4-1 and JFH1#2-3 cells. Each replicon RNA was transfected into Huh-7 cells following the selection with the medium in the presence of 500-1000 μg/ml G418 for around four weeks. The resultant cell colonies were isolated and expanded. The HCV RNA titers in cell clones carrying JFH1 replicons were not significantly different from those in established cell clones carrying genotype 1b replicons.

Plasmid construction

pSN1, the sequence of which is derived from I377NS3-3' (18), was prepared essentially as described previously (15). pSN1A was generated by inserting the region from core to NS2 of pM1LE (15) into the upstream of the coding region for NS3 in pSN1. To obtain pSNC, the EMCV IRES of pSN1A was replaced with the HCV IRES. pNNC was produced by inserting the coding region from NS3 to NS5B of pM1LE into pSNC.

Real time RT-PCR analysis

The 5'-UTR of HCV genome RNA was quantified using the ABI PRISM 7700 sequence detector (Applied Biosystems) as described previously (29).

Immunoblot analysis

Immunoblot analysis was performed as described previously (30). The primary antibodies used in this study were anti-core, anti-E2 (kindly provided by Dr. Kohara, Tokyo Metropolitan Institute of Medical Science) anti-NS3, anti-NS5A (a generous gift from Dr. Takamizawa, Osaka University), anti-NS5B (NS5B-6; kindly provided by Dr. Fukuya, Osaka University), anti-CyPA (Upstate Cell Signaling), anti-CyPB (Affinity BioReagents), and anti-tubulin (Oncogene).

Immunoprecipitation assay and RNA-protein binding precipitation assay

Immunoprecipitation and RNA-protein binding precipitation was performed as described previously (30, 31).

RNAi technique

The condition of siRNA used in this study was described previously (31). Transfection was performed using siLentFect (BIORAD) according to the manufacturer's protocol.

Isolation of replication complex

HCV replication complex was isolated from cells by treatment with 50 μg/ml digitonin at 27°C for 5 min following of 0.3 μg/ml proteinase K at 37°C for 5 min as described previously (31).

Purification of recombinant GST-fused CyPB protein

GST and GST-fused CyPB protein (GST-CyPB) expression was induced in transformed BL21 cells (Amersham) with 1 mM isopropyl-β-thiogalactopyranoside (IPTG). Cell lysate was incubated with glutathione sepharose resin (Amersham) and washed extensively. The recombinant protein was eluted by glutathione (pH 8.0) and subsequently dialyzed.

In vitro RNA binding assay

In vitro translated [^{35}S]-labeled NS5B proteins and poly-U (Amersham) or protein G sepharose resin (Amersham) as a negative control were incubated in the presence of recombinant GST-CyPB protein at 4°C for 1 hr. After washes, precipitates were fractionated by SDS-PAGE and analyzed by imaging analyzer.

Results

CsA suppressed the replication of HCV full genome replicon

We and another group have reported an anti-HCV activity of CsA using subgenomic replicons (22, 29). HCV structural proteins, especially core, have multiple functions. These

proteins interact with many cellular factors and modulate a variety of cellular functions (32). Potentially, these viral proteins could diminish or circumvent the suppression of HCV genome replication by CsA. In fact, core and E2 reportedly modulate the activity of IFN signaling (9, 25). To test this possibility, we established a full-genome HCV replicon system with cells transfected with the NN strain (NNC#2 cells (NN/1b/FL)) (Fig. 1). HCV RNA and protein productions were confirmed by real time RT-PCR and immunoblot analysis (Fig. 2A and B). In addition, we confirmed that this replication was not due to the integration of the replicon construct into the cellular genome (data not shown). Similarly, we generated other full-genome replicon cells carrying sequences from the Con1 strain at the nonstructural coding region of the replicon RNA (SN1A#2 (Con1/1b/FL) and SNC#7 cells (Con1/1b/FL)) (Fig. 1). The replicon of SN1A#2 cells (Con1/1b/FL) possesses the EMCV IRES upstream of the open reading frame for HCV proteins while that of SNC#7 (Con1/1b/FL) cells contains the HCV IRES (Fig. 1). SNC#7 cells (Con1/1b/FL) exhibited almost the same response as that of SN1A#2 cells (Con1/1b/FL) to CsA treatment (Fig. 2D). Consistent with a previous report (22), EMCV IRES is not responsible for the anti-HCV activity of CsA. We compared the sensitivity to CsA of full-genome replicons with that of subgenomic replicons. CsA strongly decreased the production of HCV proteins in both the full-genome replicon, NNC#2 cells (NN/1b/FL) and the subgenomic replicon, MH-14 cells (NN/1b/SG) (Fig. 2C). Real time RT-PCR analysis also revealed a dramatic reduction of the RNA level of full-genome replicons in NNC#2 (NN/1b/FL), SN1A#2 (Con1/1b/FL), and SNC#7 cells (Con1/1b/FL) (Fig. 2D). The IC_{50} values of CsA in NNC#2 (NN/1b/FL), SN1A#2 (Con1/1b/FL), and SNC#7 cells (Con1/1b/FL) are estimated to be 0.13, 0.19, and 0.24 μg/ml, respectively. IC_{90} of CsA in these cells is 0.68, 0.94, and 0.81 μg/ml, respectively. The CsA

dose-response curves of full-genome replicons and subgenomic replicons are similar (see Fig. 3C; SN1A#2 or SNC#7 (Con1/1b/FL) vs. SN1 (Con1/1b/SG); NNC#2 (NN/1b/FL) vs. MH-14, #50-1, or MH14#W31 (NN/1b/SG)). These results demonstrate that CsA suppresses the replication of full-genome replicons and subgenomic replicons to almost the same extent. Since concentrations of CsA up to 3 μg/ml did not affect the proliferation of any replicon cells (Fig. 2E and data not shown), the effect of CsA on replication is not due to the cytotoxic effect. In addition, we observed the reduction of production of infectious viral particles in the presence of 3 μg/ml CsA (data not shown) using the viral production system with fullgenome JFH1 RNA (27).

The JFH1 replicon was less sensitive to CsA compared with genotype 1b replicons

We compared the sensitivity of HCV replication to CsA in several subgenomic replicon cells. We used MH-14 (NN/1b/SG) and #50-1 cells (NN/1b/SG), carrying subgenomic replicons with HCV NN strain (15, 29), SN1 cells (Con1/1b/SG), carrying the Con1 subgenomic replicon (18), and sO cells (O/1b/SG), bearing the subgenomic O strain (12), as genotype 1b replicon-containing cells. We also employed JFH1#4-1 (JFH1/2a/SG) and JFH1#2-3 (JFH1/2a/SG) cell clones carrying JFH1 subgenomic replicon (13). Treatment of CsA (1 μg/ml, 7 day) drastically decreased HCV RNA in all the subgenomic replicon cells carrying HCV genotype 1b strain. HCV RNA levels in SN1 (Con1/1b/SG), MH-14 (NN/1b/SG), sO (O/1b/SG), and #50-1 cells (NN/1b/SG) decreased to 1/134, 1/219, 1/128, and 1/295, respectively (Fig. 3A). Genotype 1b replicon cells appear highly sensitive to CsA. In contrast, the effect of CsA on HCV RNA level in replicon cells containing sequences from the JFH1 strain was limited to

1/5-1/7 (Fig. 3A). These results of the response to CsA were reproduced in further additional cell clones.

The cellular characteristics of Huh-7 cell strains differ among laboratories. To exclude the possibility that differences between Huh-7 cell strains influence the sensitivity to CsA, we established genotype 1b-replicon cells based on the identical Huh-7 cell strain which was used as parental cells of JFH1#4-1 (JFH1/2a/SG) and JFH1#2-3 cells (JFH1/2a/SG). The response of the corresponding replicon cells, MH14#W31 cells (NN/1b/SG), to CsA was almost the same as that of SN1 (Con1/1b/SG), MH-14 (NN/1b/SG), sO (O/1b/SG), and #50-1 cells (NN/1b/SG) (Fig. 3C). Thus, the difference in sensitivity to CsA of JFH1 and genotype 1b strains can be attributed to the characteristic differences of the HCV strains, not the parental Huh-7 cell strain. In addition, the reduction of NS3 protein in JFH1#4-1 cells (JFH1/2a/SG) following treatment with CsA was less prominent than that in MH14#W31 cells (NN/1b/SG) (Fig. 3B).

We examined the dose-response curve of HCV RNA against the concentration of CsA (Fig. 3C). The effect of CsA in genotype 1b replicons plateaues at around 1 μg/ml, while in the dose-response curve in JFH1 replicon, the inhibition has not yet saturated (Fig. 3C). As concentrations of CsA up to 3 μg/ml did not affect the proliferation rate of any replicon cells (Fig. 3D and data not shown), the effect of CsA on replication is not due to the cytotoxic effect. The IC_{50} of CsA in MH-14 (NN/1b/SG), #50-1 (NN/1b/SG), MH14#W31 (NN/1b/SG), SN1 (Con1/1b/SG), sO (O/1b/SG), JFH1#4-1 (JFH1/2a/SG), and JFH1#2-3 cells (JFH1/2a/SG) are estimated to be 0.15, 0.18, 0.16, 0.20, 0.25, 0.67, and 0.43 μg/ml, respectively. IC_{90} is 0.86, 0.82, 0.76, 0.88, 0.92, 2.77, and 2.39 μg/ml, respectively. Similar dose response curve in JFH1 replicon was obtained in a transient replication assay using the luciferase reporter driven from a

JFH1 replicon construct (data not shown) (14).

JFH1 replicon was less sensitive to a CsA derivative, NIM811

Analysis of several CsA derivatives has revealed that the anti-HCV effect of CsA on the genotype 1b replicon is mediated by the inhibition of CyP (31). We examined the sensitivity of JFH1 replicon to CsA derivatives. CsA is known to have three major cellular targets: CyP, calcineurin (CN)/NF-AT, and P-glycoprotein (P-gp) (28, 31). A CsA derivative, NIM811, inhibits CyP and P-gp but not CN/NF-AT while another derivative, PSC833, inhibits P-gp but neither CyP nor CN/NF-AT (31). The decrease of HCV RNA in MH14#W31 cells (NN/1b/SG) with NIM811 treatment (0.5 μg/ml, 7 day) was more than an order of magnitude greater than that in JFH1#4-1 cells (JFH1/2a/SG) (Fig. 4A). The slope of dose-response curve of NIM811 treatment of JFH1 replicon was gentler than that of genotype 1b (Fig. 4B). IC_{50} of NIM811 in MH14W#31 (NN/1b/SG) and JFH1#4-1 cells (JFH1/2a/SG) is 0.17 and 0.30 μg/ml, respectively. IC_{90} is 0.46 and 0.93 μg/ml, respectively. In contrast, PSC833, which does not inhibit CyP, did not alter HCV RNA level in either genotype 1b or JFH1 replicon (Fig. 4C). Thus, a CyP inhibitor is less effective at suppressing the replication of the JFH1 replicon relative to genotype 1b replicons.

Interaction between CyPB and JFH1 NS5B

Previously, we have shown that CyPB interacts with NS5B to promote HCV genome replication and that CsA inhibits this binding in a genotype 1b replicon (31). Here, we examine the association between CyPB and NS5B in a JFH1 replicon. Immunoprecipitation

analysis revealed an interaction of CyPB with NS5B in JFH1#4-1 cells (JFH1/2a/SG) (Fig. 5A). This interaction was dissociated following the treatment of CsA, as observed with the genotype 1b replicon (Fig. 5B).

The role of CyPB in the replication of JFH1 replicon

Although we observed some differences of expression levels of endogenous CyPB among the replicon cells in the immunoblot analysis (Fig. 6A), there was no particular correlation between endogenous CyPB expression level and replication sensitivity to CsA among cells. CyPB reportedly regulates HCV genome replication of the genotype 1b replicon (31). We then explored the requirement of CyPB for the replication of JFH1 replicon with RNAi. Transfecting siRNAs designed to recognize several CyP subtypes (si-CyP(broad)) (Fig. 6B) reduced HCV RNA to less than 1/5 in MH14#W31 cells (NN/1b/SG) (Fig. 6C). Specific knock-down of CyPB, but not CyPA, (Fig. 6B) decreased HCV RNA in MH14#W31 cells (NN/1b/SG), consistent with a previous report (31) (Fig. 6C). In contrast, HCV RNA in JFH1#4-1 cells (JFH1/2a/SG) was not altered following the suppression of either endogenous CyPA or CyPB (Fig. 6B and C). We observed a weak decrease of HCV RNA levels (around 1/2) with si-CyP(broad) (Fig. 6C). These data suggests the possibility that the replication of the JFH1 replicon is independent of CyPB, in contrast to genotype 1b replicon. In the previous study, it was reported that the doubling time, saturation density, and response to cell confluency of the replicon cells carrying JFH1 was different from that carrying a genotype 1b replicon, suggesting the possibility that the coupling relationship between the replication and cell growth is different between genotype 1b and JFH1 replicon (21). The introduction of either si-CyPB or si-CyP(broad), however, had little effect on

cell growth in MH14#W31 (NN/1b/SG) and JFH1#4-1 cells (JFH1/2a/SG). And we did not observe cells being confluent in the experiment period. The above results suggest that the different response to si-CyPB among the two lines is independent of the condition of cell growth.

The role of CyPB in the RNA binding activity of JFH1 NS5B

CyPB regulates HCV genome replication of a genotype 1b replicon by promoting the RNA binding activity of NS5B (31). We examined the effect of CyPB on the RNA binding activity of NS5B in JFH1. NS5B in the replication complex was isolated from cells by treatment with digitonin/proteinase K as described previously (31). This fraction was incubated with poly-U RNA-sepharose or protein G-sepharose as a negative control for the detection of RNA binding NS5B in the replication complex. RNA-bound NS5B in this fraction from MH14#W31 cells (NN/1b/SG) was decreased drastically following treatment with CsA (Fig. 7A lanes 5 and 6). However, the reduction of RNA binding of NS5B in the replication complex of JFH1#4-1 cells (JFH1/2a/SG) was not as prominent (Fig. 7A lanes 11 and 12). We confirmed this result with in vitro RNA binding assay, in which in vitro synthesized NS5B was incubated with poly-U RNA-sepharose together with recombinant GST-CyPB. The addition of recombinant GST-CyPB increased the binding of genotype 1b NS5B to poly-U RNA (Fig. 7B and C). However, this augmentation of RNA binding was not observed with NS5B from the JFH1 strain (Fig. 7B and C). From the above results, it is suggested that the RNA binding of JFH1 NS5B is free from the regulation by CyPB.

Discussion

Until now, we and another group have utilized subgenomic replicons carrying genotype 1b NN and HCV-N strains to demonstrate that CsA suppresses HCV genome replication (22, 29). This study reveals that CsA is effective on full-genome replicons to almost the same extent. In addition, other available genotype 1b replicons, carrying Con1 and O strain, also have a high sensitivity to CsA, consistent with our proposal that HCV genotype 1b is highly sensitive to CsA. However, a fulminant-type genotype 2a replicon, JFH1, was less responsive to CsA although a high dose of CsA suppressed the replication of this strain.

CyPB interacts with genotype 1b NS5B to stimulate its RNA binding activity. In contrast, CyPB binds JFH1 NS5B, but does not regulate the function of JFH1 NS5B. This is consistent with a previous speculation that genotype 1b and JFH1 replicons utilize the same cellular factors in distinct manners (21). The NS5B sequence of NN strain has 95.0, 95.9, and 70.4 % homology to that of Con1, O, and JFH1, respectively (Fig. 8). The region 521-591 aa of NS5B, which is involved in the interaction with CyPB (31), is highly conserved among genotype 1b strains NN, Con1, and O while that of JFH1 has 21 substituted residues in this region. The proline at 540 aa, which is important for CyPB binding (31), is conserved but the adjacent residues such as isoleucine at 539 aa and alanine at 541 aa are replaced by leucine and glutamic acid, respectively, in JFH1. Through molecular interaction, CyPB seems to make the conformation of NS5B of genotype 1b strains, but not JFH1, suitable for RNA binding (31). The diverse regulation system of NS5B by CyPB among strains may be due to the difference of either the sequence or the entire conformation of NS5B. Further study is important for elucidating the

regulation mechanism of RNA binding activity of NS5B by CyPB.

Thus, replication in JFH1 replicon is independent of CyPB. Interestingly, HIV-1 strains also have a diversity of CyP-dependence on viral proliferation (3, 33). CyPA plays an important role in the life cycle of HIV-1. The interaction of HIV-1 capsid protein with CyPA that resides within the target cells of infection is critical for HIV-1 replication (7, 24). In peripheral blood mononuclear cells or Jurkat T cells, CsA suppresses the proliferation of HIV-1 group main (M) strain (3). However, certain strains of group outlier (O), such as MVP5180 and MVP9435, are resistant to CsA (3, 33), suggesting the different dependency of the replication on CyPA. Authors have suggested that MVP5180 and MVP9435 clones adapt to replicate independently of CyPA and this adaptation provides a significant replication advantage for the virus in vivo (3). In vesicular stomatitis virus (VSV) strains, a role for CyPA in viral replication also has been reported (2). CyPA is required for the infection of the VSV-NJ strain but not the VSV-IND strain. These authors proposed that during evolutionary divergence from the ancestral lineages that initially were dependent on CyPA for replication, VSV-IND may have adapted to reduce its dependency on CyPA (2). In the case of HCV, a fulminant type genotype 2a replicon JFH1 replicates independently of CyPB. It has previously been reported that JFH1 has a much higher competency of the replication in the cells compared with other strains (13). The adaptation to independence from CyPB may contribute to the high capacity of the replication of JFH1.

Although JFH1 replicon is less sensitive to CsA, high concentrations of CsA still suppress the replication of JFH1 replicon. Moreover, the introduction of the siRNA designed to recognize several CyP subtypes (si-CyP(broad)) moderately diminished HCV RNA in JFH1 replicon. We suspect that a CyP family member other than CyPB is involved in HCV genome replication.

Further analysis is needed on the role of other CyP subtypes.

As there is not yet a replicon system for a fulminant-type genotype 1b replicon or chronic-type genotype 2a replicon, we cannot conclude whether chronic-type genotype 2a replicons or fulminant-type replicons are less sensitive to CsA or not. However, there is a clinical report describing that cotreatment of patients with chronic hepatitis C with IFN and CsA results in a higher sustained virological rate (SVR) compared with treatment of IFN alone (11). In this report, the increase of SVR is prominent with patients carrying genotype 1 HCV (51.7 % vs 21.9 %) while it is relatively weak in patients carrying genotype 2 HCV (66.7 % vs 58.3 %) (11). Thus, genotype may affect the sensitivity of HCV replication to CsA. However, we cannot exclude the possibility that the diminished sensitivity to CsA is a characteristic of only the fulminant-type genotype 2a strain.

Our results suggest that sensitivity to CsA and replication dependency to CyPB is different among HCV strains. This finding is an important insight into the diversity with the mechanism of HCV genome replication and its sensitivity to anti-viral agents.

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