



SUPPORTING INFORMATION

MATERIALS AND METHODS

Generation of HCVpp. The cDNA sequence encoding the C-terminal core and full-length E1E2 proteins of HCV isolate AD78 (corresponding to amino acid residues 131 to 746 of the HCV polyprotein) was amplified from plasmid pK33QIAD78 (17) by polymerase chain reaction using AD78 specific primers (AD78-1 5'-TAT AGA TAT CAT GGG GTA CAT TCC GCT CGT C-3' (HCV nt 740 to 760) and AD78-2 5'-ATA TGA TAT CTT ACT CAG CCT GAG CTA TCA G-3' (HCV nt 2558 to 2575) and ligated into vector pCR2.1. Following digestion of pCR2.1cE1E2AD78 with EcoRV and subsequent dephosphorylation with alkaline phosphatase, the PCR fragment was cloned downstream from a human cytomegalovirus promoter in the EcoRV-digested expression vector phCMVIRE5 resulting in phCMVcE1E2AD78. To generate HCVpp, 293T cells were transfected with three expression vectors as described (19). For expression of HCV envelope glycoproteins the following expression plasmids were used: phCMVcE1E21a containing the cDNA of E1E2 for genotype 1a infectious clone H77C, phCMVcE1E21b for genotype 1b J strain, phCMVcE1E2AD78 for genotype 1b isolate AD79 and phCMVcE1E22 for strain UKN2A.2.4.

Quantitation of antibody mediated neutralization. Antibody mediated neutralization was assessed by the specific infectivity of HCVpp in the presence or absence of serum. Specific HCVpp infectivity was determined by the percentage of GFP-positive Huh7 cells following infection of HCVpp subtracted by the percentage of GFP-positive cells following infection of control pp (background). The specific neutralization was determined according to the following equation (13, 37, 39, 40): specific neutralization = 100% x [1 - (infectivity of HCVpp in the presence of serum – infectivity of control pp with serum) / (infectivity of HCVpp without serum– infectivity of control pp without serum)]. The percent of inhibition

were calculated as the mean of at least two independent experiments performed in duplicate. Only sera demonstrating a decrease in HCVpp infectivity of $\geq 50\%$ were considered inhibitory. Titers of neutralizing antibodies were determined by pre-incubating HCVpp with serial two-fold dilutions of sera (starting at a serum dilution of 1/20) followed by infection assay as described above. Samples showing an inhibition of HCVpp infection of less than 50% at a serum dilution of 1/20 were considered negative. The highest serum dilution decreasing HCVpp infectivity by $\geq 50\%$ was defined as anti-HCV-AD78 titer of the respective serum sample.

FIGURE LEGENDS SUPPORTING FIGURES

Fig. 4. Infection of Huh-7 cells by HCVpp derived from different isolates. Huh7 cells were incubated with 50 μ L of supernatant from transfected 293 cells containing HCVpp derived from strains AD78 (1b), HCV-J (1b), H77C (1a) and UKN2A.2.4 (2). HCVpp entry into Huh7 cells was determined by analysis of GFP reporter gene expression by flow cytometry as described in Fig. 1. Results are depicted as means \pm SD of five experiments. Significance between infectivity of HCVpp AD78 and non-enveloped control pseudoparticles was determined by student's t-test.

Fig. 5. Cross-neutralization of heterologous HCV strains by sera from patients with chronic hepatitis C infected with HCV-AD78. HCVpp derived from strains AD78 (1b), HCV-J (1b), H77C (1a) and UKN2A.2.4 (2) were preincubated for 1 h with serum samples from the late phase of patients with chronic HCV (dilution 1/20) as described in Fig. 1. Infection of HCVpp was determined by analysis of GFP reporter gene expression as described in Fig. 1 and 4. Individual patients are indicated by numbers corresponding to Fig. 3. Presence of cross-neutralization was defined as inhibition of infection of HCVpp derived

from HCV-J, H77C and HCV-UKN2A.2.4 by $\geq 50\%$ at a serum dilution of 1/20. Inhibition of HCVpp infection by 50% is indicated by a dotted line.

Fig. 6. Antibody-mediated cross-neutralization of heterologous HCV strains in the early phase of infection in patients with resolved and chronic hepatitis C. HCVpp derived from strains HCV-J (genotype 1b), H77C (genotype 1a) and and UKN2A.2.4 (genotype 2) were preincubated for 1 h with serum samples from the early phase of patients with resolved and chronic HCV (dilution 1/20). Infection of HCVpp was determined by analysis of GFP reporter gene expression as described in Fig. 1 and 4. Data are expressed as percent of patients with sera cross-neutralizing at least one (black bars), at least two (open bars) or three (hatched bars) heterologous HCV strains. Presence of cross-neutralization was defined as inhibition of infection of HCVpp derived from HCV-J, H77C and HCV-UKN2A.2.4 by $\geq 50\%$ at a serum dilution of 1/20.