Antibody-Dependent Enhancement of Hepatitis C Virus Infection

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Hepatitis C virus (HCV) often causes a persistent infection associated with hypergamma-globulinemia, high levels of antiviral antibody and circulating immune complexes, and immune complex disease. We previously reported that only a limited neutralizing activity to vesicular stomatitis virus or HCV pseudotype is generated in animals immunized with recombinant HCV envelope proteins and chronically infected HCV patient sera. Interestingly, when some of these neutralizing sera were diluted into a range of concentrations below those that reduced virus plaque number, an increase in pseudotype plaque formation was observed. Purified HCV E2-specific human monoclonal antibodies were used to further verify the specificity of this enhancement, and one-to-twofold increases were apparent on permissive Huh-7 cells. The enhancement of HCV pseudotype titer could be inhibited by the addition of a Fe-specific anti-human immunoglobulin G Fab fragment to the virus-antibody mixture prior to infection. Treatment of cells with antibody to Fc receptor I (FcRI) or FcRII, but not FcRII, also led to an inhibition of pseudotype titer enhancement in an additive manner. Human lymphoblastoid cell line (Raji), a poor host for HCV pseudotype infection, exhibited a four- to sixfold enhancement of pseudotype-mediated cell death upon incubation with antibody at nonneutralizing concentrations. A similar enhancement of cell culture-grown HCV infectivity by a human monoclonal antibody was also observed. Taken together, antibodies to viral epitopes enhancing HCV infection need to be taken into consideration for pathogenesis and in the development of an effective vaccine.

Hepatitis C virus (HCV) may exist in blood as free virus or complexed with antibodies. HCV infects and replicates in B cells (1, 56, 64, 72) and has been associated with B-cell lymphoproliferative disorders (23). The development of a cell culture system which more closely resembles the natural course of HCV infection is an important new tool in the analysis of this virus. However, precise quantitative determination using this system remains difficult. In this regard, the use of pseudotype or viral particle mimic as a functional model is still beneficial to the study of HCV-antibody interactions. Neutralizing antibodies are a principal component of an effective human immune response to many pathogens, yet their role in HCV infection is unclear. Immunoglobulin G1 restriction, as well as a delayed appearance of antibody responses, is observed during persistent HCV infection in patients (7). We have observed that the vesicular stomatitis virus (VSV) pseudotypes generated using HCV E1 and/or E2 chimeric glycoproteins as a surrogate model fail to efficiently neutralize sera from chronically infected patients by a large percentage (35, 44, 45, 50, 62). Results from a different study also suggested that chronically infected patients develop low levels or no neutralization of binding (of E2 to CD81) antibodies (25, 60). Investigating the nature of antigen-antibody interactions in HCV infection may lead to an understanding of the poor neutralizing performance of anti-HCV specific immunoglobulin.

Human blood contains a component(s) that facilitates murine leukemia virus (MuLV)-derived HCV pseudoparticle (HCVpp) infection, although the nature of the component remains unknown (36, 43). The facilitation of HCVpp infection observed by Lavillette et al. (36) could not be explained, because heat-treated-decomplemented sera from uninfected donors displayed the same levels of enhancement, and facilitation was not observed when the HCVpp were incubated with normal purified human immunoglobulin. Viruses from various families elicit antibodies that enhance infectivity through the binding of virus-antibody complexes to cellular Fc receptors (FcRs) via the Fc portion of the antibodies (15, 21, 24, 38, 52, 53, 55, 57). Infection by an antibody-mediated mechanism may also occur with HCV (23, 54). Antibody-dependent enhancement (ADE) of virus infection is a process by which an infectious virus may use preexisting virus-specific antibodies to increase virus infection. Antibodies may mediate enhancement of virus infection in the presence or absence of complement in vitro and are called infection-enhancing antibodies. ADE of infection has also been observed in vivo in animal models and among individuals vaccinated against certain viruses, such as flavivirus (yellow fever virus and dengue virus [DENV]), human immunodeficiency virus type 1 (HIV-1), Ebola virus, and hantavirus (69). An enhancement of HIV-1 infection in vitro has been associated with gp120/41 antibodies. This increased infection has been noted to occur through interactions with both FcRs and receptors for complement in a number of different human cell lines (17, 24, 59, 67, 68, 70). Monoclonal antibodies (MAbs) to distinct epitopes of HIV gp120 displayed either neutralizing or enhancing properties (67). The ability of sera to enhance HIV-1 infection in the presence of complement has been associated with a progression toward AIDS (17, 24, 65), and an in vivo correlate of increased viral burden and antigenemia has been noted in a simian immunodeficiency virus (SIV)/macaque model system (47). A humanized MAb to

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DENV was also found to enhance infection in a variety of FcR-bearing cells in vitro (19). Passively transferred dilutions of this antibody also increased DENV viremia in monkeys. Sequence differences among viruses may contribute to a lack of susceptibility to virus neutralization by the antibodies. The effect of sequence differences at antibody-binding sites is suggested to reduce the avidity of the interactions between the preexisting antibodies and the new DENV serotype (61). These less-avid interactions have a significant effect on the ability of the antibodies to neutralize virus infectivity. However, there is sufficient binding of antibody to the virion to trigger ADE. In addition, these low-avidity antibodies increase in titer in preference to new antibodies with high avidity for the new DENV serotype, because the preexisting memory B cells and plasma cells are more rapidly activated than naïve B cells. While the in vivo implications of ADE have not been thoroughly identified, there has been considerable concern that ADE must be taken into account in vaccine design for viral diseases caused by DENV and HIV (33, 42, 49). This is particularly important, as an ADE mechanism might be responsible for the life-threatening dengue hemorrhagic fever and dengue shock syndrome. ADE may also act for lymphocytic tropic HIV strains to adjust their tropism for macrophages, which is thought to predispose the host to persistent infection (71).

In this study, we examined whether HCV antibody and/or complement may enhance virus infection in the absence of an efficient neutralization activity. We have observed an enhancement of VSV/HCV pseudotype infectious titer in the presence of a number of chronically HCV-infected patient sera and with human MAbs (HMAbs) specific for HCV E2 envelope glycoprotein. A similar evaluation made with HMAbs using cell culture-grown HCV (HCVcc) further validated our results. These observations clearly suggested that an enhancement of HCV infection occurs by utilizing a mechanism which is dependent upon FcR-mediated activity and may be associated with a specific epitope(s) found in the HCV E2 protein.

**MATERIALS AND METHODS**

**Cells and plasmids.** Human hepatoma cells (Huh-7) and baby hamster kidney (BHK) epithelial cells were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). B-lymphoblastoid (Raji) cells were maintained in RPMI supplemented with 10% fetal calf serum. The plasmids expressing chimeric E1-G and E2-G from HCV genotype 1a (GenBank accession number M62321) under the control of a cytomegalovirus (CMV) or MuLV promoter have been described earlier (2, 3). BHK stable transfectants were selected by treatment of cells with puromycin (2 µg/ml) under the control of a CMV promoter and used for cell culture-grown HCV (HCVcc).

**Pseudotype virus.** Incorporation of HCV E1-G or E2-G chimeric glycoprotein onto VSVΔG pseudotype has been previously described (34, 45). Cells transfected with an empty vector or VSV-G alone were similarly treated with VSVΔG as a negative and positive control, respectively. Treatment of pseudotype with an antisera to VSV G did not alter virus titer, suggesting the absence of revertant VSV G in pseudotype preparation, while positive-control virus incorporating native VSV G exhibited pseudotype neutralization as previously reported (34). NMS03, sodium [2,2-bis (docosyl-oxyethyl)] propyl-5-acetoamido-3,5-dideoxy-4,7,8,9-tetra-O-(sodium-oxysulfonyl)-2-n-glycero-a-t-galacto-2-nonulopyranosid- onate (molecular weight, 1458.7), is a sulfated sialyl, and was produced by GL Synthesis at a purity of >98% (Microbix, Worcester, MA). For an additional safeguard from contaminating VSV G glycoprotein reconstitution on pseudotype virus, a discriminating concentration (1.25 µg/ml) of NMS03 (inhibiting VSVΔG to ~100%, and VSV/HCV to only 15 to 20%) was also used (44).

**Generation of HCV in cell culture.** HCV genotype 1a (clone H77) was grown in immortalized human hepatocytes (IIH) as recently described (27). Virus growth was measured from cell culture supernatant filtered through a 0.45-µm cellulose acetate membrane (Nalgene, Rochester, NY) by the fluorescence-focus formation assay. HCV titer was calculated as ~10^7Focus-forming units per ml. Virus neutralization and antibody-dependent enhancement. The assay for antibody-mediated neutralization of pseudotype infectivity of Huh-7 cells was performed as described earlier (44). The antibody dilution that caused a 50% reduction in the plaque number of pseudotype virus compared to that of a negative control was considered the neutralization titer. ADE was measured from virus plaque titer. Briefly, Huh-7 cells were grown overnight on a six-well cell culture plate. Serial dilutions of neutralizing antibody were mixed with a known number of pseudotype viruses at 37°C for 1 h. The mixture was added to the cells for virus infection and plaque formation (45). An agarose-containing medium overlay was added, and the plates were incubated for 48 h at 32°C for plaque development. The number of plaques were counted after staining with neutral red and compared to control pseudotype mixed with normal serum or an isotopematched unrelated HMAb (R04) to CMV. The experiments were done in triplicate, and the infection enhancing antibody titer was defined as the antibody dilution that resulted in a 50% or more increase in comparable plaque number. Parental VSV was used as a control against nonspecific ADE.

**Blocking FcR interactions.** A Fab fragment recognizing the Fe region of immunoglobulin (Sigma, St. Louis, MO) was used for inhibition of ADE. The Fe-specific Fab fragment was added at the time of pseudotype/antibody incubation, with the control also having the added reagent. The remainder of the experiment was carried out as described above. For determining the roles of specific FcRs, cells (1 x 10^6) were treated with antibodies directed specifically against individual cellular FcRs prior to incubation with VSV/HCV pseudotype-antibody complexes. Cells were incubated for 15 minutes in the presence of FcR-inhibiting antibodies prior to adsorption of the virus-antibody mixture. Virus plaque numbers were counted and compared to those of control ADE infections in the absence of an antibody specifically against FcR. Plaque titers were converted to percent enhancement compared to control.

**ADE of pseudotype infection and Raji cell death.** Raji cells were seeded at 1 x 10^5 cells/well in Dulbecco modified Eagle medium supplemented with 1% heat-inactivated fetal bovine serum and 50 nM phytohemagglutinin (PHA). Cells were infected with a multiplicity of infection of 0.05 (titer established on Huh-7 cell line) of VSV/HCV pseudotype virus that had been preincubated with or without the presence of serial dilutions of HMBa for 1 h at 32°C. Pseudotype virus was adsorbed to Raji cells for 2 h at 37°C. Cells were incubated for 36 h at 32°C in RPMI, supplemented with 1% heat-inactivated fetal bovine serum. The number of viable cells was determined by CellTiter-Glo luminescent cell viability assay using a Glomax 96 microplate luminometer (Promega Corp, Madison, WI) as described earlier (40, 46). Results are presented as percent viability in comparison to an unaffected control.

**RESULTS**

Enhancement of pseudotype infection by HCV-infected patient sera. A high level of occupancy or antibody coating leads to neutralization, whereas in the presence of permissive target cells, low levels of occupancy may lead to an enhancement of infection (6). This phenomenon suggests a potential problem for some viral infections or vaccines that induce low levels of neutralizing antibodies. Antibody-mediated enhancement of infection is sensitive to target cell origin and is dependent on
the types and expression levels of Fc and/or complement receptors on the target cell surface (69).

In earlier studies, we have observed a rise in pseudotype plaque number when serum dilutions fell well below a neutralizing concentration. This enhancement was markedly different than the enhancement observed with lower serum dilutions, nonneutralizing patient sera, or uninfected control sera (36). To further explore this observation, sera from HCV-infected patients were tested for their ability to enhance infection at dilutions which fell below their normal neutralizing thresholds in a cell line which is permissive for HCV infection. A pseudotype-antiserum mixture was subjected to plaque titer determination, and the calculated values (percentage of control) are shown (Fig. 1). Normal sera enhanced infectivity, and this property decreased with increasing serum dilutions. Sera from six of the eight HCV-infected patients tested exhibited an enhancing activity covering a broad range of serum dilutions. Interestingly, the sera that exhibited the strongest ability to neutralize pseudotype virus failed to exhibit any significant enhancing titer. However, the ability to successfully neutralize pseudotype virus did not correlate with an inability to also enhance infection at higher dilutions. The data from this set of experiments suggested that an inhibition of virus infection occurs when the ratio of neutralizing antibody to virus is high, whereas in a manner similar to that observed in context to dengue hemorrhagic fever, enhancement occurs when this ratio is reversed (48).

Enhancement of pseudotype infection by HMAbs. We analyzed the capacity of purified HMAbs to mediate ADE in a permissive cell line (Huh-7). These MAbs directed to the E2 glycoprotein were derived from peripheral B cells of an individual who had an asymptomatic HCV genotype 1b infection (20, 30). A total of four HMAbs were included in this study. Three of the antibodies (CBH4B, CBH4D, and CBH4G) represent domain A, and one (CBH7) represents immunogenic domain C of E2. All of these antibodies also react with HCV genotype 1a. The antibodies representing domain A do not have the ability to block HCV E2 binding to human CD81, a putative receptor of HCV, while CBH7 of domain C can block E2 binding to CD81 (31). Antibodies generated against the E2 glycoprotein enhanced VSV/HCV pseudotype infection of Huh-7 cells in a dose-dependent manner (Fig. 2). In representative experiments, the HMAbs increased the number of infected cells by as much as 180%. CBH4B, CBH4D, and CBH4G did not display neutralization, while CBH7 neutralized HCVpp infectivity at a higher antibody concentration (31). The peak enhancing activity was noted at ~2 μg/ml with three of the antibodies studied. On the other hand, an isotype-matched control MAb (R04) did not enhance pseudotype infection. The Vero cell line is a FcR-negative cell line and can be infected by VSV/HCV pseudotype to a much lower degree than Huh-7 cells. A similar experiment using the antibodies did not impart an enhanced pseudotype infection of Vero cells, as seen on Huh-7 cells (data not shown). These findings revealed that the antibodies directed against the E2 glycoprotein could mediate ADE based on the cell type used for pseudotype infection.

Complement-induced ADE is not associated with the infectivity of VSV/HCV pseudotype. Antibody-dependent enhancement of human immunodeficiency virus has been observed to follow two separate mechanisms, one of which utilizes the alternate complement pathway to increase binding efficiency to host cells (41). The addition of human complement to virus-antibody complexes was examined to identify the potential for complement to work in conjunction with antibody to further enhance pseudotype virus titer. However, our earlier work
suggested that the addition of complement led to a significant enhancement of pseudotype neutralization (44). In each instance (CBH4B, CBH4G, and CBH7), pseudotype neutralization (>50%) was apparent at an antibody concentration of 2 μg/ml and was in contrast to >100% enhancement visible utilizing antibody alone at this concentration (Fig. 3A to C).

**ADE is mediated by FcR.** The FcR-dependent antibody enhancement of infection has been demonstrated previously for several viruses. Further, the use of anti-Fc MAbs has been observed to offset the enhancing properties of parvovirus B19 antibodies (51). Here, the use of an Fc-specific Fab fragment inhibited antibody-mediated enhancement at all specific HCV antibody dilutions tested (Fig. 4). Enhancement of pseudotype infection in Huh-7 cells was inhibited by treatment with 5 μg of anti-Fc (Fab) per ml. Percent enhancements for antibodies CBH4B, CBH4D, CBH4G, and CBH7 were reduced significantly following treatment with anti-Fc (Fab) antibody and are presented using plaque counts of the experimental wells against a control incubated only with the anti-Fc (Fab) reagent.

The use of purified reagents and specific antagonistic antibodies suggest that the interaction of anti-E2 antibodies with an Fc

**FIG. 2.** Human monoclonal antibody-mediated enhancement of pseudotype infection in Huh-7 cells. A known VSV/HCV pseudotype titer (~50 PFU) was incubated with different concentrations (in micrograms per milliliter) of E2-specific HMAbs or with an isotype-matched control antibody (R04). Cells were incubated with the virus-antibody mixture for infection. Similarly, ~50 PFU of the virus in the absence of an antibody was used as a mock control. Increase in percent enhancement of infection was determined from mock control virus titer, and results are shown from three independent experiments along with standard errors (error bars).

**FIG. 3.** Serum complement plays a role in pseudotype entry. VSV/HCV pseudotypes (~50 PFU) were incubated with different concentrations (in micrograms per milliliter) of MAb CBH4B, CBH4G, or CBH7 alone or together with human complement. Virus infectivity was measured by plaque assay in Huh-7 cells. Similarly, ~50 PFU of the virus in the absence of antibody was used as a mock control. Increase or decrease in percent control was determined from mock-treated virus titer, and results are shown from three independent experiments along with standard errors (error bars).
receptor on the cell surface is a possible mechanism for the enhanced infectivity of VSV/HCV.

To further define FcR specificity for ADE of VSV/HCV pseudotype, virus was separately incubated with 2 μg/ml of enhancing HMAb for 1 h at 32°C. Fifteen minutes prior to infection, cell monolayers were treated with anti-FcR antibodies, and left as competitors after the addition of the pseudotype-antibody mixture. Cells were incubated in the presence of pseudotype virus for 1 h at 32°C prior to the addition of agarose overlay. In cells pretreated with a MAb directed specifically at Fc receptor I (FcRI) or FcRII, percent enhancement attributable to the addition of human E2-specific HMAb was reduced by ~60 to 70% for each HMAb tested. Interestingly, the use of both FcRI and FcRII antibodies led to an additive reduction of ADE that was most significant (>85% reduction) using 0.5 μg total anti-FcR antibody (Table 1). However, antibody to FcRIII did not significantly reduce the level of ADE.

**ADE of pseudotype infection correlates with Raji cell death.** Antibodies generated against the E2 glycoprotein clearly enhanced HCV infection of Huh-7 cells in a dose-dependent manner as presented above. HCV has also been shown to infect B cells and replicate in B cells (1, 56, 64, 72) and is associated with B-cell lymphoproliferative disorders (23). We examined whether an increased cytopathic effect is associated with ADE of infection from VSV/HCV pseudotype and subsequent VSV replication of the pseudotype backbone in Raji cells. For this, the effects of HMAbs on VSV/HCV pseudotype infection in the Raji cell line and the loss of cell viability as a readout for VSV growth were analyzed (Fig. 5). Antiviral antibody can mediate virus infection of a monocytic cell line via FcRII (15). This phenomenon of ADE of infection may play a role in the immunopathogenesis of Aleutian mink disease parvovirus infections in adult mink (15, 28, 29). We analyzed the capacity of the HMAbs (CBH4B and CBH7) to mediate ADE (Fig. 5). Since it is difficult to perform a plaque formation assay in association with a suspension cell line, we determined cell death by VSV-mediated lysis following ADE of pseudotype entry into Raji cells. In representative experiments, each of the HMAbs increased cell death by as much as 80% over cell death using an untreated infected control. Of particular importance, the infectivity of the VSV/HCV pseudotype was poorer in Raji cells than in Huh-7 cells, while Raji cells are relatively competent for infection by parental VSV. These findings further suggested that antibodies directed to the E2 glycoprotein could mediate ADE in Raji cells and could provide a mechanism of B-cell infection by HCV.
ADE of HCVcc. To test the relevance of ADE in relation to cell culture-grown HCV, we infected immortalized human hepatocytes (IHH) with HCV genotype 1a (clone H77) in the presence or absence of E2-specific HMAbs. HCVcc genotype 1a was used in a fluorescent-focus formation assay as previously described (3). CBH5 is known to have a neutralizing activity on HCVpp and can block E2 binding to CD81 (31). The CBH5 antibody strongly neutralized HCVcc (65 to 98%) over a range of 2 to 4 μg/ml (Table 2). Further dilution of this neutralizing antibody did not result in any visible sign of ADE. In contrast, a different MAb (CBH7) enhanced fluorescent-focus formation by HCVcc (155 to 195%) over a range of 2 to 4 μg/ml (Table 2), below which neither virus neutralization nor enhancement was detected. A representative photograph of neutralization/enhancement of HCVcc infection by specific MAb is shown in Fig. 6. The neutralization efficiency of HCVcc genotype 2a by CBH5 has been shown to be much higher than that by CBH7 (30). Thus, our results suggested that a neutralizing epitope is located on a different domain than that of antibody directed to virus enhancing activity. These findings provide evidence that both neutralizing and enhancing epitopes exist on the E2 envelope glycoprotein of HCV.

**DISCUSSION**

The nature of HCV-specific antibodies generated in humans for the neutralization or enhancement of pseudotype as a surrogate of HCV infection was examined in Huh-7 and Raji cells. Both these human cell lines of distinct origin have Fc receptors on their surfaces, although the level of expression in hepatocytes is likely lower (4, 11, 12, 13, 16, 58, 63). In this study, the enhancement of infection by sera from HCV-seropositive patients was noted. A similar evaluation was also made with HMAbs and HCVcc genotype 1a for validation of some of our observations from the pseudotype virus. Results from our study characterized HCV-specific antibody-dependent enhancement of infection. HCV E2 has multiple epitopes recognized by infectivity-enhancing and -neutralizing antibodies. The purified HMAbs used in this study were characterized according to their ability to inhibit a CD81-HCV E2 interaction (20). Later, it was observed that the antibodies that displayed neutralization of HCVpp infection (CBH5 and CBH7) also inhibited the association between CD81 and HCV E2.

### TABLE 1. Enhancement of VSV/HCV pseudotype infection by HMAbs

<table>
<thead>
<tr>
<th>MAb used</th>
<th>Enhancement by antibody alone (%)</th>
<th>Concn of anti-FCR antibody (μg)</th>
<th>Enhancement in the presence of anti-FeR reagents (%)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Anti-CD16 (FcRIII)</td>
</tr>
<tr>
<td>CBH4B</td>
<td>115.90</td>
<td>0.5</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>1</td>
<td>109.10</td>
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<tr>
<td>CBH4D</td>
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<td>87.6</td>
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<td></td>
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<td>0.5</td>
<td>96.7</td>
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<td>94.8</td>
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<td>0.5</td>
<td>110.3</td>
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<td></td>
<td>1</td>
<td>111.0</td>
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<sup>a</sup> ND, not determined.
glycoprotein. Additionally, the antibodies that failed to neutralize HCVpp infection could be associated with an epitope not involving CD81 and HCV E2 interaction (31). The epitopes recognized by the HMAbs used in this study did not include those which recognize HVR1. However, a series of purified hypervariable domain 1 of HCV E2 (HVR1) mimotope-specific rabbit immunoglobulin G that had exhibited a limited level of VSV/HCV pseudotype neutralization ability (44) failed to enhance pseudotype infectivity (data not shown).

We have observed that heat-inactivated human sera from a pool of individuals that had not been exposed to HCV was able to facilitate VSV/HCV pseudotype infection by as much as 60% at a lower serum dilution. This process was observed in the absence of known HCV-specific factors and was titrated out completely in a linear manner by 1/200 dilution. The use of heat-inactivated serum from HCV-exposed subjects contrasts with the normal serum enhancement by imparting a low to moderate level of pseudotype virus neutralization at similar dilutions. However, upon further dilution of this infected sera beyond the level at which it retained its neutralizing function, a progressive level of pseudotype virus enhancement was observed, but not with normal human sera. Earlier studies suggested that antibody concentrations that fall below levels associated with neutralization induce ADE with other viruses. For example, sera from individuals infected with HIV-1 displayed an ability to neutralize in vitro infections of HIV-1, but at subneutralizing concentrations, these same sera enhanced infection in permissive cells (73). Further, the cross protective antibody response of DENV-1 sera for DENV-2 infection was lost at higher dilutions, with manifestations of ADE present (8). Enhancement of DENV-2 infection was also observed in a rhesus monkey model using passive transfer of dengue virus immune sera, and an association between increased viral burden and disease severity was noted (22, 37). Recently, the use of the rhesus monkey model could be reduced by altering the Fc region of the antibody to limit binding to Fc receptors and was completely abrogated by a deletion in the CH2 region, reinforcing an in vivo correlate for ADE. We examined three HCV-infected patient sera by the addition of exogenous human complement, and none of these sera significantly altered virus plaque counts (K. Meyer and R. Ray, unpublished observation). This result is unlike those observed with HMAbs, indicating although such antibodies may be generated in infected humans, the production of complement-fixing antibodies is not a significant feature in patients with chronic HCV infections and does not occur at a concentration that would affect the course of infection. The levels of specific complement components are depleted (10), while specific C4 activity is significantly lower in patients with chronic HCV infections (14). In our earlier study (44), we noted the importance of C4 in complement-mediated neutralization of VSV/HCV pseudotype. Examining functional complement levels in chronically HCV-infected patients and the ability of these patients to produce significant levels of complement-fixing antibodies will be important for implications of the disease course and prophylaxis. The results of this study have implicated the HVR1 peptide sequence and other regions of E2 for HCV-mediated pathogenesis. Consequently, the results suggest that complex functional classes of epitopes exist on the HCV E2 glycoprotein, some mediating only neutralization, others potentially involved with ADE and immune complex formation, and some having mixed characteristics. It is possible that nonneutralizing epitopes might dominate by inducing antibodies that interfere with neutralization and accentuate disease (9, 18), and the results appear to suggest this possibility.

The use of MAb derived from humans in a similar assay allowed for the identification of HCV-specific molecules which enhanced the level of infectivity of the VSV/HCV pseudotype virus. The HMAbs (CBH4B, CBH4D, CBH4G, and CBH7) were observed to display their ability to enhance VSV/HCV pseudotype infection by as much as twofold in a susceptible cell line (Huh-7). Further, an increase in cell death of a poorly receptive B-cell line (Raji) up to sixfold highlighted this ability to upregulate infection. However, the use of an FcR-negative cell line (Vero) allowed for no enhancement of pseudotype infection, despite a similar level of specific neutralization that was also apparent with human sera or HCV HMAb (data not shown). To further confirm the specificity of this observation, we utilized a Fab fragment directed specifically against Fc against various concentrations of the HMAbs, and an inhibition of enhancement was observed.
In agreement with our earlier work (44), the addition of human complement sera to the ADE discriminating HMAbs led to neutralization of pseudotype virus at the dilutions used. In contrast, using a panel of FcR-specific antibodies able to inhibit recognition of a specific FcR by the HMAbs exhibited FcR-specific inhibition of enhancement of the VSV/HCV pseudotype. Using FcRII-specific MAbs, this FcR has been identified as having a central role in the process of ADE for West Nile virus and in infection by severe acute respiratory syndrome coronavirus (5, 26, 55), while FcRI has been implicated in ADE associated with HIV-1 (66). ADE associated with DENV infection has been demonstrated to occur with both FcRI (32) and FcRII (39). In experiments conducted with VSV/HCV, we observed an inhibition of ADE to HCV-specific HMAbs using either antibody to FcRI or FcRII, but not to FcRIII. Interestingly, an additive response was observed using both FcRII and FcRIII.

The HMAbs used in this study were part of a group of antibodies for which discrete domains on HCV2E have been established by competitive assay. CBH7 represents immunogenic domain C, CBH5 is identified as being from the neutralizing immunogenic domain B, and CBH4B, CBH4D, and CBH4G represent immunogenic domain A as previously mapped (31). Olsen et al. (53) studied the ability of certain epitopes of the S protein of feline infectious peritonitis virus to enhance or neutralize virus infection and were able to categorize these abilities with different epitopes. Interestingly, the results presented here using VSV/HCV and HCV with a limited number of E2-specific MAbs conveyed a similar finding. The development of HCVcc allowed for a test of HCV HMAb-dependent enhancement of infection. HCVcc of the genotype 1a (clone H77) used by our laboratory appeared to be significantly less sensitive to neutralization than CBH5 antibody compared to the results seen in a different lab using genotype 2a (clone JFH1) (30). However, the use of HCVpp as a surrogate model for both the 1b and 2a strain in the same study indicated that the 2a strain may well be markedly more sensitive to neutralization by HMAbs from domain B than even the genotype from which they were raised. Thus, the neutralization sensitivity of the diverse HCV genotypes could have an impact upon future vaccine design. Although there are no indications that ADE may trigger increased severity of HCV infection at this time, it may be a mechanism by which infection by different HCV genotypes may be enhanced or as a contributing mechanism by which a chronic infection is maintained. Our observations may prove important with regards to the future prospects of prophylaxis and design of a vaccine against HCV infection.

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