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Functional Analysis of Cell Surface-Expressed Hepatitis C Virus E2 Glycoprotein

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Functional Analysis of Cell Surface-Expressed 
Hepatitis C Virus E2 Glycoprotein

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Hepatitis C virus (HCV) glycoproteins E1 and E2, when expressed in eukaryotic cells, are retained in the endoplasmic reticulum (ER). C-terminal truncation of E2 at residue 661 or 715 (position on the polyprotein) leads to secretion, consistent with deletion of a proposed hydrophobic transmembrane anchor sequence. We demonstrate cell surface expression of a chimeric glycoprotein consisting of E2 residues 384 to 661 fused to the transmembrane and cytoplasmic domains of influenza A virus hemagglutinin (HA), termed E2661-HATMCT. The E2661-HATMCT chimeric glycoprotein was able to bind a number of conformation-dependent monoclonal antibodies and a recombinant soluble form of CD81, suggesting that it was folded in a manner comparable to “native” E2. Furthermore, cell surface-expressed E2661-HATMCT demonstrated pH-dependent changes in antigen conformation, consistent with an acid-mediated fusion mechanism. However, E2661-HATMCT was unable to induce cell fusion of CD81-positive HEK cells after neutral- or low-pH treatment. We propose that a stretch of conserved, hydrophobic amino acids within the E1 glycoprotein, displaying similarities to flavivirus and paramyxovirus fusion peptides, may constitute the HCV fusion peptide. We demonstrate that influenza virus can incorporate E2661-HATMCT into particles and discuss experiments to address the relevance of the E2-CD81 interaction for HCV attachment and entry.

Enveloped viruses acquire their lipid membranes by budding through host cellular membranes (reviewed in reference 35). The majority of enveloped viruses bud at the plasma membrane. However, several viruses assemble and bud at internal membranes such as those of the endoplasmic reticulum (ER) (e.g., rotaviruses), ER-Golgi intermediate compartments (e.g., coronavirus), or the Golgi complex (e.g., bunyaviruses). This behavior generally reflects the targeting of the viral glycoproteins (gps) within subcompartments of the ER or Golgi complex. In the latter cases, viruses are released from infected cells either by cell lysis or after transport through the cellular secretory pathway to the cell surface.

Hepatitis C virus (HCV), the major cause of non-A, non-B hepatitis, is an enveloped virus classified in the Flaviviridae family (reviewed in references 3 and 39). The genome encodes two putative envelope gps, E1 (polypeptide residues 192 to 383) and E2 (residues 384 to 746), which are released from the viral polyprotein by signal peptidase cleavage(s) (13, 18, 43). Both gps are heavily modified by N-linked glycosylation and are believed to be type I integral transmembrane proteins, with C-terminal hydrophobic anchor domains.

Expression of the E1E2 gps in mammalian cell lines demonstrates their ER retention with no cell surface gp expression detectable (8, 46, 47). Immunoelectron microscopic studies localized the gps to the ER (7, 8). We (10) and others (4) reported the presence of ER retention “signals” within the C-terminal regions of both E1 and E2 gps, explaining these observations. Consistent with these data, truncation of E2 at its C-terminus leads to its secretion from expressing cells (26, 30, 45, 47). These observations are consistent with a model of HCV particle morphogenesis occurring by budding into the ER, as reported for other members of the Flaviviridae.

When expressed in tissue culture cells, the E1 and E2 gps interact to form noncovalently linked complexes, whose size is consistent with E1E2 heterodimers (6, 8). In addition to these noncovalently associated E1E2 complexes, a significant proportion of E1 and E2 are present in disulfide-linked aggregates, which are believed to result from a nonproductive folding pathway (1a, 6, 8, 13). Since HCV cannot be propagated efficiently in vitro, it has been difficult to study “native” E1E2 gp forms as they exist on the virus particle. It is critical when studying the biological activity of the HCV gps to distinguish between molecules that undergo productive folding and assembly and those that follow a nonproductive pathway(s) resulting in misfolding and aggregation (7). Recently, Dubuisson and colleagues reported a number of conformation-dependent monoclonal antibodies (MAbs) (H2 and H53) which specifically recognize nondisulfide-bridged E2, both alone and when complexed with E1, allowing the study of gp complexes which may represent “native” prebudding forms of the HCV gp complex (4, 6, 30).

gps exposed on the virus surface mediate entry into target cells. This process requires binding of the virus particle to a receptor(s) present at the surface of the host cell, followed by fusion of the viral and cellular membranes. For viruses such as influenza virus and the flavivirus tick-borne encephalitis virus, particles internalize after receptor binding and fuse with the endosomal membranes. The low pH within the endosomal compartment induces a major structural rearrangement of the gps, resulting in exposure of a fusion peptide which destabilizes membranes, leading to fusion (reviewed in references 11, 17, and 50). The mechanism by which HCV enters target cells is currently unknown; however, the E2 gp is thought to be responsible for initiating virus attachment to a receptor on po-
tential host cells (42). Indeed, a soluble form of a C-terminally truncated E2 gp was used to identify CD81 as a putative receptor for HCV (36). CD81 is a broadly expressed protein and is reported to be involved in a variety of biological responses including adhesion, morphology, proliferation, activation, and differentiation of T-, B-, and other cell types (reviewed in reference 23).

Generation of viral pseudotypes is one of the most widely used methods for assaying functional receptors, allowing attachment, penetration, and uncoating to be studied. Recent reports (28, 30, 33) of the partial stomatitis virus and VSV) expressing chimeric HCV E2 gpus, comprising the putative E2 ectodomain fused to the transmembrane and cytoplasmic domains of VSV G protein, allowed entry into target cells suggested that the ectodomain of E2 was sufficient to confer viral attachment and entry (22, 28). We were interested in studying the antigenic conformation of E2 expressed at the cell surface and whether such a protein could be induced CD81-dependent cell fusion. Here, we demonstrate cell surface expression of a chimeric gp consisting of E2 residues 384 to 661 fused to the transmembrane and cytoplasmic domains of influenza A virus hemagglutinin (HA) (E2661-HATMCT). These data are consistent with a previous report demonstrating cell surface expression of truncated versions of E2 fused to the transmembrane domain of CD4 or a glycosylphosphatidylinositol anchor (4). The E2661-HATMCT chimeric protein possesses an additional Gly-Ala amino acid pair at the junction of the ectodomain (E2 sequence) and transmembrane domain (HA sequence). When expressed in antigen conformation, consistent with an acid-mediated fusion mechanism. However, E2661-HATMCT was unable to induce cell fusion of CD81-positive HEK cells after neutral- or low-pH treatment. We demonstrate that influenza virus can incorporate E2661-HATMCT into particles and discuss possible strategies to address the relevance of the HCV-CD81 interaction for HCV attachment and entry.

MATERIALS AND METHODS

Materials. MAbs specific for E1 (3/8d and 3/8ow), E2 (1/39, 6/82a, and 6/16), CD81 (5A6 [33]), and glutathione S-transferase (GST) (2/18) were raised by standard methods. MAbs specific for conformational epitopes (H2, H31, H33, H44, H50, H53, H60, and H61) were a gift from J. Dubuisson (Institut Pasteur de Lille) or with 100 μl of tissue-culture supernatant or anti-E2 conformational MAbs; H2, H31, H33, H44, H50, H53, H60, and H61 at 1/500 dilution) for 1 h at room temperature, and washed three times with P/F/A. Immu-

Flow-cytometric analysis. HEK cells were transfected as described above. At 48 h posttransfection, the cells were harvested with PBS containing 0.2% EDTA and washed with PBS twice. They were incubated for 30 min at room temperature in PBS containing 1% FCS and 0.05% sodium azide (P/F/A). Vi-

Expression and purification of GST-CD81EC2. The human CD81 E1C2 was made from a gel-purified Henvi-R1a fragment, coding for amino acids 116 to 202, of the cDNA clone and ligated to pGEX-2T (Pharmacia) which had been im-

Cell surface expression of HCV E2

PCB with plasmid pBRTM/HCV1-3011 (kindly supplied by C. M. Rice, Wash-

Indirect immunofluorescence. HEK (293) cells were grown in DMEM sup-

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of the ectodomain or transmembrane and cytoplasmic domains of influenza A virus haemagglutinin (HA) (E2661-HATMCT). These data are consistent with a previous report demonstrating cell surface expression of truncated versions of E2 fused to the transmembrane domain of CD4 or a glycosylphosphatidylinositol anchor (4). The E2661-HATMCT chimeric protein possesses an additional Gly-Ala amino acid pair at the junction of the ectodomain (E2 sequence) and transmembrane domain (HA sequence). When expressed in antigen conformation, consistent with an acid-mediated fusion mechanism. However, E2661-HATMCT was unable to induce cell fusion of CD81-positive HEK cells after neutral- or low-pH treatment. We demonstrate that influenza virus can incorporate E2661-HATMCT into particles and discuss possible strategies to address the relevance of the HCV-CD81 interaction for HCV attachment and entry.
fect cells were treated similarly. Following overnight incubation, the cells were fixed with methanol-acetone and E2 or NP antigen was visualized by indirect immunofluorescence as described above. To visualize nuclear DNA, a mountant containing propidium iodide (Vectorshield; Vector Laboratories) was used. The cells were visualized with a confocal microscope (Bio-rad).

Generation and analysis of pseudotyped influenza viruses. COS-7 cells were electroporated either with empty vector, pCDM8, or with 15 μg of plasmid pE2661-HATMCT as described elsewhere (2). Following electroporation, the cells were resuspended in DMEM containing 10% FCS and 10 μM HEPES (pH 7.4) and allowed to recover at 37°C overnight. They were then infected with influenza A virus AP8/34 at a multiplicity of infection of 3. After 24 h, supernatants were collected and clarified of cellular debris by centrifugation at 15,000 rpm in a Beckman SW55 rotor. To confirm that the released virus contained the E2661-HATMCT protein, virus was purified by centrifugation at 45,000 rpm in a Beckman SW55 rotor through a 1-ml cushion of 30% sucrose in NTE (100 mM NaCl, 10 mM Tris-HCl [pH 7.8], 1 mM EDTA). Virus pellets were resuspended in 10 μl of NTE and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting to detect the incorporation of mNTE and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting to detect the incorporation of mNTE.

Proteins were visualized following exposure to enhanced chemiluminescence detection reagents (Amersham Life Sciences) and photographic film. Evanston, Ill.), followed by an anti-mouse HRP-conjugated secondary antibody. Mouse MAb, 14C2 (kindly supplied by R. A. Lamb, Northwestern University, Evanston, Ill.), followed by an anti-mouse HRP-conjugated secondary antibody. Proteins were visualized following exposure to enhanced chemiluminescence detection reagents (Amersham Life Sciences) and photographic film.

RESULTS

Truncated E2 with transmembrane and cytoplasmic domains of influenza virus HA is expressed at the cell surface. Since C-terminal truncation of E2 results in protein secretion from the cell (29, 30, 45, 47), we reasoned that addition of a transmembrane domain to such a truncated form may result in localization at the plasma membrane. To test this hypothesis, cDNA encoding the chimeric gps was constructed, consisting of the E2 ectodomain (from amino acids 384 to 661 or 715) fused to the transmembrane and cytoplasmic domains of influenza A virus HA (Fig. 1). Since the E2 gp acts as a chaperone for E1 folding (30), we were interested in determining any effects of coexpression of the full-length E1 protein on both E1 and E2 localization. Plasmids encoding both E1 and the chimeric E2 gps were therefore constructed (Fig. 1). All plasmids contained endogenous signal sequences to direct translocation to the ER, including polyprotein residues 364 to 383 for E2 chimeras and 171 to 191 for E1-encoding plasmids.

HEK (293) cells were transfected with the plasmids shown in Fig. 1, and 48 h posttransfection the cells were fixed, or without Triton X-100 permeabilization, to monitor internal and cell surface-expressed antigen, respectively. Indirect immunofluorescence was performed with MAb specific for both E1 and E2 proteins. The results are summarized in Table 1. As expected, full-length E1 and E2 could not be detected at the cell surface whereas E2661-HATMCT could be detected. Coexpression of E1 did not result in E1 expression at the cell surface, nor did it have any detectable effect(s) on E2661-HATMCT expres-

![Figure 1](http://jvi.asm.org/)

**Fig. 1.** Schematic representation of the proteins expressed in these studies. HCV E1 or E2 sequences were fused to the transmembrane and cytoplasmic domains of influenza A virus HA protein. The amino acid position on the HCV polyprotein is indicated above the bars. Signal sequences are indicated by solid boxes, while the HA sequence is shown by hatching. These chimeric proteins were cloned in the eukaryotic expression vector pCDM8.

**TABLE 1. Summary of indirect immunofluorescence observed for E1 and E2 localization on transiently expressing cells.**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Anti-E1</th>
<th>Anti-E2</th>
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<td>Internal</td>
<td>Surface</td>
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<td>Vector</td>
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<td>pE2</td>
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</tr>
<tr>
<td>pE1E2</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>pE2661-HATMCT</td>
<td>++</td>
<td>–</td>
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<tr>
<td>pE1E2661-HATMCT</td>
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<td>pE2715-HATMCT</td>
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<tr>
<td>pE1E2715-HATMCT</td>
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*The intensity of fluorescence across a number of fields is indicated.*

Recognition of cell surface E2 by conformation-dependent anti-E2 MAb: the effect of low-pH treatment. We were inter-

![Image](http://jvi.asm.org/)

**TABLE 2. Summary of indirect immunofluorescence observed for E1 and E2 localization on transiently expressing cells.**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Anti-E1</th>
<th>Anti-E2</th>
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<tbody>
<tr>
<td>Vector</td>
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<td>pE2</td>
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<td>pE1E2715-HATMCT</td>
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*The intensity of fluorescence across a number of fields is indicated.*
These data are consistent with a bent assay for MAb recognition of low-pH-treated soluble E2. Similar results were observed by enzyme-linked immunosorbent assay. After being treated at pH 5.0 or 7.0, the cell-bound GST-CD81EC2 was lower than that of the anti-E2 MAbs for E2. However, we cannot exclude the possibility that the EC1 loop of CD81 (lacking from this recombinant fusion protein) influences the affinity of EC2 for E2. These data confirm that cell surface-expressed chimeric E2 can bind a recombinant form of CD81, with the binding site residing between residues 384 and 661, and that E2661-HATMCT is active in the (putative) receptor-binding function.

E2 at the cell surface does not induce acid-mediated cell-cell fusion. Fusion of cells expressing influenza virus HA protein by acid treatment has been well characterized (reviewed in references 11, 17, and 50). Since flaviviruses have been reported to induce syncytia following low-pH treatment. Influenza virus-infected cells were identified by using an antibody specific for nucleoprotein (NP). Influenza virus-mediated cell fusion was easily detected, with large syncytia forming around NP-positive cells following low-pH treatment (Fig. 4B) but not after neutral-pH treatment (Fig. 4A). The nuclei were visualized with propidium iodide. After neutral- or low-pH treatment, no cell-cell fusion of cells expressing E2 at their surface was observed (Fig. 4C). As a positive control for the assay, HEK cells were infected with influenza A virus strain A/WSN/33 at different multiplicities of infection. This strain was chosen because cleavage of HA0 to HA1 and HA2, a necessary prelude to fusion, does not require trypsin treatment. Influenza virus-infected cells were identified by using an antibody specific for nucleoprotein (NP). Influenza virus-mediated cell fusion was easily detected, with large syncytia forming around NP-positive cells following low-pH treatment (Fig. 4B) but not after neutral-pH treatment (Fig. 4A). The syncytia were most evident at high multiplicities of infection, in line with previous observations that membrane fusion may be dependent upon the local density of fusion protein (5). However, the U87 glial cell line is a more sensitive indicator cell for studying both influenza virus- and human immunodeficiency virus-mediated cell fusion (24); hence, the experiment detailed above with E2661-HATMCT were repeated in this cell line. Comparable results were obtained, such that no E2661-HATMCT-mediated cell fusion was observed; however, influenza virus-
induced fusion was observed at all multiplicities of infection tested (data not shown). These data indicate that under conditions which support cell-cell fusion by the influenza virus HA protein, the chimeric E2661-HATMCT gp does not induce any detectable cell fusion.

E2661-HATMCT can be incorporated into influenza virus particles. Influenza A viruses do not incorporate significant levels of host cell proteins into their envelopes. However, possession of HA transmembrane and cytoplasmic tail sequences has previously been shown to direct the incorporation of foreign proteins into influenza virus particles (31, 52). Since E2661-HATMCT was expressed at the cell surface and contained the relevant HA sequences, we tested the ability of influenza A virus to incorporate the chimeric gp expressed in COS cells. It is important to note that we (9) and others (36) have previously shown that E2 is unable to bind to COS-expressed CD81, such that high level expression of E2661-HATMCT can be achieved without receptor ligand complex formation. COS-7 cells were electroporated with pE2661-HATMCT (Fig. 5A and B, lanes 2 and 4) or empty vector (lanes 1 and 3). Cells were infected with influenza virus 24 h after transfection (multiplicity of infection, 3; lanes 1 and 2), and the extracellular progeny virus was harvested after a further 24 h. Virions were separated from host cell membrane fragments by ultracentrifugation through a high-density sucrose cushion and characterized for their constituent proteins by Western blotting. The influenza virus protein M2, a minor component of influenza virus, was visible, confirming the presence of influenza A virus particles derived from infected cells (Fig. 5B). E2 antigen was detected in a lysate derived from pE2661-HATMCT-transfected cells (Fig. 5A, lane 4), indicating that expression had occurred in these cells. Furthermore, this protein was incorporated into influenza virus particles, since it was present in progeny virions from cells transfected with pE2661-HATMCT (Fig. 5A, lane 2). The E2661-HATMCT in a lysate of expressing cells and that incorporated into influenza virus virions was compared (Fig. 5C). Since influenza virus virions bud through the plasma membrane, the chimeric molecule would be expected to undergo modification with complex glycans during transport through the secretory transport system. Consistent with this, the E2661-HATMCT present in a lysate from expressing cells migrated more rapidly in SDS-PAGE (Fig. 5C, lane 1) than did that present in influenza virus virions (lane 2).
DISCUSSION

The current understanding of HCV gp function is limited by the lack of a tissue culture system supporting efficient replication of the virus. From studies with transient-expression systems, it is believed that gps E1 and E2 localize to the ER in infected cells (4, 6, 8). By analogy to other flaviviruses, virus morphogenesis may involve budding into the ER and subsequent transport of viral particles through the host cell secretory pathway before release into the extracellular space. Modification of flavivirus E and prM protein glycans by trimming and terminal addition suggests that virions do indeed move through an exocytosis pathway similar to that used for host gps (27, 32).

In this report we describe a truncated form of the HCV E2 gp fused to the transmembrane and cytoplasmic domains of the influenza A virus HA protein. This chimeric protein was expressed at the cell surface, where it was able to bind a number of conformation-dependent MAbs and a recombinant soluble version of the putative HCV receptor, CD81 (Table 1; Fig. 2 and 4). These data suggest that the chimeric gp is folded in a manner comparable to E2 present in native E1E2 complexes and that it is in a form able to bind the putative receptor, CD81. Low-pH treatment of cell surface-expressed E2 resulted in a conformational change(s). However, neutral- or low-pH treatment of CD81-positive cells expressing the chimeric gp at the cell surface did not result in cell fusion.

If HCV virions are indeed transported through the host cell secretory pathway, then E2_{661-HATMCT} should resemble E2 on the surface of virions. Understanding the way in which E2 is glycosylated may help our understanding of E2 conformation and structure. Inhibition of core glycosylation by tunicamycin prevents E2 from folding correctly and being recognized by the conformation-dependent MAbs (1a). Since E2_{661-HATMCT} reacts with such MAbs and since H2 and H53 react with noncovalently associated E1E2 heterodimers (4, 6), this indicates that E2_{661-HATMCT} has a conformation similar to that adopted in E1E2 complexes.

A proteolytic cleavage is a common posttranslational modification of viral membrane proteins (reviewed in reference 21). For example, during virion transit, the prM protein of flaviviruses is cleaved by the host protease furin within a post-Golgi acidic compartment (15, 38, 48). This cleavage is required for the acquisition of virion infectivity. No proteolytic cleavage was detectable in deglycosylated E2_{661-HATMCT}, since no size differences were observed when expressing cells were treated with or without brefeldin A, an inhibitor of the secretory transport system (data not shown). This suggests that, unlike prM, HCV E2 does not undergo proteolytic cleavage as a step in virus maturation.

Previous work has shown that truncation of E2 to residue 661 results in a molecule that is more readily exported from the cell than is a molecule truncated at residue 715 (4, 26, 30). The additional residues could reduce the efficiency of E2 folding and hence of secretory transport. Our data is consistent with this hypothesis, since E2_{661-HATMCT} was detected more readily on the surface of expressing cells than was E2_{715-HATMCT} (Table 1). Given the reported chaperone role of E2 in E1 folding, we were interested in determining whether

FIG. 4. Cell-cell fusion is not mediated by E2_{661-HATMCT} under conditions which permit HA-mediated fusion. HEK cells were infected with influenza A virus (A and B) or transfected with plasmid pE2_{661-HATMCT} (C). Cell monolayers were treated at pH 5.0 (B and C) or pH 7.0 (A) and visualized by indirect immunofluorescence with anti-E2 antibodies for transfected cells, or anti-NP MAb for influenza virus-infected cells, with propidium iodide to visualize nuclei. These micrographs show representative fields from the examined samples.
cell surface expression of E2_{661-HATMCT} would lead to expression of E1 at the cell surface (30). However, E1 was not detected at the cell surface under any circumstances; furthermore, E1 coexpression did not affect the level of E2 transport to the plasma membrane.

It is thought that after receptor-mediated endocytosis, flavivirus entry into target cells proceeds via an acid-mediated fusion event, where the viral envelope fuses with an endosomal membrane. Acid treatment of the flavivirus envelope protein E in mature virions results in a conformational change (14, 16, 20, 40). This conformational change is irreversible and results in mature virions releasing from mock-transfected cells (31). Hence, it is not surprising that E2 expressing the HCV genome incorporated efficiently into influenza virus particles (31). Hence, it is not surprising that E2 expressing the HCV genome incorporated efficiently into influenza virus particles (31). Hence, it is not surprising that E2 expressing the HCV genome incorporated efficiently into influenza virus particles (31).

We were unable to demonstrate any E2_{661-HATMCT}-mediated cell-cell fusion (Fig. 4 and data not shown). However, the conditions used in this assay, although compatible with influenza virus HA-mediated fusion, might not support E2-mediated fusion. The production of polykaryotic cells is dependent upon the density of the fusion protein at the cell surface (5), and the expression method used may not result in a sufficient accumulation of cell surface E2_{661-HATMCT} to support fusion. Alternatively, the lipid composition of HEK and U87 cell membranes may not be compatible with E2-mediated fusion (12, 49). In any event, the lack of detectable polykaryons is not definitive evidence that HCV E2 does not have a fusogenic activity, since variant influenza viruses, herpesviruses, and paramyxoviruses exist that do not cause syncytium formation even though they are active in their fusion function (50). Another explanation is that E1 is required, or indeed is responsible, for the fusion event. Since we were unable to demonstrate any fusion activity for E2_{661-HATMCT}, we examined the sequence of E1 for a putative fusion peptide. Interestingly, recombinant E1 protein is secreted when truncated after amino acid 340, only if an internal deletion between residues 262 and 290 is also present (29). This internal deletion spans a hydrophobic domain, possibly containing a fusion peptide, that could act as a transmembrane anchor when E1 is truncated at residue 340, preventing its secretion. Viral fusion peptides may act as transmembrane anchor domains, converting normally soluble proteins into membrane-bound ones (34). Most fusion peptides are composed of 16 to 26 relatively hydrophobic amino acids (50). The sequence of the internal hydrophobic domain in E1 is relatively highly conserved, with changes usually being conservative (Fig. 6A) (25). Alignment of this region of HCV E1 with the putative fusion peptide from flavivirus E proteins (41) revealed several similarities (Fig. 6B). Two Cys residues are completely conserved between all sequences analyzed. The structural implications of this are unclear, but if these residues were involved in disulfide bonds, the putative fusion peptide may be constrained in some fashion. An Asp residue is present in all the representative HCV sequences and some of the flavivirus sequences. The presence of acidic residues in the fusion peptides of some low-pH-activated viral fusion proteins has been noted previously (51). Two Cys residues are conserved within these putative fusion domains. The Gly residues within the E1 sequences have a spacing similar to that observed in the fusion peptides of the paramyxoviruses, at positions 3, 7, and 12 and at positions 3, 7, and 13 for the majority of HCV sequences analyzed to date (Fig. 6C). In the paramyxovirus F proteins, the Gly residues are believed to be important for the structure of the fusion peptide (19). Given the similarities between the internal hydrophobic region of HCV E1, the putative (flavivirus) and known (paramyxovirus) fusion peptides, we propose that this region may comprise the HCV fusion peptide.

Some enveloped viruses are promiscuous in regard to the proteins they will incorporate into their membrane, while others appear to use specific signals in the sequences of their envelope proteins to discriminate between viral and cellular proteins present at the site of budding. Influenza virus utilizes the transmembrane and cytoplasmic tail sequences to select its major envelope protein, HA, during particle formation (31). Hence, it is not surprising that E2 expressing the HCV E1 with the putative fusion peptide could function independently to mediate binding and entry into target cells (22). The entry of these chimeric HCV sequences has been used extensively to study many viral gpS. VSV particles expressing either chimeric HCV E1 or E2 gpS were recently reported to confer VSV entry, suggesting that the gpS could function independently to mediate binding and entry into target cells (22). The entry of these pseudotyped viruses could be inhibited by sera from chimpanzees immunized with the homologous HCV gpS; however, the entry was not shown...
To be CD81 dependent. Clearly, it will be important to demonstrate whether CD81, either alone or with additional factors, can function as the HCV receptor in allowing pseudotyped virus-cell attachment and entry. Since CD81 is so widely expressed, it is unlikely to be the sole factor determining HCV liver tropism. We are now in an ideal position to answer these questions by studying the receptor requirements for attachment, entry, and uncoating of influenza viruses expressing chimeric HCV gpl.

ACKNOWLEDGMENTS

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FIG. 6. A putative fusion peptide within the E1 protein. Numbers above the alignments indicate the amino acid position within the HCV-1 polyprotein. (A) Alignment of the internal hydrophobic domain of representative HCV genotypes (2). (B) The HCV putative fusion peptide contains similarities to the predicted fusion peptide from flavivirus E glycoprotein. Shown are alignments of the HCV-1 sequence with representative paramyxovirus glycoprotein sequences: HPIV, human parainfluenza virus; SV, simian virus; CDV, canine distemper virus; NDV, Newcastle disease virus (19). Gly residues are shaded.