Epitope mapping of envelope glycoprotein E1 of hog cholera virus strain Brescia

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Four antigenic domains (A, B, C and D) on envelope glycoprotein E1 (gp51-54) of hog cholera virus strain Brescia have been specified by using 13 monoclonal antibodies (MAbs) that recognize non-conserved and conserved epitopes. It was shown that the non-conserved epitopes map to the N-terminal half of E1 by analysis of chimeric E1 proteins of strains Brescia and C. Conserved epitopes, however, could not be mapped using this approach. Here we describe mapping of both conserved and non-conserved epitopes on E1 by the use of an extensive set of single and double deletion mutants of E1 of strain Brescia. Deletion mutants were transiently expressed in COS1 cells and analysed by immunostaining with the 13 MAbs directed against strain Brescia and four MAbs directed against strain C. All MAbs bound to the N-terminal half of E1, i.e. amino acids 690 to 866 encoded by the sequence of strain Brescia. Domain B and one epitope in domain C are located between residues 690 and 773. Other epitopes in domain C are located on an extended region, i.e. between residues 690 and 800. Conserved epitopes of domain A are mapped between residues 766 and 866, whereas the only non-conserved epitope in this domain is located between residues 766 and 813. Domain D, represented by one MAb, is located in the same region as this non-conserved epitope of domain A, i.e. between residues 766 and 800. The results suggest the presence of two distinct antigenic units on E1, one consisting of domains B and C and the other consisting of domain A.

Introduction

Hog cholera virus (HCV), bovine viral diarrhoea virus (BVDV) and border disease virus (BDV) belong to the genus Pestivirus. This genus, which was formerly assigned to the Togaviridae, has recently been reclassified within the family Flaviviridae (Francki et al., 1991). The genomes of pestiviruses consist of an RNA molecule of positive polarity and are about 12.5 kb in size (Renard et al., 1985; Moormann & Hulst, 1988). The genomes of two HCV strains, Alfort (Meyers et al., 1989) and Brescia (Moormann et al., 1990a), and three BVDV strains, NADL (Collett et al., 1988a), Osloss (Renard et al., 1987) and SD-1 (Deng & Brock, 1992), have been cloned and sequenced. Each contains a single large open reading frame (ORF) encoding a polyprotein of approximately 4000 amino acids (aa), which is processed co- or post-translationally (Collett et al., 1988b). The 5'-terminal part of the ORF encodes one non-structural protein (Thiel et al., 1991) and four structural proteins, the nucleocapsid C and three glycoproteins, E2 (gp44-46), E3 (gp31) and E1 (gp51-54), which are associated with the envelope of the virus (Moormann et al., 1990a; Wensvoort 1989a; Stark et al., 1990).

The major antigenic protein of HCV is envelope glycoprotein E1 (Wensvoort et al., 1990). The immune response against E1 alone is sufficient for protection, because vaccination with a recombinant pseudorabies virus (PRV) which expresses the E1 gene of HCV strain Brescia protects swine against hog cholera (van Zijl et al., 1991).

Monoclonal antibodies (MAbs) against HCV have been isolated and are directed against E1 (Wensvoort et al., 1986) or envelope glycoprotein E2 (Wensvoort, 1989a; Weiland et al., 1992). Based on competitive binding studies and antigen capture assays, MAbs directed against E1 of HCV strain Brescia have been grouped in the four distinct antigenic domains A, B, C and D (Fig. 1a; Wensvoort, 1989b). Domain A consists of three subdomains (A1, A2 and A3) of which A1 and A2 are conserved among all tested HCV strains, but only MAbs directed against A1 are neutralizing. Subdomain A3, as well as domain D, is neither neutralizing nor conserved, but domain D differs from subdomain A3 in that it does not compete for binding with MAbs to domain A. Furthermore, domain D is conserved in strain Brescia and the Chinese vaccine strain C (Bognár & Mészáros, 1963), whereas subdomain A3 is not. Domains B and C are neutralizing but not conserved.

Epitope mapping of gp53, the E1 equivalent of BVDV, showed that a less conserved antigenic domain is located at the ultimate N terminus of the protein (Paton et al., 1991).
It was suggested that domains are defined by discontinuous sequences of amino acids. We also obtained indications for discontinuous or conformational epitopes on E1, because in a Pepscan analysis (Geysen et al, 1984) none of the nonapeptides reacted with E1-specific MAbs (R. H. Meloen, unpublished). These data suggested that for mapping of epitopes on E1 care should be taken to conserve the antigenic sites on the protein. Transient expression in COS1 cells has been used successfully in mapping of discontinuous epitopes on glycoprotein D of herpes simplex virus type 1. In that study, detection of epitopes on truncated proteins was done by Western blot analysis (Cohen et al., 1988). In an earlier report, we used in vivo eukaryotic expression of chimeric E1 proteins of strains Brescia and C to map non-conserved epitopes (van Rijn et al., 1992). Amino acid differences of non-conserved epitopes are located in the N-terminal half of E1, whereas domains B and C are located directly downstream of the signal peptide, and non-conserved subdomain A3 is located C-terminally of domains B and C (see Fig. 1b). In this report we used deletion mutants of E1 in order to map the boundaries of non-conserved as well as conserved epitopes on E1.

Methods

Construction of deletion mutants of E1. Plasmids pPRb2 and pPRc34 have been described previously and contain cDNA sequences encoding E1 genes of HCV strains Brescia and C (van Rijn et al., 1992). The E1 genes in plasmids pPRb2 and pPRc34 are located downstream and in frame with transcription and translation signals of expression vector pEVhisD12. Plasmid pEVhisD12 is one member of a set of vectors that contain promoter/enhancer sequences of the immediate early gene of human cytomegalovirus followed by a translation initiation codon in different reading frames (Peeters et al., 1992).

To investigate the importance of the N-terminal signal peptide in detection of transiently expressed E1, exactly eight codons (encoding aa 683 to 690) in the sequence encoding the proposed signal peptide of C strain E1, which includes the predicted cleavage site located between residues Gly-689 and Arg-690 (Moormann et al., 1990 a), were deleted by digesting pPRc34 with SpeI and Nhel before refugation. The resulting plasmid was designated pPRc37. The role of the transmembrane region (TMR) was tested by generating an expression plasmid containing E1 without a TMR. Therefore, the E1 gene of strain C was amplified (PCR in standard conditions: 94°C for 1 min, 55°C for 1 min, 72°C for 1 min) with primers A (5' AGATTTGGATCCTAAAGTATTAAGAGGACAGG 3') and B (5' AGATTTGGATCCGAATTCGGAAGTATCTGGAGTGG 3'). After BamHI digestion, the fragment was ligated in pEVhisD12 (pPRc35). In pPRc35, the region from aa positions 1031 to 1063 encoding the C-terminal hydrophobic region (proposed anchor or TMR) was deleted.

Because most MAbs are directed against E1 of strain Brescia, the E1 gene of this strain was used to map epitopes. To create a unique BgII site in the E1 gene of strain Brescia, the BgII site in the expression vector was destroyed. To this end the BgII site of pEVhisD11 was filled in, resulting in expression vector pPR1, in which cloning sites downstream of the destroyed BgII site are in the same frame as in pEVhisD12. Cloning of the E1 gene harbouring the EcoRI-SalI fragment of pPRb2 in pPR1 resulted in pPR9, which contains unique Nhel and BgII sites in the E1 gene.

Deletions in the C-terminal half of the E1 gene were introduced by replacing the BgII-SalI fragment of pPRb9 by the Apal-SalI fragment or the Apal-SalI fragment of pPRc34, resulting in a deletion of the

<table>
<thead>
<tr>
<th>Table 1. Primers used to amplify defined parts of the E1 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primer sequence</strong></td>
</tr>
<tr>
<td>P1 5' GG.GCC.GAA.GGG.CTA.GTC.ACC.ACC 3'</td>
</tr>
<tr>
<td>P2 5' AT.GGG.ACC.GTG.CTA.GCC.ATC.TGC 3'</td>
</tr>
<tr>
<td>P3 5' CC.TTT.AAA.GTG.CTA.GCA.CTT.AAT 3'</td>
</tr>
<tr>
<td>P4 5' CT.TTA.CCC.ACA.CTA.GTC.AGA.TTC 3'</td>
</tr>
<tr>
<td>P5 5' AT.ACG.AGC.CCA.CTA.GTC.AAG.GGA 3'</td>
</tr>
<tr>
<td>P6 5' TA.GGG.TGG.ACA.CTA.GTT.ATA.GAG 3'</td>
</tr>
</tbody>
</table>

*a* Nhel, SpeI and BgII sites are underlined. Mismatches with respect to the Brescia sequence are underlined twice. Reading frame and positions with respect to the nucleotide sequence of HCV strain Brescia are indicated.
enzymes were purchased commercially and used as described by the supplier. Transformation and maintenance of plasmids were in the bacterial strain DH5α (Hanahan, 1985).

All cloning procedures were carried out essentially according to Sambrook et al., (1989). Restriction enzymes and DNA modifying enzymes were purchased commercially and used as described by the suppliers. Transformation and maintenance of plasmids were in Escherichia coli strain DH5α (Hanahan, 1985).

Transfection and immunoperoxidase monolayer assay (IPMA). Transfection of COS1 cells (ATCC) was carried out essentially as described (van Rijn et al., 1992). Briefly, COS1 cells were grown in Earle's medium with 5% fetal bovine serum (FBS). Subconfluent monolayers in 2 cm² wells were washed three times with PBS (8% NaCl, 1-15% Na₂HPO₄, 0.2% KH₂PO₄ and 0.2% KCl). Cells were transfected by adding 0.5 ml medium (without FBS), which was followed by the transfection mixture. The transfection mixture consisted of 0.2 μg plasmid DNA in 50 μL of a 1:1 mixture of the DNA solution and a 1:12.5 dilution of lipofectin (Gibco BRL) in water. Plasmid DNA was isolated from overnight cultures and purified by phenol extraction. Medium above the monolayer was constantly gently mixed during addition of the transfection mixture. After overnight incubation at 37 °C, 0.5 ml of medium containing 20% FBS was added, and the incubation was continued for another 24 h. Subsequently, transfected monolayers were washed three times with PBS, dried for 45 min at 37 °C and frozen at -20 °C for 45 min. Frozen cells were fixed for 5 min with 4% paraformaldehyde (in PBS) and washed three times with 0.1% NaCl.

The IPMA to detect E1 was performed as described by Wensvoort et al. (1986). If a negative result was obtained, transfection was checked by a second immunostaining of the same monolayer with MAbs directed against other intact epitopes.

## Results

### Comparison of amino acid sequences of E1 of pestiviruses

In the previous study we demonstrated that non-conserved epitopes on E1 could be mapped by an IPMA of COS1 cells transfected with plasmids that contain hybrid E1 genes of HCV strains Brescia and C (van Rijn et al., 1992). However, hybrid E1 genes could not be used for mapping conserved epitopes and for the accurate mapping of the boundaries of the non-conserved epitopes. To this end we constructed deletion mutants of E1 of strain Brescia.

In order to predict the location of conserved epitopes, and thus the appropriate sites for deletion, we first compared the available pestivirus E1 sequences and the E1 sequence of strain C, of which the complete sequence will be published elsewhere (Fig. 3).

Remarkably, all 15 cysteine residues in E1 are conserved in pestiviruses, whereas the overall similarity of this protein between HCV and BVDV is among the lowest (about 60%) when all pestivirus gene products are compared (Moormann et al., 1990b; Weiland et al., 1990). The conservation of cysteines suggests that the global structure of E1 of HCV and gp53 of BVDV is similar and agrees with the presence of conformational or discontinuous epitopes on E1. By comparing the sequence of HCV and BVDV strains, five regions in E1 of HCV were identified that could contain HCV-specific epitopes: aa 739 to 760, aa 795 to 851, aa 905 to 941, aa 943 to 974 and aa 976 to 1016 (Fig. 3). The region between residues 795 and 851 encloses a hydrophobic sequence (residues 806 to 826) (Eisenberg et al., 1984; Rao & Argos, 1986), suggesting that this part is not very antigenic. The region between residues 979 and 1016 is conserved in both HCV and BVDV. However, MAbs directed against conserved epitopes on E1 of HCV strain Brescia do not recognize BVDV (Wensvoort et al., 1989).
**Table 2. Results of IPMAs of COS1 monolayers transfected with E1 expression plasmids**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Genotype</th>
<th>Conserved MAbs</th>
<th>MAbs specific to Brescia</th>
<th>MAbs specific to C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pPRb9</td>
<td>Brescia</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>pPRc34</td>
<td>C</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>pPRc37</td>
<td>Signal</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>pPRc35</td>
<td>-TMR</td>
<td></td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* Original E1 genes and regions that were deleted are indicated. Immunostaining of transfected monolayers was carried out with E1-specific MAbs raised against strains Brescia and C.

Fig. 3. Comparison of amino acid sequences of envelope glycoprotein E1 of pestiviruses. Dashed aa sequences are from nucleotide sequences of HCV strains Brescia (Moormann et al., 1990), Alfort (Meyers et al., 1989) and C (the complete nucleotide sequence of which was published elsewhere) and BVDV strains NADL (Collett et al., 1988), Osloss (Renard et al., 1987) and SD-1 (Deng & Brock, 1992). Differences and positions are shown with respect to the amino acid sequence of strain Brescia. Conserved regions in HCV strains are underlined and hydrophobic regions are underlined twice. Conserved cysteines in HCV and BVDV strains are indicated by asterisks.

Thus, this region is not a likely candidate for Brescia-specific epitopes of E1. The remaining three conserved regions in HCV contain several non-conservative aa differences in the sequences of BVDV strains, and thus conform to the requirements for harbouring conserved epitopes of HCV.

**Epitopes on E1 of HCV strain Brescia**

Plasmids pPRb9 and pPRc34 contain the E1 genes of HCV strains Brescia and C, and include the sequences encoding the proposed signal peptide and TMR. Cells transfected with these plasmids can be specifically immunostained by MAbs recognizing non-conserved epitopes (Table 2). To examine the effect of the N-terminal signal peptide and the TMR on immunological detection of E1 in transfected cells, we constructed plasmids pPRc37, a derivative of pPRc34 in which eight codons were deleted from the proposed signal peptide, and pPRc35, in which the proposed anchor or TMR is deleted (see Methods). COS1 cells transfected with plasmids pPRc35 and pPRc37 were not immunostained by MAbs, demonstrating the importance of signal peptide and TMR for detection of transiently expressed E1 (Table 2).

Since the presence of a signal peptide and a TMR of E1 are essential for detection of E1 in an IPMA, we deleted only internal sequences of E1 of HCV strain Brescia. Two mutants were constructed with deletions in the C-terminal half of E1; one deletion corresponds to aa 866 to 962 (pPEh10) and a larger deletion runs from positions 866 to 1007 (pPEh11). Expression of epitopes after transfection of the deletion mutants of E1 was established by immunostaining with 13 MAbs directed against E1 of strain Brescia and four conserved MAbs directed against E1 of strain C. COS1 cells transfected with pPEh10 and pPEh11 were immunostained by all MAbs, indicating that aa residues between 866 and 1007 are not essential for recognition of the conserved and non-conserved epitopes on E1 (Table 3). In addition, these results suggest that aa residues between 866 and 1007 are not involved in the conformation or accessibility of these epitopes. The N-terminal half of E1, between residues 690 and 866, appears to be the most antigenic part of the protein.

To map epitopes more precisely, we investigated the N-terminal part of E1 in detail. Internal deletions were constructed downstream of the proposed cleavage site of the signal peptide (aa position 690), which corresponds to an NheI site. Defined parts of the sequence of E1 were amplified with a set of primers containing NheI or SpeI sites (Table 1) and primer B (Fig. 2). Ligation of these amplified NheI/SpeI–BgII fragments to pPRb9, which had been digested with NheI and BgII, resulted in small deletions flanking the NheI site. The deletion plasmids were designated pPEh12 to pPEh17. The mutant E1 protein of plasmid pPEh12 containing the smallest deletion (residues 693 to 716) was recognized by MAbs to domains A and D, but not by those to domains B and
Table 3. Results of IPMAs of COS1 monolayers transfected with E1 expression plasmids harbouring one internal deletion

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Schematic representation of deletions in E1</th>
<th>Deletion*</th>
<th>A1/A2</th>
<th>A3</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Strain C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pPEh10</td>
<td></td>
<td>866 to 962</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>pPEh11</td>
<td></td>
<td>866 to 1007</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pPEh12</td>
<td></td>
<td>693 to 716</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pPEh15</td>
<td></td>
<td>692 to 766</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ND†</td>
</tr>
<tr>
<td>pPEh16</td>
<td></td>
<td>692 to 799</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pPEh17</td>
<td></td>
<td>692 to 824</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pPEh22</td>
<td></td>
<td>813 to 864</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pPEh18</td>
<td></td>
<td>800 to 864</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pPEh19</td>
<td></td>
<td>773 to 864</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pPEh20</td>
<td></td>
<td>740 to 864</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pPEh21</td>
<td></td>
<td>721 to 864</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Deleted amino acids are indicated with respect to the aa sequence of strain Brescia.
† Immunostaining of transfected monolayers was carried out with E1-specific MAbs raised against strains Brescia (MAbs bx) and C (MAbs cx).
† ND, Immunostaining of transfected monolayer not done.

C (Table 3). Obviously, essential aa residues of domains B and C are located between aa 693 and 716 and domains A and D are located C-terminally of domains B and C on E1. Binding of MAbs of domains A and D was not affected when the aa sequence between residues 693 and 766 was deleted (pPEh15). However, binding was completely abolished by deleting the flanking sequence up to 799 or 824 (pPEh16 and pPEh17). Essential aa residues of domains A and D appear to be located downstream of position 766. In conformity with the IPMA results using mutants pPEh10 and pPEh11 which indicated that epitopes are located upstream of aa position 866, we can conclude that essential aa residues of domains A and D are located between positions 766 and 866.

In a similar way, plasmids containing E1 genes with deletions adjacent to and upstream of the BgII site (corresponding to aa position 864) were constructed, resulting in plasmids pPEh18 to pPEh22 (Table 3). Binding of MAbs to subdomains A1 and A2 was abolished by the smallest deletion tested (from positions 813 to 864, pPEh22). Other MAbs (domains B, C, D and subdomain A3) still immunostained cells transfected with pPEh22, indicating that mutant E1 encoded by this plasmid is translated properly in detectable amounts. Apparently essential aa residues for subdomains A1 and A2 are located in the sequence of aa 813 to 864, whereas epitopes in subdomain A3 and domains B, C and D are still intact and located N-terminally of position 813. Deletion of 13 extra codons, between aa positions 800 and 813 from pPEh22, abolished binding of MAb b12 (subdomain A3), indicating the location of essential aa residues for subdomain A3 (pPEh18). MAb b6 representing domain B and MAb b5, member of domain C, still recognized E1 with a deletion from positions 773 to 864 (pPEh19). Thus, epitopes of MAb b5 and MAb b6 are located between aa 690 and 773. Furthermore the IPMA results with pPEh18 and pPEh19 indicated that deletion of aa 773 to 800 abolishes binding of MAbs b1, b8 (domain C) and b13 (domain D). Further extension of the deletion towards the N terminus (from aa 773 to 740 or 721, compare pPEh19 with pPEh20 or pPEh21, respectively) resulted in negative immunostaining by all MAbs that recognize domains B and C.

Comparison of the results of single deletions in Table 3 suggests that aa sequences at both sides of domains A and D can be deleted without affecting these epitopes. To investigate this we combined several single deletions resulting in plasmids pPEh23 to 26, and examined the binding of MAbs. The mutant E1 proteins of pPEh23 and pPEh24 should contain all the essential aa residues of domain D (pPEh15 and pPEh18), and the mutant E1 of pPEh25 should contain all essential aa residues of both subdomain A3 and domain D (pPEh15 and pPEh22). However, transiently expressed mutant E1s were not detected in cells transfected with pPEh23 to 25 (Table 4). This suggests that the conformation or accessibility of epitopes of MAbs b12 and b13 is disturbed in deletion mutants pPEh23 to 25. To analyse domains A and D, the deletions of pPEh11 and pPEh14 were combined (pPEh26). Based on pPEh11 and pPEh15, pPEh26 should contain all epitopes of domains A and D. Immunostaining by MAbs b3, b4 and c8 indicated expression of mutant E1 of pPEh26. However, other MAbs of domains A and D and MAbs c1, c4 and c11 did not immunostain cells transfected with pPEh26.
Table 4. Results of IPMAs of COS1 monolayers transfected with E1 expression plasmids harbouring two internal deletions

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Schematic representation of deletions in E1</th>
<th>Deletion*</th>
<th>Domain and MAb†</th>
</tr>
</thead>
<tbody>
<tr>
<td>pPEh23</td>
<td></td>
<td>692 to 766+</td>
<td>A1 b2 b3 b4 b7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>800 to 864</td>
<td>A2 a11</td>
</tr>
<tr>
<td>pPEh24</td>
<td></td>
<td>693 to 746+</td>
<td>A3 b12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>800 to 864</td>
<td>D b13</td>
</tr>
<tr>
<td>pPEh25</td>
<td></td>
<td>692 to 766+</td>
<td>C strain cl c4 c8 c11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>813 to 864</td>
<td></td>
</tr>
<tr>
<td>pPEh26</td>
<td></td>
<td>693 to 746+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>866 to 1007</td>
<td></td>
</tr>
</tbody>
</table>

* Deleted aa residues are indicated with respect to the aa sequence of strain Brescia.
† Immunostaining of transfected monolayers was carried out with E1-specific MAbs raised against strains Brescia (MAbs bx) and C (MAbs cx).
‡ ND, Immunostaining of transfected monolayer not done.

(Table 4). Mutant E1 of pPEh26 is obviously not recognized by any of the MAbs to domains A and D.

Epitopes of MAbs raised against strain C on E1 of strain Brescia

Six MAbs raised against E1 of strain C have been isolated (Wensvoort, 1989a). Two of these MAbs (c2 and c6) are specifically directed against E1 of strain C, whereas four MAbs (c1, c4, c8 and c11) recognize conserved epitopes in all 41 HCV strains tested. The epitopes on E1 of MAbs c2 and c6 were mapped to the ultimate N-terminal 105 aa residues and are therefore characterized as ‘domain B/C-like’ (van Rijn et al., 1992). We employed our set of deletion E1 plasmids to map conserved epitopes that were yet to be assigned to a specific domain. It was demonstrated that the MAbs c1, c4, c8 and c11 can be allocated to the conserved subdomains A1 or A2 (Table 3). Since these four C-strain MAbs are neutralizing (Wensvoort, 1989a), we conclude that they belong to the conserved and neutralizing subdomain A1. This is in agreement with findings of Wensvoort (1989a) who showed that MAbs c1, c4, c8 and c11 firstly compete with MAbs of domain A, secondly bind to all HCV strains tested and, finally, do not recognize BVDV and BDV strains.

Discussion

In an earlier report, we showed that detection of transiently expressed E1 in COS1 cells by IPMA occurs with high specificity and is indistinguishable from detection of virus-encoded E1 expressed in SK6 cells (van Rijn et al., 1992). Here we used this assay in order to map epitopes on deletion mutants of E1. It appeared that for detection of transiently expressed E1 in COS1 cells constructs had to contain a proper signal peptide and TMR (Table 2). Instability of incorrectly processed E1 caused by lack of the signal peptide, or secretion of E1 caused by the absence of the TMR, will reduce the amount of E1 in transfected cells and is probably the cause of negative IPMA results.

Binding of MAbs to mutant E1s with internal in-frame deletions in the C-terminal half of E1 showed that all epitopes are located in the N-terminal half of E1. By using escape variants of BVDV strains NADL and Oregon C24V, it has been demonstrated that aa substitutions are located in the N-terminal half of gp53, the homologue of HCV E1, suggesting that the antigenic sites of gp53 are also located in the N-terminal half of the protein (Paton et al., 1992). Further evidence supporting the antigenicity of the N-terminal half of E1 in particular was obtained by immunostaining transfected monolayers with sera of swine vaccinated with PRVE1 recombinant M205 (van Zijl et al., 1991). This monospecific polyvalent serum against E1 was used to check our expression of E1 mutants with large in-frame deletions of the N-terminal part, which stained negatively with all of the MAbs. However, only cells transfected with plasmids encoding the N-terminal part of E1 were stained by this pig serum, indicating that most antibodies in the serum are directed against the N-terminal half of E1 (data not shown).

Our studies with deletions in the N-terminal half of E1 resulted in mapping of the four antigenic domains A, B, C and D (Fig. 4). Domain A is located between aa positions 766 and 866. The region between aa 795 and 851 is highly conserved in HCV strains (Fig. 3). This region is a likely candidate for highly conserved subdomains A1 and A2, although part of this aa sequence, between residues 806 and 826, is hydrophobic and therefore probably not very antigenic. Non-conserved subdomain A3 was mapped between aa residues 766 and 813. Within this sequence, variation between strain Brescia and strains Alfort and C is seen at nine aa positions. Since MAb b12 does not bind to...
strains Alfort and C, we suppose that the epitope of this MAb comprises one or more of these variable aa residues. In a similar way, one or more variable aa residues within the map of non-conserved epitopes of domains B and C must be involved in the specific binding of these MAbs. Apparently, epitopes in domain C can be divided into two classes, one consisting of MAb b5 and one consisting of MAbs b1 and b8 (Table 3), which confirms that MAb b5 differs from MAbs b1 and b8 (Wensvoort et al., 1989). Finally, domain D is located between residues 766 and 800. MAb b13 of domain D recognizes strains Brescia, Alfort and C (Wensvoort et al., 1989). Indeed, a part of the region in which domain D was mapped is conserved in these HCV strains.

The IPMA results of double deletion mutants were not in agreement with the results predicted from our studies with the single deletion mutants. For instance, mutant E1 of pPeH26 was expected to contain all essential aa residues of epitopes in domains A and D and epitopes of conserved MAbs directed to E1 of strain C. However, only MAbs b3, b4 and c8 stain COS1 cells transfected with this plasmid (Table 4). This suggests that the presence of all essential aa residues of epitopes/domains on truncated E1 of pPeH26 does not necessarily result in detection of COS1 cells expressing this double mutant of E1. The conformation or accessibility of epitopes is probably changed by the combination of deletions flanking a particular epitope. However, one has to be careful in drawing conclusions about the absence of epitopes in cases where mutant E1s are not detected by any of the MAbs because in such instances the possibility of instability of mutant E1 cannot be excluded. On the other hand, from positive IPMA results it can be concluded that all essential aa residues of the investigated epitopes are present in the studied mutant of E1.

Until now, the function of the cysteines in E1 was unclear. Surprisingly, the deletion mutant of pPeH11, in which nine of 15 codons for cysteines are deleted, was recognized by all MAbs (Table 3). Thus, the nine cysteines in the C-terminal half of E1 are not essential for the conformation and accessibility of epitopes on the N-terminal half of E1. It has been shown that E1 forms homodimers as well as heterodimers with E3 (gp31) and that cysteines are involved in this dimerization process (Wensvoort et al., 1990; Thiel et al., 1991). Here, we showed that the N-terminally located cysteines are probably involved in the antigenic structure of E1. It thus seems likely that one or more of the nine C-terminal cysteines of E1 are involved in the intermolecular interaction with E1 or E3. Whatever the function of these conserved cysteines may be, it can be concluded that none of them is essential for a correct conformation of epitopes on the N-terminal half of E1.

Six cysteines are located in the antigenic N-terminal half of E1, in which all epitopes were mapped. Deletion of a relatively small region, containing one cysteine (Cys-693), in the vicinity of the cleavage site of the signal peptide resulted in negative IPMA results with all MAbs of antigenic domains B and C (Table 3, pPEh12). This suggests involvement of Cys-693 in the conformation of two antigenic domains. IPMA results of plasmids pPEh15 and pPEh16 showed that deletion of 33 amino acids (containing Cys-792) results in negative immunostaining with all MAbs of domains A and D and with the conserved MAbs directed against strain C (Table 3). This indicates an essential role of Cys-792 for antigenic domains A and D, probably via formation of a disulphide bond. Furthermore, domain A can be deleted without affecting immunostaining with MAbs of domains B and C (pPEh18), and domains B and C can be deleted without affecting detection of domain A (pPEh12 to 15). The part of E1 on which antigenic domains B and C are located and which contains two cysteines seems to be formed independently of domain A. Conversely, the part containing domain A, which contains four cysteines, is formed independently of domains B and C. It thus seems that the six conserved cysteines in the N-terminal half of E1 can be assigned to two separate structures on E1. In neutralization tests, only a combination of MAbs of subdomain A1 and of domains B or C have shown a synergistic effect, whereas two MAbs of subdomain A1 or two MAbs of domains B and C showed no enhancement of neutralization (Fig. 1; Wensvoort, 1989b). This pattern of synergism of neutralizing sites correlates perfectly with the two separate structures on E1 and suggests that these units are not only formed but also function independently. It is likely that both units are involved in infection, in binding to a receptor of the cell, as both units harbour epitopes of neutralizing MAbs. Evidence for such a receptor has been obtained for gp53 of BVDV (Xue & Minocha, 1993).
From the results presented here, several conclusions can be drawn. Firstly, IPMA in combination with transient expression in COS1 cells is an excellent approach for mapping epitopes on envelope glycoprotein E1 of HCV in vivo. Secondly, the most antigenic part of E1 is the N-terminal half of the protein. The C-terminal half of E1 located upstream of the TMR is not involved in conformation and accessibility of epitopes in the N-terminal half of transiently expressed E1. Thirdly, the following antigenic domains were mapped: domain A is located between aa residues 766 and 866, with the non-conserved subdomain A3 located between residues 766 and 813; non-conserved domain B is located between residues 691 and 773; non-conserved domain C is between residues 691 and 800, with one epitope of domain C (MAb b5) located between aa 691 and 773; domain D is mapped between residues 766 and 800.

References


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