The cloning, sequencing and expression of a major antigenic region from the feline calicivirus capsid protein

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RNA purified from the feline calicivirus (FCV) F9 vaccine strain was used to prepare a cDNA library in the expression vector λgt11. The library was screened for expression of FCV antigen using a rabbit antiserum prepared against purified FCV. A 330 bp cDNA clone was identified and used as a probe to obtain a larger overlapping clone of 1369 bp. Comparative sequence analysis with the CFI and F4 strains showed that the clones were derived from the 3' open reading frame encoding the capsid protein. The region encoded by the 330 bp clone was shown to be variable in the three strains compared, and therefore the probable location of major antigenic variation. This clone was expressed in a bacterial system and antiserum to the recombinant protein was used in immunoblots to confirm that this clone was derived from the gene encoding the capsid protein. From these immunoblots, several other capsid-related polypeptides were identified. Comparison with immunoblots using post-vaccination cat sera showed the antibody response in the cat was directed mainly against the capsid protein. Antiserum to the recombinant protein was shown to be effective in neutralizing the infectivity of FCV, indicating that at least one major neutralizing epitope had been cloned.

The Caliciviridae are a group of non-enveloped viruses with cup-shaped morphology, possessing a positive-sense ssRNA genome of about 8 kb. Characterized members of this group include feline calicivirus (FCV), San Miguel sealion virus and vesicular exanthema of swine virus (VESV). Relatively few studies on the molecular biology of this group have been carried out. FCV has been the most studied virus of this group, although recently the causative agent of rabbit haemorrhagic disease has been identified as a calicivirus and the complete sequence has been reported (Neill et al., 1991). ORFs of 2004 bp have been identified for the CFI and F4 strains, encoding proteins of 73467 and 73588, respectively. An ORF of 2012 nucleotides for the F9 strain, encoding a protein of 73441, has also been identified. These ORFs have been shown to encode the mature capsid protein of 62K to 65K for the CFI and F9 strains (Neill et al., 1991; Tohya et al., 1991b; Carter et al., 1992). The demonstration of the negative-sense strand for some of these subgenomic RNAs would indicate that caliciviruses probably transcribe and translate these RNAs for gene expression (Neill & Mengeling, 1988; Carter, 1990). Sequence analysis of the FCV genome by Neill (1990) identified an open reading frame (ORF) in the CFI strain which encodes an amino acid sequence with similarity to three picornavirus non-structural proteins, the 3D RNA-dependent RNA polymerase, the 3C cysteine protease and the 2C polypeptide. Termination of this ORF occurs approximately 2400 bp from the 3' end of the genomic RNA. The nucleotide sequence of the 3' end has recently been determined for the CFI, F4 and F9 strains of FCV (Neill et al., 1991; Tohya et al., 1991b; Carter et al., 1992). ORFs of 2004 bp have been identified for the CFI and F4 strains, encoding proteins of 73467 and 73588, respectively. An ORF of 2012 nucleotides for the F9 strain, encoding a protein of 73441, has also been identified. These ORFs have been shown to encode the mature capsid protein of 62K to 65K for the CFI and F9 strains (Neill et al., 1991; Carter et al., 1992), and antiserum to the protein expressed by the F9 strain has been shown to react with both the mature capsid protein and its precursor of 76K (Carter et al., 1992).

The FCVs are recognized as consisting of a single serotype, although demonstrable antigenic variation between strains has been shown by using polyclonal antisera (Povey, 1974). Similarly, antigenic variation has been shown between strains using a panel of neutralizing monoclonal antibodies to the F4 strain (Tohya et al., 1991a), and of the seven neutralizing epitopes identified four have been shown to exist on the capsid protein. This implies considerable variation of the capsid protein
between strains, and may explain the observation that vaccination with the F9 strain protects against only 54% of field isolates (Knowles et al., 1990).

The determination of the nucleotide sequence of the capsid protein is important in studying the antigenic structure and variability between strains of FCV. In this study, a cDNA expression library was constructed from FCV genomic RNA, from which we have isolated two clones encoding the major part of the capsid protein. The FCV F9 strain (Bittle et al., 1960) was used to infect the feline embryonic lung cell line (Flow). Virus was purified from infected cells as described by Neill & Mengeling (1988) and the purity of the virus was checked by electron microscopy. RNA from purified FCV was obtained from infected cells as described by Neill & Mengeling (1988) and the purity of the virus was checked by electron microscopy. RNA from purified FCV was obtained using the guanidinium thiocyanate extraction protocol (Maniatis et al., 1982) and used to construct a cDNA library using the Invitrogen library construction system (Advanced Protein Products). A modification of the procedure of Gubler & Hoffman (1983) was used for cDNA synthesis and the cDNA was subsequently cloned into the expression vector pBluescript SK+ (Stratagene). Single-stranded templates were prepared and both strands were sequenced using a microtitre plate sequencing system (Amersham). Each nucleotide was sequenced a minimum of four times.

A clone of 1369 bp was sequenced and a single continuous ORF was identified. Comparison with available sequence data for FCV (Neill et al., 1991; Tohya et al., 1991 b; Carter et al., 1992) showed that this clone was derived from the gene encoding the capsid protein. Its location in the proposed genomic structure of FCV (Neill et al., 1991) is shown in Fig. 1. The deduced amino acid sequence was compared with the correspond-
region 2 where the identity is reduced to 76.4%. The remaining sequence, from amino acids 307 to 455 (region 3) shows 94.6% identity. Similar comparisons of the F9 and CFI strains show 97.1%, 78.7%, and 92.6% identity in regions 1, 2, and 3 respectively. Comparison of the F4 and CFI strains reveals even greater variation, with amino acid identities of 96.6%, 71.1%, and 93.2% for regions 1, 2, and 3 respectively. A similar comparison between the CFI and the F9 strains for the capsid precursor proteins reveals the same pattern of variation as described with an additional region showing a smaller degree of variation (13%) towards the N terminus; however, this region is cleaved during maturation (Carter et al., 1992). Antigenic variation between FCV strains occurs primarily in the capsid protein (Tohya et al., 1991a), and therefore region 2 is likely to be the source of major antigenic variation between the three strains.

As a result of the analysis of amino acid conservation between the three strains of FCV, a second cDNA clone of 330 bp in length representing most of variable region 2 (amino acids 192 to 301) was expressed in *E. coli* using the high level plasmid expression vector pET3cp* (Rosenberg et al., 1987; Mackett et al., 1990). Expression of the cloned fragment is under control of the φ10 promoter from bacteriophage T7, and gives rise to a fusion protein containing the first 12 amino acids encoded by T7 gene 10. The resulting plasmid, pET3 FCV1, was introduced into *E. coli* BL21, which has a lysogenic copy of the T7 RNA polymerase gene under the control of a lacZ promoter (Rosenberg et al., 1987). Fig. 3 shows the expression of the FCV clone in *E. coli* as analysed by SDS–PAGE (Laemmli, 1970) before and after induction of expression by the addition of IPTG, and shows high level expression of a polypeptide of approximately 15K. Immunoblots with the rabbit antiserum PFCV gave a strong reaction with the expressed 15K recombinant polypeptide in the induced *E. coli* lysate, indicating that the cloned fragment was of FCV origin. Preparative SDS–PAGE was used to purify the recombinant polypeptide (Leppard et al., 1983) before its use for the production of hyperimmune rabbit antiserum. This antiserum was shown to react with the 65K capsid protein by immunoblotting with purified FCV, therefore confirming that the expressed clone was derived from the gene encoding the capsid protein (Fig. 4.). The smaller polypeptides detected are therefore capsid-related, and

Fig. 3. Expression of the FCV cDNA clone in *E. coli*. Protein extracts from *E. coli* induced to express recombinant protein from plasmid pET3 FCV1 were analyzed by SDS–PAGE. (a) Coomassie blue-stained polyacrylamide gel. Lanes 1 and 2 show uninduced *E. coli* extract; lanes 3 and 4, *E. coli* extracts after a 5 h induction period. (b) Western blot of (a) probed with rabbit antiserum PFCV. The expressed protein band is indicated by a dash. Lane *M*, Mr markers, sizes of which are indicated to the left.

Fig. 4. Western blot analysis of purified FCV probed with (a) rabbit antiserum PFCV, (b) rabbit antiserum to purified recombinant capsid protein expressed from pET3 FCV1 and (c) post-vaccination cat serum. The sera were tested at dilutions, 10<sup>-4</sup> and 10<sup>-5</sup> (lanes 2 and 3, respectively). Pre-immune rabbit serum was tested in lanes 1 in (a) and (b), and pre-vaccination cat serum was tested in lane 1 in (c), both at a dilution of 10<sup>-3</sup>. Positions of Mr, markers, sizes of which are indicated to the left.
may represent additional processing and/or degradation products of the capsid protein. Immunoblots of the rabbit antiserum PFCV with purified FCV showed a similar pattern, indicating that the major immune response was directed against the capsid protein. No reaction was seen with the rabbit antiserum in immunoblots with mock-infected cell lysates (results not shown). Similarly, serum from a cat vaccinated with FCV strain F9 (Intervet) showed an immunoblotting pattern similar to that of the recombinant antiserum with purified FCV, indicating that the immune response following vaccination is directed primarily against the capsid protein. The post-vaccination cat serum also reacted strongly with the purified recombinant protein as judged by immunoblotting (Fig. 5), indicating that a highly antigenic region of FCV had been cloned and expressed.

Regions of variability in closely related picornaviruses have been shown to be concentrated in areas of the genome encoding the surface residues of the capsid protein, which are involved in determining the antigenic properties of the virus (Rossmann et al., 1985). Regions of sequence variation between serotypes of picornaviruses have also been shown to correspond to neutralizing epitopes located in variable region 2. Therefore, antigenic variation of important neutralizing epitopes may result in reduced protection following vaccination with one strain only, as has been demonstrated in neutralization studies with field isolates of FCV (Knowles et al., 1990). Further knowledge of the antigenic variation between strains will be important for the future development of more effective vaccines.

We would like to thank Dr Rosalind Gaskell, University of Liverpool, for supplying the FCV strain, and Dr Alan Curry for assistance with the electron microscopy work. We also acknowledge the assistance of Dr J. R. Arrand and the staff in the Department of Molecular Biology at the Paterson Institute for Cancer Research. This work was funded by the North Western Regional Health Authority clinical research project grant number 642/8118.

References


(Received 19 July 1991; Accepted 28 April 1992)