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

Malcolm Guiver,1,2* Edward Littler,1† E. Owen Caul3 and Andrew J. Fox2

1 The Paterson Institute for Cancer Research, The Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester M20 9BX, 2 Manchester Public Health Laboratory, Withington Hospital, Nell Lane, Manchester M20 8LR and 3 Bristol Public Health Laboratory, Myrtle Road, Kingsdown, Bristol BS2 8EL, U.K.

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 (Meyers et al., 1991). Unlike the picornaviruses with which they were once grouped, caliciviruses possess a single capsid protein of 60K to 70K. Subgenomic RNAs are produced during replication and up to eight have been described for FCV (Carter, 1990). 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

1 Present address: Department of Molecular Sciences, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.
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 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 pgtl 1. Aliquots of the library were plated onto a lawn of Escherichia coli Y1090 and expression was induced by placing a nitrocellulose filter previously soaked in 10 mM IPTG on to the agar surface. Plaque lifts were probed with hyperimmune rabbit antiserum to purified FCV virions (antiserum PFCV) and selected clones were subcloned into the phagemid 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-

![Diagram](image-url)  
**Fig. 1.** Location of the sequenced 1369 bp cDNA clone in the proposed genome structure of the FCV (Neill et al., 1991). Hatched area indicates the region expressed in pET3 FCV1. The three picornavirus-like non-structural proteins are also shown.

... Poly(A) 
330 bp  
1369 bp

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 comparison of the nucleotide sequence deduced from the cDNA encoding part of the FCV F9 strain capsid protein with that of the F4 strain (Tohya et al., 1991 b) and the CFI strain (Neill et al., 1991) is shown in Fig. 2. The F9 strain shows 89-8% and 90-3% identity with the F4 and CFI strains respectively, and the F4 strain (Tohya et al., 1991) shows 88-3% identity with the CFI strain over the whole length of the predicted amino acid sequence. Variation between the three strains is predominantly in a region between amino acids 176 and 306 (region 2). Comparison of the sequence between amino acids 1 and 175 of the F9 and F4 strains, up to the start of the variable region, reveals 96% identity, compared to...
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, $10^{-4}$ and $10^{-5}$ (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 $10^{-3}$. Positions of Mr markers are indicated to the left.

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 (Laemmlli, 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
neutralization of the virus by rabbit antiserum to the recombinant capsid protein would indicate the presence of at least one neutralizing epitope within the expressed clone representing variable region 2. However, during purification of the expressed recombinant capsid protein, the denaturing conditions may preclude the identification of conformational neutralizing epitopes that may also be located in this region. Therefore, protection in the cat following vaccination is predominantly in response to neutralizing epitopes located in the capsid protein, at least one of which is 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.

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