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Potential for broad-spectrum protection against feline calicivirus using an attenuated myxoma virus expressing a chimeric FCV capsid protein

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Abstract

It has previously been demonstrated that recombinant myxoma viruses expressing FCV capsid protein are capable of eliciting protective responses against virulent FCV challenge, following vaccination, in cats. An attempt was made to produce a bivalent myxoma recombinant expressing the capsid protein genes of both FCV strains F9 and LS015. The FCV capsid protein genes were inserted into the myxoma growth factor gene (MGF) locus, and the serine protease inhibitor (SERP 2) gene locus. Subsequent recombination between myxoma-FCV viruses resulted in a recombinant expressing a chimeric form of the capsid protein. Nonetheless, cats immunised with this myxoma-FCV recombinant demonstrate high levels of serum neutralising antibodies against both F9 and LS015 strains. Such a chimeric vaccine may provide effective protection against a wide range of FCV strains.

Keywords: Vaccination; Myxoma virus; Feline calicivirus

1. Introduction

Feline calicivirus (FCV) is associated with acute oral and upper respiratory tract disease in the cat [1,2]. FCV infection may also lead to an acute febrile lameness syndrome [3]. Neurological disorders have also, albeit rarely, linked to infection with FCV [4]. Additionally, there have been outbreaks reported in which FCV has caused a severe febrile haemorrhagic disease in cats [5,6] a condition now termed virulent systemic disease [7]. Vaccines are available, which may be either based on live attenuated or inactivated preparations, however, there are concerns that antigenic variation is leading to the emergence of new field strains for which vaccines do not offer protection [5,8,9]. Pursuing a bivalent approach may offer a vaccine with the potential to provide cross-protection against a broader range of field strains. Studies have shown that vaccination with two strains of FCV can provide bivalent immune serum with high levels of cross-neutralisation against diverse field isolates [10].

We have previously demonstrated the potential of myxoma virus as a vector for FCV vaccination [11]. The same vector system could be used to express the capsid protein gene of two different strains of FCV to provide a bivalent vaccine. We chose to express the capsid proteins of the strains F9, the most commonly used vaccine strain, and LS015, which is a strain shown previously to have a different neutralisation profile [10].

In addition to examining the protective properties of such a bivalent recombinant, following virulent FCV challenge, the generation of such a recombinant virus allows us to explore further the myxoma vector system and possibly to extend its use. The insertion sites chosen for the capsid protein genes were in the virulence factor gene loci, MGF and SERP 2. The functions of both these myxoma virus gene products have been well characterised [12–15]. Both genes are non-essential for the growth of myxoma virus in vitro and when deleted produce viruses with significantly attenuated virulence in vivo [12,14]. Hitherto there have been no reports of myxoma viruses deleted for both MGF and

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SERP-2. Although it would be possible to include both F9 and LSO15 capsid protein genes at a single locus the use of both loci simultaneously enables assessment of the viability of a virus containing both deletions. A recombinant vector providing multiple insertion sites would be of benefit for the generation of vaccines containing multiple antigens, or additional, immunostimulatory molecules.

Replication and recombination are strongly linked in poxviruses and it has been proposed that homologous recombination may be involved in the resolution of replicative intermediates of the viral genome [16]. The insertion of two FCV capsid protein genes in a single recombinant myxoma virus is potentially problematic due to the high degree of nucleotide similarity. The level of homology between LSO15 and F9 is 81%, however, long stretches of identity within the processed capsid protein gene are infrequent. The longest stretch of identical sequence is 41 bp from position 1936–1976, in the complete capsid protein gene. Other such stretches of sequence are less than 18 bp in length. So, although the possibility of uncontrolled homologous recombination could not be dismissed it was considered that the incidence of such recombination may not be unacceptably high.

This paper confirms that the propensity of myxoma virus to undergo homologous recombination should be taken into account in the construction of recombinants expressing related antigens. Nevertheless in this instance the resultant virus has the potential to form a broad-spectrum vaccine against FCV.

2. Materials and methods

2.1. Cells and virus stocks

Crandell-Rees feline kidney (CRFK) and rabbit kidney (RK-13) cells were maintained in M6B8 medium containing 5% fetal calf serum (Gibco BRL, Paisley, UK), 2 mM L-glutamine, penicillin (100 IU/ml), and streptomycin (100 μg/ml). Myxoma virus strain MR24 was isolated from an infected rabbit and attenuated by continued passage in RK-13 cells (Baxendale et al. unpublished data). Feline calicivirus strain LS015 was provided by Prof. R.M. Gaskell University of Liverpool. The F9 strain of FCV was taken from the seed stock of commercially available FCV vaccine TriCat (Intervet). All myxoma viruses were propagated in RK-13 cells. FCVs were propagated in CRFK cells.

2.2. Construction of plasmids

Previously (McCabe et al., 2002), the MGF locus had been chosen as a site of insertion of foreign material, in this study the SERP2 locus was chosen as a second for insertion of target genes. The flanking sequences necessary for construction of the recombinant viruses were generated by PCR using the following primers NS31 5′ GC-CAATTGCATGCGATTATGGAGTTTTCAAGC 3′ and NS32 5′ GCGGTACCGCGGTCACGTACCGCCTGCATAAAC 3′ amplify the upstream flanking region. The primers NS33 5′ GGCTGCAGCTAGCCACGTTGCGATACGTACATCTC 3′ and NS34 5′ GCAAGCTTTAATACGACGGGATGCATCGTACC 3′ amplify the downstream flanking region. The SERP flanking sequences were inserted into pUC18 to produce plasmid pSERP AB

The recombinant plasmid p22-GFP was constructed, using standard procedures, in pUC 18 using the MGF flanking sequences. The early/late promoter sequence has been described previously [17]. The 240 bp repeated sequence used to flank the GFP is derived from a non-coding segment in rabbit haemorrhagic disease virus, which was present in a plasmid used in an other project. The purpose of this sequence is simply to provide a region of homology for the exclusion of the GFP gene.

Plasmid p22-GFP was used as the standard vector into which the target genes were inserted.

The processed capsid gene of FCV LS015 was amplified from a sub-genomic cDNA clone, (pI.18 LSO15 full), using primers NS23 5′ GGATCGATGCGCTGATGATGGATCCAT 3′ and NS24 5′ GGGGACTAGTATTCATAATTTTGTCATTAC 5′ and cloned into the pE/Lpoly vector [McCabe et al] as a *Cla*I/*Spe*I fragment (sites underlined). The capsid sequence was then subcloned as an *Sst*II/*Spe*I fragment from the pE/Lpoly background into p22GFP. Finally, the expression cassette, *Sst*II/*Nhe*I, from the p22GFP LS015 plasmid was inserted into the pSERP AB vector.

2.3. Generation of the recombinant myxoma virus

The recombinant poxvirus was constructed using standard protocols. The recombinant myxoma virus MS0013 [11], containing the F9 FCV processed capsid protein gene at the MGF gene locus was used as the starting strain. The plasmid pSERP AB LS015 linearised with the restriction enzyme *Sap*I was used to provide the second feline calicivirus gene. Initial selection of the potential F9/LS015 capsid expressing double recombinant virus was achieved by monitoring for GFP expression. Subsequently, duplicates of GFP positive foci were screened for FCV F9 capsid expression using the F9-specific monoclonal antibody IG9 (Novacastra). Briefly, a series of 96-well tissue culture plates seeded with RK-13 cells were infected with virus from the infection/transfection at a range of dilutions. After 2–3 days, GFP positive foci were picked, and these stocks of virus freeze/thawed three times. A sample of each stock was used to infect fresh RK-13 cells, which after a further 2–3 days were fixed with ice-cold methanol and stained first with the IG9 monoclonal and then with an anti-mouse IgG FITC labelled second antibody. Viral stocks positive for FCV F9 by immunofluorescence were then subjected to further rounds of purification, until the stocks were 100% recombinant. Then single GFP negative foci were selected to provide the final recombinant virus. This final recombinant was subjected to PCR with primers NS31 and