Monoclonal Antibodies to Feline Calicivirus

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SUMMARY

We have prepared monoclonal antibodies to the capsid protein of feline calicivirus (FCV). These antibodies are directed against two close but distinct epitopes, only one of which is involved in virus neutralization. We have used these antibodies and immune cat serum in immunoprecipitation and Western blotting experiments and have identified novel proteins in FCV-infected cells which we term P78, P41, P35 and P29. The number and sizes of FCV proteins now known resemble those made by other caliciviruses.

The caliciviruses are a group whose members show the same distinctive morphology (Schaffer et al., 1980). The non-enveloped virus particles are roughly spherical and have a characteristic surface pattern of 32 cup-shaped depressions from which the virus group derives its name. The capsid is constructed from one major polypeptide which varies in size between members but lies in the Mr range 60K to 70K (reviewed by Schaffer, 1979).

The replication strategy of the caliciviruses is not yet fully elucidated, but it is clear that the positive-stranded genome gives rise to a smaller RNA intracellularly (Black et al., 1978). Cells infected with San Miguel sea lion virus (SMSV) or vesicular exanthema of swine virus (VESV) contain several virus-induced polypeptides, and there is general agreement that, in the absence of any special measures to inhibit proteolytic processing, up to six proteins can be distinguished. Apart from the capsid protein, two species of higher Mr (80K and 100K to 135K) termed P80 and P135 are synthesized. Neither of these appears to be a direct precursor of the capsid protein (Black & Brown, 1978; Fretz & Schaffer, 1978). In addition three lower Mr proteins (40K, 35K and 29K) termed P40, P35 and P29 have been identified (Fretz & Schaffer, 1978).

The type virus for the calicivirus group is VESV, but work with this strain is tightly controlled due to its economic importance as a pathogen of livestock. Feline calicivirus (FCV) however is widely available and used in the vaccination of domestic cats. The vaccine strain F9 of this virus was kindly provided by Professor O. Jarrett (Veterinary School, University of Glasgow, U.K.), and can be readily propagated in the feline kidney cell line CRFK (Flow Laboratories).

Of the proteins described above, only P68 (the capsid protein), P80 and P40 have previously been reported in FCV-infected cells. However it has been inferred from immunoprecipitation experiments that FCV may specify two proteins of 80K although these were not resolved. An additional polypeptide of 15K was observed (Komolaffe et al., 1980). We have confirmed the presence of two protein bands at approximately 80K as well as the capsid protein and P40 using serum from immunized cats in immunoprecipitation experiments (Fig. 1). In addition we routinely observe two proteins of Mr 35K and 29K (Fig. 1). At late times after infection we have identified a 40K protein which is related to the capsid protein. In our experiments the capsid protein appears slightly smaller (62K) than in previous reports. These observations bring the number and sizes of the known FCV polypeptides into close agreement with those of other caliciviruses, but we did not find an equivalent of the 100K protein.

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Fig. 1. Immunoprecipitation of infected cell proteins. Infected and mock-infected cells were labelled with [35S]methionine (20μCi/ml) for 3 h immediately following virus adsorption. Lysates were prepared and processed for immunoprecipitation as previously described using aprotinin and PMSF to inhibit proteolytic cleavage (Baczko et al., 1984). Proteins were analysed by electrophoresis through a 10% polyacrylamide gel and detected by fluorography. Infected cell extracts (lanes 1, 3, 5 and 7) and mock-infected cell extracts (lanes 2, 4, 6 and 8) were treated with monoclonal antibodies 1G9 (lanes 1 and 2) and 1E1 (lanes 3 and 4) or with polyclonal serum from an immunized cat (lanes 5 and 6) or no antibody (lanes 7 and 8).

Fig. 2. Western blotting with monoclonal antibodies. Infected cell cultures were harvested at 6 (lane 2) or 24 (lane 1) h post-infection and processed for electrophoresis. Mock-infected cells were treated in a similar manner. Proteins were then separated on a 10% polyacrylamide gel, transferred to nitrocellulose filters and detected using monoclonal antibodies as described (Samson et al., 1986). (a) Proteins probed with antibody 1G9; (b) probed with 1E1. Lanes 1, FCV-infected cells 24 h harvest; lanes 2, FCV-infected cells 6 h harvest; lanes 3, mock-infected cells. The capsid protein, and the additional 40K polypeptide formed at late times, are indicated.

It seemed appropriate to produce monoclonal antibodies in order to examine the relationships between the polypeptides. Such antibodies would also be of use in the investigation of strain variation and analysis of protein structure and function. Monoclonal antibodies were therefore produced by the following method. Twelve mice were bled from the orbital sinus before immunization and the blood was stored for later assay. Mice were inoculated in the footpad on day 1 of the procedure using 25 μl of CRFK cell antigen emulsified in Freund’s complete adjuvant. Four of the mice received uninfected cell antigens and the other eight received infected cell antigen harvested 24 h post-infection. The stimulus was repeated on day 7 post-inoculation when each mouse received 50 μl of antigen without adjuvant intraperitoneally and 50 μl intravenously. The final inoculation was made on day 20 and consisted of 25 μl of antigen without adjuvant intravenously, 25 μl of antigen in adjuvant per footpad, and 100 μl subcutaneously to the neck. The mice were bled again on day 27 and tested for the development of antibodies in an ELISA. All mice immunized showed an increased reactivity to uninfected cell antigens, but those which had received the infected cell lysates discriminated well between infected and non-infected cell antigens.

The protocol for the production of monoclonal antibodies was the same as that employed to produce such antibodies specific for respiratory syncytial virus, and has already been reported (Routledge et al., 1985). In brief, spleens were removed from selected animals and lymphocytes were fused with NS1 myeloma cells using polyethylene glycol. Fused cells were selected in HAT medium and the media from any hybridoma cells that grew were tested by ELISA for the presence of antibodies specific for FCV. Positive cells were cloned by dilution in the presence of
spleen feeder cells and re-tested for antibody secretion. In this way two hybridoma cell lines, 1G9 and 1E1, were obtained which secreted high levels of FCV-specific antibody. Each was inoculated into the peritoneal cavities of pristane-primed mice from which ascites fluid could be recovered.

The monoclonal antibodies described above were tested in immunoprecipitations and both were found to react with the capsid protein (Fig. 1). Both also recognized this protein in Western blots (Fig. 2). The abilities of these antibodies to neutralize FCV were therefore examined in a plaque reduction assay using CRFK cells. Although both antibodies were present at high titre in ELISAs, 1G9 was found to have an excellent neutralizing capability whereas neutralization by 1E1 could not be detected. Consequently it can be concluded that there are at least two distinct epitopes on this protein, only one of which is associated with virus neutralization. Further evidence for the physical separation of these epitopes was provided by Western blot experiments in which infected cells were harvested after the completion of the virus replication cycle. These cells contained a 40K peptide which was recognized well by antibody 1G9, but not by 1E1 (Fig. 2, lanes 1). This band was also detected in immunoprecipitation experiments conducted in the absence of protease inhibitors (data not shown), and is thus most likely to result from proteolysis of the capsid protein.

The relative positions of the two epitopes were then examined by testing the antibodies for additive binding. This test, developed by Routledge et al. (1986), examines the effect of allowing the antibodies to bind to the antigen under conditions in which at least one of them is saturating. Provided sufficient of the second antibody is added, a significant increase in absorbance should be detected by an ELISA. In order to ensure that these conditions were met, this test was performed by testing the two antibodies, both separately and in combination, in an ELISA (Fig. 3). In this way the absorbance values expected from the binding of the individual antibodies could be determined and compared with the value obtained from the mixture at each dilution. However, there were no differences in the total amount of antibody that could be bound when the antigen was saturated (Fig. 3). The two epitopes concerned therefore appear to be sufficiently close together for binding of one antibody effectively to exclude the other.
Short communication

Calicivirus proteins almost certainly mature by a process of proteolytic cleavage, and an 86K precursor to mature capsid protein has been identified in SMSV-infected cells using procedures which inhibit protein processing (Fretz & Schaffer, 1978). Since the monoclonal antibodies described here react with epitopes which are stable to the denaturing effects of SDS-polyacrylamide gel electrophoresis, they should also detect any precursors to the capsid protein, but we have not observed these in infected cells. This indicates that any modifications must closely follow peptide synthesis. Experiments are in progress to elucidate this process more fully.

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REFERENCES


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