Polypeptides of Feline Leukaemia Virus: Identification of p15(E) and p12(E)

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SUMMARY

Antiserum to the p15(E) polypeptide of Rauscher murine leukaemia virus (R-MuLV) precipitated two proteins from purified virions of feline leukaemia virus (FeLV) with apparent mol. wt. of 18 500 and 15 500 on SDS-polyacrylamide gels. These proteins have been designated p15(E) and p12(E), in line with the nomenclature for MuLV proteins. Like the analogous protein of MuLV, FeLV p15(E) was found to be disulphide-linked to the virion glycoprotein, gp70. FeLV p15(E) was sensitive to digestion of intact virus particles with the proteolytic enzyme, bromelain, indicating that this protein is on the outer surface of the virion.

An analysis of cat sera for precipitating activity for FeLV p12(E) showed this only in sera from cats which had recovered from FeLV infection and had virus-neutralizing activity.

Virion surface components are of particular interest since some are involved in the initial interaction of the virus with its host cell and since they act as targets for virus-neutralizing antibodies. For feline leukaemia virus (FeLV), the virion proteins which have been characterized are the four internal structural proteins, p27, p15, p12 and p10, which are products of the FeLV gag gene (Graves & Velicer, 1974; Khan & Stephenson, 1977) and the virion glycoprotein, gp70 (Bolognesi et al. 1974). The demonstration that murine leukaemia virus (MuLV) particles carry an additional surface protein, p15(E), stimulated this analysis of FeLV isolates for similar constituents.

MuLV p15(E) was first recognized when virion protein profiles on SDS-polyacrylamide gel electrophoresis (SDS–PAGE) and gel filtration in 6 M-guanidine hydrochloride (GuHCl) were compared. The newly identified protein was eluted in the void volume fraction from the 6 M-GuHCl column but showed a mol. wt. of about 15 000 by SDS–PAGE (Ikeda et al. 1975). More recently it has been recognized that MuLV virions can contain two very similar components in this mol. wt. range, p15(E) and p12(E). MuLV p12(E) may be a proteolytic cleavage product of p15(E) since it shows a similar but less complex tryptic peptide pattern and accumulates relative to p15(E) on chasing after pulse labelling (Karshin et al. 1977). MuLV p15(E) and p12(E) are components of purified virion envelopes (Van de Ven et al. 1978). Since p15(E) and p12(E) are synthesized in infected cells on a large precursor together with the major virion glycoprotein, gp70 (Famulari et al. 1976; Karshin et al. 1977), they are defined as products of the MuLV env gene.

To characterize components of FeLV which were antigenically cross-reactive with MuLV p15(E) labelled proteins from purified virion preparations of FeLV and lysates of FeLV-infected cells were immunoprecipitated. Cells were labelled with 3H-leucine (50 μCi/ml) for 18 to 24 h. Virus was harvested from the supernatant and purified by sucrose density gradient centrifugation. The cells were washed twice in phosphate-buffered saline and lysed in detergents by the procedure described by Van Zaane et al. (1976). The purified virus preparations were also solubilized in this detergent mixture (1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, plus 1% Aprotinin, a protease inhibitor) and spun at 100000 g to remove insoluble and aggregated material immediately before the
Fig. 1. (a) Identification of FeLV p15(E) and p12(E) by immunoprecipitation and SDS–PAGE. 
24 h harvests of FeLV-producing cells labelled with ³H-leucine (50 μCi/ml) were used as the 
source of purified FeLV-ABC/KT (lane A). A detergent lysate of FL74 cells labelled with ³H-
leucine (see text) was immunoprecipitated with antiserum to R-MuLV p15(E) (kindly provided by 
Dr W. D. Hardy, Jun., Sloan-Kettering Cancer Center, New York) for lane B. Lanes C to H are 
immunoprecipitated from purified, detergent-disrupted FeLV preparations. Lanes C, D, G and H 
are precipitates of FeLV-ABC/KT, while lanes E and F are of FeLV-A/Rickard (F422) (Rickard 
et al. 1969). In lane D, the disrupted virus was pretreated with 10 mM-dithiothreitol at 37 °C for 
90 min followed by iodoacetamide (20 mM) at 4 °C for 90 min. After dialysis, proteins were 
immunoprecipitated as for the other samples. Lanes C, D, E and G were precipitated with anti-
serum to R-MuLV p15(E), while lanes F and H were precipitated with monospecific antiserum 
to FeLV p27. (b) Removal of p15(E) by protease digestion of intact virions. Panel A: purified 
FeLV-ABC/KT (2 mg protein) was incubated in tris-HCl pH 7.4, 0.1 M-NaCl, 0.001 M-EDTA 
containing 20 mM-mercaptoethanol at 37 °C for 16 h. After incubation the virus was banded on 
20 to 50% sucrose gradients before pelleting at 100000 g for 1 h and solubilizing for electro-
phoresis. Densitometer traces of the SDS–polyacrylamide gels are illustrated. Panel B: virus 
was treated identically, except that 1 mg/ml bromelain was added to the incubated mixture. 
Mol. wt. markers used were: bovine serum albumin (68 000), ovalbumin (43 000), chymotrypsinogen 
(25 000) and cytochrome c (12 000). All chemicals were purchased from Sigma (London), Poole, 
Dorset, U.K.
addition of antisera. After incubation at 0 °C for 1 h, immune complexes were collected
by the addition of fixed Staphylococcus aureus (Kessler, 1975), washed four times
and separated on SDS–polyacrylamide gels using the buffer system described by Laemmli

Fig. 1 (a) shows a comparison by SDS–PAGE of the intracellular FL74 (Theilen et al.
1969) proteins precipitable by rabbit antiserum to R-MuLV p15(E) (lane B) and proteins
of purified FeLV-ABC/KT (lane A) labelled with 3H-leucine. Three specifically precipi-
tated bands could be seen: one of mol. wt. 80000 and two of low mol. wt. (18500 and
15500) which could be seen to co-migrate with two of the virion proteins. The two low mol. wt.
proteins, which we have designated p15(E) and p12(E), could also be precipitated from
FeLV-ABC/KT with the anti-MuLV p15(E) serum (lanes C, D and G). The high mol. wt.
protein which was precipitated from cells by the anti-p15(E) serum was of somewhat
higher mol. wt. than the virion glycoprotein, gp70. No monospecific serum to gp70 was
available to test the 80000 mol. wt. protein, but this protein could be precipitated by cat
sera which reacted almost exclusively with the virion glycoproteins (data not shown).
This observation, along with the fact that the 80K protein is distinct from the series of
precursors precipitated by antisera to the FeLV gag proteins, suggests that the high mol.
w. intracellular protein is analogous to the MuLV env precursor which is cleaved to

Co-precipitation of gp70 with p15(E) and p12(E) was seen with most FeLV preparations,
including all of those of FeLV-ABC/KT (Fig. 1 a, lanes C and G). This co-precipitation
of gp70 could be abolished by pretreatment of disrupted virus with a reducing agent
dithiothreitol) and an alkylating agent (iodoacetamide), as shown in Fig. 1(a) (lane D).
This shows that the co-precipitation occurs due to the disulphide linkage of gp70 to at
least one of the small proteins. A preparation of FeLV-A/Rickard (F422) (Rickard et al.
1969), which lacked p15(E), also did not co-precipitate gp70 (Fig. 1 a, lane E). Subsequent
analyses of this virus showed variable, though generally small or undetectable amounts
of p15(E). Where p15(E) was seen, co-precipitation of gp70 was also observed (data not
shown). This suggests that in FeLV, as in MuLV (Bolognesi et al. 1978), only p15(E) is
covaletly linked to gp70.

Two proteins of FeLV that are analogous to p15(E) and p12(E) of MuLV were character-
ized. In the light of this information the nomenclature of the low mol. wt. proteins of
FeLV should be reconsidered. In a review, Schafer & Bolognesi (1977) described a com-
ponent of FeLV which cross-reacted with MuLV p15(E) as p15. We suggest that that
protein was one of the two proteins which are described in this paper and that the nomen-
cature of the envelope proteins of FeLV should parallel that of their MuLV analogues.
A protein was isolated which shows a mol. wt. of 15000 by gel filtration in 6 M-GuHCl
and 14500 on SDS–polyacrylamide gels, and can be assigned to the FeLV gag gene since
antiserum prepared against it precipitates intracellular precursors which are also pre-
cipitable with antisera to p27 (J. Neil, unpublished results). This protein we designate p15
in Fig. 1(a). Although p10 and p12 are not resolved on our gels, previous studies have
shown that these proteins migrate very close together on GuHCl gel filtration and SDS–
polyacrylamide gels (Graves & Velicer, 1974; Khan & Stephenson, 1977). This supports
our designation of the 15000 mol. wt. gag protein as p15. Schafer & Bolognesi (1977)
describe as p12 a protein with similar electrophoretic mobility to our p15. It is clear from
that work and from studies on MuLV proteins that the mobility of the low mol. wt. virus
proteins relative to one another by SDS-PAGE varies from one mammalian virus to
another. Consequently it is important to assess the serological reactivity of each com-
ponent before it is incorporated into a consistent system of nomenclature.

Neutralization of FeLV by antiserum to Friend MuLV p15(E), in the presence of
Table 1. *Cat sera tested for precipitating activity to FeLV p12(E)*

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<tr>
<th>Serum</th>
<th>VN†</th>
<th>Viraemia</th>
<th>IF‡</th>
<th>Other details</th>
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<td>+</td>
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<td>Leukaemic</td>
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</table>

* Sera were selected to give representatives from as many 'classes' of natural and experimental responses as possible (Hardy et al. 1977). The indirect immunofluorescence test for antibodies to FeLV cell-surface antigens and the procedure for the virus neutralization test have been published (Riggs, 1971; Essex et al. 1971; Russell & Jarrett, 1978). In each case, a positive result is recorded for a titre greater than 1/8.

Viraemia was assessed by plating plasma samples on S+L- indicator cells (Jarrett & Russell, 1978). Positive precipitation of FeLV p12(E) was recorded where a visible band was seen after fluorography on pre-flashed Kodak X-ray films (Laskey & Mills, 1975). Immune precipitation conditions were identical to those used in Fig. 1.

† VN, Virus neutralization.
‡ IF, Indirect membrane immunofluorescence.

Complement, provided evidence for the existence and surface location of an analogous component of FeLV (Fischinger et al. 1976). However, our analysis with antisera to R-MuLV p15(E) did not reveal such neutralization (data not shown). A probable explanation is that the cross-reactive determinants recognized by the serum used in this study were not exposed on the surface of FeLV particles, in contrast to the anti-Friend MuLV p15(E) serum used by Fischinger et al. (1976).

A more direct approach to establishing a surface location for FeLV p15(E) was proteolytic digestion of intact virions with bromelain. This technique showed p15(E) to be as sensitive to digestion as gp70 (Fig. 1b). The effect of the treatment on p12(E) could not be assessed here since it co-migrated with p15. However, subsequent experiments have shown p12(E) to be as sensitive to digestion as p15(E) (data not shown). It can be concluded from these experiments that p15(E) and p12(E) are available to the proteolytic enzyme at the virion surface, as is gp70.

In view of the definition of new low mol. wt. proteins of FeLV, it was decided to determine whether cats respond immunologically to these components. Using a preparation of FeLV-A/Rickard (F422) which contained p12(E) but no detectable p15(E), a series of 18 cat sera were tested for their ability to precipitate the newly characterized protein. This virus was chosen since the analysis could have been complicated by the co-precipitation of disulphide-linked gp70-p15(E) complexes.

The results of this analysis are summarized in Table 1. It was concluded that only cats which have been exposed to FeLV and are immune, as judged by the absence of viraemia and the presence of virus-neutralizing antibodies, possess antibodies which precipitate p12(E). This is in contrast to the situation in mice, many strains of which show 'natural' antibodies which react with MuLV p15(E) (Ihle et al. 1975). This is assumed to reflect the expression of endogenous MuLV and the resultant immune response (Ihle et al. 1976).
Extrapolating to FeLV, our results suggest that no endogenous FeLV pI5(E)/pI2(E) is expressed in cats. If the FeLV-related sequences which are endogenous in cats (Okabe et al. 1976; Levin et al. 1976) include the genetic information for pI5(E)/pI2(E), it is anticipated that this is not commonly expressed. This study indicates that an antibody response to pI2(E) correlates with immunity to FeLV infection, although it must be stated that not all cats which showed neutralizing antibodies had detectable precipitating activity for pI2(E). Further work should establish the significance of the immune response of cats to FeLV pI5(E) and pI2(E) for the outcome of FeLV infection.

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REFERENCES


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Short communications


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