Isolation of a FAIDS Upregulated Protein from Infected Feline Lymphoid Cell Lysates by Preparative SDS Gel Electrophoresis

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Introduction

Feline acquired immunodeficiency syndromes (FAIDS) can be induced in cats by either of two feline retroviruses: one an oncornavirus, feline leukemia virus (FeLV), and the other a lentivirus, feline immunodeficiency virus (FIV).[1] FIV closely resembles human and simian immunodeficiency viruses (HIV and SIV) in the lentivirus lineage, ultrastructural morphology, molecular compositions, and immunosuppressive potential. Therefore, FIV-FAIDS is an excellent small animal model for human AIDS.[1]

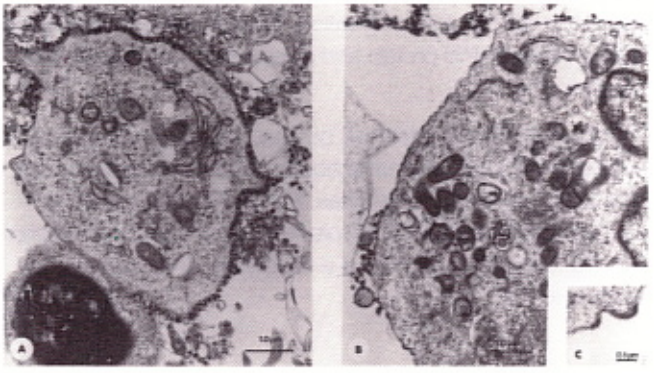

Fig. 1. Electron micrograph of FIV infected lymphoid cells. A. Whole virus surrounding infected cells. B. Virus budding from the cell surface. C. Close-up of virus budding from cell surface.

We have derived the lymphoid cell line, FL-4, of mixed peripheral blood lymphocyte origin. FL-4 cells are interleukin-2 independent, require no uninfected cell supplementation, and spontaneously produce large amounts of FIV.[2] Feline vaccinations were performed with chemically-inactivated whole FL-4 cells which do not produce whole virus but do present a full complement of viral antigens on their cell surface. The whole-cell vaccine elicited humoral responses to all of the viral proteins and significantly protected cats against FIV infection. Control cats, immunized with chemically-inactivated uninfected cells (FeT1 cells) were not protected from FIV infection.

Immunoblot analysis indicated that sera from FL-4 vaccinated cats contains antibody to an 85 kd protein that is not of viral origin. Control cat sera did not demonstrate antibody to this protein. SDS-PAGE analysis demonstrates the presence of the 85 kd protein in FeT1 cell lysates. However, the same protein is expressed in significantly higher amounts by FL-4 cells. This suggests that this protein may be upregulated by FIV (see Figure 2).

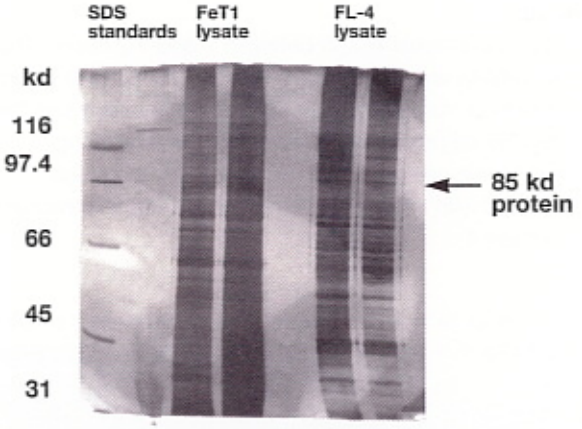

Fig. 2. Silver stained analytical SDS-PAGE mini gel showing separation of proteins from lysates derived from non-infected (FeT1) and infected (FL-4) lymphoid cells. The upregulated 85 kd protein of interest is indicated.

We report here how the Model 491 Prep Cell was used to purify this 85 kd protein in order to characterize its role during viral infection.

Methods

SAMPLE PREPARATION

Cell lines used were FeT1 (uninfected lymphoid cells) and FL-4 (FIV-infected lymphoid cells). Cell lysates: cells (4×10^8) were washed three times with PBS. Cell pellets were suspended in lysis buffer (0.375 M Tris-HCl, pH 8.6, containing 0.1% SDS, 0.04% Tween 20, 0.04% NP-40) for 10 minutes. Cells were microfuged for 5 minutes at 14,000 rpm and supernatants were taken. Supernatants were microfuged for 5 minutes at 14,000 rpm to insure the cleanliness of the preparation. The concentration of protein in the sample, as determined by the Bio-Rad Protein Assay, was approximately 25 mg/ml.

Analytical SDS-PAGE was used to determine the acrylamide concentration (%T) that would best separate the 85 kd protein from its nearest contaminants in the prep cell. For this application a monomer concentration of 7% was indicated.

PREPARATIVE SDS-PAGE—EXPERIMENTAL CONDITIONS

Two milliliters of the protein sample containing 50 milligrams total protein were added to an equal volume of standard SDS-PAGE reducing buffer, heated at 95 °C for 5 minutes, and loaded directly onto a gel in the Model 491 Prep Cell for purification.

4 ml total sample volume
50 mg total protein loaded
Gel tube–37.0 mm OD
7% resolving gel (50 ml) with 4% stack (5 ml)
40 mA constant current
2.5 ml fractions collected
Elution rate–1.0 ml/min
Length of the run–7 hours

Results

Fractions of interest eluted between 7 and 7.2 hours. The 85 kd protein was collected in fractions 70–80 (see Figure 3). Immunoblots run on fractions 75 and 80 revealed that the purified 85 kd protein was free of any contaminating immunogenic cellular or viral proteins. Sera from cats vaccinated with infected FL-4 cells was used in immunoblots. See Figure 4.

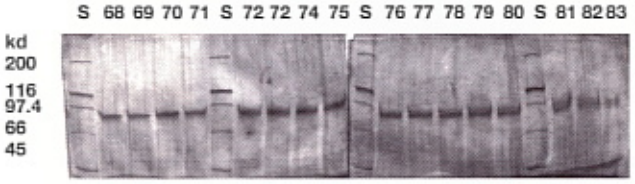

Fig. 3. Aliquots from prep cell fractions 68–83, which contained the purified 85 kd protein, were analyzed on silver stained SDS-PAGE mini gels to determine relative purity. SDS-PAGE Silver Stain Standards were run in every fifth fraction.

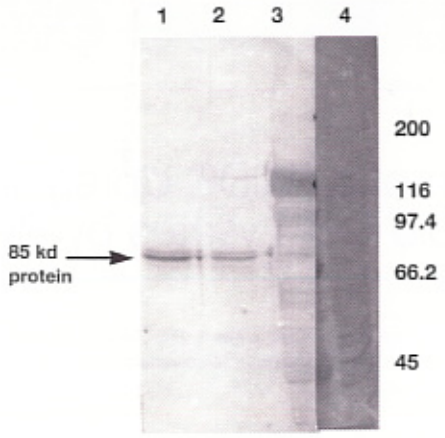

Fig. 4. Immunoblot analysis demonstrates the relative purity of the 85 kd protein isolated in prep cell fractions 68–80. Blots were developed with primary antibodies derived from the sera of cats vaccinated with FIV infected FL-4 cells. Lanes 1 and 2, prep cell fractions 75 and 80. Lane 3, immunogenic cellular and viral proteins derived from whole cell lysates of infected FL-4 cells. Lane 4, Coomassie® blue stained SDS-PAGE analysis of cellular and viral proteins derived from whole cell lysates of infected FL-4 cells.

Of the 50 milligrams total protein separated with the Model 491 Prep Cell, 220 micrograms of purified 85 kd protein, or 0.4% of the total, was recovered in fractions 70–80. This represents approximately 225-fold purification. The difference in molecular weight between this protein and its nearest contaminant was less than 2%.

The purified 85 kd protein will be used for the production of polyclonal and monoclonal antibodies, sequencing, and other structural and functional studies. Purified 85 kd protein will also be used to elucidate its role in FIV infection. Such studies will determine if this protein is a candidate for a subunit vaccine for FIV.

References

1 Oswald, J., Yamamoto, J. K. and Neil, J. C., Feline Immunodeficiency Virus as a Model for AIDS Vaccination, AIDS, supplement 1, S163–S165 (1990).
2 Yamamoto, J. K., et al., Intervirology, 32, 361–375 (1991).

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