Hosie, M.J. et al. (2005) *Vaccination with an Inactivated Virulent Feline Immunodeficiency Virus Engineered to Express High Levels of Env.* Journal of Virology, 79 (3). pp. 1954-1957. ISSN 0022-538X

http://eprints.gla.ac.uk/2985/

Deposited on: 30 August 2006

Enlighten – Research publications by members of the University of Glasgow
http://eprints.gla.ac.uk

Vaccination with an Inactivated Virulent Feline Immunodeficiency Virus Engineered to Express High Levels of Env

Margaret J. Hosie[1*], Dieter Klein[2], James M. Binley[3], Thomas H. Dunsford[1], Oswald Jarrett[1], James C. Neil[1], Elzbieta Knapp[2], Simone Giannecchini[4], Donatella Matteucci[4], Mauro Bendinelli[4], James A. Hoxie[5] and Brian J. Willett[1]

[1]*Retrovirus Research Laboratory, Institute of Comparative Medicine, University of Glasgow, Bearsden Road, Glasgow, G61 1QH, UK*

[2]*Institute of Virology, Department of Pathobiology, University of Veterinary Medicine, Veterinarplatz 1, A-1210 Vienna, Austria*

[3]*The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla CA 92037*

[4]*Retrovirus Center and Virology Section, Department of Experimental Pathology, University of Pisa, Pisa, Italy*

[5]*Department of Medicine, University of Pennsylvania, Biomedical Research Building II/III University of Pennsylvania, 421 Curie Blvd, Philadelphia, PA. 19104.*

Running title: Control of a virulent FIV by inactivated viral vaccine

*corresponding author

Dr MJ Hosie

Retrovirus Research Laboratory

Institute of Comparative Medicine

University of Glasgow

Bearsden Road

Glasgow G61 1QH

United Kingdom

Email: m.hosie@vet.gla.ac.uk

Tel: +44 141 330 3274, Fax: +44, 141 330 5602

Abstract

An inactivated virus vaccine was prepared from a pathogenic isolate of feline immunodeficiency virus containing a mutation that ablated an endocytic sorting signal in the envelope glycoprotein, increasing expression on virions. Cats immunized with inactivated preparations of this modified virus exhibited strong ELISA titers to Env. Evidence of protection following challenge demonstrated the potential of this approach to lentiviral vaccination.

Although several animal model systems have been developed to test lentivirus vaccines [18] and numerous immunogens have been evaluated, the mechanisms underlying protective immunity and the essential components of an effective vaccine remain unclear. FIV infection of the domestic cat is a useful model since it represents an immunosuppressive lentivirus in its natural host [18,23]. Vaccine-induced protection against FIV infection has been achieved using several immunogens, but protection has been restricted mainly to viruses of low virulence [8], with both inactivated virus vaccines and molecularly derived vaccines having been ineffective at protecting cats against homologous challenge with a pathogenic isolate[9,11]. Previous studies have indicated that Env is an important component of efficacious vaccines, to stimulate cytotoxic T cells (CTL) and neutralizing antibodies (VNA)[5,10]. To attempt to improve immunogenicity, our strategy was to prepare a vaccine from a pathogenic clone of FIV-GL8 engineered to express high levels of Env by means of a mutation preventing Env endocytosis. In this way we aimed to reproduce high levels of Env in a native conformation.

First we confirmed that FIV Env was endocytosed from the cell surface, as had been demonstrated for SIV Env [20]. Immunofluorescence microscopy of FIV-infected CrFK cells incubated at 4°C with the anti-FIV Env monoclonal antibody vpg71.2 [22] demonstrated only low surface expression compared with control antibody recognising CD29 (4B4). However, when cells were incubated with antibody at 37°C, a marked increase in fluorescence was noted in intracellular sites (Figure 1a). These data indicated that at 37°C Env was transiently expressed on the cell surface and then internalized. In contrast, no change in the level of fluorescence was seen on the cells incubated with the control antibody at 4 or 37°C.

The observation that FIV Env was rapidly endocytozed from the surface of infected cells, similar to other lentivirus Envs [17], led us to identify the tyrosine-containing endocytosis motif GYTVI, located between positions 820 and 824 of the *env* gene of the $GL8_{Mya}$

molecular clone [9], corresponding to the GYxxΦ motif conserved in all SIV and HIV Envs [3,4](Figure 1b). To test the effect of ablating this motif in FIV *env*, we constructed the mutant $GL8_{YI}$ based on the $GL8_{Mya}$ molecular clone by polymerase chain reaction (PCR)-mediated mutagenesis, incorporating the mutation Y821I. Stocks of $GL8_{YI}$ were prepared following transfection in 3T3 cells using Superfect transfection reagent (QIAgen) followed by recovery into Mya-1 cells [19]. Evaluation of Env surface expression by FACS on Mya-1 cells infected with either $GL8_{YI}$ or the wild type GL8 clone ($GL8_{WT}$) revealed that although $GL8_{YI}$ and $GL8_{WT}$ produced similar levels of FIV p24 when measured by ELISA (data not shown), surface expression of Env was markedly greater in cells infected with $GL8_{YI}$ (49%) compared to $GL8_{WT}$ (2.6%). Furthermore, we compared the Env content of $GL8_{WT}$ and $GL8_{YI}$ by adsorbing equal amounts of the two viruses onto *Galanthus nivalis* lectin (GN)-coated microwells and comparing the ability of the adsorbed virions to bind FIV immune sera, using the method described previously[6]. Although similar amounts of p24 were present by immunoblotting (data not shown), $GL8_{YI}$ bound considerably more antibody than $GL8_{WT}$, indicating a higher Env content in the mutated virions (Figure 1c). Subsequently, an inactivated virus vaccine was prepared from paraformaldehyde-treated culture fluids of $GL8_{YI}$-infected Mya-1 cells as described previously [12].

Eight 11-week-old kittens were randomly divided into 2 groups of 4. One group of kittens (V1 to V4) was immunized subcutaneously at 0, 3 and 7 weeks with 250 µg inactivated $GL8_{YI}$ virus in 0.5 ml PBS and 0.5 ml MF 59.0 citrate adjuvant. The controls (C1 to C4) received 0.5 ml PBS and 0.5 ml MF 59.0 citrate adjuvant at the same times. At week 10, three weeks following the third inoculation, no virus could be isolated from peripheral blood mononuclear cells (PBMC), confirming that the inactivated virus vaccine did not contain any residual infectivity for cats.

FIV gp140 Env, consisting of the entire surface unit and the ectodomain of the transmembrane domain, was used as antigen in an ELISA to measure anti-Env antibodies

in cat sera. The *env* gene of the FIV-GL8 molecular clone $GL8_{414}$ [9] was cloned by PCR into pPPI4 [2] using KasI and BstB1, with the 3' primer containing a C-terminal D7324 epitope tag [1]. An antigen-capture ELISA was developed, based on one described previously [1] in which supernatant containing FIV Env (approximately 1 μg/ml) derived from 293T cell transfections was captured using sheep antibody raised against the D7324 epitope tag (Cliniqa, CA). Bound antibody was detected using goat anti-cat IgG Fc alkaline phosphatase conjugate (Accurate Chemical and Scientific, Westbury, NY) at 1:5000 and the signal was amplified and developed as described previously[1]. $GL8_{YI}$ vaccinates developed anti-Env antibodies, detectable by ELISA in all vaccinates (Table 1), with the highest titer in vaccinate V4.

To test whether the anti-Env antibodies were neutralizing, VNA were measured against 10 50% tissue culture infectious doses ($TCID_{50}$) of the GL8 molecular clone using MBM cells as previously described[6], with or without prior adsorption of test sera with MBM or Mya-1 cells. Vaccinates V1, V2 and V4 developed VNA against the GL8 challenge virus, provided that the sera were absorbed with either MBM or Mya-1 cells prior to testing. It has been reported that the presence of antibodies to substrate cells can mask detection of VNA in vaccinated cats sera[6] and in this study absorption of sera with Mya-1 cells eliminated this in 2/4 sera whereas absorption of sera with the substrate MBM cells revealed neutralizing antibodies in 3/4 sera (Table 1).

To assess whether immunization with $GL8_{YI}$ would protect against virulent challenge, all eight cats were challenged intraperitoneally with 10 50% infectious doses (ID_{50}) of virus derived from the GL8 molecular clone at week 10. The challenge virus was prepared from the $GL8_{414}$ [9] molecular clone of FIV by transfection of the murine fibroblast cell line 3T3 using Superfect transfection reagent (QIAgen, Valencia,CA.). 72 hours post transfection, supernatants were harvested, 0.45μm filtered and used to infect the IL2-dependent feline T cell line Q201 [21]. The infected cultures were monitored visually for cytopathicity and for the production of FIV p24 by enzyme-linked immunosorbent assay

(ELISA, PetCheck FIV antigen ELISA, IDEXX Corp., Portland, Maine, USA). Supernatants were collected at peak cytopathicity/ p24 production, 0.45µm filtered, dispensed into 1ml aliquots and stored at –70°C. Previously we had demonstrated that infection of cats with a similar dose of this challenge stock led to a high proviral burden that was maintained throughout the acute phase of infection. Coincident with the sharp rise in proviral load was a marked decline in the CD4:CD8 ratio [13], consistent with the pathogenic potential of the GL8 molecular clone challenge stock.

Cultures of PBMC isolated from samples from all cats taken 3 weeks post challenge were positive by FIV p24 antigen ELISA after 7 days in culture, indicating that all of the vaccinated and control cats became infected following challenge. However, we noted that a smaller proportion of PBMC was infected in three vaccinates compared to the remaining vaccinate (cat V3) and the control cats (data not shown) and so, to provide more quantitative information on the viral burdens following challenge, we examined proviral loads in PBMC at intervals until 21 weeks post challenge by real-time PCR[7] using oligonucleotides designed to detect a variety of FIV A-subtype isolates as described[14-16] (Figure 2a). The mean proviral load was consistently lower in the vaccinates than the controls, although the difference did not reach statistical significance because of the high proviral load of vaccinate V3 (Figure 2b). Furthermore, the peak proviral loads in PBMC of 3/4 vaccinates were markedly lower than those of the controls (Figure 2c). Supporting evidence that the virulent challenge was controlled in 3/4 vaccinates was provided by the analysis of tissues sampled post mortem, 21 weeks after challenge (data not shown). The highest proviral burden in splenocytes was in vaccinate V3, consistent with the high proviral load in the PBMC of this cat throughout the study. In contrast, the proviral loads of the remaining vaccinates were lower compared to the controls in both splenocytes and PBMC, although again these differences were not statistically significant. Proviral DNA was detected in cells isolated from the mesenteric lymph node (MLN) from all of the control cats but only a single vaccinate (cat V3). The high proviral loads detected in cat V3 were inconsistent with the remaining three vaccinates, providing no evidence of even partial protection against the challenge; indeed

the viral and proviral loads measured in this cat usually exceeded the loads in the unvaccinated, control cats, suggesting that the infection may have been enhanced. Taken together, these data indicate that a larger vaccine trial is merited to test the applicability of this approach to lentiviral vaccines in general.

The mutated virus preparation did express increased levels of Env and immunization induced anti-Env responses in 4/4 cats and VNA in 3/4 cats. The pilot challenge study led to encouraging results and, although vaccination did not prevent infection with the pathogenic GL8 molecular clone, the course of infection was modified in the cats that developed VNA, with decreased viral burdens compared to the controls. Vaccinate V3, which developed very high viral and proviral loads following challenge, did not develop VNA. It is tempting to speculate, therefore, that the VNA detected in the remaining three vaccinates played a role in reducing the viral burden. Thus the data indicate that Env is an important constituent of an effective lentiviral vaccine and encourage further studies to optimize immune responses against virulent isolates, since the GL8 molecular clone is pathogenic and achieves high virus loads similar to those described for other pathogenic strains (our unpublished observations). It is possible that the quality of the humoral immune response generated against Env was sub optimal, since the vaccine appeared to induce VNA inefficiently. Therefore, future studies might address methods of improving the quality of the VNA responses since the generation of protective immune responses in the domestic cat against challenge with the GL8 isolate would provide encouragement for the testing of similar strategies to increase the quantity of Env on HIV-1 or HIV-2 virions.

Figure Legends

Figure 1. (a) Immunofluorescence of FIV-infected CrFK cells incubated with antibodies detecting either FIV Env or CD29 for 1 h at either $4^{o}C$ or $37^{o}C$. (b) Conservation of endocytosis motif between feline and primate lentiviruses. (c) Relative Env content in the $GL8_{WT}$ (■) and $GL8_{YI}$ (□) clones was determined by measuring the ability of virions to bind anti-FIV antibody. Equal amounts of gradient-purified virions of the two clones were adsorbed onto GN-coated microwells and then probed for the ability to bind IgG from cat sera diluted 1:100. Cat sera from a GL8-infected cat, a PET-infected cat, and a serum pool from 5 uninfected control cats were used. The experiment was repeated twice with comparable results.

Figure 2. Proviral loads in vaccinated (open symbols) and control (closed symbols) cats measured at intervals post challenge by real-time PCR. The proviral loads of the individual cats (V3 represented by Δ) are shown in (a), the mean loads +/- SEM of the vaccinates (O) and the controls (•) are shown in (b) and the peak proviral loads in PBMC from vaccinated (□) and control (■) cats are shown in (c).

Table 1. Anti Env antibody responses and neutralizing activity of day of challenge vaccine and control sera.

Serum	Anti-Env titer Determined by ELISA	VNA titer[1]		
		Cells used for sera adsorption		
		None	MBM	Mya-1
Vaccinated				
V1	14 000	<8	128	128
V2	5 000	<8	16	64
V3	10 000	<8	<8	<8
V4	80 000	<8	16	<8
Control				
C1	<1 000	<8	<8	<8
C2	<1 000	<8	<8	<8
C3	<1 000	<8	<8	<8
C4	<1 000	<8	<8	<8

[1] VNA titers were measured on untreated sera as well as cell-adsorbed sera as indicated and are expressed as the reciprocal of the highest serum dilution that gave 50% inhibition of reverse transcriptase production by 10 50% $TCID_{50}$ GL8 clone mixed with the corresponding dilution of a pool of 10 normal cat sera. The experiment was repeated twice, with comparable results.

Reference List

1. **Binley, J. M., P. J. Klasse, Y. Cao, I. Jones, M. Markowitz, D. D. Ho, and J. P. Moore**. 1997. Differential regulation of the antibody responses to Gag and Env proteins of human immunodeficiency virus type 1. J. Virol. **71**:2799-2809.

2. **Binley, J. M., R. W. Sanders, B. Clas, N. Schuelke, A. Master, Y. Guo, F. Kajumo, D. J. Anselma, P. J. Maddon, W. C. Olson, and J. P. Moore**. 2000. A recombinant human immunodeficiency virus type 1 envelope glycoprotein complex stabilized by an intermolecular disulphide bond between the gp120 and gp41 subunits is an antigenic mimic of the trimeric virion-associated structure. J. Virol. **74**:627-643.

3. **Boge, M., S. Wyss, J. S. Bonifacino, and M. Thali**. 1998. A membrane-proximal tyrosine-based signal mediates internalization of the HIV-1 envelope glycoprotein via interaction with the AP-2 clathrin adaptor. J. Biol. Chem. **273**:15773-15778.

4. **Bowers, K., A. Pelchen-Matthews, S. Honig, P. J. Vance, L. Creary, B. S. Haggarty, J. Romano, W. Ballensiefen, J. A. Hoxie, and M. Marsh**. 2000. The simian immunodeficiency virus envelope glycoprotein contains multiple signals that regulate its cell surface expression and endocytosis. Traffic **1**:661-674.

5. **Flynn, J. N., P. Keating, M. J. Hosie, M. Mackett, E. B. Stephens, J. A. Beatty, J. C. Neil, and O. Jarrett**. 1996. Env-specific CTL predominate in cats protected from FIV infection by vaccination. J. Immunol. **157**:3658-3665.

6. **Giannecchini, S., D. Del Mauro, D. Matteucci, and M. Bendinelli**. 2001. AIDS vaccination studies using an ex vivo feline immunodeficiency virus model: reevaluation of neutralizing antobody levels elicited by a protective and a nonprotective vaccine after removal of antisubstrate cell antibodies. J. Virol. **75**:4424-4429.

7. **Heid, C. A., J. A. Stevens, K. J. Livak, and P. M. Williams**. 1996. Real time quantitative PCR. Genome Research **6**:986-994.

8. Hesselink, W., Sondermeijer, P., Pouwels, H., Verblakt, E., and Dhore, C. 1999. Vaccination of cats against feline immunodeficiency virus (FIV): a matter of challenge. Vet.Microbiol. 69[1-2], 109-110.

9. **Hosie, M. J., T. H. Dunsford, D. Klein, B. J. Willett, C. Cannon, R. Osborne, J. Macdonald, N. Spibey, N. Mackay, O. Jarrett, and J. C. Neil**. 2000. Vaccination with inactivated virus but not viral DNA reduces viral load following challenge with a heterologous and virulent isolate of feline immunodeficiency virus. J. Virol. **74**:9403-9411.

10. **Hosie, M. J. and J. N. Flynn**. 1996. Feline immunodeficiency virus vaccination: characterization of the immune correlates of protection. J. Virol. **70**:7561-7568.

11. **Hosie, M. J., R. Osborne, G. Reid, J. C. Neil, and O. Jarrett**. 1992. Enhancement after feline immunodeficiency virus vaccination. Vet. Immunol. Immunopathol. **35**:191-198.

12. **Hosie, M. J., R. Osborne, J. K. Yamamoto, J. C. Neil, and O. Jarrett**. 1995. Protection against homologous but not heterologous challenge induced by inactivated feline immunodeficiency virus vaccines. J. Virol. **69**:1253-1255.

13. **Hosie, M. J., B. J. Willett, D. Klein, T. H. Dunsford, C. Cannon, M. Shimojima, J. Neil, and O. Jarrett**. 2002. Evolution of replication efficiency following infection with a molecularly cloned feline immunodeficiency virus of low virulence. J. Virol. **76**:6062-6072.

14. **Klein, D., P. Janda, R. Steinborn, M. Muller, B. Salmons, and W. H. Gunzburg**. 1999. Proviral load determination of different feline immunodeficiency virus isolates using real-time polymerase chain reaction: influence of mismatches on quantification. Electrophoresis **20**:291-299.

15. **Klein, D., C. Leutenegger, P. Bahula, R. Hofmann-Lehmann, R. Gold, B. Salmons, H. Lutz, and W. H. Guenzburg**. 2001. Influence of preassayand sequence variations on viral load determination by a multiplex real-time reverse transcriptse-polymerase chain reaction for feline immunodeficiency virus. J. AIDS and Human Retrovirology **26**:8-20.

16. **Leutenegger, C., D. Klein, R. Hofmann-Lehmann, C. Mislin, U. Hummel, J. Boni, F. Boretti, W. H. Guenzburg, and H. Lutz**. 1999. Rapid FIV provirus quantitation by PCR using the TaqMan fluorigenic real time detection system. J. Virol. Meths. **78**:105-116.

17. **Marsh, M., A. Pelchen-Matthews, and J. A. Hoxie**. 1997. Roles for endocytosis in lentiviral replication. Trends in Cell Biol. **7**:1-4.

18. **Miller, R. J., J. S. Cairns, S. Bridges, and N. Sarver**. 2000. Human immunodeficiency virus and AIDS: insights from animal lentiviruses. J. Virol. **74**:7187-7195.

19. **Miyazawa, T. M., T. Furuya, S. Itagaki, Y. Tohya, E. Takahashi, and T. Mikami**. 1989. Establishment of a feline T-lymphoblastoid cell line highly sensitive for replication of feline immunodeficiency virus. Arch. Virol. **108**:131-135.

20. **Sauter, M. M., A. Pelchen-Matthews, R. Bron, M. Marsh, C. C. Labranche, P. J. Vance, J. Romano, B. S. Haggarty, T. K. Hart, W. M. Lee, and J. A. Hoxie**. 1996. An internalization signal in the simian immunodeficiency virus transmembrane protein cytoplasmic domain modulates expression of envelope glycoproteins on the cell surface. J. Cell. Biol. **132**:795-811.

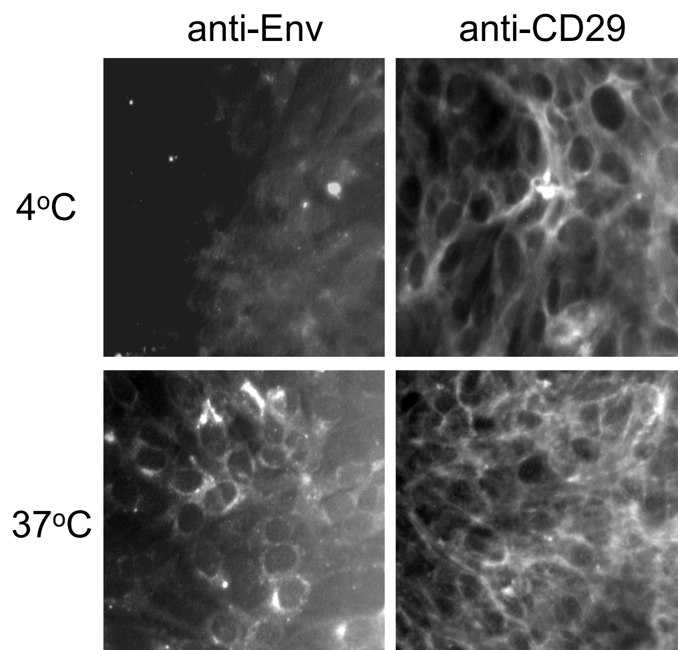
21. **Willett, B., M. J. Hosie, T. Dunsford, J. C. Neil, and O. Jarrett**. 1991. Productive infection of helper T lymphocytes with FIV is accompanied by reduced expression of CD4. AIDS **5**:1469-1475.

22. **Willett, B. J., M. J. Hosie, J. C. Neil, J. D. Turner, and J. A. Hoxie**. 1997. Common mechanism of infection by lentiviruses. Nature **385**:587.

23. **Willett, B. J., J. N. Flynn, and M. J. Hosie**. 1997. FIV infection of the domestic cat: an animal model for AIDS. Immunol. Today **18**:182-189.

Fig 1 Hosie et al

a)



b)

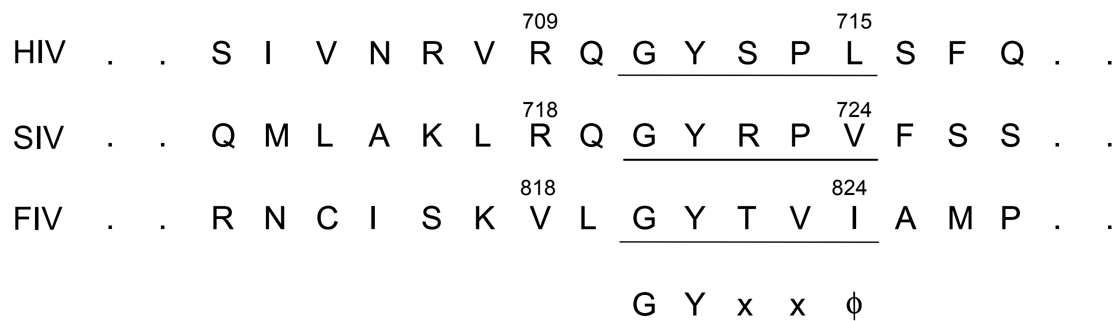


Fig 2 Hosie et al

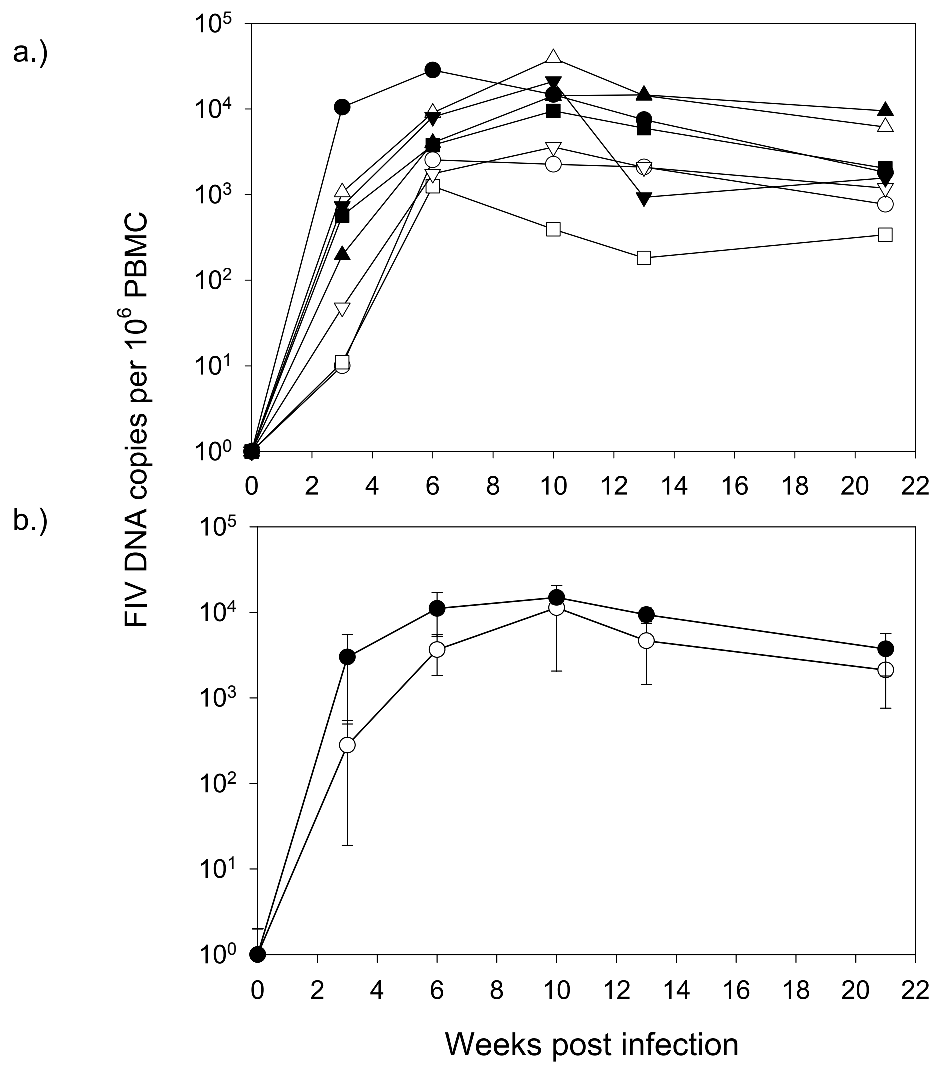


Fig 2 Hosie et al

c.)