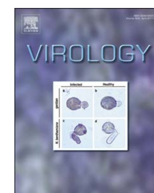
Virology 506 (2017) 19–27

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro



Latent murine leukemia virus infection characterized by the release of non-infectious virions

Stefano Boi[1], Erik Van Dis[1], Ethan J. Hansen, Kyle Rosenke, Karin E. Peterson, Morgan E. Ferrell, Leonard H. Evans*

Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840 United States

ARTICLE INFO

Keywords:
Retroviruses
MuLV
Latency
Replication
Virions

ABSTRACT

Clonal cell lines derived from cultures infected with a polytropic MuLV release vastly different levels of infectious virions ranging from undetectable to very high. Low producing clones release an overwhelming proportion of non-infectious virions containing retroviral RNA but deficient in the Env protein. Non-infectious virion production is not due to an inability of the cells to support infectious MuLV production or to an inherent replicative defectiveness of the proviruses. Reinfection of the lowest producing lines with the polytropic or an ecotropic MuLV results in enormous increases in the specific infectivity of the released virions. This indicates a reversible state of retroviral latency characterized by the release of non-infectious virions that is likely the result of insufficient levels of Env protein required for infectivity. The latency state described here may have important roles in in vivo retroviral infections including alterations of the immune response and the production of defective interfering particles.

1. Introduction

Infection of mice of susceptible strains with exogenous ecotropic murine leukemia viruses (MuLVs) or expression of endogenous ecotropic MuLVs results in the generation of host-range variants derived by recombination with endogenous retroviral sequences (Chattopadhyay et al., 1982; Hartley et al., 1977; Ruscetti et al., 1981; van der et al., 1981). These variants utilize a different cell surface receptor for infection and are capable of infecting cells from several species compared to ecotropic MuLVs that only infect murine cells; hence the designation, polytropic MuLVs. Mixed infection of mice with retroviruses of different properties can profoundly affect the replication of the viruses and the outcome of the infection. Mixed infections generated by inoculation of mixtures of ecotropic and polytropic MuLVs (Evans et al., 2006) result in an enhanced incidence and tempo of disease. This is accompanied by nearly complete pseudotyping of the polytropic genome within ecotropic virions and a striking increase (10^2-10^3-fold) in the *in vivo* replication of the polytropic MuLV but not the ecotropic MuLV. In contrast, ecotropic MuLV replication is not elevated in mixed infections. *In vitro* studies of ecotropic and polytropic mixed infections mirror many of the *in vivo* observations (Rosenke et al., 2012). The polytropic genome is highly pseudotyped within ecotropic virions and the replication of the polytropic virus is highly elevated (30-100-fold) compared to cells infected with the polytropic MuLV alone.

In the present study, we have found that the release of infectious polytropic MuLV from chronically infected cells is not uniform. Individual clonal cell lines from polytropic MuLV-infected cultures release comparable levels of virions but exhibit vast differences in the release of infectious particles. This is very likely the result of insufficient incorporation of Env proteins into the virions. Superinfection of the low-producing cells by another retrovirus, ecotropic or polytropic, results in enormous (10^4-10^5-fold) increases of infectious polytropic virus production. These observations do not reflect the presence of replication-defective mutants harbored by the clonal cell lines nor an inability of the clonal cell lines themselves to properly synthesize and assemble polytropic Env proteins. Our results suggest a unique context of the initial polytropic virus infection leading to a state that results in the suppression of Env protein levels in the cell. This can result in a state of reversible latency characterized by the release of non-infectious virions containing retroviral RNA transcripts.

* Corresponding author.
E-mail address: levans@niaid.nih.gov (L.H. Evans).
[1] SB and EV contributed equally to this study and share first authorship.

http://dx.doi.org/10.1016/j.virol.2017.03.004
Received 20 January 2017; Received in revised form 6 March 2017; Accepted 8 March 2017
0042-6822/ © 2017 Published by Elsevier Inc.

2. Materials and methods

2.1. Cells, viruses and vectors

NIH 3T3 cells were used for the propagation of viruses and assays of MuLVs. All cells were maintained in tissue culture media supplemented with glutamine (2 mM), penicillin (100 units/ml), streptomycin (25 μg/ml), gentamycin (50 μg/ml), and amphotericin B (2 μg/ml). NIH 3T3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine serum. The ecotropic MuLV, F-MuLV$_{57}$ (Oliff et al., 1980), and the polytropic MuLVs Fr98 (Portis et al., 1995) were obtained as virus stocks after transfection of NIH 3T3 cells with plasmids encoding each provirus and subsequent infection of Mus dunni cells (Lander and Chattopadhyay, 1984). NIH 3T3 cells were infected with Fr98 at a multiplicity of infection (MOI) of 2, grown to confluency, seeded at a low cell number on 100 mm tissue cultures dishes, allowed to grow to individual colonies and subsequently expanded to obtain clonal cell lines. The remaining cells from the culture were plated on new dishes and examined by cell-surface fluorescence with mAb 514 to confirm extensive infection.

Fr98 encoding the red fluorescent protein (Fr98-RFP) was constructed by ligation of a synthesized nucleic acid (GenScript USA, Inc.) containing the encephalomyocarditis virus IRES sequence (Bochkov and Palmenberg, 2006) followed by an RFP sequence from pTurboRFP-N (Evrogen). The synthesized nucleic acid was designed to contain Fr98 sequences at the 5′ (103 bp) and 3′ (65 bp) termini that included the Cla1 and Blp1 sites, respectively and was ligated into the Fr98 genome between the unique Cla1 and a Blp1 restriction sites following the *env* gene (Portis et al., 1995). Fr98-RFP can be assayed by direct fluorescence of the RFP in the cytoplasm of infected cells or by using antibodies reactive to the SU protein which is displayed on the cell surface.

2.2. Infectivity assays and monoclonal antibodies

Infectious MuLVs were quantified using monoclonal antibodies (mAb) specific for the Env proteins of the viruses in a focal immunofluorescence assay (FIA) (Sitbon et al., 1985). Briefly, NIH 3T3 cells were seeded and infected with serial dilutions of samples containing 8 μg/ml of polybrene (Sigma Aldrich #52495). The cells were allowed to grow to confluence (5 days) and the monolayers were incubated with mAbs (~0.1 mls per dish of media harvested from hybridoma cells lines producing the mAb), rinsed and then incubated with a fluorescein isothiocyanate-conjugated (FITC) goat anti-mouse Immunoglobulin to bind the mAb on the infected cells. Foci of infected cells were detected and quantified by fluorescence microscopy. In the case of Fr98-RFP, foci were detected by fluorescence microscopy without antibodies. The mAbs employed in the assays included mAb 48, which is specifically reactive with F-MuLV (Chesebro et al., 1981), mAb 514 (Chesebro et al., 1983) which reacts specifically with polytropic MuLVs and mAb 573 (Evans et al., 2014) that is reactive with all MuLVs tested.

Pseudotyping of polytropic genomes within ecotropic virions was assessed by infectivity assays using uninfected target cells and cells which were chronically infected with the ecotropic MuLV, F-MuLV$_{57}$. The chronically-infected cells are completely refractory to infection by virions utilizing the same receptor including virions containing pseudotyped polytropic genomes (Rein, 1982). The degree of pseudotyping was assessed by comparisons of infectivity on uninfected target cells, susceptible to both pseudotyped and non-pseudotyped polytropic virions, to infectivity on chronically-infected cells, susceptible to only non-pseudotyped polytropic virions.

2.3. Cell-surface staining and flow cytometry

Clonal cells line releasing different levels of the polytropic virus, Fr98, were treated with PBS containing 0.002 M EDTA to detach the cells from the tissue culture plate. The cells were incubated with mAb 514 reactive with the Env protein of Fr98 followed by incubation with FITC-conjugated goat anti-mouse Ig. Data were collected by a LSRII (BD Biosciences) flow cytometer and were analyzed using FlowJo (Tree Star).

2.4. Quantification of cellular and virion RNA

Chronically infected clonal cell lines were cultured in 60 mm diameter tissue culture dishes. Total cellular RNA was extracted with Trizol reagent (Invitrogen) or Quick-RNA™ MiniPrep Plus (Zymo Research) according to the manufacturer's instructions. RNA was treated with DNAse I (Ambion) for 30 min at 37 °C to remove any genomic DNA contamination and purified using RNA cleanup columns (Zymo Research). RNA concentrations were quantified by measuring optical absorption at 260 nm. Reverse transcription was performed using RT Supermix (Bio-Rad) according to the manufacturer's instructions. The RT reaction mixture was then inactivated at 85 °C for 5 min, chilled at 4 °C, and the cDNA samples diluted fivefold in RNase-free

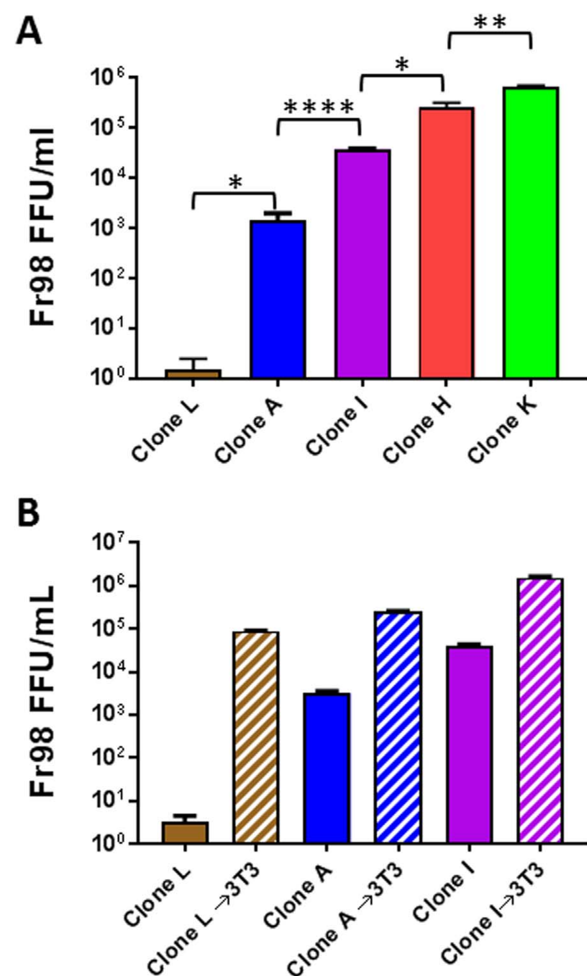


Fig. 1. Clonal cell lines releasing different levels of infectious Fr98 viruses and the replicative ability of the released viruses. A. Clonal cell lines were obtained from 3T3 cells chronically infected with Fr98. Fr98 infectivities released during an 8 h interval from confluent cultures were assessed by the FIA using mAb 514 or mAb 573. Each bar represents the means and SEMs of 4 determinations. Independent assays were normalized to the highest producing clonal cell line (Clone K). **B.** NIH 3T3 cells were infected with viruses harvested from the Fr98-infected clonal cell lines. When the newly infected 3T3 cells from each clonal cell line were completely infected they were seeded onto new dishes and grown to confluency. Viruses released from the newly-infected 3T3 cells during an 8 h period were assessed by the FIA and compared to viruses released from the original clonal cell lines under the same conditions. Each bar represents the means and SEMs of 2–4 determinations.

Download English Version:

https://daneshyari.com/en/article/5675102

Download Persian Version:

https://daneshyari.com/article/5675102

Daneshyari.com