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Review paper

Methods for assessing feline immunodeficiency virus infection, infectivity and purification

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ABSTRACT

Infection of cats with the feline immunodeficiency virus (FIV) recapitulates many aspects of infection of humans with HIV, including highly activated but ineffectual immune responses. Infected hosts remain seropositive for life, and detection of antibodies is the mainstay of diagnosis. However, to quantify virus for research or prognosis, viral proteins, nucleic acids or enzymes, are typically measured by ELISA, PCR or activity, respectively. While such assays are in wide use, they do not distinguish whole, infectious viral particles from defective or disrupted viruses. Titers of infectious viral particles may be estimated from tissue culture infectious doses or by enumerating cell-associated viral proteins, viral transcriptional activity or formation of syncytia. To analyze the viral proteome and the incorporation of host components into viral envelopes, pure lentiviral preparations are required. Methods for purifying lentiviruses include ultracentrifugation to separate particles by size, mass and/or density; chromatography to separate particles by charge, affinity or size; and additional removal of extraviral proteins and exosomes through subtilisin digestion or immunoaffinity. This article reviews advantages and disadvantages of different approaches to purification of lentiviruses with special reference to suitability for FIV, and highlights effects of purification on immune responses and immune assays.

Contents

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1. Introduction

Shortly after human immunodeficiency virus (HIV) was identified as the cause of the acquired immunodeficiency syndrome (AIDS) in people, a virus was described that causes similar clinical signs in domestic cats (Pedersen et al., 1987). This virus was determined to also be a lentivirus and termed feline immunodeficiency virus (FIV). FIV infects domestic cats and many species of non-domestic cats with prevalence ranging from <3% (North America) to 40% (Japan) in domestic cats, and nearly 100% in some non-domestic feline populations (Ishida et al., 1989; Lorentzen and Caola, 2008).

FIV structure, genetic organization and induction of host immune response are very similar to those of HIV; therefore, FIV in cats is a useful animal model for the study of HIV pathogenesis, vaccination, and therapy (Overbaugh et al., 1997; Elder et al., 2010; Yamamoto et al., 2010). Much knowledge has been gained concerning the immune response to FIV, and the molecular biology and pathogenesis of FIV, since the virus was first described in 1987. Albeit, certain laboratory techniques that have been vital to study of lentiviruses such as HIV either have not been applied or remain to be optimized for FIV. In this article we review approaches to total and infectious virus quantification, and methods for viral concentration and purification.

2. Background

The morphology of FIV is similar to that of other lentiviruses. The FIV virion is 100–125 nm in diameter and consists of an approximately spherical shell composed of myristylated matrix proteins (MA, p14) surrounded by an envelope largely derived from the host cell membrane (Pedersen et al., 1987; Zhang and Nguyen, 2008). The envelope contains knob-like projections composed of transmembrane (TM, gp40) and surface unit (SU, gp95) glycoprotein trimers. The SU is anchored to the viral envelope via the TM unit. The mature virion has a conical core composed of major capsid proteins (CA, p24) and surrounds an electron-dense nucleoid consisting of two equal copies of single-stranded genomic RNA (Miyazawa et al., 1994), which are associated with nucleocapsid proteins (NC, p7). Also contained within the virion are the viral protease, reverse transcriptase, dUTPase and integrase (Fig. 1) (Wagaman et al., 1993).

In addition to containing proteins encoded by the viral genome, lentiviruses acquire host cell proteins incorporated into the envelope and internal virion compartments. Many such host proteins have been characterized for HIV, and incorporation of similar proteins likely also occurs during FIV replication. The precise manner in which HIV acquires host proteins is incompletely understood, but it appears to occur in the cytoplasm during viral assembly and at the plasma membrane during budding (Cantin et al., 2005; Ott, 2008). Cells normally generate membrane-bound vesicles from endosomal compartments, which, if released into the extracellular milieu are termed exosomes. Exosomes, in turn, may fuse with neighboring cell membranes and thus contribute to intercellular trafficking. Retroviral proteins alter vesicle formation from endosomal compartments for selective enrichment or exclusion of viral and other proteins, and lipids such as cholesterol (Gould et al., 2003; Nguyen et al., 2003). Intracellular vesicles containing viral components may result in targeting to specific cell membrane areas such as lipid rafts. Budding into plasma membrane-connected intracellular compartments is more prominent in monocyte/macrophages and dendritic cells (DC) than in T cells, suggesting that exosomes containing viral components vary in frequency depending on the host cell origin (Deneka et al., 2007; Welsch et al., 2007; Bennett et al., 2009). Since exosomes may be of similar size as virions, they are frequent contaminants of purified virus preparations.

3. Viral detection assays

Most assays for viral quantification detect the presence of a viral protein or glycoprotein, viral RNA, or viral enzyme such as reverse transcriptase. In general, the assays do not discriminate between intact virions, microvesicles containing virion components, or non-membrane bound viral proteins; hence higher concentrations of viral components may not correlate to higher concentrations of infectious particles. Fig. 2 summarizes the viral replication cycle relative to the detection assays described below.

3.1. p24 ELISA

An ELISA for the detection of the viral core p24 antigen was developed shortly after the discovery of FIV, and has been available commercially until recently (Tilton et al.,