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Cell-specific adaptation of two flaviviruses following serial passage in mosquito cell culture

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Abstract

West Nile Virus (WNV) is a mosquito-borne flavivirus that was introduced into the U.S. in the New York City area in 1999. Despite its successful establishment and rapid spread in a naive environment, WNV has undergone limited evolution since its introduction. This evolutionary stability has been attributed to compromises made to permit alternating cycles of viral replication in vertebrate hosts and arthropod vectors. Outbreaks of a close relative of WNV, St. Louis encephalitis virus (SLEV), occur in the U.S. periodically and are also characterized by limited genetic change overtime. We measured both phenotypic and genotypic changes in WNV and SLEV serially passaged in mosquito cell culture in order to clarify the role of an individual host cell type in flavivirus adaptation and evolution. Genetic changes in passaged WNV and SLEV were minimal but led to increased relative fitness and replicative ability of the virus in the homologous cell line C6/36 mosquito cells. Similar increases were not measured in the heterologous cell line DF-1 avian cells. These phenotypic changes are consistent with the concept of cell-specific adaptation in flaviviruses.

Keywords: West Nile virus; St. Louis encephalitis virus; Flavivirus; Viral fitness; Virus evolution

Introduction

West Nile Virus (WNV) is a mosquito-borne flavivirus that was introduced into the U.S. in the New York City area in 1999. Since its introduction, WNV has spread rapidly across the U.S. and into Canada, Mexico, and Central and South America (Lanciotti et al., 1999; Dupuis et al., 2003; 2005; Austin et al., 2004). Outbreaks of its close relative, St. Louis encephalitis virus (SLEV), occur in the U.S. periodically (Chandler et al., 2001; Day and Stark, 2000). Understanding the adaptability and selective pressures that drive genetic and phenotypic changes in these viruses is crucial to predicting their ability to persist and re-emerge.

Both WNV and SLEV are maintained in nature in enzootic cycles in which they are transmitted between ornithophilic mosquitoes and avian hosts. Non-avian vertebrates become infected as a result of feeding by infected vectors, but virus is generally not perpetuated in this manner, due to the low level of viremia in most non-avian, vertebrate hosts (Kramer and Bernard, 2001). Evolutionary pressures on the virus, therefore, are applied predominately by the mosquito and avian environments.

RNA viruses have the capacity for rapid evolution due to their high mutation rates, short replication times, and large population sizes (Drake and Holland, 1999). Despite this, rapid rates of evolution have not been observed with arboviruses (Cilnis et al., 1996; Jenkins et al., 2002; Weaver et al., 1992). It has been shown, for example, that WNV has undergone limited evolution in the years during which it has been circulating in the U.S. (Davis et al., 2005; Ebel et al., 2001, 2004). The case of WNV is particularly interesting because the virus's genotypic stability has not compromised its ability to succeed in new environments. One hypothesis for this genetic conservation is that the alternate cycles of viral replication in vertebrate hosts and arthropod vectors constrain evolution (Scott et al., 1994; Weaver et al.,

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1992). This implies that compromises in replicative ability are made regularly by virus populations, in both the arthropod vectors and vertebrate hosts, due to differential selection in each. Specifically, mutations exclusively advantageous to either host are purged by purifying selection if they are detrimental to replication in the alternative host; positive selection then generally results from the infrequent mutations that result in coadaptation. Reduced positive selection and increased purifying selection in vector-borne RNA viruses have been reported previously (Holmes, 2003; Jerzak et al., 2005; Woelk and Holmes, 2002). Further phenotypic and genotypic evidence for replicative compromises has been provided by previous studies including those on the flavivirus dengue 2 (Chen et al., 2003) and the togaviruses Sindbis (SINV) (Greene et al., 2005) and eastern equine encephalitis (EEEV) (Cooper and Scott, 2001; Weaver et al., 1999). However, different results were obtained when studies were performed with the rhabdovirus vesicular stomatitis virus (VSV) (Novella et al., 999; Zarate and Novella, 2004).

We assessed individual relative fitness, replicative ability, and genetic alterations of both WNV and SLEV during serial passage in C6/36 cell culture in order to better characterize the evolutionary pressures and adaptive ability of flaviviruses replicating in mosquito cells. Specifically, we sought to clarify the extent to which these flaviviruses are capable of cell-specific adaptation and the degree to which this adaptation alters both fitness in other cell types and genetic sequences. We hypothesized that adaptation to a single cell type would lead to decreases in viral fitness in the bypassed cell. Understanding the spectrum of phenotypic changes and degree of genetic alteration in virus serially passaged in a single host cell type will help us to understand the evolutionary stability observed in virus cycling in nature.

Results

Changes in relative fitness of WNV and SLEV following serial passage in mosquito cells

Competition assays to measure the relative fitness of WNV and SLEV were based on co-infection of three different cell lines with a mixture of test (biologically cloned virus) and control (monoclonal antibody [MAb] resistant mutant; MARM) virus. Growth curves were analyzed to confirm that replication of WNV and SLEV and their respective MARM is similar in each cell line (Fig. 1). Time of virus harvest for competition assays in each cell line was selected based on the time at which the peak titers of the test and control viruses were similar. Consequently, WNV competition assays were carried out for 48, 72, and 96 h post inoculation (hpi) in Vero (mammalian), DF-1 (avian), and C6/36 (mosquito) cells, respectively, and SLEV competition assays were harvested at 96 hpi in C6/36 cells and 48 hpi in DF-1 and Vero cells (Fig. 1). Although titers of SLEV and its MARM began to diverge at 48 hpi in DF-1 cells, the assays were harvested at a time at which neither strain had a significant replicative advantage.

The results of competition assays measuring the relative fitness values of WNV and SLEV in three different cell lines are summarized in Fig. 2. Changes in unpassaged virus: MARM ratios were minimal during competition. Ratios fluctuated slightly in all cell lines for both viruses but were never significantly different from input ratios in any round of competition (data not shown). Values were adjusted such that a relative fitness value of 0 was assigned to these controls. Therefore, relative fitness values accurately represent changes resulting from passaging such that a positive value represents

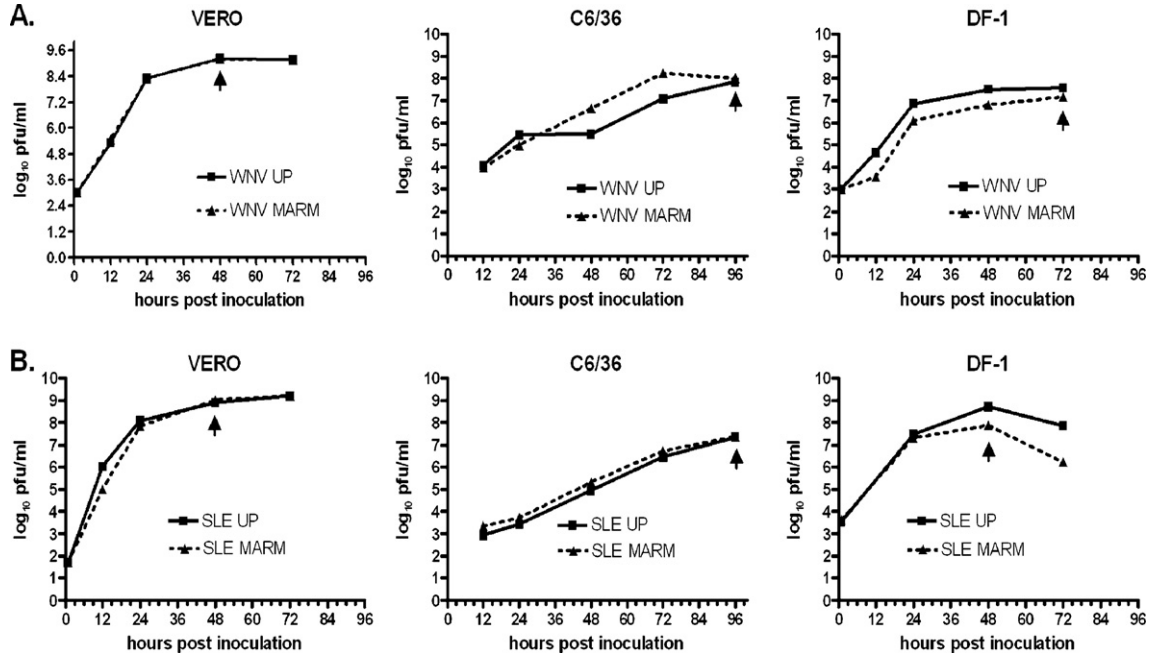

Fig. 1. Replication in different three cell lines of WNV (A) and SLEV (B) unpassaged biological clones (UP) and monoclonal antibody resistant mutants (MARM). Multiplicity of infection for all growth curves is 0.1 PFU/cell. Arrows indicate the time of harvest for competition assays.