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SYMPOSIUM: Herpesvirus Infections of the Nervous System

An Animal Model of Varicella Virus Infection

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Varicella-zoster virus (VZV) causes chickenpox in children; establishes latency in cranial nerve, dorsal root, and autonomic ganglia; and reactivates decades later to produce zoster. VZV produces disease only in humans. Although attempts to produce disease and study VZV latency in experimentally infected animals have resulted in virus in trigeminal or dorsal root ganglia, no clinical signs of infection or reactivation developed. In contrast, simian varicella virus (SVV) produces a naturally occurring exanthematous disease in non-human primates that mimics human varicella. Experimental inoculation of non-human primates causes similar, if not identical, clinical and pathological changes observed in monkeys naturally infected with SVV. Like VZV, SVV becomes latent in ganglia and reactivates, often with entire body rash. SVV and VZV encode antigenically related polypeptides. Both virus genomes have been sequenced and shown to be colinear, sharing up to 75% DNA homology. During latency, an SVV homolog of one of the five VZV genes transcribed in latently infected human ganglia has been detected in monkey ganglia. Preliminary studies in which monkeys were inoculated intratracheally with SVV revealed the presence of viral DNA and RNA in multiple tissues, including blood mononuclear cells, months after experimental infection. These findings differed from the expected restricted localization of the virus DNA to ganglia only and the expected limited viral gene expression, and probably reflect the high virus load delivered intratracheally compared to natural SVV infection in monkeys. Nevertheless, clinical, pathological, and molecular similarities between SVV and VZV indicate that SVV infection in non-human primates has considerable potential as an animal model for human varicella.

Introduction

Varicella-zoster virus (VZV) causes chickenpox (varicella) in children. After primary infection, VZV becomes latent in cranial, dorsal root and autonomic ganglia (11) and reactivates decades later to produce zoster, primarily in the elderly. Zoster, characterized by pain and a vesicular rash involving 1-3 dermatomes, is common, with more than 300,000 new cases annually in the United States. The incidence after age 60 is 8-to-10-fold greater than in people under 60 (15). As the American population ages, the incidence of zoster-associated morbidity and mortality among the elderly will increase. Furthermore, between 40-45% of zoster patients over age 60 develop postherpetic neuralgia (PHN), pain that persists more than 4 to 6 weeks after acute zoster. PHN is the most common complication of zoster. Zoster patients also develop stroke from large vessel granulomatous arteritis, encephalitis from diffuse small vessel arteritis, myelitis, zoster paresis, and even pain without rash (zoster sine herpete). These neurologic complications of zoster are also increased in the rapidly aging and immunocompromised, especially AIDS, populations.

Other closely related viruses such as herpes simplex types 1 and 2 have been used in experimental animal models to study latency and pathogenesis. However, VZV produces disease only in humans. Thus, studies on VZV pathogenesis, latency, and reactivation have been hampered by the lack of a useful animal model. Attempts to produce disease by experimental infection of animals have led to seroconversion without clinical signs (22, 26, 27, 37, 42). Experimental subcutaneous inoculation of the Oka VZV (vaccine strain) into the breast of chimpanzees produces viremia and mild rash restricted to the site of inoculation (5). VZV DNA was detected in blood mononuclear cells during the 10-day incubation period. Although the mild varicella observed resembles the low-level infection seen in some children vaccinated with VZV, latency was not studied in this model. Attempts to study VZV latency in guinea pigs after corneal inoculation revealed virus in trigeminal ganglia by electron microscopy during acute infection (29), but no reactivation. This indicated that guinea pigs are less than an ideal model to study VZV latency.

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Features	VZV	SVV
Primary infection		
incubation period	7-21 days	7-9 days
rash	vesicular	vesicular
dissemination	rare	frequent
mortality	rare	frequent
Latency		
site	ganglia	ganglia
viral burden	5-260 genomes/10^5 cells	unknown
transcription	genes 21, 29, 62, 63	gene 21;others yet to be analyzed
translation	genes 4, 21, 29, 62, 63	unknown
Reactivation	yes	yes
Viral immunology	cross-reactivity to SVV	cross-reactivity to VZV
Cell culture	cell-associated	cell-associated
	low titer	low titer
	multinucleated foci	multinucleated foci
Genomic characteristics		
structure	double-stranded linear	double-stranded linear
size	124,884 bp	124,139 bp
molecular weight	80 x 10^6 daltons	80 x 10^6 daltons
density (G + C)	46.0%	40.4%

Table 1. Clinical and virologic features of VZV infection in humans and SVV infection in primates.

Additionally, non-specific dermatitis has been shown to develop in some animals (25). Adult rat neurons of dissociated ganglia were initially shown to be permissive for VZV after *in vitro* infection. VZV immediate-early, early, and late gene transcripts were detected, but the VZV immediate-early gene 63 was most abundant (23). In adult rats experimentally infected with VZV, no clinical symptoms were observed, but viral nucleic acid and protein were detected in neurons of dissociated dorsal root ganglia at multiple intervals for 9 months after inoculation (33). However, these ganglia were cultured 3 to 12 days before analysis, and it is not clear whether the data reflect latency or reactivation of VZV due to culture conditions. A later study of rats experimentally infected with VZV revealed VZV gene 63 protein only in neurons of latently infected dorsal root ganglia (7). In contrast to the above studies, Annunziato *et al.* (1) detected VZV DNA in both satellite cells and neurons of dorsal root ganglia from latently infected rats harvested 1 and 3 months after inoculation. Thus, rats may be suited to some studies of VZV latency, but not reactivation.

Finally, a severe combined immunodeficient (SCID-hu) mouse model that carries human fetal thymus and liver implants (SCID-hu) demonstrated the T cell tropism of VZV (24). Infectious VZV was recovered from human lymphocytes circulating in SCID-hu mice up to 21 days after infection. Failure to recover infectious virus correlated with a decline in CD4+ and CD8+ cells. FACS analysis of VZV-infected human T cells from the thymus/liver implants revealed viral proteins in CD4+, CD8+ and CD4+CD8+ cells. The disadvantage of this model is that the implanted tissue is infected externally rather than by viremia.

Simian varicella virus

Simian varicella virus (SVV) produces a naturally occurring exanthematous disease of non-human primates that mimics human varicella (28, 36). Acute clinical (25, 28) and pathological (8, 28, 39) changes produced by SVV infection of non-human primates are similar to those in human varicella (Table 1). Simian varicella is characterized by an incubation period of one or more weeks followed by fever and a papulovesicular rash of skin and mucous membranes. However, naturally occurring simian varicella is typically more severe than human varicella, resulting in a high mortality rate (36). Like VZV, SVV infection causes viremia, and infectious virus can be recovered from blood mononuclear cells (4, 36, 41). Occasionally, rash becomes hemorrhagic, a poor prognostic sign (34). SVV infection frequently disseminates. Lung and liver are the most severely affected organs, similar to the findings in disseminated human varicella in immunosuppressed patients (32). Histologic examination of skin and viscera from primates with varicella reveals foci of hemorrhagic necrosis and inflammation, with eosinophilic intranuclear inclusions (4, 41). Like VZV, SVV becomes latent in sensory ganglia at multiple levels of the neuraxis (17, 18). To date, latency has only been observed in monkeys experimentally infected with SVV. Ganglia from naturally infected monkeys await analysis.

Evidence for reactivation in SVV-infected monkeys is limited, but reactivation has been observed in monkeys exposed to social and environmental stress (36). Both the 1968 and 1974 outbreaks of varicella in *Erythrocebus patas* monkeys at the Delta Regional Primate Research Center were attributed to reactivated SVV (34). Although VZV reactivation in humans (zoster) is generally localized to 1-3 dermatomes, SVV reactivation often appears as a whole-body rash. Limited forms of zoster in monkeys are often obscured by fur, and the duration of rash is generally less than one week (K. Soike, personal communication). VZV has been isolated from zoster lesions (38) and SVV has been isolated from skin vesicles after reactivation (36). Neither virus has been isolated from blood in otherwise healthy immunocompetent humans or non-human primates, respectively.

SVV and VZV encode antigenically related polypeptides, and SVV-specific antibodies cross-react with human VZV in serum neutralization and complement fixation tests (Table 1) (9, 10, 35). Although VZV does not cause disease in non-human primates, it has been used to immunize and protect monkeys from SVV infection (9). To date, there is no evidence that SVV can infect or cause disease in humans. However, because nearly all adults in North America are VZV-seropositive and because VZV can immunize and protect monkeys from SVV infection, it seems likely that humans exposed to VZV are protected against SVV infection.

Molecular virology

Both VZV and SVV are enveloped, double-stranded DNA viruses. The genomes of both viruses are colinear (30, 40), similar in size, and share 70-75% DNA homology (3, 6, 12, 13) (Table 1). The entire SVV genome has been sequenced (14). It is 124,139-base pairs (bp) in size, 745-bp shorter than VZV DNA, and its G+C content is 40.4%. SVV DNA contains a 104,104-bp unique long (U_L) component bracketed by 8-bp inverted repeats (IR_L) and a unique short (U_S) component composed of a 4909-bp region bracketed by 7557-bp inverted repeats (IR_S). Each of the 69 SVV open reading frames (ORFs) shares extensive homology to corresponding VZV genes. The only major difference between SVV and VZV DNA is found at the leftward terminus (14), where SVV lacks a VZV ORF 2 homolog and encodes an 882-bp ORFA that is absent in VZV, but has over 40% identity to the SVV and VZV ORF 4 (21). Like VZV, SVV and transfected SVV DNA produce a cytopathic effect in tissue culture, characterized by the formation of syncytia preceding cell lysis, a low virus titer, and cell-associated virus (3, 34, 36).

Experimental infection

The clinical, pathological, and molecular virological similarities between SVV and VZV observed to date have provided a basis for the use of SVV infection of non-human primates as an animal model for human VZV infection. Experimental inoculation of non-human primates with SVV causes the same clinical and pathological changes seen in naturally infected monkeys (8, 16, 31, 39). After primary infection, SVV-specific antigen and nucleic acid can be detected in liver, lung, spleen, adrenal gland, kidney, lymph node, bone marrow, and ganglia at all levels of the neuraxis (8, 28, 31, 39). Experimental SVV infection causes viremia, and infectious virus can be recovered from blood mononuclear cells starting at day 2 post-inoculation until day 11 (8, 16, 36, 39). Like other organs, monkey ganglia become infected with SVV before rash appears. We have shown that SVV enters the ganglia as early as 6 days post-infection, and that intravenous infection results in a higher proportion of infected ganglia (63%) than after intratracheal inoculation (13%), pointing to the role of hematogenous spread in ganglionic infection (19).

Ganglia from monkeys that recover from primary SVV-induced varicella are latently infected. Using PCR, we detected SVV DNA in trigeminal, cervical, and thoracic ganglia, but not in brain, at 5 months after primary SVV infection of a 6-year-old female African green monkey (18). The detection of various regions of the SVV genome and at multiple levels of the neuraxis, parallels VZV latency in humans (18, 20). Furthermore, the SVV homolog of the VZV gene 21 transcript (one of five VZV genes known to be transcribed in latently infected human ganglia) has been found in latently infected monkey ganglia (2). Other SVV-specific transcripts expressed during latency await analysis.

To study SVV pathogenesis further, we recently inoculated adult African green monkeys intratracheally with 10^3-10^4 plaque-forming units of SVV, and searched for evidence of the virus. Surprisingly, we found that in contrast to a latent infection, the experimental inoculation conditions resulted in the presence of viral DNA and RNA in multiple organs, including blood, for months. SVV-specific DNA and transcripts corresponding to immediate-early, putative early and late SVV ORFs were found in liver, lung and ganglia of most monkeys at multiple intervals during a 12 month study period. SVV DNA was also detected in blood mononuclear cells from these experimentally infected monkeys at multiple intervals for up to 2 years (unpublished results), indicating that these animals were viremic. Further analysis of SVV-infected blood mononuclear cells revealed that CD4+ and CD8+, but not CD14+ and CD20+ cells harbored the viral genome. SVV-specific transcripts, infectious virus, and SVV viral particles were not found in blood mononuclear cells, suggesting that infection is either abortive or below the level of detection.

These results most likely reflect the high virus load delivered intratracheally compared to natural SVV infection in monkeys. Inoculation with a high titer of virus by this route does not lead to varicella latency as it is currently understood in humans, *i.e.* viral DNA restricted to ganglia and viral transcription limited to 4-5 genes. Ganglia and other tissues from experimentally infected monkeys must be compared virologically with

those of SVV-seropositive monkeys months after naturally occurring infection. Used properly, SVV infection in non-human primates has considerable potential as an animal model that is likely to further our understanding of human varicella.

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