Replication of two porcine parvovirus isolates at non-permissive temperatures

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Accepted March 31, 1990

Summary. Previous studies have shown that replication in vitro of the porcine parvovirus (PPV) isolate, KBSH, was restricted at 39 °C but not at 37 °C. In contrast, replication of the Kresse isolate was restricted at 37 °C but not at 39 °C. In this study, Kresse and KBSH isolates were passaged up to ten times in swine testicle (ST) cells at non-permissive temperatures, and at subsequent passage viral protein synthesis, viral DNA synthesis, and progeny virus were evaluated. KBSH became adapted for replication at 39 °C upon serial passages, displaying an appreciable increase in viral progeny, viral polypeptides, and viral DNA concentration. This finding was also observed with Kresse virus isolate continuously passaged at 37 °C. Neither isolate became adapted for replication at 32 °C. In an attempt to examine the effect of in vitro passage at non-permissive temperatures on pathogenicity in swine, KBSH passaged 10 times either at 37 °C or 39 °C was inoculated into swine fetuses. Two of four fetuses inoculated with 39 °C-passaged KBSH were dead and hemorrhagic or mummified. All four fetuses inoculated with 39 °C-KBSH contained viral antigen and viral DNA. In contrast, fetuses inoculated with 37 °C-passaged KBSH, or with cell culture fluid were normal in appearance. Viral antigen and viral DNA were not demonstrated in fetuses inoculated with 37 °C-KBSH or cell culture fluids. These findings suggest the possibility that the ability to replicate at 39 °C is associated with virulence in swine fetuses.

Introduction

Porcine parvovirus (PPV), a member of the autonomous parvoviruses, causes reproductive failure in susceptible pregnant sows [14, 18]. A number of isolates of PPV have been made worldwide, some of which show obvious differences in pathogenicity [7, 16, 21].

In a previous study, four PPV isolates, NADL-8, NADL-2, KBSH, and Kresse, were compared for their replicative properties in vitro at either 32 °C,
37 °C, or 39 °C to explain the differences observed in the replication of these isolates in swine [5]. NADL-8 and NADL-2 are isolates pathogenic only to mid-term gestation fetuses [18, 23] when inoculated in utero. These two isolates showed similar replication patterns at 32 °C, 37 °C, and 39 °C. However, replication of KBSH, an isolate which is not pathogenic to swine fetuses [21], was restricted at 39 °C, but not at 32 °C or 37 °C. The replication of Kresse, an isolate which is pathogenic both to mid- and late-term fetuses as well as to young pigs [3, 16], was favored at 39 °C and restricted at 32 °C and 37 °C.

Temperature sensitive (ts) mutants have been described for pseudorabies virus [2, 8], measles virus [6], coxsackie virus B3 [10], fowl plague virus [22], and paroviruses, H-1 [26], Kilham rat virus [27], and PPV [9, 13]. H-1 parovirus ts mutant synthesizes a nonsense capsid protein defective in hemagglutination and exhibits decreased synthesis of viral DNA, but not RF-DNA [26]. Ts mutants isolated from nitrous acid-treated KRV [27] show two distinct groups, one restricted in single stranded (SS) viral DNA and capsid protein production and the other restricted in accumulating RF-DNA. Low temperature adapted ts PPV mutants were shown to be effective when employed as modified live vaccines [9, 13]. Revertants of ts mutants have also been described and a possible mechanism for revertants has been suggested in various viruses [1, 15, 17, 24].

In order to understand the relationship between permissive temperatures for virus replication and their pathogenicity in vivo, virus isolates were serially passaged in vitro at non-permissive temperatures. Kresse and KBSH isolates were serially passaged at non-permissive temperatures, 37 °C or 39 °C. These passaged viruses were subsequently evaluated for their abilities to replicate in vitro in ST cells and in vivo following in utero exposure to swine fetuses. An important consideration in this study is that the mean body temperature of swine is 39.2 °C in difference to 36.8 °C for humans.

Materials and methods

Cells and viruses

An established cell line, swine testicle (ST) cells, was employed throughout this study for the propagation of PPV in vitro. Conditions for the culture of ST cells were followed as previously described [20]. The isolates of PPV chosen for this study included KBSH, obtained from Dr. P. Tattersall (Yale University, New Haven, CT) and Kresse from Dr. J. Kresse (NVSL, Ames, IA). KBSH, previously passaged approximately 300 times in KB cells at 37 °C [11], had been propagated twice in ST cells at 37 °C in our laboratory before use in this study. Kresse, originally isolated from skin lesions of young pigs [15], was propagated twice in ST cells and once in swine fetuses. Using these isolates, stock virus was prepared in ST cells at 37 °C as previously described [5].

Continuous passages of isolates at non-permissive temperatures

Kresse and KBSH isolates were passaged in ST cells either at 32 °C, 37 °C, or 39 °C and changes in virus progeny and virus antigen were evaluated. ST cells seeded at $1 \times 10^6$ cells per 25 cm$^2$ flask were infected at an m.o.i. of 1. Infected cells were harvested at 5 days