African swine fever (ASF) is a highly lethal, hemorrhagic disease of domestic pigs for which animal slaughter and area quarantine are the only methods of disease control. African swine fever virus (ASFV), the causative agent of ASF, is a large, enveloped, double-stranded DNA virus which is the only member of the newly named Asfarviridae (11). Although the icosahedral morphology of the ASFV virion resembles that of icosahorviruses, both the genomic organization, which includes terminal cross-links and inverted terminal repeats, and its cytoplasmic replication strategy suggest some relationship with the Poxviridae (16, 28, 41).

In domestic pigs, ASF occurs in several disease forms, ranging from highly lethal to subclinical infections, depending on contributing viral and host factors (7, 23, 34). ASFV infects cells of the mononuclear-phagocyte system, including fixed-tissue macrophages, and specific lineages of reticular cells in the spleen, lymph node, lung, kidney, and liver (7, 21–23, 25). This ability to replicate and induce marked cytopathology in these cell types in vivo appears to be critical for ASFV virulence. The viral and host factors responsible for the differing outcomes of infection with highly virulent strains and strains of lesser virulence are largely unknown.

ASFV is the only known DNA arbovirus (6, 8). The perpetuation and transmission of this virus in nature involve the cycling of virus between two highly adapted hosts, Ornithodoros ticks and wild pigs (warthogs and bushpigs) in Sub-Saharan Africa (30, 31, 45, 49). In areas of ASF enzooticity, adult warthogs are typically nonviremic, although most are seropositive (19, 29, 31, 40, 44), and virus has been isolated from lymph nodes (19, 31). Young warthogs are most likely infected through feeding by infected Ornithodoros porcinus porcinus ticks. Infection in young warthogs is subclinical, with viremic titers ranging from 2 to 3 log$_{10}$ 50% hemadsorption doses (HAD$_{50}$)/ml (45, 46), a level sufficient to infect a low percentage of naïve ticks (33, 44). The sylvatic ASFV cycle is further maintained by transovarial and venereal transmission in ticks (32, 37). The mechanism of ASFV transmission from the sylvatic cycle to domestic pigs is most likely through infected Ornithodoros ticks feeding on pigs (31, 44), since direct contact with infected warthogs rarely results in transmission to pigs (10, 19, 24, 44).

Previous studies have described experimental infections of O. porcinus porcinus ticks with different ASFV isolates (17, 18, 20, 33, 36). Infection is characterized by the establishment of a long-term, persistent infection with relatively high levels of virus replication occurring in a number of different tissues and organs. These data suggest that ASFV infection of its natural arthropod host represents a well-adapted and possibly co-evolved biological system. However, differences in infection rates, infectious doses, and persistent infections have been observed when ticks were exposed to different ASFV isolates (17, 33). The viral and host factors and the mechanisms responsible for the differing outcomes of infections with different ASFV isolates are unknown.

ASFV is genetically complex; its genome of 170 to 190 kbp contains 160 or more open reading frames (ORFs), and ap-
proximately 100 proteins have been observed in virus-infected cells (2, 8, 12, 13, 39, 43). The ASFV genome contains a central conserved region and variable terminal regions (5, 16, 41, 42). Swine virulence and host range genes, including the NL, UK, and MGF genes, have been identified in the terminal variable regions of the ASFV genome. The terminal variable regions comprise the left 46-kbp and the right 12.5-kbp ends of the genome and contain at least five multigene families (MGFs): MGF100, MGF110, MGF300, MGF360, and MGF530 (3, 4, 9, 15, 47, 50).

MGF360 and MGF530 genes do not show similarity to other genes or motifs in current databases. Individual MGF genes are conserved among ASFV isolates (with 45 to 100% corresponding amino acid identity), and they are transcribed early in infection (38, 50). The functions of individual MGF genes or the different multigene families in virus-host interactions remain poorly understood.

It was recently shown that MGF360 and MGF530 genes perform an essential macrophage host range function that involves the promotion of infected-cell survival (52), and Neilan et al. reported that MGF360 and MGF530 genes also function in pig virulence (27).

Here, we show that ASFV MGF360 genes are significant tick host range determinants and are required for the replication and generalization of infection in the tick host. Impaired virus replication of the MGF360 gene deletion mutant Pr4Δ3-C2 in the tick midgut likely accounts for the absence of the generalized infection that is necessary for the natural transmission of virus from tick to pig.

MATERIALS AND METHODS

Cell cultures and viruses. Primary porcine blood macrophage cell cultures were prepared from debrinated swine blood as previously described (14). Briefly, heparin-treated swine blood was incubated at 37°C for 1 h to allow sedimentation of the erythrocyte fraction. Mononuclear leukocytes were separated by flotation over a Ficoll-Paque (Pharmacia, Piscataway, N.J.) density gradient (1.079 specific gravity). The monocyte/macrophage cell fraction was cultured in plastic Primaria tissue culture flasks (Falcon; Becton Dickinson Labware, Franklin Lakes, N.J.) containing RPMI 1640 medium with 30% L929 supernatant and 20% fetal bovine serum for 48 h (37°C in 5% CO2). Adherent cells were detached from the plastic by using 10 mM EDTA in phosphate-buffered saline and then reseeded into Primaria T25 6- or 96-well dishes at a density of 5 × 104 cells per ml for use in assays 24 h later.

The pathogenic ASFV strain Pretoriuskop96/4 (Pr4) was isolated from O. porcinus porcinus ticks collected from warthog burrows in Kruger National Park, Republic of South Africa, in 1996 (20). The ASFV gene deletion mutant Pr4Δ35 was constructed as previously described (52).

Construction of recombinant ASFV Pr4 viruses containing deletions in MGF360 and MGF530 genes. Gene deletion mutants Pr4Δ3-C1, Pr4Δ3-C2, Pr4Δ5-1, and Pr4Δ5-2 were generated by homologous recombination of ASFV Pr4 genomes and recombinant transfer vectors following infection and transfection of macrophage cell cultures (53). Flanking DNA fragments to the left and right of the MGF360 and MGF530 ORFs were amplified by using primer sets, each of which introduced a BamHI restriction site adjacent to the MGF360/530 ORFs and a BglII restriction site (left- or right-flank fragment) at the opposite end. The primer set for Pr4ΔΔ-C1 was as follows: left-flank forward primer, 5’-TGCTTAAAGATCCCTCTTGACTCCT-3’; left-flank reverse primer, 5’-GGATCCACTCATGTGTTAAAAAGATATACCTC-3’; right-flank forward primer, 5’-GGAGGATCCAGGGGAAGGCAACATCGT-3’; and right-flank reverse primer, 5’-AAAGATCTCGTCCCTCTTCCTACCGT-3’. The primer set for Pr4Δ5-2 was as follows: left-flank forward primer, 5’-TTGCTTAAAGATCCCTCTTGACTCCT-3’; left-flank reverse primer, 5’-GGATCCACTCATGTGTTAAAAAGATATACCTC-3’; right-flank forward primer, 5’-GGAGGATCCAGGGGAAGGCAACATCGT-3’; and right-flank reverse primer, 5’-AAAGATCTCGTCCCTCTTCCTACCGT-3’. The primer set for Pr4ΔΔ-C2 was as follows: left-flank forward primer, 5’-AAAGATCTCGTCCCTCTTCCTACCGT-3’; right-flank forward primer, 5’-GGAGGATCCAGGGGAAGGCAACATCGT-3’; and right-flank reverse primer, 5’-AAAGATCTCGTCCCTCTTCCTACCGT-3’. The primer set for Pr4Δ5-2 was as follows: left-flank forward primer, 5’-AAAGATCTCGTCCCTCTTCCTACCGT-3’; right-flank forward primer, 5’-GGAGGATCCAGGGGAAGGCAACATCGT-3’; and right-flank reverse primer, 5’-AAAGATCTCGTCCCTCTTCCTACCGT-3’.

\[ \text{p7GUS} \]

FIG. 1. Characterization of ASFV MGF360 and MGF530 gene deletion mutants Pr4Δ3-C1, Pr4Δ5-1, Pr4Δ3-C2, Pr4Δ5-2, and Pr4Δ35. (A) Diagram of MGF360 and MGF530 gene regions in parental Pr4 and deletion mutants. Transfer vectors and recombinants with gene deletions were constructed as described in Materials and Methods. LVR, left variable region; CVR, central variable region; RVR, right variable region. (B) Growth characteristics of ASFV Pr4 and recombinant viruses Pr4Δ3-C1, Pr4Δ5-1, Pr4Δ3-C2, Pr4Δ5-2, and Pr4Δ35 in swine macrophage cell cultures. Primary macrophage cell cultures were infected (MOI = 1), and at the indicated times postinfection, duplicate samples were titrated for virus yield. These data are the means of results of two independent experiments. TCID50, 50% tissue culture infectious dose.

\[ \text{p7GUS} \]
TABLE 1. ASFV titers in O. porcinus porcinus ticks following oral inoculation

<table>
<thead>
<tr>
<th>Expt and virus&lt;sup&gt;a&lt;/sup&gt;</th>
<th>1 DPI</th>
<th>7 DPI</th>
<th>14 DPI</th>
<th>21 DPI</th>
<th>28 DPI</th>
<th>35 DPI</th>
<th>56 DPI</th>
<th>77 DPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pr4</td>
<td>3.8 ± 0.3</td>
<td>5.8 ± 0.4</td>
<td>6.0 ± 0.5</td>
<td>7.0 ± 1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr4Δ35</td>
<td>3.9 ± 0.2</td>
<td>3.9 ± 0.3 (66)</td>
<td>4.0 ± 0.2 (66)</td>
<td>4.8 ± 0.6 (66)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Expt 2

| Pr4                      | 2.9 ± 0.3 | 5.5 ± 0.2 | 6.3 ± 0.1 |
| Pr4Δ5-1                 | 2.5 ± 0.3 | 4.8 ± 0.2 | 6.0 ± 0.1 |
| Pr4Δ5-2                 | 2.5 ± 0.2 | 6.2 ± 0.1 | 6.4 ± 0.1 |
| Pr4Δ3-C1                | 2.3 ± 0.7 | 4.8 ± 0.4 | 6.6 ± 0.0 |
| Pr4Δ3-C2                | 2.8 ± 0.3 | 3.2 ± 0.1 (63) | 4.3 ± 0.1 (75) |

Expt 3

| Pr4                      | 2.8 ± 0.4 | 4.7 ± 0.1 | 4.2 ± 0.1 | 6.3 ± 0.2 |
| Pr4Δ3-C2                | 3.3 ± 0.2 | 4.0 ± 0.2 | 3.1 ± 0.1 | 4.3 ± 0.1 |

Expt 4

| Pr4                      | 4.9 ± 0.1 | 5.4 ± 0.1 | 6.8 ± 0.2 |
| Pr4Δ3-C2                | 4.9 ± 0.9 | 4.0 ± 0.2 | 5.0 ± 0.4 |

<sup>a</sup> A virus dose of 10<sup>6</sup> HAD<sub>50</sub>/ml was used for each experiment except experiment 4, in which a dose of 10<sup>8</sup> HAD<sub>50</sub>/ml was used.

as products of a double-crossover recombination event by using PCR and Southern blot analyses, as described previously (51, 53).

Infection of ticks. Groups of O. porcinus porcinus ticks (48) were exposed to ASFV Pr4 wild-type or recombinant viruses by allowing them to feed on an artificial membrane feeder placed in heparinized pig blood containing virus with a known titer. Ticks were allowed to feed to repletion before being removed from the membrane and placed in a holding container. Only fully fed ticks were used for subsequent experiments.

Virus titration. Individual whole ticks were ground in 0.5 ml of RPMI 1640 medium supplemented with twice the normal level of antibiotics in sterile tubes with plastic pestles (Pellet; Kontes). Samples were stored at −70°C. Immediately prior to titration, the samples were thawed at 37°C, sonicated for 1 min, and centrifuged for 3 min at 10,000 × g. Supernatants were serially diluted and then added to porcine macrophage cell cultures. Titers were calculated by the method described above.

Reverse transcription-PCR analysis. A group of stage N1 ticks (n = 2000) was exposed to blood meals containing 10<sup>3</sup> HAD<sub>50</sub> of Pr4 or Pr4Δ3-C2 virus/ml. At 10 days postfeeding, ticks were snap-frozen in liquid nitrogen, and total RNA was extracted by using a ToTALLY RNA kit (Ambion, Austin, Tex.). Poly(A) RNA was purified with a MicroPoly(A) kit (Ambion) and reverse transcribed with a RETROscript kit (Ambion) according to the manufacturer’s instructions. The resulting cDNAs were then amplified by PCR for 30 cycles (94°C for 10 s; 50°C for 1 min; 60°C for 1 min) with a final 10-min incubation at 72°C. The primers used were as follows: 3HL forward primer, 5′-GGTTCGTGTTAGTGCAGG-3′; 3HL reverse primer, 5′-TCGGACCAAAAGGTGTTGGG-3′; 3IL forward primer, 5′-TGCCAYCTCATTAAGATCCTG-3′; 3IL reverse primer, 5′-CGCGCTGAAAAACGTGCTTACTA-3′; and 3IL reverse primer, 5′-GTGGTTGCTTTACAAAAAGTGTTG-3′.

Amplified products were run on a 0.7% agarose gel under standard conditions and cloned into the TA cloning vector pCR 2.1 (Invitrogen). Eight independent clones of each amplification product were completely sequenced with an Applied Biosystems 377 automated DNA sequencer.

Ultrastructural analysis. Nympath ticks (stage N3) were orally exposed to high titer (10<sup>6</sup> HAD<sub>50</sub>/ml) of Pr4Δ3-C2 and Pr4Δ3-C3. At 7, 21, 42, and 90 days postfeeding, three ticks from each group were processed for transmission electron microscopy as previously described (20). Seventy- to 90-nm sections were collected on single-slot grids coated with Formvar, stabilized with carbon (Electron Microscopy Sciences, Fort Washington, Pa.).

FIG. 2. TID<sub>50</sub> of Pr4 and MGF360/530 gene deletion mutants. N1 ticks (n = 8) were fed blood meals containing serial 10-fold dilutions of ASFVs, and virus isolation and/or titration was performed at 28 DPI. TID<sub>50</sub> were expressed as log<sub>10</sub> HAD<sub>50</sub> of virus per milliliter that resulted in a 50% infection rate.

FIG. 1. Percent infected at 10<sup>d</sup> HAD<sub>50</sub> /ml.

TABLE 2. Virus titers in tissues from O. porcinus porcinus ticks infected with Pr4 and MGF360/530 gene deletion recombinants

<table>
<thead>
<tr>
<th>Source of tissue samples (n = 3)</th>
<th>Mean virus titer in tissue (log&lt;sub&gt;10&lt;/sub&gt; HAD&lt;sub&gt;50&lt;/sub&gt;/ml) ± SEM of virus strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pr4</td>
<td>Pr4Δ3-C1</td>
</tr>
<tr>
<td>Hemolymph&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.26 ± 0.12</td>
</tr>
<tr>
<td>Midgut&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.32 ± 0.40</td>
</tr>
<tr>
<td>Reproductive tissue&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.26 ± 0.48</td>
</tr>
<tr>
<td>Salivary gland&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.26 ± 0.35</td>
</tr>
<tr>
<td>Salivary fluid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.66 ± 0.10</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sample taken at 42 days postfeeding.

<sup>b</sup> Tissue dissected at 108 days postfeeding.

<sup>c</sup> Sample taken at 267 days postfeeding.
tron Microscopy Sciences, Fort Washington, Pa.), and photographed with a Philips 410 electron microscope operated at 80 kV. At least five different sagittal sections from each of the three ticks were examined. The ticks were sampled most extensively from the anterior hemocoel, which contained large portions of the midgut, salivary glands, and coxal glands.

RESULTS

Construction and growth characteristics of recombinant ASFV MGF360 and MGF530 gene deletion mutants in porcine macrophage cell cultures. ASFV MGF360 and MGF530 gene deletion mutants were constructed from the pathogenic African isolate Pr4 by homologous recombination of the parental genome and engineered recombination transfer vectors in primary swine macrophage cell cultures, as described in Materials and Methods. The deletions introduced into Pr4 removed genomic regions from the left variable region, which contained members of the MGF360 and/or MGF530 genes, and inserted in their place a 2.4-kbp p72GUS reporter gene cassette (Fig. 1A). Deletion mutant Pr4Δ35 was constructed by deleting a 10,163-bp region from Pr4 as previously described (52). The deletion removed six MGF360 ORFs (3CL, 3DL, 3EL, 3HL, 3IL, and 3LL) and two MGF530 ORFs (3FR and 3NR). In Pr4Δ5-1 and Pr4Δ5-2, the deletions removed a single MGF530 ORF, 3FR and 3NR, respectively. Recombinant viruses Pr4Δ3-C1 and Pr4Δ3-C2 contained deletions of three MGF360 genes, 3CL, 3DL, and 3EL and 3HL, 3IL, and 3LL, respectively. Recombinant viruses expressing GUS were obtained at a frequency of approximately 1 in 5,000. Independent primary plaques were purified to homogeneity. Genomic DNAs from parental virus and deletion mutants were analyzed by Southern blotting and PCR to characterize genomic changes. Results obtained verified the predicted genomic structure of the recombinant Pr4 viruses and showed that the mutants were free of contaminating parental virus (data not shown).

The growth kinetics and viral yields of ASFV Pr4 MGF360 and MGF530 gene deletion mutants were compared to those of the Pr4 parental virus by infecting primary porcine macrophage cell cultures (MOI = 1). Unlike recombinant virus Pr4Δ35, which exhibited a significant 100- to 1,000-fold growth defect compared with the growth of the parental strain Pr4 (52), deletion mutants Pr4Δ3-C1, Pr4Δ3-C2, Pr4Δ5-1, and Pr4Δ5-2 exhibited unaltered growth characteristics in macrophage cell cultures (Fig. 1B).

MGF360 genes 3HL, 3IL, and 3LL affect growth of ASFV in O. porcinus porcinus ticks. To determine the roles of ASFV MGF360 and MGF530 genes in tick host range, Ornithodoros ticks were infected with mutants Pr4Δ35, Pr4Δ3-C1, Pr4Δ3-C2, Pr4Δ5-1, and Pr4Δ5-2. Nymphal O. porcinus porcinus ticks (stages N1 and N3) were fed blood meals containing 10^6 HAD_{50} of mutant or parental ASFV/ml. At various times postinfection, whole ticks (n = 8) were titrated for infectious virus. Pr4Δ35 exhibited a reduced infection rate (66 versus 100% for parental virus) and a significant growth defect in Ornithodoros ticks (Table 1, experiment 1). In contrast to parental virus Pr4, where a 100-fold increase over the initial titer was observed at 3 weeks postinfection, viral titers in Pr4Δ35-infected ticks showed no increase at 5 weeks postinfection. Low levels of Pr4Δ35 replication (~10^3 HAD_{50}/ml) were detected at 77 days postinfection (DPI); however, these titers were 100-fold lower than those observed for Pr4-infected ticks. These data indicate that ASFV MGF360 and MGF530 genes contained within this region affect viral replication in O. porcinus porcinus ticks.

To further define specific MGF360/530 genes within the left variable region of the ASFV genome responsible for this growth defect, viral deletion mutants lacking individual MGF360 genes or multiple MGF360 genes were tested for their ability to infect ticks. Groups of N1 ticks were infected with 2 x 10^6 HAD_{50} of either recombinant virus Pr4Δ3-C1, Pr4Δ3-C2, Pr4Δ5-1, or Pr4Δ5-2 or parental virus Pr4 per ml. Titration of individual whole ticks (n = 8) at 1, 28, and 56 DPI demonstrated indistinguishable growth characteristics for wild-type Pr4 and deletion mutants Pr4Δ3-C1, Pr4Δ5-1, and Pr4Δ5-2 (Table 1, experiment 2). In these groups, mean virus titers increased to approximately 5 log_{10} HAD_{50}/ml at 28 DPI, reaching a peak titer of 6.5 log_{10} HAD_{50}/ml at 56 DPI. In contrast, MGF360 gene deletion mutant Pr4Δ3-C2 exhibited a

![FIG. 3. (A) Low-power electron micrograph of an N2 tick midgut after exposure to a blood meal containing 10^6 TCID_{50} of Pr4/ml at 3 weeks postfeeding. Two ASFV-infected PDC with virus factories (arrowheads) are present in the midgut lumen. Free granules (large arrows) are remnants of disrupted PDC. (B) A high-magnification view of the infected PDC attached to the midgut wall with an extensive virus factory (VF) and mature virus particles (arrowhead). Bars, 10 (A) and 1 (B) μm.](http://jvi.asm.org/)
significant growth defect. At 28 and 56 DPI, Pr4Δ3-C2 titers were 100- to 500-fold lower than those observed for Pr4 and the other deletion mutant viruses. It is interesting that, as for ASFV Pr4Δ35, Pr4Δ3-C2 titers increased only at a late time postinfection (56 DPI). In two independent experiments, Pr4Δ3-C2 exhibited a significant growth defect as early as 7 DPI (Table 1, experiments 3 and 4). In both experiments, Pr4Δ3-C2 titers showed little to no increase whereas Pr4 titers increased 50- to 100-fold by 7 DPI. Thus, ASFV MGF360 genes 3HL, 3IL, and 3LL perform a tick host range function affecting early aspects of virus replication in the tick.

Fifty percent tick infectious doses (TID50) were determined for the wild-type and recombinant Pr4 viruses (Fig. 2). Ticks (stage N1) were exposed to blood meals containing serial 10-fold dilutions of ASFV, and virus isolation and/or titration was performed at 28 DPI. The TID50 for the Pr4, Pr4Δ3-C1, Pr4Δ5-1, and Pr4Δ5-2 viruses was 4.5 log10, while the TID50 for the Pr4Δ3-C2 gene deletion mutant was significantly increased 10- to 25-fold (5.8 log10).

The expression of MGF360 genes 3HL, 3IL, and 3LL during tick infection was evaluated by reverse transcription-PCR with RNA extracted from Pr4- and Pr4Δ3-C2-infected ticks at 10 DPI. PCR amplification of Pr4 cDNA with specific primers for the MGF360 genes 3HL, 3IL, and 3LL resulted in products of the expected sizes of 720, 600, and 940 bp, respectively. Sequence analysis confirmed that these amplicons corresponded to the expected Pr4 MGF360 genes (data not shown). As expected, PCR amplification products were not detected in ticks infected with Pr4Δ3-C2. These data indicate that the MGF360 genes 3HL, 3IL, and 3LL are transcribed in ASFV-infected ticks within the first 10 DPI, a time that is critical for efficient establishment of infection (20).

MGF360 genes 3HL, 3IL, and 3LL are required for efficient persistence and generalization of ASFV infection in O. porcinus porcinus ticks. To determine the level of host restriction observed for Pr4Δ3-C2, generalization of infection, virus transmission, and long-term persistence of infection were examined in Ornithodoros ticks. Groups of adult ticks (n = 10) were infected with parental virus Pr4 and MGF360/530 gene deletion mutants (105 HAD50/ml). The mean weight of the tick blood meals was 140 ± 10 mg, indicating a mean exposure dose of 1.1 x 107 HAD50 of ASFV per tick per ml. At various times postinfection, tick tissues (three ticks per group) were dissected and triturated. Virus titers in tissues are shown in Table 2. Tissue involvement and mean virus titers were similar for Pr4 and gene deletion mutants Pr4Δ3-C1, Pr4Δ5-1, and Pr4Δ5-2. In contrast, Pr4Δ3-C2 was not detected in any tissues examined. At 42 DPI, hemolymph samples contained on average 4.2 log10 HAD50 of virus per ml in all tick groups (except those exposed to Pr4Δ3-C2), indicating successful generalization of infection. The failure to isolate virus from the hemolymph of Pr4Δ3-C2-infected ticks is consistent with the low level of virus replication in whole ticks described above. These data indicate a defect in Pr4Δ3-C2 in critical early events in the midgut that are necessary for successful generalization of ASFV infection in Ornithodoros ticks (20). The absence of detectable Pr4Δ3-C2 in the salivary and coxal glands, coxal fluid, and salivary secretion suggests that low levels of primary virus replication in the midgut were not sufficient for successful generalization of infection.

The Pr4Δ3-C2 growth defect in ticks could not be rescued by increasing the infectious dose 1.000-fold (108 HAD50/ml). High-dose infection of ticks with Pr4Δ3-C2 was atypical. Ultrastructural analysis demonstrated significant differences in the cellular distributions of mature virus particles in midgut cells. At 21 DPI, both the phagocytic digestive cells (PDC) and less-differentiated epithelial cells showed the presence of virus factories (31 positive cells of 185 examined) with large numbers of mature virions in Pr4-infected ticks (Table 3 and Fig. 4). In contrast, in Pr4Δ3-C2-infected ticks, only a small number of virus-containing PDC were observed (3 positive cells of 398 examined), and no evidence of Pr4Δ3-C2 virus infection was found in undifferentiated midgut epithelial cells (Table 3 and Fig. 4). Differences were seen in Pr4Δ3-C2-infected salivary and coxal glands at 90 DPI. Significantly, no virus particles were observed in the coxal glands of Pr4Δ3-C2-infected ticks, while in Pr4Δ3-C1-infected tissues, mature virions with an intact plasma membrane appeared in the extracellular space of the filtration membrane and along the internal membranes of connecting tubule cells (data not shown). Interestingly, mature Pr4Δ3-C2 particles were present in the salivary gland; however, virions were localized exclusively in the connective tissue surrounding the salivary gland. In Pr4Δ3-C1-infected ticks, virus particles were present not only in the connective tissue but also in electron-dense secretory granules. These data indicate a role for MGF360 gene function in other aspects of generalized infection and tick tissue tropism.

DISCUSSION

Here we have shown that MGF360 genes in the left variable region of the ASFV genome encode a novel tick host range determinant required for efficient replication and generalization of infection in the tick host.

The ASFV MGF360 and MGF530 gene deletion mutant Pr4Δ35 failed to replicate efficiently in Ornithodoros ticks. Pr4Δ35, which lacks six MGF360 and two MGF530 genes in the left variable region of the genome, exhibited a 100- to 1,000-fold reduction in viral replication in infected ticks (Table 1), indicating that the MGF360 and MGF530 genes within this region affect tick host range.

By using deletion mutants lacking individual or multiple MGF genes, the minimal set of MGF360/530 genes required for tick host range was mapped. The deletion mutant Pr4Δ3-C2, which lacked three MGF360 genes, exhibited markedly reduced viral replication in ticks. In addition, Pr4Δ3-C2 repli-

FIG. 4. (A) Low-power electron micrograph of an N2 tick after exposure to a blood meal containing 108 TCID50 of Pr4Δ3-C2/ml at 3 weeks postfeeding. A single infected PDC is found among growing and expanding undifferentiated cells. The typical electron-lucent agranular area of a virus factory (arrows) is present. (B) A higher magnification of the factory region showing developing virus forms (arrowheads) but no mature virions. Bars, 10 (A) and 1 (B) μm.
that they are of significance for viral virulence (27, 52). Initial
that MGF360 and MGF530 genes promote infected-cell sur-
function in this early event. The impaired virus replication of Pr4Δ3-C2 in tick midgut likely accounts for the observed lack of generalized infec-

The Pr4Δ3-C2 growth defect in ticks could not be rescued by increasing the infectious viral dose. Interestingly, some general-

ASVF MGF360 and MGF530 genes do not show similarity to other known genes or motifs in current databases. Individual MGF genes are conserved among ASVF isolates (with 45 to 100% corresponding amino acid identity) (27). Amino-terminal regions of predicted MGF360 proteins do share similarity with corresponding regions of MGF530 ORFs (50). The align-

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We thank Aniko Zsak, Eric Shedlosky, and the PIADC animal care staff for excellent technical assistance.

REFERENCES


primary swine macrophage cultures (1). The inability of MGF360/530 mutant Pr4Δ35 to suppress an IFN response in infected cells may account for its growth defect in macrophage cell cultures. Although IFN response had not been associated with viral resistance in arthropods, these genes may be in-


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ASVF MGF360 GENES AFFECT VIRUS INFECTION IN TICKS 2453


