Glycoprotein I of Varicella-Zoster Virus Is Required for Viral Replication in Skin and T Cells

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Varicella-zoster virus (VZV) glycoprotein I (gI) is dispensable in cell culture; the SCIDhu model of VZV pathogenesis was used to determine whether gI is necessary in vivo. The parental and repaired viruses grew in human skin and thymus/liver implants, but the gI deletion mutant was not infectious. Thus, gI is essential for VZV infectivity in skin and T cells.

The purpose of these experiments was to assess the role of gI in VZV infectivity in vivo. The approach was to evaluate infectivity in SCIDhu mice with thymus/liver implants that contain human T cells and skin implants that contain all of the differentiated cell types found in the dermal and epidermal layers of human skin. Our prediction was that VZV mutants lacking gI would replicate, although more slowly than viruses derived from intact Oka strain cosmids, as was observed in vitro. However, we found that gI expression is essential for VZV replication in both skin and T cells, which are necessary cellular targets during the pathogenesis of primary and recurrent VZV infection in the human host.

Recovery of infectious VZV from skin implants. The gI deletion mutant virus, rOkaΔgI, has a slow-growth phenotype in cell culture compared to the rOka recombinant and the restored virus, rOka-gI@Avr, that expressed gI from a nonnative site (Fig. 1) (7). To determine whether gI was important for growth in differentiated human tissue in vivo, we investigated the relative capacity of these viruses to grow in human skin implants (Fig. 2). All procedures involving SCID mice were performed in accordance with state and federal regulations and were approved by the Committee for the Humane Use of Animals at SUNY Upstate Medical University (CHUA protocol no. 667). The SCIDhu model of VZV pathogenesis is described in detail elsewhere (8). Before inoculation into skin implants, all VZV strains were passaged three times in MRC-5 or HEL cells (primary human lung fibroblasts). VZV is highly cell associated and loses infectivity when stored frozen, thus, the inocula were freshly prepared and titers were determined on October 14, 2017 by guest http://jvi.asm.org/ Downloaded from
deletion virus, six implants inoculated with rOkaΔgI were harvested at day 21 and four implants were harvested at day 28. Comparison of the infectious virus yield per implant and the mean PFU/implant showed no detectable replication of rOka/H9004gI compared with that of rOka or rOka-gI@Avr (Fig. 2A) (sensitivity of the infectious focus assay was 10 PFU per specimen). Infectious VZV was not detected even at the late day 28 time point in any of the skin implants infected with rOka/H9004gI (data not shown). VZV was recovered from implants infected with rOka, with a range of infectious virus titers, which is the typical growth pattern observed when SCIDhu skin implants are inoculated with vaccine Oka (9). Importantly, the yield of infectious virus from implants infected with the restored strain, rOka-gI@Avr, was comparable to that with rOka. The fact that rOka-gI@Avr replicated like rOka indicates that the location of ORF67 within the VZV genome is not critical for skin virulence, as long as gI is produced during replication.

Immunoblot analysis of VZV-infected skin implants. Immunoblotting was done to ensure that the inability to recover infectious VZV from implants infected with rOkaΔgI corresponded to an absence of viral protein synthesis (Fig. 2B). Protein extracts from skin implants were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes (Millipore, Inc., Bedford Mass.), and stained with amido black (1% naphthol blue black, 45% methanol, 10% acetic acid) to reveal total protein before immunoblot analysis was performed. The high-titer polyclonal human antisera used in these experiments bound to VZV proteins in the 70- to 110-kDa range, representing viral glycoproteins predominantly and were detected using Immun-eStar chemiluminescence reagent (Bio-Rad, Inc., Hercules, Calif.). Viral proteins were detected in all of the skin implants infected with rOka and rOka-gI@Avr that had also yielded virus in the infectious focus assay. Results from three representative samples are shown in Fig. 2A. As expected, VZV antigens were undetectable in five of six implants infected with rOkaΔgI. A faint trace of viral protein was visualized in lane 1 but this was probably due to overflow of the sample from the adjacent well.

PCR analysis of VZV-infected skin implants. To confirm the genotype of the inocula and the viruses recovered from infected implants, DNA was extracted from skin implants infected with rOka, rOka/H9004gI, and rOka-gI@Avr and analyzed by PCR. Approximately 100 ng of DNA was used for PCR detection of VZV and the housekeeping gene β-actin (Stratagene, San Diego, Calif.). Primer sequences and annealing locations (Fig. 1) were derived from VZV Dumas (3). Reaction conditions were 94°C for 1 min, then 35 cycles of 94°C for 30 s, 60°C for 30 s, and 68°C for 1 min, followed by a final 10-min extension at 68°C. The sensitivity of this assay is sufficient to detect 10 pg of VZV DNA. The expected sizes of these PCR products are listed in Fig. 1.

PCR analysis of the rOka-, rOkaΔgI-, and rOka-gI@Avr-infected cell preparations used to inoculate the skin implants confirmed that the input viruses were correct (Fig. 2C). In six skin samples infected with rOka and harvested at day 10, the PCR products were identical to the inoculum (data not shown). Similarly, DNA recovered from six skin implants infected with rOka-gI@Avr had the appropriate PCR pattern. VZV DNA was not detected by PCR analysis with 10 skin implants infected with rOka/H9004gI.

Recovery of infectious VZV from thymus-liver implants. The capacity of VZV laboratory strains and cosmid-generated VZV mutants to replicate in human T cells does not always parallel their growth in skin. For example, the ORF66 kinase

![FIG. 1. Viruses and PCR primers. Arrow boxes indicate the direction of transcription of ORFs 65, 66, 67, and 68. The short vertical lines show the positions of AvrII sites. Facing arrows show the approximate annealing sites of PCR primer pairs that span the AvrII site and ORF67. The sequences and annealing sites of all primers are listed.](http://jvi.asm.org/)

<table>
<thead>
<tr>
<th>VZV strain</th>
<th>Location of ORF67 (gl)</th>
<th>AvrII gl</th>
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<tbody>
<tr>
<td>rOka</td>
<td>65-66-67-68</td>
<td>1.1 kb</td>
</tr>
<tr>
<td>rOkaΔgI</td>
<td>65-66-68</td>
<td>1.1</td>
</tr>
<tr>
<td>rOka-gI@Avr</td>
<td>65-66-67-68</td>
<td>2.6</td>
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<table>
<thead>
<tr>
<th>Primer name</th>
<th>Annealing site</th>
<th>Primer sequence</th>
</tr>
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<tbody>
<tr>
<td>gl upper</td>
<td>113304</td>
<td>5'-TTTGCCTTTGCGTGTTGATGGA-3'</td>
</tr>
<tr>
<td>gl lower</td>
<td>117112</td>
<td>5'-TATGCCGGTATGTTATCTGC-3'</td>
</tr>
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<td>AvrII upper</td>
<td>116194</td>
<td>5'-CCACACAAACATCACCTG-3'</td>
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<td>AvrII lower</td>
<td>117317</td>
<td>5'-TTACCACCGTCCATCA-3'</td>
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<tr>
<td>Human β-actin</td>
<td></td>
<td>5'-CTAGAAGCATTGGCGTGGACGATGGAGG-3'</td>
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was required for full infectivity in T cells but not skin, whereas vaccine Oka grows more slowly in skin but is not different from parent Oka in its growth in T-cell implants (9, 10). Therefore, we tested rOkaΔgI in SCIDhu mice with thymus/liver implants to determine whether this protein was also necessary for replication in T cells. Thymus/liver implants were inoculated with rOka (8.0 × 10^6 infected cells/ml), rOkaΔgI (2.5 × 10^6 infected cells/ml), or rOka-gI@Avr (3.3 × 10^5 infected cells/ml) and

FIG. 2. Replication of VZV rOka, rOkaΔgI, and rOka-gI@Avr in human skin. (A) Six implants infected with rOka or rOka-gI@Avr were harvested at 10 days postinfection, and six implants infected with rOkaΔgI were harvested at 21 days postinfection. The number of PFU/implant was determined in an infectious focus assay. Each point represents one human skin implant, and the bar indicates the average PFU/implant. Where points overlap, the number of implants analyzed is given in parentheses. (B) Immunoblot analysis of three skin implants infected with rOka or rOka-gI@Avr and six implants infected with rOkaΔgI. VZV proteins in the 70- to 110-kDa range were detected with high-titer human polyclonal antiserum. (C) PCR analysis of the gI region of viruses recovered from skin implants. Primers spanning the AvrII restriction site, gI, or β-actin were used, and the sizes of the fragments produced are given in kilobases (center). These results represent similar data obtained from three separate experiments.
FIG. 3. Replication of VZV rOka, rOka-gI, and rOka-gI@Avr in human T cells. Thymus/liver implants infected with rOka (black), rOka-gI (zeros), and rOka-gI@Avr (white) were harvested at 7, 14, and 21 days after infection. The number of PFU/implant was determined using an infectious focus assay. Each bar represents the mean and standard deviation from three to five implants from two separate experiments.

The failure of the ΔgI mutant to replicate in skin and T cells confirms that VZV genes that are dispensable in cell culture helps to explain why removing ORF67 is lethal in vivo. The full and partial gI deletion mutants rOkaΔgI, rOkaΔgI-C, and rOkaΔgI-N showed significant decreases in syncytium formation and infectious virus yields in melanoma cells, suggesting an inhibition of cell-cell spread (7). Wang et al. found that the TGN cisternae in human fibroblasts infected with these gI deletion mutants became adherent, viral envelopment was impaired, and virions did not reach post-Golgi structures (15). These studies demonstrated a dramatic effect of gI deletion on virion assembly and egress.

Our interpretation of the requirement for gI expression in differentiated skin and T cells is that the interactions between gI and gE are necessary for VZV replication in vivo (1, 11, 12, 14, 16). These glycoproteins form heterodimers, and gE endocytosis is enhanced substantially in the presence of gI, suggesting that gI regulates the intracellular trafficking of the gE-gI complex (11). In the absence of gI, infected cells showed an unusual punctate distribution of gE on plasma membranes, reduced syncytium formation, and diminished synthesis of the mature 94-kDa form of gE (7). VZV gE is the most abundant viral glycoprotein produced in infected cells (5), and our recent experiments document that gE is essential for VZV replication in vitro (7a). However, these experiments show that gE expression alone is not sufficient for VZV replication in human skin or T cells and that expression of both gE and gI is necessary for VZV virulence.

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REFERENCES


