Naturally Acquired Simian Varicella Virus Infection in African Green Monkeys

Ravi Mahalingam,1 Vicki Traina-Dorge,2 Mary Wellish,1 John Smith,1 and Donald H. Gilden1,3*

Departments of Neurology1 and Microbiology,2 University of Colorado Health Sciences Center, Denver, Colorado 80262, and Department of Microbiology, Tulane National Primate Research Center, Covington, Louisiana 704332

Received 29 March 2002/Accepted 21 May 2002

Simian varicella virus (SVV) infection of primates shares clinical, pathological, immunological, and virological features with varicella-zoster virus infection of humans. Natural varicella infection was simulated by exposing four SVV-seronegative monkeys to monkeys inoculated intratracheally with SVV, in which viral DNA and RNA persist in multiple tissues for more than 1 year (T. M. White, R. Mahalingam, V. Traina-Dorge, and D. H. Gilden, J. Neurovirol. 8:191-205, 2002). The four naturally exposed monkeys developed mild varicella 10 to 14 days later, and skin scrapings taken at the time of the rash contained SVV DNA. Analysis of multiple ganglia, liver, and lung tissues from the four naturally exposed monkeys sacrificed 6 to 8 weeks after resolution of the rash revealed SVV DNA in ganglia at multiple levels of the neuraxis but not in the lung or liver tissue of any of the four monkeys. This animal model provides an experimental system to gain information about varicella latency with direct relevance to the human disease.

RESULTS AND DISCUSSION

The two monkeys that had been inoculated intratracheally with SVV developed diffuse varicella 10 to 12 days later. The four monkeys that were caged with each of the intratracheally infected monkeys developed a mild rash 2 weeks later. Analysis of the DNA extracted from skin scrapings of one intratrache-
ally inoculated monkey and monkeys 190 and 191, which were caged with the inoculated monkey, revealed SVV ORF 63 sequences in the skin of all three monkeys (Fig. 1), indicating that the rash in the simulated natural infection was caused by SVV.

In the four monkeys that developed varicella after natural exposure, blood MNCs were obtained once a week for the next 2 months. SVV DNA was detected in one monkey (no. 190) 10 days later (data not shown), consistent with the peak viremic phase seen days before and at the time of varicella infection (9). SVV DNA was not detected in blood MNCs at any other times or at necropsy from any of the four naturally infected monkeys (data not shown). It is unclear why the three other naturally infected monkeys that developed varicella were not viremic and why MNCs of only one monkey revealed SVV DNA. Perhaps the milder disease, indicated by minimal rash in the naturally infected monkeys compared with extensive rash in the intratracheally inoculated monkeys, accounts for this.

Six to 8 weeks after resolution of the rash, the four naturally infected monkeys were sacrificed and ganglionic and nonganglionic tissues were harvested. Pools of ganglia were prepared from multiple dermatomes (cervical, thoracic, lumbar, and sacral) of two naturally infected monkeys (no. 165 and 166) and from one intratracheally inoculated monkey. PCR analysis of DNA extracted from these pools of ganglia and from the lungs and livers of all three monkeys revealed sequences specific for SVV ORF 63 in all of the samples from the intratracheally inoculated monkey, consistent with earlier observations that SVV is found in multiple tissues months after experimental infection (13). SVV DNA was detected in pooled sacral ganglia of one naturally infected monkey (no. 165) and in pooled thoracic and pooled sacral ganglia of another naturally infected monkey (no. 166) but not in the lung or liver tissue of either monkey (Fig. 2 and Table 1).

Because pooling multiple ganglia for DNA extraction might have diluted any low-abundance SVV DNA present in individual ganglia from each monkey, we examined DNA extracted from both individual and pooled ganglia of two naturally infected monkeys (no. 190 and 191) (Table 1). When analyzed individually, one of the trigeminal ganglia from monkey no. 190, but neither of the two trigeminal ganglia from monkey no. 191, revealed SVV DNA. Five of eight cervical ganglia from

### Table 1. PCR detection of SVV DNA in ganglia of naturally infected monkeys

<table>
<thead>
<tr>
<th>Monkey no.</th>
<th>Trigeminal (Ind&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>Cervical</th>
<th>Thoracic</th>
<th>Lumbar</th>
<th>Sacral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pooled</td>
<td>Ind</td>
<td>Pooled</td>
<td>Ind</td>
<td>Pooled</td>
</tr>
<tr>
<td>165</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/1</td>
<td>ND</td>
<td>0/1</td>
<td>ND</td>
</tr>
<tr>
<td>166</td>
<td>ND</td>
<td>0/1</td>
<td>ND</td>
<td>1/1</td>
<td>ND</td>
</tr>
<tr>
<td>190</td>
<td>1/2</td>
<td>1/2</td>
<td>5/8</td>
<td>4/4</td>
<td>ND</td>
</tr>
<tr>
<td>191</td>
<td>0/2</td>
<td>0/2</td>
<td>0/8</td>
<td>3/6</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ind, individual ganglia.
<sup>b</sup> ND, not done.
monkey no. 190, compared to none of eight cervical ganglia from monkey no. 191, contained SVV DNA. Because of their small size, individual thoracic ganglia were not tested. In both monkeys, SVV DNA was found in two of four lumbar ganglia, but not in sacral ganglia. Overall, SVV DNA was detected in 8 of 17 ganglia in monkey no. 190 and in 2 of 20 ganglia of monkey no. 191, both of which were naturally infected.

In pools of two to four ganglia from monkeys 190 and 191, SVV DNA was detected in cervical ganglia in one of two pools from monkey no. 190 but not in either of two pools from monkey no. 191 (Table 1). In thoracic ganglia, SVV DNA was present in four of four pools from monkey no. 190 and in three of six pools from monkey no. 191. None of the three pools of lumbar ganglia from either monkey revealed SVV DNA. Together, the results obtained from PCR analysis of individual and pooled ganglia indicate that SVV DNA was distributed along the trigeminal, cervical, thoracic, and lumbar regions in monkey no. 190 and only in the thoracic and lumbar regions in monkey no. 191. Overall, the detection of SVV DNA in ganglia but not in the lung or liver tissue from any of four naturally infected monkeys suggests the presence of latent infection in the ganglia.

It was previously shown that intratracheal inoculation of SVV results in persistence of viral DNA in multiple tissues for many months (13). In contrast, in the naturally infected monkeys described herein, virus DNA was restricted to the ganglia. The virological differences could reflect the large amount of virus administered intratracheally compared to a presumed smaller aerosol inoculum in the naturally exposed monkeys. Taken together, SVV infection of monkeys provides a unique animal model that closely resembles human VZV infection. Our model will allow analysis of the physical state of varicella nucleic acid in latently infected ganglia (e.g., cell type and extent of transcription and translation) and experiments to reactivate the virus from latency.

ACKNOWLEDGMENTS

This work was supported in part by NIH grants NS 32623 and AG 06127.

We thank Marina Hoffman for editorial review and Cathy Allen for preparing the manuscript.

REFERENCES


