Introduction

Despite its eradication, smallpox remains a biothreat. While elements of the US military’s Global Response Force service troops are vaccinated with clonal, live vaccinia virus (VACV), safety concerns limit its further use in groups like first responders. There is a need for an effective but better tolerated, single dose, live replicating smallpox vaccine. Sequence analysis with horsepox virus (HPXV)1,2, and suggest that modern VACV diverged in the core viral sequence, and in the accumulation of deletions in the left and right inverted terminal repeats (ITRs) (Figs. 1 and 2). However, it is unknown if the early HPXV-like vaccines, which protected against smallpox, exhibited different safety and efficacy profiles compared to modern VACV. To assess the tolerability and vaccine activity of scHPXV, four groups of macaques were vaccinated with two different doses of scHPXV, one dose of synthetic VACV (sVACV), or vehicle prior to challenge with monkeypox virus (MPXV).

HPXV and VACV are closely related (Fig. 1). HPXV is an environmental isolate, while VACV vaccines have a variety of telomere deletions (presumably from passage) of approximately 10.7 kB from the left ITR and approximately 5.5 kb from the right ITR (Fig. 2). Consequently, HPXV has a genome sequence, including the terminal hairpin sequences and repeat regions in the inverted terminal repeats (ITRs). This sequence is very similar to VACV (strain ACAM 2000) and has been deposited in Genbank (Accession # MN974381). The sVACV genome sequence was also deposited into Genbank (Accession # MN974381).

Cynomolgus macaques (non-human primates/NHPs) (4 per group), were deposited into Genbank (Accession # MN974381). scHPXV and sVACV were assembled using synthetic DNA fragments as described previously in Noyce RS et al.3 A laboratory isolate of VACV was sequenced using Illumina sequencing technologies to obtain a complete genome sequence, including the terminal hairpin sequences and repeat regions in the inverted terminal repeats (ITRs) (Figs. 1 and 2). However, it is unknown if the early HPXV-like vaccines, which protected against smallpox, exhibited different safety and efficacy profiles compared to modern VACV.

Experimental Procedures

scHPXV and sVACV were assembled using synthetic DNA fragments as described previously in Noyce RS et al. A laboratory isolate of VACV was sequenced using Illumina sequencing technologies to obtain a complete genome sequence, including the terminal hairpin sequences and repeat regions in the inverted terminal repeats (ITRs). This sequence is very similar to VACV (strain ACAM 2000) and has been deposited in Genbank (Accession # MN974381). Cynomolgus macaques (non-human primates/NHPs) (4 per group), were vaccinated via scarification using a bifurcated needle with 4x10^6 PFU ("high dose") or 5x10^5 PFU scHPVX and the 3 sVACV animals were revaccinated with 2.4x10^5 PFU sVACV. All but one of the sVACV animals subsequently produced a take. A laboratory isolate of VACV was sequenced using Illumina sequencing technologies to obtain a complete genome sequence, including the terminal hairpin sequences and repeat regions in the inverted terminal repeats (ITRs). This sequence is very similar to VACV (strain ACAM 2000) and has been deposited in Genbank (Accession # MN974381).

Table 1. Experimental Design for scHPXV Dose-Range Finding Study in NHP.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Vaccine (Day 0)</th>
<th>Route of Vaccine Administration</th>
<th>IT Challenge Dose (Day 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>4</td>
<td>scHPXV 4x10^6 PFU</td>
<td>Scarification</td>
<td>N/A</td>
</tr>
<tr>
<td>Group 2</td>
<td>2</td>
<td>scHPXV 5x10^6 PFU</td>
<td>Scarification</td>
<td>N/A</td>
</tr>
<tr>
<td>Group 3</td>
<td>3</td>
<td>scVACV 5x10^6 PFU</td>
<td>I.t.</td>
<td>N/A</td>
</tr>
<tr>
<td>Group 4</td>
<td>4</td>
<td>Vehicle</td>
<td>N/A</td>
<td>5x10^5 PFU</td>
</tr>
</tbody>
</table>

*A second vaccination by scarification (following procedures and dose volume used on Day 0) was given to four animals that did not show evidence of a take at the vaccination site by Day 7: one scHPXV animal was revaccinated with 2x10^5 PFU scHPXV and the 3 sVACV animals were revaccinated with 2x10^4 PFU sVACV. Revaccination of these four animals occurred on Day 14.

Figure 1. Phylogenetic relationships between VACV strains and HPXV

Figure 2. Similarities between VACV strains and HPXV

Results

Two different doses of scHPXV vaccine were tested: 4 of 4 animals in the 4x10^6 PFU dose, and 3 of 4 animals in the 5x10^5 PFU dose groups exhibited a “take” at Day 7 after a single vaccination. A take is a biomarker of protective immunity. In the sVACV arm only 1 of 4 animals exhibited a take after a single vaccination. The animals that did not present a take were revaccinated on Day 14: the one scHPXV animal was revaccinated with 2x10^5 PFU scHPXV and the 3 sVACV animals were revaccinated with 2.4x10^5 PFU sVACV. All but one of the sVACV animals subsequently produced a take. Tolerability was comparable for scHPXV and sVACV (Figs. 5-6). After MPXV challenge (Figs. 7-9) no lesions were seen in any of the 8 animals vaccinated with scHPXV (Fig. 9). One animal in the sVACV arm died from unrelated causes, but while the 3 remaining animals all had takes, 2 still showed lesions by Day 69 (Fig. 9). Clinical signs of systemic monkeypox infections were seen in all 4 vehicle animals by Day 69.

scHPXV is Tolerated During Vaccination of Macaques

Figure 3. Weights of Macaques During Vaccination Period

Figure 4. Representative Images of Vaccination Site on Day 7:

Groups 1—4: scHPXV @ 4.0x10^6 PFU, scHPXV @ 5.0x10^6 PFU, sVACV @ 8x10^4 PFU, sVACV @ 8x10^5 PFU

Conclusions

- HPXV virus is closely related to VACV vaccines
- HPXV is an environmental isolate and molecular analysis indicates that HPXV has not undergone deletions (with “complete” left and right ITRs)
- HPXV has additional genes, relative to VACV vaccines, that could be related to host range and immune interactions
- Molecular analysis suggests HPXV is closer to the vaccine discovered by Dr. Edward Jenner than modern VACV swarms related to host range and immune interactions
- One or more may serve as antigens for protective immunity
- Protection of scHPXV and sVACV was comparable (all 8 scHPXV-vaccinated animals and all 3 VACV-vaccinated animals survived and recovered)
- scHPXV had higher rates of “take” (4/4 high dose and 3/4 low dose animals) than sVACV (1/3 animals) after a single vaccination (“Take” is a biomarker of protective and sterilizing immunity)
- scHPXV induced sterilizing immunity (no lesions) in all 8 animals (4 high dose and 4 low dose), while sVACV (low dose) provided sterilizing immunity to only one of three animals
- scHPXV’s additional genes, relative to VACV may modulate host immune interactions and one or more may serve as antigens for protective immunity