Correspondence

DNA Sequence Variability in Oka Vaccine Isolates

To the Editor—A recent article by Loparev et al. [1] describes the analysis of vaccine rashes after the administration of Oka varicella vaccine. The authors note the high concordance with our own data [2, 3]. However, one area of difference is their finding that most vaccine rashes are mixed, whereas we have found the opposite, that most vaccine rashes are monomorphic. We would like to correct the assertion that these differences arise because our viruses were cultured. Of 86 rash genotypes sampled from lesions that we have reported in 3 articles, only 20 were passaged, most of which dated from the 1980s. The remaining 66 rashes were uncultured, and the majority of these (42) were monomorphic [2–4].

There are several possible explanations for mixtures occurring in some samples (in both studies). The most obvious is that clinicians sampling rashes will swab >1 lesion, thus mixing the viruses from both. This possibility is acknowledged by the authors, and, until samples verified to come from individual lesions are tested, this remains the most likely explanation. Even in the small subset of our samples that were passaged, we doubt that this procedure explains the monomorphic results. Repeated passage of the Oka varicella vaccine preparation in human embryo lung cells does not appreciably alter the composition of the mixture (table 1). In our first publication, all 15 viruses reported, which had been passaged between 2 and 5 times, were monomorphic, suggesting either monoclonal or oligoclonal starting material.

Although we agree with Loparev et al. that no single vaccine variant is responsible for vaccine adverse events, we have recently produced strong statistical genetic evidence for the selection of 4 single-nucleotide polymorphisms (SNPs) in vaccine-associated rashes (P < .001, corrected for multiple sampling) [3]. Two of these code for amino acid changes in the IE62 protein, and in both of these cases the wild-type residue is selected. Loparev et al. analyzed only one of these loci but gratifyingly found it to be present in all vaccine rashes and, interestingly, to be consistently monomorphic, despite mixtures occurring at other loci.

In general, there is remarkable consistency between allele frequencies in rash viruses reported by Loparev et al. and those reported by us. The wild-type SNP frequencies reported by Loparev et al. tended to be slightly lower than what we found. This may result in part from what we assume, from the data, to have been their method of calculating allele frequencies, in which mixed residues were included in the dominator but the component of the mixture that was wild type was not included in the numerator. The effect of this is to underestimate the total percentage of wild-type residues at loci where mixtures occur.

Like Loparev et al., we found many SNPs to be wild type in >50% of postvaccination rashes. We hypothesized that, stochastically, in the absence of biological selection, allele frequencies would be identical in the vaccine and rashes (allowing for variation of the method). This appears to be the case except at 4 loci described in our article, at which the allele frequency in the vaccine rashes was significantly different from that in the original vaccine. For this reason, we would be cautious in concluding that there are functional constraints on the remaining alleles in the pathogenesis of vaccine adverse events. However, more work to analyze vaccine pathogenicity is ongoing. Finally, although our earlier results also showed 4 loci to be fixed for vaccine mutations, more recent work making use of plaque purification of the Merck Oka vaccine has found that only 3 of these loci were fixed for vaccine mutations; position 10705 in open reading frame 62 actually has a low frequency of the wild type (2/43 strains) in the vaccine [3].

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Table 1. Comparison by pyrosequencing of single-nucleotide polymorphism (SNP) frequencies in the Oka vaccine preparation before and after 10 passages in human embryo lung cells.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Vaccine</th>
<th>Vaccine passage 10</th>
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<tbody>
<tr>
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<tr>
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<td>105356</td>
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<td>62</td>
<td>107136</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>62</td>
<td>107797</td>
<td>70</td>
<td>82</td>
</tr>
</tbody>
</table>

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3. Quinlivan ML, Gershon AA, Al Bassam MM, et al. Natural selection for rash-forming ge-
notypes of the varicella-zoster virus detected within immunized human hosts. Proc Natl Acad Sci USA 2007; 104:208–12.

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Reply to Breuer et al.

To the Editor—We agree with Breuer et al. [1] that our 2 studies concur in many important respects, including the identification of the same subset of monomorphic wild-type markers (at similar frequencies) among isolates of vaccine adverse events. As they point out, the relative frequency of mixtures of strains in clinical specimens from confirmed adverse events is the most salient difference between our respective observations. Although we acknowledge that specimens obtained by sampling multiple lesions are one possible source for the observation of mixed strains from lesions caused by the Oka vaccine, we do not agree that this is the likeliest explanation. Most of the specimens that we analyzed for the study consisted of lesion swabs or macular scrapings, and for these specimens it is not possible to rule out the sampling of >1 lesion. However, a review of our specimen accession log revealed that 5 of the specimens that we analyzed (from 4 varicella cases and 1 zoster case) were single scabs dislodged from crusted lesions. In all 5 of these cases, mixtures of vaccine strains were observed. As such, sampling protocols may contribute to this phenomenon, but they cannot be the only explanation.

Moreover, the specimen collection protocol that we provide to health care professionals for varicella zoster virus (VZV) testing requests clearly recommends unroofing and swabbing the base of a single lesion for individual shipment, so it is likely (albeit unprovable) that most of the swab specimens that we receive are collected in that fashion.

There are potentially important differences in our respective technical approaches that could explain the disparity. In our hands, the pyrosequencing method, although easy to perform and amenable to automation, was limited in its ability to detect allelic mixtures. This was particularly true for mutations occurring at selected loci in open reading frame (ORF) 10, ORF55, and ORF62. The limit of detection of mixed bases for pyrosequencing is dependent on the ability to create an optimized dispensation order, which in turn depends on both the nucleotide mutation targeted for detection and the flanking nucleotide sequence. In a variety of publications that have addressed this issue, pyrosequencing was unable to reliably detect mutant alleles present in a mixture at levels of <5% [2–5]. By contrast, the fluorescent resonant energy transfer–based real-time polymerase chain reaction (PCR) method used in our laboratory to detect allelic variants was capable of readily resolving allelic mixtures that were not apparent when pyrosequencing was used. We concede that various sequencing approaches may perform differently among operators; nonetheless, we observed that direct DNA sequencing was the most accurate approach for primary screening of mixed alleles for most of the VZV vaccine single-nucleotide polymorphisms (SNPs) located in problematic regions.

In addition, as we stated in our article, “[r]esults of DNA sequencing were separately confirmed using fluorescent resonance energy transfer–based LightCycler real-time PCR (Roche Diagnostics); for this purpose, acceptor probes (end-labeled with CybR Red 704) and fluorescein-labeled detection probes overlapping the SNP were used. The presence of >1 peak revealed the presence of ≥2 variants in clinical isolates” (p. 504) [6]. Another difference between our approaches is the method used to purify DNA. We evaluated 7 alternative methods for DNA purification, including the miniprep method used by Breuer et al., and we found the automated, magnetic bead–based method that we used in our study to be superior to all of them. Although differences in the efficiency of DNA purification are unlikely to matter for routine VZV DNA detection or for determining DNA sequence of a pure wild-type isolate, it could affect the ability to detect allelic variants represented at a low percentage in a mixture of strains [4].

It has been well established that all Oka vaccine preparations are made up of a mixture of probably a large number of variant VZV strains [7, 8]; as such, multiple variants would be expected to establish latency in an immunized person. On the basis of the universally observed strain mixtures among those specimens that we are certain represent material from an individual lesion represented in our study, our prediction is that the observation of mixtures of strains in Oka vaccine adverse events will prove to be the rule rather than the exception. We are currently planning studies to reinforce the observations that we reported.

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