LETTER

REPLY TO JENSEN AND KOWALIK:

Consideration of mixed infections is central to understanding HCMV intrahost diversity

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Jensen and Kowalik (1) have reported that intrahost variation in human cytomegalovirus (HCMV) approaches levels similar to those of hepatitis C virus, with fast mutation rates mooted as one explanation (2). While we discuss that HCMV mutation rates were postulated as an explanation for high diversity, the focus of our work is on observed inconsistencies in nucleotide diversity between and within patients (3). Jensen and Kowalik did calculate HCMV mutation rates to be similar to mouse CMV but maintained that this could underestimate the true levels (2). In contrast, our study shows that, in the absence of mixed infections, HCMV is no more diverse than other DNA viruses, and considerably less so than chronic RNA viruses. This simple conclusion is different than that of Jensen and Kowalik and had not been stated prior to our publication.

Their previous work concluded that diversity was similar in mixed and single HCMV infections and correlated with selection (4). Yet, in the presence of pervasive recombination or mixed infection, calculation of selection is unreliable and likely to be upwardly biased. Their use of consensus sequence principle component analysis (PCA) to identify mixed and single infections is also not optimal. PCA clusters by polymorphisms, ignoring haplotypes, and thus identifies mixed infections only where the constituent viruses are highly divergent, underestimating their true number. We did not cite their work as we did not have the space to adequately discuss the discrepancies in our approaches.

We agree that HCMV compartmentalization can exist, for example, within the vitreous humor of the eye (5). However, our analyses were conducted on HCMV from blood, where Jensen and Kowalik argue diversity is highest (6), so it is unlikely that our lower diversity estimates were an artifact of compartmentalization. Jensen and Kowalik also mention the importance of bottlenecks in generating diversity, arguing that severe bottlenecks can both decrease (1, 5) and increase (2) diversity, depending on the environment. Where we observe high diversity, we detect both haplotypes from the earliest time point, indicating that the initial infection bottleneck had already occurred. We agree that recombination renders phylogenetic (and indeed selection) analyses inaccurate. The RL11 region has the advantage of being in stronger linkage disequilibrium (7) and thus less affected by recombination. Notwithstanding, figure 4 in our paper (3) clearly demonstrates that the pairwise differences we detected between haplotypes are not limited to the RL11D region but extend to multiple regions across the genome (3).

To summarize, Jensen and Kowalik postulate multiple complex models, which have themselves evolved over the years, to explain HCMV intrahost diversity (4, 6, 8). In contrast, we observe that the measure of HCMV intrahost diversity can be explained by mixed infections involving genetically distant viral strains. We do not exclude the contribution of mutation or recombination to HCMV evolution over short or longer periods in our PNAS paper (3) or in our previous publications (7, 9). Importantly, our methods can be applied to other pathogens, and our conclusions for HCMV are now supported by the findings of other leaders in the field of HCMV genetics (5, 10).

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