Lausannevirus bilevel set-points

Linda Mueller, Hector Moreno, Stefan Kunz* and Gilbert Greub
Institute of Microbiology, University Hospital Centre and University of Lausanne, Lausanne, Switzerland

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Dear Editor,

The nucleo-cytoplasmic large DNA viruses (NCLDV) are a highly diverse group of viruses in terms of shape, size, genetic content and host range. Eleven families have been described so far [1]. Among them, the family of Marseilleviridae includes an amoebae-resisting virus discovered in Lausanne from a Seine river water sample; the Lausannevirus [2]. Besides its life cycle, host range and genomic features that have been characterized, its replicative strategy is still unclear.

Two mode distinguish viral replicative strategies; the “hit-and-run”, during which viruses actively replicate and propagate, and the “slow-and-low” strategy, during which viruses replicate continuously but at a low level. The first mechanism comprises viruses that display a high burst of replication such as Influenza, Poxviruses or Ebola. Oppositely, the second strategy is adopted by viruses such as Epstein-Barr virus (EBV), Human herpesvirus 6 (HHV-6), or cytomegalovirus (CMV), which, generally remain latent until reactivation, and possibly associated immunosuppression. A third case comprising viruses commonly moving from latency to productive replication has been described. Human Immunodeficiency Virus type 1 (HIV-1) and bacteriophage-λ are example of viruses capable to switch from productive replication to latency [3]. Such reactivation is generally triggered by a variety of factors, including stress, fever and/or sunlight. Similarly, a set-point change may occur after years of HIV infection, generally when the CD4 lymphocytes counts drop below 200 cells/mm³. Such HIV set-point change is due to genetic switches. Indeed, diminution in the “Trans-Activator of Transcription” (Tat) protein act as molecular switch and drives HIV-1 from productive replication to latency.

Interestingly, our previous work on Lausannevirus showed fluctuations in its population size when co-cultured in amoebae in presence of the intracellular bacterium E. lausannensis [4]. We previously found that among the 8 subcultures propagated, 4 showed a high viral replication (10⁶ DNA copies/µl) i.e. productive replication, 3 a low replication (10² DNA copies/µl), i.e. latency and 1 exhibited an intermediate level (10⁴ DNA copies/µl).

To further investigate and characterize the different isolates obtained during co-cultures of A. castellanii and E. lausannensis, we performed additional experiments in A. castellanii but in absence of E. lausannensis, and monitored the replication along time.

E. lausannensis was eliminated by filtration from the 8 (A-H) Lausannevirus subcultures, which were further co-cultured for one month in A. castellanii. Population sizes were quantified after each passage and each subculture was propagated in triplicate.

Population size remained constant for each isolate, but two set-points, attributable to two opposite replication strategies, lytic cycles and latency, were clearly differentiated (Fig. 1). From the 3 biological replicas of each subculture, 11 replicates exhibited high set-points (>10⁵ viral DNA copies/µl), whereas 13 showed low set-points (<10⁴ viral DNA copies/µl). Thus, confirming fluctuations in Lausannevirus population, and the existence of bilevel set-points. Interestingly, biological replicas from two isolates, C and G, showed different set-points among triplicates. We hypothesized that the viral input could determine the set-point maintained along the passages. To test this hypothesis, high-level replicates (C3, and all replicas of D and F subcultures) were diluted to a concentration of 10² DNA copies/µl after two to three weeks of co-culture and then re-inoculated in A. castellanii. Results showed that after dilution, high set-points were restored (Fig. 1, subcultures C3, D and F).

Importantly, whole genome sequencing was assessed in all subcultures and triplicates after one month of co-culture as well as SNPs/indels calling. However, results prevented us to link SNPs/indels detected in the Lausannevirus subcultures with high

*Deceased on 10.01.2020.
or low set-points level. In fact, genetic analysis revealed that the same SNPs/Indels were present in both, high and low set-points levels. This suggested that the presence of the observed mutations does not affect the viral replication and the set-point level. As example, mutations 195,949 (insertion of T) and 195,970 (deletion of G) appears in all the observed replicates, regardless their replication capacity. Such mutations are located in two close ORFs (LAU_0251 and LAU_0252), probably encoding for a putative restriction endonuclease split gene [4]. All the triplicates of subcultures B and F, as well as replicate 3 of subculture C and G carry these mutations and exhibited high set-points. These mutations were also observed in replicate 2 of subculture C and replicates 1 and 2 of subculture H, exhibiting low level set-points. Additionally, our previous publication [4] showed that after one year of co-culture in A. castellanii within E. lausannensis, subculture D carried these mutations and exhibited an intermediate level set-point.

Overall, these results suggest that the bilevel set-points observed in Lausannevirus subcultures are not due to SNPs and/or indels, and therefore, the underlying mechanism remains elusive. Thus, the next step will focus on Lausannevirus transcription investigation, to establish whether differential expression of some specific proteins may drive Lausannevirus replication fate, as previously observed for HIV-1 and bacteriophage-λ [5].

**Transparency declaration**

The authors declare no conflicts of interest.
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