

April 17, 2021

Dear Colleagues,

I am writing this letter to accompany our manuscript, entitled, “*Co-evolutionary analysis implicates TLR9 in the restriction papillomavirus infection*”. We were motivated by recent findings from our lab and others that papillomaviruses evade detection by the innate immune system by avoiding the cytoplasm during infection. However, this made us think that the viral DNA may still be detected in the endosome, specifically by TLR9. Below I summarize our study to help you evaluate if it would be appropriate for consideration at *mBIO*.

In brief, biology has been performing experiments on the form and function of papillomavirus genomes for 600 million years. Papillomaviruses are thought to coevolve with their hosts; however, there is no direct evidence for this. We reasoned that if TLR9 restricts papillomaviruses, we should identify signatures of CpG depletion in the context of TLR9 recognition motifs. We have previously used 'molecular archeology' to demonstrate that mucosal papillomaviruses deplete TpC dinucleotides to avoid triggering APOBEC3. This analysis was complicated by the observation that all papillomaviruses have a drastic depletion of CpG dinucleotides. However, the bat immune system, including TLR9, is under selective pressure to alter how it interacts with (viral) infections. Therefore, if papillomaviruses coevolve with their hosts, we would expect that bat-associated PV genomes would allow us to detect an interaction between PVs and TLR9. Specifically, we demonstrate that the TLR9 of a suborder of bats (yangochiroptera) is under Darwinian selection. In response, papillomaviruses that infect these yangochiroptera bats (but not other bats) show a further depletion in CpG dinucleotides.

Our key findings are as follows:

- We isolate and characterize two novel papillomaviruses that infect yangochiroptera bats.
- The TLR9 molecule of bats in this sub-order (yangochiroptera) is under selective evolutionary pressure. These mutations likely change the target of TLR9 recognition.
- Papillomaviruses associated with a specific sub-order of bats have a dramatically depleted CpG content.
- Yangochiroptera bat papillomaviruses are specifically depleted in CpG dinucleotides in the context of a TLR9 recognition motif.

To our knowledge, these data represent the first evidence that: 1) TLR9 may antagonize papillomavirus infection; 2) this is the first direct evidence that papillomaviruses coevolve with their hosts.



Our goal in this study has been to use molecular archeology to identify novel pathways involved in the papillomavirus lifecycle. Specifically, we focused on evasion of detection by TLR9. Since TLR9 would activate an antiviral interferon response, it makes sense that these viruses evolved ways to counteract this system. Unlike other larger viruses, papillomaviruses do not have the genomic 'real estate' to encode novel proteins/functions. However, by depleting TLR9 recognition motifs, these viruses can avoid detection in the first place.

Notably, while this study focuses on bat viruses and the bat immune system, we propose that all papillomaviruses, including the oncogenic viruses, depleted CpG dinucleotides to avoid detection by TLR9. However, we need the signal boost provided by yangochiroptera TLR9 evolution to dissect these interactions.

We think that the nature of the questions we have asked, our multi-disciplinary approach, and, most importantly, our results will be of interest to the broad readership of *mBIO* because, in our minds, the mark of an important study is not simply what is discovered, but also the new and exciting questions that arise that we did not even know to ask. Certainly, we find ourselves excited about the questions that our results provoke, and we think your readers will be too. We believe that these findings will be of interest to researchers studying virus-host interactions and researchers focused on the innate immune system. We are excited to follow up on these studies and demonstrate the effect of TLR9 on human PV infection.

Regarding potential reviewers, people with expertise in molecular evolution and include Dr. Jeremy Kamil (jkamil@lsuhsc.edu) at the Louisiana State University Health Sciences Center Shreveport and Dr. Piet Maes (piet.maes@kuleuven.be) at the University of Leuven. We also suggest Dr. Arinjay Banerjee (Arinjay.banerjee@usask.ca), a recent tenure track faculty member at McMaster University who has considerable expertise in the (evolution of) the bat immune system and viral infections. Finally, Drs. Karl Münger (Karl.Munger@tufts.edu) and Lou Laimins (l-laimins@northwestern.edu) have conducted seminal work in papillomavirus-host interactions. I believe that these colleagues would provide a valuable perspective on the significance of the work.

Sincerely,



Koenraad Van Doorslaer
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