

## WIIR-139

**Genetic dissection of IFN antagonistic functions of rabies virus phosphoprotein: Inhibition of IRF3/7 activation is crucial for pathogenicity**

Rieder Martina<sup>1</sup>, Brzózka Krzysztof<sup>1</sup>, Pfaller Christian K.<sup>1</sup>, Cox James H.<sup>2</sup>, Stitz Lothar<sup>2</sup>, Conzelmann Karl-Klaus<sup>1</sup>

<sup>1</sup>Max von Pettenkofer Institute & Gene Center, Munich, Germany

<sup>2</sup>Friedrich Loeffler Institute, Tübingen, Germany

The rabies virus (RV) phosphoprotein P is a powerful antagonist of the host innate immune response interfering with both RIG-like receptor-mediated IFN induction and IFN-mediated JAK/STAT signaling. In addition, P is an essential cofactor of the viral polymerase and is required for encapsidation of viral RNA into nucleoprotein during replication. By site-directed mutagenesis, we have identified a domain of P required for efficient inhibition of IFN induction. Phosphoproteins lacking amino acids (aa) 176 to 181, 182 to 186, or 176 to 186 were severely compromised in counteracting phosphorylation of IRF3 and IRF7 by TBK1 or IKKi while retaining the full capacity of preventing nuclear import of activated STATs and of supporting virus transcription and replication. Recombinant RV carrying the mutated phosphoproteins (SAD  $\Delta$ Ind1, SAD  $\Delta$ Ind2, and SAD  $\Delta$ Ind1/2 viruses) activated IRF3 and beta IFN (IFN- $\beta$ ) transcription in infected cells but still blocked STAT-mediated expression of IFN-stimulated genes. Due to a somewhat higher transcription rate, the SAD  $\Delta$ Ind1 virus activated IRF3 more efficiently than the SAD  $\Delta$ Ind2 virus. After intracerebral injection into mouse brains at high doses, the SAD  $\Delta$ Ind1 virus was completely apathogenic for wild-type (wt) mice, while the SAD  $\Delta$ Ind2 virus was partially attenuated and caused a slower progression of lethal rabies than wt RV. Neurovirulence of IFN resistant RV thus correlates with the capacity of the virus to prevent activation of IRF3 and IRF7.