Virulence factor W of emerging Hendra virus forms nuclear fibrils that contribute to the repression of antiviral innate immune response

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Hendra and Nipah virus (HeV and NiV) are emerging zoonotic pathogens belonging to the Henipavirus genus and causing lethal respiratory syndrome and encephalitis. Their high virulence, case fatality rate in humans up to 100%, and absence of vaccine or therapeutic treatment classify these viruses among pathogens of the highest biosafety level. HeV and NiV inhibit the host immune response to allow viral replication and subsequent pathogenesis development, by using specialized viral proteins called virulence factors. Notably, the W protein counteracts innate immunity via different mechanisms, e.g., by downregulating proinflammatory NF-κB pathway.

However the molecular basis of these functions is poorly described. We recently demonstrated that W is able to form amyloid-like fibrils. Our hypothesis is that fibrillation is a viral strategy and the molecular support by which W antagonizes the immune response.

Using a version of W tagged with a tetracysteine peptide, I was able to detect the presence of W filaments in the nuclei of transfected cells. The abundance of these filaments was highly reduced when using a W variant with all its cysteine residues mutated to serine (W^{CallS}), thus unable to form disulfide bridges. To determine whether filamentation was required to mediate innate response inhibition, we performed luciferase assays using a reporter cell line expressing Firefly luciferase under the control of a NF-κB-dependent promoter, taking advantage of the Bright-Glo™ Luciferase Assay System (E2620). Cells were transfected with wild-type W or W^{CallS} expression plasmids and stimulated 24 h later with interleukin-1β to activate the NF-κB pathway. Luciferase activity was measured 5h post-stimulation via luminescence quantification. While a >15-fold induction of NF-κB pathway was detected in cells transfected with an empty vector upon stimulation, this induction was significantly inhibited in the presence of W, even below basal luciferase activity. Strikingly, NF-κB induction was partly restored with W^{CallS}, with luciferase activity five times higher than that of wild-type W condition. These results indicate that the propensity to form filaments is correlated to the ability of W to inhibit proinflammatory NF-κB pathway. Viral fibrillation may thus be a key process for Henipaviruses and a valuable therapeutic target.

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