Biological Design Doctoral Defense

Vaccinia Virus' E3 Protein Inhibits Cellular Recognition of Canonical dsRNA and ZRNA

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Abstract

Poxviruses such as monkeypox virus (MPXV) have the potential to be emerging zoonotic diseases. Compared to MPXV, Vaccinia virus (VACV) has reduced pathogenicity in humans and can be used as a partially protective vaccine against MPXV. While most orthopoxviruses have E3 homologues with highly similar N-termini, the MPXV homologue, F3, has a start codon mutation leading to an N-terminal truncation of 37 amino acids. The VACV protein E3 consists of a dsRNA binding domain in its C-terminus which must be intact for pathogenicity in murine models and replication in cultured cells. The N-terminus of E3 contains a Z-form nucleic acid (ZNA) binding domain and is also required for pathogenicity in murine models. Poxviruses produce RNA transcripts that extend beyond the transcribed gene which can form double-stranded RNA (dsRNA). The innate immune system easily recognizes dsRNA through proteins such as protein kinase R (PKR). When we compared a vaccinia virus with a wild-type E3 protein (VACV wt) to one with an E3 N-terminal truncation of 37 amino acids (VACV E3Δ37N), phenotypic differences appeared in several cell lines. In HeLas and certain murine embryonic fibroblasts (MEFs), dsRNA recognition pathways such as PKR become activated during VACV E3Δ37N infections, unlike VACV wt. However, MPXV does not activate PKR in HeLas or MEFs. Additionally, our investigation determined that MPXV produces less dsRNA than VACV. We made VACV E3Δ37N more similar to MPXV by selecting mutants that produces less dsRNA. By producing less dsRNA, VACV E3Δ37N no longer activated PKR in HeLa cells or MEFs restoring the wild-type phenotype. Furthermore, in other cell lines such as L929 (also a murine fibroblast) VACV E3Δ37N but not VACV wt infection leads to activation of DNA-dependent activator of IFN-regulatory factors (DAI) and induction of necroptotic cell death. Using the same low dsRNA mutants, we demonstrate that DAI activation and necroptotic induction is independent of classical dsRNA. Finally, we look at spread in an animal model and replication in cell lines where both the PKR and DAI pathways are intact. In these cases, inhibition of both pathways is required for VACV E3Δ37N to replicate.

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