The Non Structural 3 (NS3) protein of Bluetongue virus interferes with the innate antiviral response

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Introduction

➢ Bluetongue is a non contagious and arthropod-borne viral disease of both domestic and wild ruminants. In 2006, BTV-8 reached Northern Europe and caused a significant epizooty with important economical losses (221M€ in France in 2008).

➢ The Bluetongue virus (BTV) belongs to the *Reoviridae* family and *Orbivirus* genus. The viral genome is composed of 10 segments of double-stranded RNAs which each encodes for one protein.

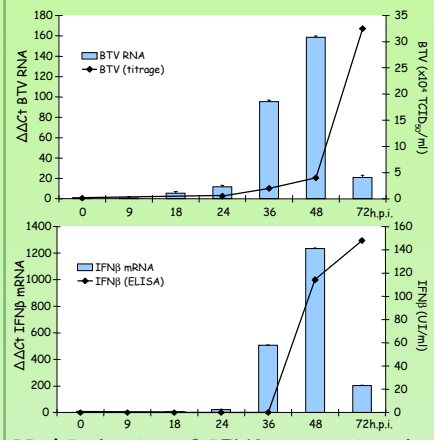
➢ BTV is a potent interferon inducer, but little is known about the antiviral response triggered by the infected host.

➢ The Non Structural 3 (NS3) protein (from the segment 10) is glycosylated and associated with the intracellular and plasma membranes. NS3 is essential for the trafficking and the release of the neovirions.

Results

BTV induces IFNβ synthesis through the RIG-I-Like receptors (RLR)-MAVS pathway in A549 cells

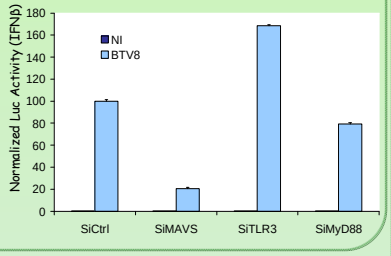
I / BTV infection induces the expression of IFNβ



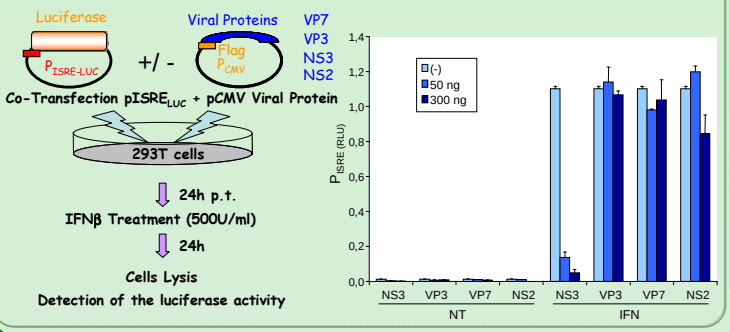
A virulent BTV8 strain (3.10^5 $TCID_{50}$ /ml) isolated during the outbreak in France in 2006 replicates in human epithelial cells (A549) and concomitantly induces the production of IFNβ transcripts which peaks at 48h p.i.

II / Induction of IFNβ expression through the RLRs pathway

Use of specific siRNA directed against the mitochondrial RLR adaptor MAVS leads to a significant decrease of the IFNβ promoter activation upon BTV infection. Similar results were obtained following viral dsRNA transfection (data not shown).

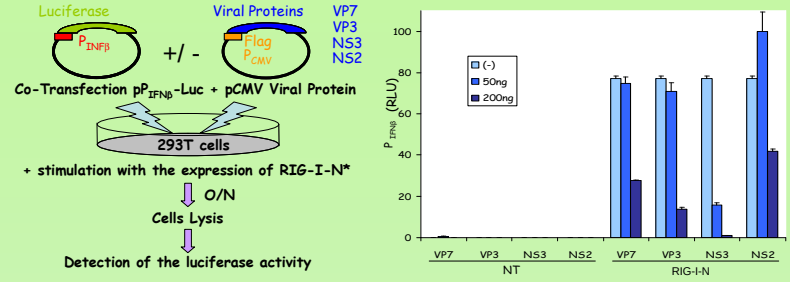


NS3 protein inhibits the type I signalling pathway



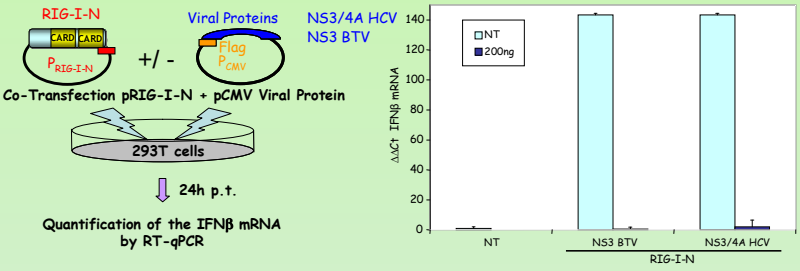
BTV NS3 protein inhibits the expression of IFNβ

I / Inhibition of the IFNβ promoter activation by NS3 protein



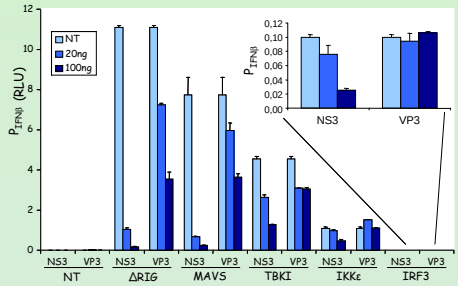
* RIG-I-N is a constitutive active form of RIG-I, composed of the two N-terminal CARD domains.

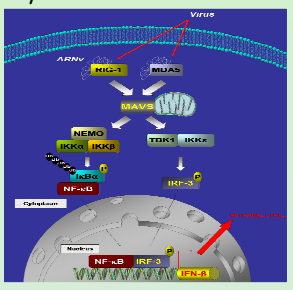
II / NS3 protein inhibits the transcription of the IFNβ gene



III / NS3 acts downstream to the MAVS protein

IFNβ reporter assays were realised towards overexpression of MAVS, TBK1, IKKε or IRF3 which act downstream to the cytoplasmic RNA sensors RIG-I/MDA5 to activate the IFN promoter activity.





NS3 might also inhibits the activation of IFN through the NFκB or AP1 pathway.

Conclusions

➢ BTV infection induces the production of IFNβ *via* the RLRs pathway in non immune cells.

➢ IFNβ expression is downregulated by the BTV NS3 protein at the transcriptional level.

➢ NS3 interferes downstream of the mitochondrial MAVS adaptor protein in the RIG-I-like receptor signalling pathway.

Perspectives

➢ Determine the mechanism of inhibition of NS3 protein on the IFN pathways.

➢ Map the domain(s) of NS3 protein involved in its role as an IFN-I antagonist.

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