Comparison between how different negative sense RNA viruses interact with the IFN system

Master thesis of Markus Schneider[1]
Supervisors: Ralf Küppers[1] and Richard E. Randall[2]
[1]Institute of Cell Biology (Cancer Research), Medical School, University of Duisburg-Essen, Essen, Germany; [2]School of Biology, University of St Andrews, Fife KY16 9ST, Scotland, UK

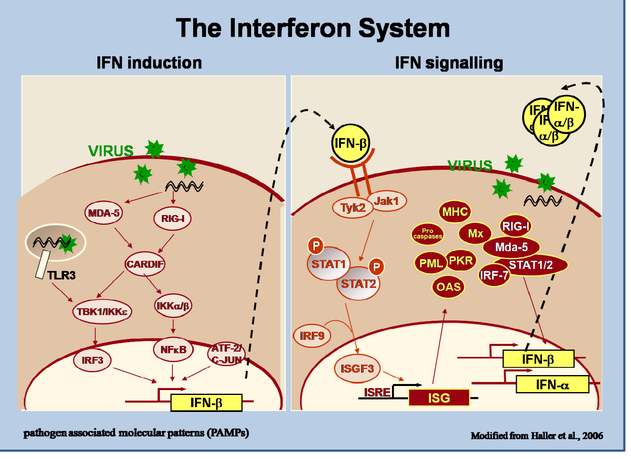
Introduction:

Interferons (IFNs) are a group of secreted cell signalling glycoproteins that elicit distinct antiviral effects. As one part of the innate, non-specific arm of immunity, the IFN system is one of the first barriers viruses have to overcome. Till now IFNs are grouped into three different classes, called type I, II and III IFNs. In contrast to type II and III IFNs type I IFN, especially INF-α and -β, are induced in direct response to viral infection. The induction of IFN-α/β occurs through the recognition of pathogen-associated molecular patterns (PAMPs) by both Toll-like receptors (TLRs) and RIG-I like receptors (RLRs). The most important inducer of IFN is dsRNA, as dsRNA is naturally not found in cells and all viruses usually produce dsRNA during their replication cycle or as the viral genome itself. dsRNAs can be detected by TLR3, present only on special cells, like dendritic cells and macrophages, and RLRs, such as MDA5 and RIG-I, which are present in most cell types. After activation of TLR3, MDA5 or RIG-I a subsequent signalling cascade leads to the activation of NF-κB and IRF-3, both required for the induction of IFN-β. Thus, IFN-β is produced and secreted by virus-infected cells and warns the body/neighbouring cells of the dangerous intruders and cause the cells to activate potent antiviral mechanisms, which limit further growth and spread of incoming virus. These mechanisms are essential for the initial control of virus infections and buy time for the host to establish an adaptive immune respond. IFN have the unique ability to induce an antiviral state in infected and uninfected cells through the activation of the JAK/STAT signalling pathway which lead to the expression of hundreds of IFN-stimulated genes (ISGs), such as Protein kinase R (PKR), the family of 2′-5′ OAS/RNaseL, and the Mx proteins. However, all these proteins either limit or even completely abolish viral replication by interfering with viral or cellular processes such as protein synthesis, making it difficult for the virus to spread.

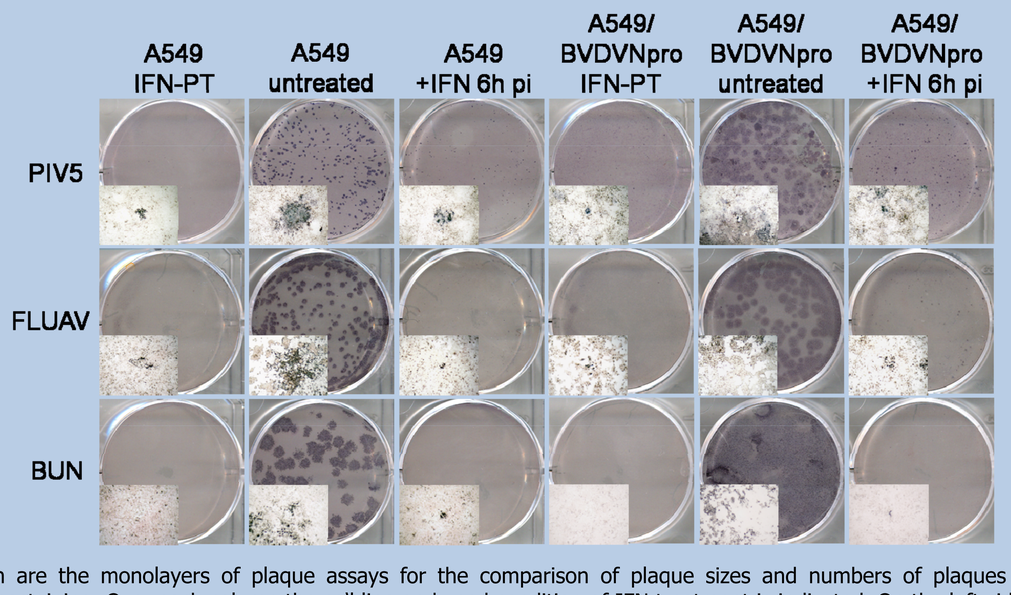
Thus, to survive in nature, all viruses must have some strategy to circumventing the IFN response. There are five main ways how viruses achieve this goal, namely by (i) interfering globally with host-cell gene expression and/or protein synthesis; (ii) minimizing IFN induction by limiting the production of viral PAMPs and/or by specifically blocking IFN-induction cascades; (iii) inhibiting IFN signalling; (iv) blocking the action of IFN-induced enzymes with antiviral activity; and (v) having a replication strategy that is (largely) insensitive to the action of IFN. Very often viruses also use combinations of these strategies. The three viruses used in this study, namely Parainfluenza virus type 5 (PIV5), influenza A (FLUAV) virus and Bunyamwera virus (BUN), all belong to the group of negative sense RNA viruses. They are all enveloped and in general similar in their structure. The small virus genomes encode mainly for structural proteins, which are very similar, and not for many luxury proteins influencing the IFN response. However, the known IFN evasion mechanisms of the three viruses are diverse: PIV5 encodes for the so called V protein which limits IFN induction by interaction with MDA5. Further the V protein blocks IFN signalling by targeting STAT1 for proteasome-mediated degradation. The FLUAV encodes the multifunctional protein NS1. NS1 limits IFN induction by interaction with RIG-I, inhibits the action of PKR, sequesters dsRNA and therefore prevents activation of 2′-5′ OAS/RNaseL pathway and inhibits the cellular pre-mRNA processing and export. BUN encodes for a protein called NSs which inhibits cellular mRNA transcription by blocking the activity of RNA polymerase II. Thus, although similar in structure and limited in coding capacity of the genome the three viruses seem to deal completely different with the IFN response.

Aim of this study:

In the last few years much has been learnt about how viruses interact with the IFN system to circumvent it and allow virus replication. It became obvious that all viruses must have some sort of evasion mechanism to be able to replicate in cells with a functional IFN system. However, till today the exact mechanisms of the interaction between viruses with the IFN system are still unknown, such as the importance of MDA5 and RIG-I for the detection of certain virus infection and subsequent induction of IFN or which proteins are responsible for inhibition of virus replication. Therefore, to get more insight of how viruses interact with the IFN system in general the replication capabilities of PIV5, FLUAV and BUN were compared in IFN competent and IFN compromised cells (BVDVNpro cells). In addition, the cells were treated with IFN on different time points regarding virus infection to study the interaction of the viruses with cells already in an IFN-induced antiviral state. Further the importance of MDA5 and RIG-I for the induction of IFN in response to infections with certain viruses was studied. Also the role of MxA during virus infections was studied. For PIV5 and FLUAV additional immunofluorescence studies were performed to further elucidate the events at the edge of a plaque developing and the role of MxA. This all should give rise to new insight how viruses interact with the IFN system.

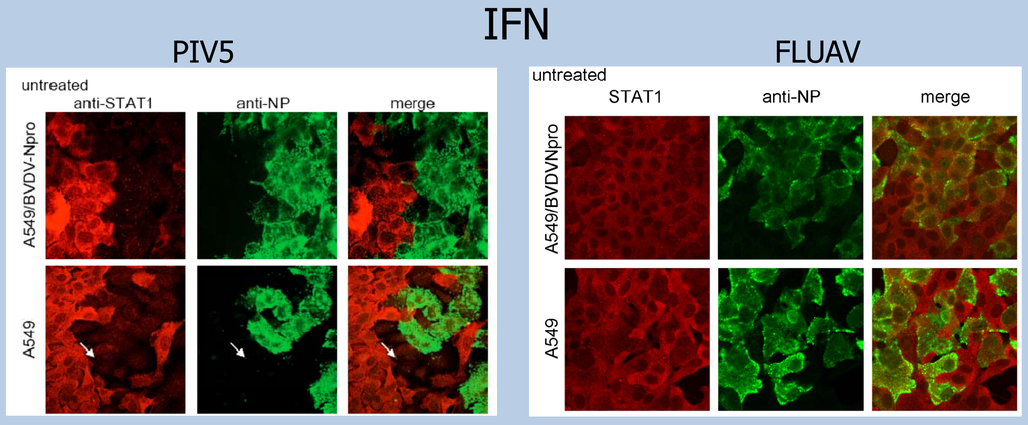


General interaction with the IFN response



Shown are the monolayers of plaque assays for the comparison of plaque sizes and numbers of plaques after immunostaining. Over each column the cell line and used condition of IFN treatment is indicated. On the left side the used virus to infect the cells is indicated for each row. For each monolayer a magnification of a single plaque is shown in the bottom left corner. If no plaque was found a picture was taken from uninfected monolayer or if the plaques were too big a picture from the edge of a plaque was taken.
IFN-PT: Cells were treated with IFN prior to infection
+IFN 6h pi: Cells were treated 6 h post infection

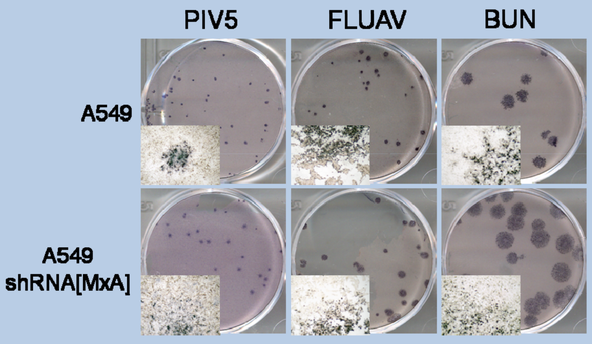
Viral cytoplasmic body formation and STAT1 degradation at the edge of a PIV5 but not FLUAV plaque developing in presence of IFN

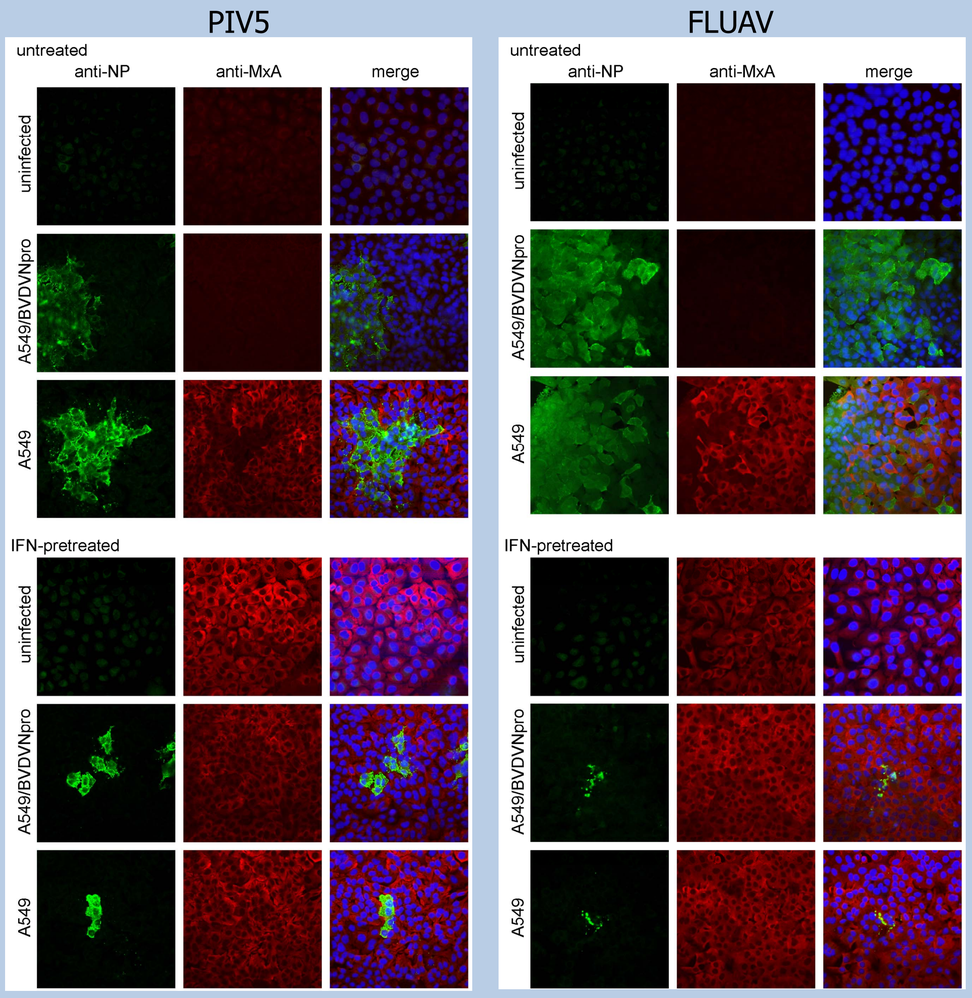


Cells were infected with PIV5 or FLUAV. At 4 days post infection cells were fixed and co-immunostained for STAT1 and viral NP. Cells were visualised using a Zeiss LSM 5 Exciter confocal microscope. Arrows highlight a cell at the edge of a plaque in which viral cytoplasmic bodies can be detected and in which STAT1 has been degraded.

Role of MxA

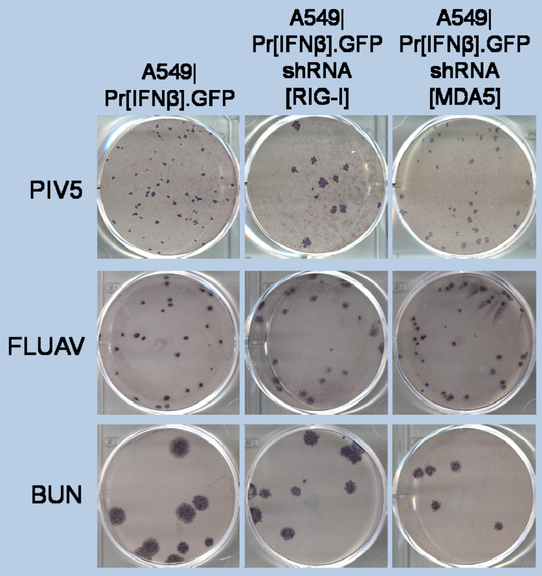
Shown are the monolayers of plaque assays for the comparison of plaque sizes and numbers of plaques after immunostaining. Over each column the used virus to infect the cells is indicated. On the left side the cell line is indicated. For each monolayer a magnification of a single plaque or the edge of a plaque is shown in the bottom left corner.





Cells were infected with PIV5 or FLUAV. At 4 days post infection cells were fixed and co-immunostained for MxA and viral NP. Cells were visualised using a Zeiss LSM 5 Exciter confocal microscope.

Role of MDA5 and RIG-I



Shown are the monolayers of the plaque assays for the comparison of the plaque sizes and number of plaques after immunostaining. Over each column the cell line is indicated. Next to each row the used virus to infect the cells is indicated.

Conclusion

General interaction with the IFN system:

- Common:
  - All viruses replicate fine in untreated cells
  - The mechanisms of all viruses to circumvent the IFN system is not absolute as witnessed by the development of bigger plaques in IFN compromised cell
- PIV5:
  - Can infect cells already in an IFN-induced antiviral state but replication is slowed down
  - Degrades STAT1 and forms cytoplasmic bodies at the edge of a plaque developing, strikingly cells positive for cytoplasmic bodies are negative for STAT1 → functional viral V protein degrades STAT1
  - Model:
    - Initially formation of viral cytoplasmic bodies in cells already in an IFN-induced antiviral state → STAT1 degradation → cell leaves antiviral state → normal virus replication
- FLUAV:
  - Has more difficulties but is able to infect cells already in an IFN-induced antiviral state as witnessed by the massive reduction in plaque size and number in IFN treated cells
  - Infections by single virus particles can be prevented in cell already in an IFN-induced antiviral state as no normal virus replication occurs
  - Model:
    - In established infections uninfected cells are subjected by large numbers of incoming virus → overrunning of cell defences → infection
- BUN:
  - Cannot replicate in cells already in an IFN-induced antiviral state as witnessed by abolished plaque development in IFN treated cells → must replicate fast to infect many cells before IFN response react

Role of MxA:

- PIV5:
  - No effect on replication
- FLUAV:
  - MxA has negative effect on virus replication, but virus can replicate in cells positive for MxA → MxA cannot block virus replication completely
- BUN:
  - MxA has negative effect on virus replication

Role of MDA5 and RIG-I:

- PIV5 + FLUAV:
  - RIG-I more important for IFN induction than MDA5 as shown by bigger plaque development in RIG-I knockdown cells
- BUN:
  - No changes in plaque sizes and therefore no conclusion

Acknowledgements: Thanks to Dan Young for his help during the project.