uni | eth | zürich

Inhibition of Semliki Forest Virus Replication by the Interferon System

Stefan Deuber and Jovan Pavlovic

Institute of Medical Virology, University of Zurich

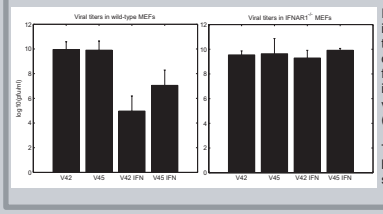
Introduction

The potency of the interferon (IFN) system is a critical parameter in the development of many virus-borne diseases. Given all the strategies applied by viruses, it seems to be essential for a virus to develop effective mechanism(s) to circumvent the actions of the IFNs.

Here we investigate two related strains of Semliki Forest Virus (SFV) that differ in their sensitivity with respect to the IFN system. While the attenuated strain (V42) is strongly inhibited by IFN, the virulent strain (V45) is shown to be more resistant to the antiviral effect of IFN. However, the molecular fundamentals of the IFN-mediated protection against SFV are not known.

The aim of the project is to functionally characterize the mechanism by which SFV is inhibited by IFN and to identify the involved viral sequences.

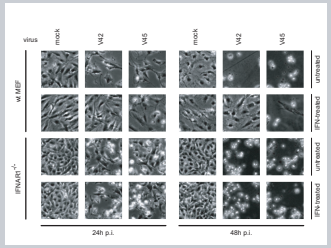
1 The attenuated SFV strain shows a strong sensitivity to IFN, whereas the viral titer of the virulent strain is less affected



In order to determine the IFN sensitivity of the investigated SFV strains, untreated and IFN-treated murine embryo fibroblasts (MEFs), derived from wild-type mice and mice lacking a functional IFN receptor (IFNAR1-/- MEFs), were infected with 2 multiplicity of infection (MOI) and viral titers were measured 24 hours post infection (hpi).

The viral titer of the attenuated strain is reduced by 5 orders of magnitude whereas the virulent strain is diminished only by a factor of 1000.

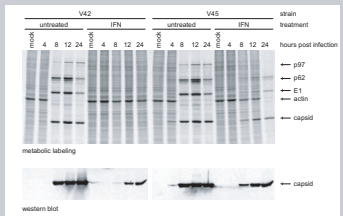
2 The cytopathic effect (CPE) induced by SFV is greatly reduced in IFN-treated cells infected with the avirulent strain



To investigate whether the induction of the CPE is affected by IFN, untreated and IFN-stimulated murine cells were infected with 2 MOI of the viruses. Pictures were taken 24 and 48 hpi.

Even while the attenuated strain is still replicating to some extent, the induction of the CPE is greatly reduced in the presence of IFN.

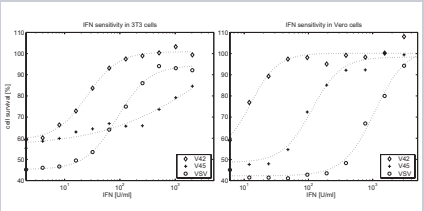
3 IFN prevents the induction of the host shut-off by the attenuated but not the virulent strain



SFV normally induces a host shut-off several hours after infection. In order to determine the behaviour in the presence of IFN, untreated and IFN-treated wild-type MEFs were infected with both strains and pulse-labeled for 15 min at different time points to reveal newly synthesized proteins.

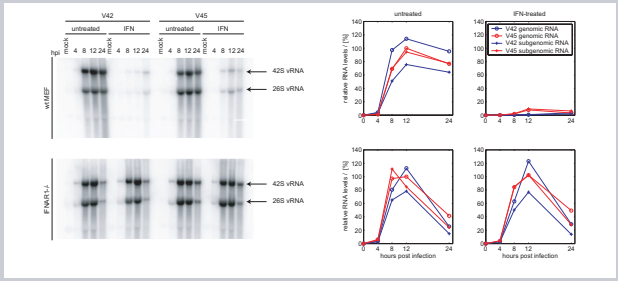
Only in the absence of IFN, the attenuated strain induces a host shut-off (4-8 hours p.i.), whereas the virulent strain shuts down the cellular translation machinery even in the presence of IFN, although delayed.

4 Very small amounts of IFN are required to protect the cells from a cytolytic infection by the avirulent strain



In 3T3 cells only 25 U/ml IFN were needed to protect 50% of the cells from cell lysis by V42. IFN-sensitive VSV required 90 U/ml to achieve the same effect. In contrast, not even the highest IFN concentration tested (2048 U/ml) was sufficient to protect 50% of the cells against V45. To obtain the same inhibition in Vero cells 13 U/ml and 113 U/ml human IFN were determined for V42 and V45, respectively. Interestingly, much more IFN was required to inhibit VSV in Vero cells probably due to a more powerful induction of CPE in Vero cells than 3T3 cells.

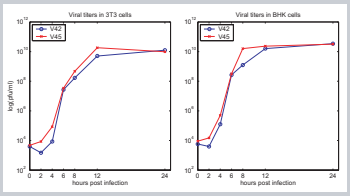
5 Inhibition of viral replication correlates with reduced accumulation of viral RNA



To measure viral transcription, untreated and IFN-treated wt MEFs and IFNAR1-/- MEFs were infected by either virus with 2 MOI. Total RNA was isolated at different time points after infection and separated on an agarose gel. The probe against the 3' end of the viral RNA detects the 42S vRNA as well as the 26S vRNA.

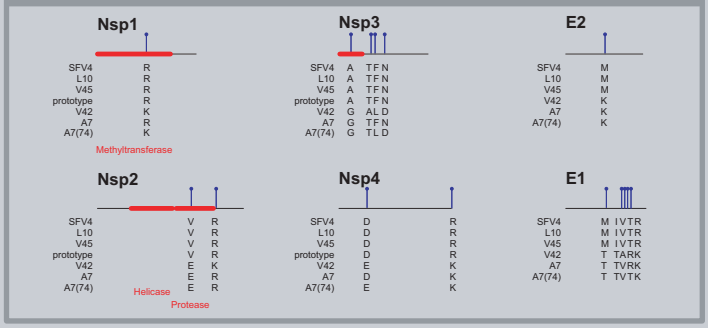
Quantification of the bands revealed higher levels of the 26S vRNA for the virulent strain. The ratio of 42S/26S vRNA levels differ by a factor of two between the two strains. Sequencing of the subgenomic promoter revealed a mutation at the transcriptional start site.

6 The growth curve of the avirulent strain shows a small delay phase early in infection

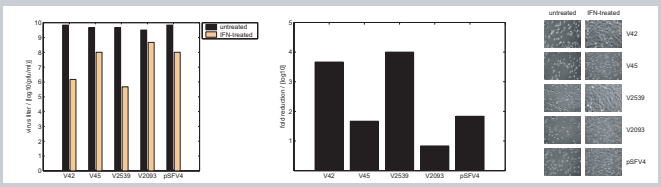


We were asking whether the avirulent strain had a general disadvantage in virus growth in the absence of exogenously added IFN and investigated therefore single-step growth curves of either strain. In two cell lines tested (3T3 and BHK cells) both strains grew almost identically. Only during the first 4-6 hours of infection a slight delay could be observed for the IFN-sensitive strain. This small delay is not due to the IFN system since it was also seen in BHK cells which lack IFN genes.

7 Detected mutations were distributed over the whole genome



8 Construction of infectious clones with the same IFN phenotype as the parental virus



Recombinant infectious clones derived from either strain were constructed and showed the same IFN-phenotype as the parental virus. Recombinant V2539 derived from V42 was strongly inhibited by IFN whereas V2093 was even a little bit more resistant than the parental V45 strain or the available clone pSFV4.

Conclusions

The inhibition of SFV by IFN seems to take place early after infection since only small amounts of viral RNAs are synthesized. The inability to induce a host shut-off by the avirulent strain appears to be a consequence of the diminshed efficacy to replicate in the presence of IFN. Since both viral strains replicate almost equally in the absence of IFN we suggest that a specific sequence or mutation determines the differential IFN sensitivity. We were successful in rescuing recombinant viruses with the same characteristics as the parental viruses. This offers now the opportunity to characterize the involved sequences by swapping segments between the clones.