A single mutation in the mammalian orthoreovirus S1 gene is responsible for increased interferon sensitivity in a virus mutant selected in Vero cells

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ABSTRACT

In a previous study, a mammalian orthoreovirus mutant was isolated based on its increased ability to infect interferon-defective Vero cells and was referred to as Vero-cells-adapted virus (VeroAV). This virus exhibits reduced ability to resist the antiviral effect of interferon. In the present study, the complete genome sequence of VeroAV was first determined. Reverse genetics was then used to identify a unique mutation on the S1 gene, overlapping the σ1 and σ1s reading frame, resulting in increased sensitivity to interferon. A virus lacking σ1s expression consecutive to mutation of its initiation codon was then shown to exhibit a further increase in sensitivity to interferon, supporting the idea that σ1s is the viral protein responsible. This identification of a new determinant of reovirus sensitivity to interferon gives credentials to the idea that multiple reovirus genes are responsible for the level of interferon induction and susceptibility to the interferon-induced antiviral activities.

Keywords: Reovirus; Interferon; Reverse genetics

Highlights:

•A Vero-cell-adapted reovirus exhibits increased sensitivity to interferon.

•Sensitivity was assigned to a mutation in the S1 gene altering both σ1 and σ1s proteins.

•Abolishing σ1s protein expression further increase sensitivity to interferon.

•This reveals a new function for the small σ1s protein.

•This stresses the multigenic nature of the reovirus determinants of interferon control.

Introduction

In the last few years, it has been shown that multiple virus genes are often involved in the control of the innate immune response mediated by the interferon signaling network (**Fensterl et al., 2015; Beachboard and Horner, 2016; Schulz and Mossman, 2016; Garcia-Sastre, 2017**). Different viruses were also shown to exhibit many sequence changes when grown under conditions where the selective pressure to keep viral moderators of the interferon response is removed (**Perez-Cidoncha et al., 2014; Hernández-Alonso et al., 2015; Garijo et al., 2016; Weber-Gerlach et al., 2016; Du et al., 2018**).

The interferon response is well known to be of critical importance as a determinant of viral pathogenicity and a better understanding of viral determinants involved is thus fundamental for epidemiological surveillance as well as choosing the appropriate treatment option. It is also well accepted that the interferon response often plays a role in determining the ability of a virus to discriminate between parental and transformed/cancer cells; the latter frequently, but not always, exhibiting a reduced interferon response (**Randall and Goodburn, 2008; Naik and Russell, 2009; Katsoulidis et al., 2010; Kaufman et al., 2015; Ebrahimi et al., 2017; Matveeva and Chumakov, 2018).** Since a plethora of viruses are presently considered as possible oncolytic viruses for cancer treatment (**Ilkow et al., 2014; Miest and Cattaneo, 2014; Pikor et al., 2015; Turnbull et al., 2015**), this aspect could be of importance. A better understanding could lead to the selection of better optimized and appropriate virus strains for given cancer cell types (**Pikor et al., 2015; Sanjuán and Grdzelishvili, 2015**).

Mammalian orthoreovirus (herein referred to as reovirus) is among the viruses presently under study for their possible use as anticancer agents; it presents the advantage of exhibiting a natural tropism for cancer cells (**Black and Morris, 2012; Maitra et al., 2012; Clements et al., 2014; Chakrabarty et al., 2015**). The interferon response does appear to be at least partly

involved in the oncoselectivity of reovirus (**Strong et al., 1998; Rudd and Lemay, 2005; Shmulevitz et al., 2010**). Another advantage of reovirus as a putative agent in virotherapy is that it is believed to be essentially nonpathogenic in humans. However, this does not preclude the possible emergence of novel viral strains of increased pathogenicity, as suggested in the last few years (**Kohl and Kurth, 2015; Thimmasandra Narayanappa et al., 2015; Yang et al., 2015**). The importance of the interferon response in viral pathogenicity does not need to be further stressed. This is also the case in reovirus whose pathogenicity in the heart and brain of animal models is clearly linked to its ability to control, or not, the interferon response (**Sherry et al., 1998; Goody et al., 2007; Zurney et al., 2007; Tyler et al., 2010; Dionne et al., 2011; Irvin et al., 2012; Wu et al., 2018**). Also, a recent study has raised the possibility that the virus contributes to the development of celiac disease in humans and, once again, there is a likely possibility that the interferon-mediated immune response is involved (**Bouziat et al., 2017**).

It thus appears to be essential to gain a further understanding of the viral determinants that control induction of the interferon response and the sensitivity of different viral isolates to this response. This is one of many approaches that could lead to next-generation reovirus variants that will be either more potent or more specific in their oncolytic effect (**Mohamed et al., 2015; Kemp et al., 2016**). In this context, a reovirus mutant was previously obtained by the establishment of persistence and thus long-term growth of the virus in Vero cells (**Jabre et al., 2013; Sandekian and Lemay, 2015b**). These cells are known to be deficient in production of interferon, interferon regulatory factor 3 (IRF3) and interferon-induced protein kinase PKR (**Desmyter et al., 1968; Emeny and Morgan, 1979; Park et al., 2006; Chew et al., 2009**). As somewhat expected, the resulting Vero-cell-adapted virus (VeroAV) was both adapted for better growth in Vero cells and exhibited an increased sensitivity to interferon compared to the original

wild-type virus (**Jabre et al., 2013; Sandekian and Lemay, 2015b**); however, this last aspect was not examined in detail.

In the present study, the complete genome sequence of VeroAV was first determined and revealed actual amino acids substitutions in four virus genes. The plasmid-based reverse genetics (**Kobayashi et al., 2007, 2010**) was then applied to introduce each of these mutant genes in the original wild-type interferon-resistant genetic background. This allowed to confirm a strongly increased interferon sensitivity with no difference in interferon secretion and the phenotype was assigned to the S1 gene that harbors two separate mutations. The mutation responsible for increased interferon sensitivity was then shown to be located in the region of the S1 gene corresponding to the amino-proximal region of σ1 where its reading frame overlap with that of σ1s. Introduction of a stop codon in the σ1s-encoding reading frame of an otherwise wild-type virus also increases interferon sensitivity indicating that σ1s is the most likely determinant involved in the virus mutant. The σ1s protein thus needs to be added to the many reovirus proteins shown to be involved in the control of either induction and/or sensitivity to the interferon response.

Results

Sequencing of the VeroAV genome

To determine the differences in sequence between the Vero-cell-adapted virus (**Jabre et al., 2013; Sandekian and Lemay, 2015b**) and the original serotype 3 Dearing (T3/Human/Ohio/Dearing/55) laboratory strain ($T3D^S$), the whole coding sequence of the genome was obtained. Reverse transcription-PCR on the semi-purified viral genome from both the mutant and wild-type laboratory stocks was used to generate PCR products that were directly

subjected to sequencing, as described in Materials and Methods and previously used in the laboratory. In a previous study, the sequence of S1 and M2 was solely reported (**Sandekian et al., 2015b**).

Overall, four genes were found to exhibit nonsynonymous mutations compared to the wild-type resulting in a total of 7 amino acids substitutions on 5 proteins since one of the two mutations overlaps the two reading frames in S1 **(Table 1)**. Only three additional silent nucleotide substitutions were found between the wild-type and VeroAV **(data not shown)**. All sequences were deposited in GenBank: the wild-type $T3D^S$ sequences were previously deposited under accession numbers KP208804 to KP208813 and sequences for VeroAV were added in the present study under accession numbers MK238541, MK238542 and MK246415 to MK246422.

Reverse genetics rescue and characterization of VeroAV

To confirm that the amino acid substitutions in the four VeroAV genes (S1, M1, M2 and M3) are actually responsible for the difference in interferon sensitivity between the two viruses, they were introduced in the $T3D^S$ background made of the other 6 genes. Cells were infected with the resulting virus and supernatants recovered to measure secreted β and α-interferons, as described in Materials and methods.

The rescued VeroAV induced similarly low amount of interferon as the control rescued wild-type, $T3D^S$ (**Fig.1**). This is consistent with previous observations using a less direct approach where the ability of infected cells supernatants from the original $T3D^S$ and VeroAV were shown to exhibit a similar ability to protect against another virus (**Jabre et al., 2013**).

Interferon sensitivity was then examined first by the decrease of the virus titer in the presence of a near-saturating (200 international units [IU] per ml) concentration of type I

interferon (mouse ß-interferon without carrier protein, PBL Assay Science). As previously shown (**Sandekian and Lemay, 2015a; Lanoie and Lemay, 2018**), the rescued T3D^S was quite resistant under these conditions. In contrast, the rescued VeroAV was essentially a hundredfold more sensitive (**Fig. 2**), as previously observed with the original VeroAV (**Jabre et al., 2013**). Secondly, the sensitivity to different concentrations of interferon was determined as described in Materials and methods and as used before (**Sandekian and Lemay, 2015a; Lanoie and Lemay, 2018**). Briefly, consecutive twofold dilutions of interferon were prepared from 250 IU/ml to approximately 4 IU/ml in single wells of a microplate; cell lysis in each well (4-5 days post-infection at a MOI of 0.05) was measured by transparency following methylene blue-staining compared with control well containing mock-infected cells. Again, the rescued VeroAV was significantly more sensitive than the wild-type T3D^S (**Fig. 3**); using this approach, the concentration of interferon needed to decrease cell lysis from 100% (in the absence of interferon) to 50% was more than 250 IU/ml for T3D^S but less than 2.5 IU/ml for VeroAV.

Introduction of different VeroAV genes in the T3D^S background

Having confirmed that the four genes are actually sufficient to increase sensitivity to interferon, the three genes M1, M2 and M3 of VeroAV were then separately introduced in the wild-type T3D^S background from which VeroAV was originally derived. In addition, a combination of both M2 and S1 of VeroAV was also introduced in the same background. This was necessary to examine the effect of S1 since it was previously shown that this mutant gene is poorly compatible with the wild-type M2 gene thus rendering analysis impossible due to strikingly reduced viral titer and virus replication (**Sandekian and Lemay, 2015b**).

The introduction of either the M1, M2 or M3 gene by themselves had no effect on the interferon sensitivity using either the reduction of titer assay or the interferon dilution assay (**Fig.**

2 and Fig.3 upper panel). In contrast the introduction of S1 (with M2) resulted in a virus as sensitive as VeroAV (**Fig. 2 and Fig.3 upper panel**), indicating that the S1 gene is the only determinant of this sensitivity.

Introduction of single mutations in the S1 gene

Having established that the S1 gene is responsible for the increased interferon sensitivity of VeroAV, each of the two mutations on this gene (resulting in Q78P and N198K amino acids substitutions in σ1) were solely introduced. When interferon sensitivity was assessed, the Q78P substitution was found to be both necessary and sufficient to fully reconstitute the interferon sensitivity of VeroAV (**Fig. 2 and Fig.3 lower panel**).

The involvement in interferon sensitivity of the virion binding region of the viral attachment protein appears unlikely. However, the mutation resulting in Q78P substitution on σ1 also overlaps the reading frame for the small nonstructural σ1s in S1, the Q78P substitution in σ1 thus also results in a N59H substitution in σ1s.

Effect of σ1s on interferon sensitivity

The previous data indicate that the amino acid substitution in the small σ1s protein is most likely responsible for increased interferon sensitivity of VeroAV, and suggest the importance of σ1s in interferon control. In order to further support this idea, the initiation codon for the σ1s protein was mutated from ATG to ACG on the plasmid in order to prevent its synthesis. This was done without affecting the σ1-encoding sequence, thus generating a virus harboring σ1 from the wild-type $T3D^S$ in the absence of any σ1s expression, as described by others, (**Boehme et al., 2009**). Although σ1s was shown to be nonessential to viral replication per

se (**Rodgers et al., 1998**), other data indicate that it plays important roles in cell-cycle arrest and apoptosis consecutive to viral infection (**Poggioli et al., 2000, 2001; Boehme et al., 2013**). Recently, an additional role in the efficient synthesis of viral proteins was also observed, at least with some viral strains and cell types (**Phillips et al., 2018**). In the present study, the $T3D^S$ virus lacking σ1s ($T3D^S$-σ1s-K/O) was examined for its ability to kill and lyse L929 cells at different multiplicity of infection, as described in previous studies (**Lanoie and Lemay, 2018**). This revealed a significant decrease in the cytopathic effects of σ1s-K/O virus, especially at low multiplicity of infection (data not shown). This indicates that the σ1s protein does have a more important role in viral replication, and/or effect on the host cell, than initially suspected. This rendered difficult the analysis of interferon sensitivity by the interferon dilution method but the diminution of viral titer could still be used and revealed that this virus is actually a further hundredfold more sensitive to interferon than the virus mutant (**Fig. 4**). This was also observed either in the presence or absence of the M2 gene of VeroAV, confirming the sole role of the mutation corresponding to Q78P of σ1 (N59H in σ1s) of VeroAV S1 in interferon sensitivity of the virus mutant and the important role of σ1s as an anti-interferon determinant.

Discussion

Efforts are currently underway to further understand reovirus determinants of pathogenesis and oncolytic activity and the interferon response is clearly central in these properties of various strains or isolates. Such efforts should possibly lead to the development of better adapted viruses for anticancer virotherapy applications (**Mohamed et al., 2015; Kemp et al., 2016**). In previous works, viruses were selected in the laboratory using combinations of chemical mutagenesis, high-passage virus stocks and selection of new viruses. The use of viral

persistence in cells exhibiting giving properties could be an interesting approach to select new, more selective, viruses for virotherapy applications (**Kim et al., 2010**).

Using this viral persistence strategy, the Vero-cell-adapted virus (VeroAV) was previously obtained in the laboratory. This virus exhibits an adaptation at a level of increased binding to sialic acid while also showing increased sensitivity to interferon (**Jabre et al., 2013; Sandekian and Lemay, 2015b**). This is in accordance with the fact that Vero cells are defective in interferon response. Interestingly, selection of a virus mutant with increased affinity for sialic acid in murine erythroleukemia cells (tryptophan to basic arginine substitution at position 202) as in the case of VeroAV in Vero cells (asparagine to basic lysine substitution at position 198) previously led to the identification of a σ1s knockout virus (**Rodgers et al., 1998**). It is tempting to speculate that the gain in infectivity due to increased sialic acid binding somehow compensates for the loss of σ1s in these viruses. Accordingly, the sole introduction of the N59H amino acid substitution and especially the introduction of a stop codon in the σ1s reading frame, was observed to reduce host-cell lysis in the present study. Further work will be needed to determine the impact of σ1s in different viral genetic backgrounds and different cell types.

Induction of and sensitivity to interferon are two interrelated phenomena, as previously shown for example during reovirus infection (**Sherry et al., 1998**) but they can also be viewed as somewhat independent from each other in some cases. Previous work in the laboratory has shown that a virus selected for increased sensitivity to interferon (**Rudd et al., 2005**) does not induce increased interferon secretion (**Sandekian and Lemay, 2015a**). Similarly, this virus does not exhibit apparent differences in the observed changes of cellular gene expression during infection compared to the parental wild-type virus (**Boudreault et al., 2016 and unpublished data**). More recently, comparisons were also made between $T3D^S$, the virus laboratory stock, and $T3D^K$, the

virus recovered from the original reverse genetics plasmids (**Kobayashi et al., 2007**). Increased interferon secretion was attributed to both the μ2 and λ1 protein while increased sensitivity is also partly dependent of μ2 with λ2 also involved (**Lanoie and Lemay, 2018**).

In previous works, the VeroAV genome was only partly sequenced and it was established that both the μ1 and σ1 proteins are altered (**Jabre et al., 2013; Sandekian et al., 2015b**). Since μ1 was altered in a way that affects viral disassembly, it was hypothesized that this could also affect its interaction with its binding partner σ3 (**Jabre et al., 2013**). This protein has long been considered to be important in the control of the interferon-induced protein kinase PKR (**Jacobs and Langland, 1998; Samuel, 1998; Schiff, 2008; Sherry, 2009; Schiff**). Although, at the time, this appeared as a logical explanation to explain increased interferon sensitivity exhibited by VeroAV, it turned out not to be the case.

This involvement of σ1s in the interferon sensitivity phenotype was somewhat surprising. The protein was recently shown to be involved in the efficiency of protein synthesis, although not in the same cell type and not the same virus strain (**Phillips et al., 2018**). It is also known that the protein has at least a partial nuclear localization (**Rodgers et al., 1998; Hoyt et al., 2004; Boehme et al., 2011, 2013**), suggesting that it may affect the expression of certain genes. Alternatively, the protein might interact with a factor important for the interferon response, beyond induction of interferon per se, as shown for the μ2-IRF9 or μNS-IRF3 interactions (**Zurney et al., 2009; Stanifer et al., 2017**). The mutant σ1s is still expressed but was found at a reduced level compared to the wild-type by immunoblotting (data not shown). Considering that the interferon sensitivity is at least a hundredfold higher when σ1s is completely absent, even a small reduction in the level of the protein could well be enough to explain the phenotype; however, an additional loss of function cannot be excluded at this point. Clearly the availability

of a virus mutant with an intermediate phenotype could facilitate further study of σ1s impact on the interferon response and related effect on viral pathogenesis. Interestingly, approximately 50% of the 120 amino acids of σ1s, including asparagine 59, are conserved in 98% or more of the 55 type 3 viral sequences recovered from the NCBI database (data not shown). In contrast,very limited sequence conservations was found between serotypes 1, 2 and 3 (data not shown). It will thus be of interest to compare the effect, if any, of σ1s in other viral serotypes and to identify the common structure involved.

The σ1s protein is well known for its importance in viral propagation *in vivo* (**Boehme et al., 2009, 2011; Nygaard et al., 2013**). It is tempting to speculate that this is due to its role in the control of the interferon response and it will be of interest to pursue these studies. The availability of viruses differing specifically in one viral determinant of interferon induction or sensitivity should certainly contribute to a better understanding of the importance of interferon in viral pathogenesis.

The σ1s protein should thus be added to σ3, μ2, μNS, λ1 and λ2, as proteins probably involved in either the induction of or sensitivity to the interferon response (**Sherry et al., 1998; Sherry 2009; Sherry et al., 2009; Zurney et al., 2009; Irvin et al., 2012; Stebbing et al., 2014; Sandekian and Lemay 2015a; Stanifer et al., 2017; Lanoie and Lemay, 2018**).

It thus appears that reovirus is one further example of virus coevolution with the innate immune response leading, as previously mentioned, to a loss of the ability of the virus to resist interferon when this selective pressure is removed (**Perez-Cidoncha et al., 2014; Hernández-Alonso et al., 2015; Garijo et al., 2016; Weber-Gerlach et al., 2016; Du et al., 2018**). Interestingly, rotavirus, another dsRNA virus of the Reoviridae family, also uses at least three of

its proteins in the control of the interferon response (**Sherry 2009; Sherry et al., 2009; Arnold et al., 2013; López et al., 2015**).

This also raises the possibility of combining these determinants in order to further modulate the interferon response. This could lead to further applications of the virus not only as an oncolytic agent but also as a gene or vaccine vector, as proposed previously by various authors (**Roner and Joklik, 2001; Rouault and Lemay, 2003; Kobayashi et al., 2007; Brochu-Lafontaine and Lemay, 2012; Demidenko et al., 2013; Mohamed et al., 2015; van den Wollenberg et al., 2015; Boehme et al., 2016; Kemp et al., 2016; Eaton et al., 2017; Stuart et al., 2017**).

Materials and methods

Cell lines and viruses

L929 mouse fibroblasts and Vero cells were originally obtained from the American type culture collection (ATCC®). The baby hamster kidney cell line (BHK) stably expressing the T7 RNA polymerase has been described was a generous gift from the laboratory of Dr. John Hiscott (Lady Davis Research Institute, Montréal, Canada). All cell lines were grown in minimal Eagle medium (MEM) with 5% fetal bovine serum.

Wild-type laboratory stock of reovirus type 3 ($T3D^S$) was previously described (**Lanoie and Lemay 2018**; **Sandekian and Lemay 2015a,b**) and recovered by introduction of the appropriate mutations in the plasmids encoding the wild-type virus from Dr. Terry Dermody's laboratory ($T3D^K$). The Vero-cell-adapted virus (VeroAV) was also previously described (**Jabre et al. 2013; Sandekian and Lemay 2015b**). Viruses harboring various combinations of genes from $T3D^S$ and VeroAV were obtained by reverse genetics, as described below. All virus stocks

were routinely grown on L929 cells and virus titer determined by $TCID_{50}$, as described (**Danis and Lemay 1993**).

Sequencing of the whole VeroAV genome

Viral genomic RNA was prepared and fragments for sequencing were generated by RT-PCR, as previously described (**Brochu-Lafontaine and Lemay, 2012**), except that Vertrel was used in replacement of Freon to prepare reovirus virions (**Mendez et al., 2000**).

Reovirus reverse genetics

The plasmids separately harboring each of the cDNA corresponding to the 10 genes of reovirus serotype 3 Dearing, $T3D^K$, under the transcriptional control of the T7 promoter were originally obtained from the laboratory of Dr. Terence Dermody (Vanderbilt University, Nashville, Tennessee) (**Kobayashi et al. 2007**). The recovery of the wild-type laboratory stock $T3D^S$ was initially achieved by first submitting each plasmid to site-directed mutagenesis for each of the gene segment differing between $T3D^S$ and $T3D^K$ (**Lanoie and Lemay 2018; Sandekian and Lemay 2015a**).

The 4 plasmids encoding proteins that differ between the wild-type $T3D^S$ and VeroAV were modified to reconstitute the 4 VeroAV genes; two of them (S1 and M2) were already described (**Sandekian and Lemay 2015b**), while the other two (M1 and M3) were obtained by site-directed mutagenesis.

The S1 gene of $T3D^S$ was further modified by site-directed mutagenesis to separately introduce each of the two amino acids substitutions in σ1. In another construct, the initiation codon for the small σ1s protein encoded in a second reading frame, was mutated (from ATG to

ACG on the plasmid) in order to prevent the synthesis of this second protein, as described by others (**Boehme et al. 2009**); this construct was referred to as knockout (KO). Sequences of all primers used in mutagenesis are available upon request.

Plasmids were then used to recover infectious virus by the reverse genetics approach using transfection in BHK cells expressing the T7 RNA polymerase (**Kobayashi et al. 2010**) and rescued viruses were propagated as described before (**Brochu-Lafontaine and Lemay 2012; Sandekian et al. 2015a,b**).

Determination of interferon induction

ELISA assay was performed on different dilutions of the tissue culture medium to determine the concentration of both β-interferon and all subtypes of α-interferon (Verikine mouse interferon beta and alpha ELISA kit, PBL Assay Science).

Determination of interferon sensitivity

Mouse type I ß-interferon was obtained from PBL interferon source. Interferon sensitivity was determined on mouse L929 cells using different approaches. In a first approach, decreased virus titer at near-saturating (200 IU/ml) concentration on L929 cells was measured by $TCID_{50}$, as previously used (**Sandekian and Lemay 2015a; Lanoie and Lemay 2018**). Secondly, twofold dilutions of interferon were prepared from 250 IU/ml to approximately 4 IU/ml in single wells of a 96 wells microplates seeded with L929 cells; cells were then infected with the different viruses at a MOI of 0.05, incubated for 4–5 days before being fixed and stained with methylene blue, essentially as described (**Sandekian and Lemay 2015a; Lanoie and Lemay 2018**).

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References

Arnold, M.M., Sen, A., Greenberg, H.B., Patton, J.T., 2013. The battle between rotavirus and its host for control of the interferon signaling pathway. PLoS Pathog. 9, e1003064. http://dx.doi.org/10.1371/journal.ppat.1003064.

Beachboard, D.C., Horner, S.M., 2016. Innate immune evasion strategies of DNA and RNA viruses. Curr. Opin. Microbiol. 32, 113–119. http://dx.doi.org/10.1016/j.mib.2016.05.015.

Black, A.J., Morris, D.G., 2012. Clinical trials involving the oncolytic virus, reovirus: Ready for prime time? Expert Rev. Clin. Pharmacol. 5, 517–520. http://dx.doi.org/10.1586/ecp.12.53.

Boehme, K.W., Guglielmi, K.M., Dermody, T.S., 2009. Reovirus nonstructural protein σ1s is required for establishment of viremia and systemic dissemination. Proc. Natl. Acad. Sci. U.S.A. 106, 19986–19991. http://dx.doi.org/10.1073/pnas.0907412106.

Boehme, K.W., Frierson, J.M., Konopka, J.L., Kobayashi, T., Dermody, T.S., 2011. The reovirus σ1s protein is a determinant of hematogenous but not neural viral dissemination in mice. J. Virol. 85, 11781–11790. http://dx.doi.org/10.1128/JVI.02289-10.

Boehme, K.W., Hammer, K., Tollefson, W.C., Konopka-Anstadt, J.L., Kobayashi, T., Dermody, T.S., 2013. Nonstructural protein σ1s mediates reovirus-induced cell cycle arrest and apoptosis. J. Virol. 87, 12967–12979. http://dx.doi.org/10.1128/JVI.02080-13.

Boehme, K.W., Ikizler, M., Iskarpatyoti, J.A., Wetzel, J.D., Willis, J., Crowe, J.E., Jr, Labranche, C.C., Montefiori, D.C., Wilson, G.J., Dermody, T.S., 2016. Engineering recombinant reoviruses to display gp41 membrane-proximal external-region epitopes from HIV-1.

mSphere 1, e00086—16–15. http://dx.doi.org/10.1128/mSphere.00086-16.

Boudreault, S., Martenon-Brodeur, C., Caron, M., Garant, J.-M., Tremblay, M.-P., Armero, V.E. S., Durand, M., Lapointe, E., Thibault, P., Tremblay-Létourneau, M., Perreault, J.-P., Scott, M.S., Lemay, G., Bisaillon, M., 2016. Global profiling of the cellular alternative RNA splicing landscape during virus-host interactions. PLoS ONE 11, e0161914–21. http://dx.doi.org/10.1371/journal.pone.0161914.

Bouziat, R., Hinterleitner, R., Brown, J. J., Stencel-Baerenwald, J. E., Ikizler, M., Mayassi, T., Marlies, M., Kim, S.M., Discepolo, V., Pruijssers, A.J., Ernest, J.D., Iskarpatyoti, J.A., Costes, L.M.M., Lawrence, I., Palanski, B.A., Varma, M., Zurenski, M.A., Khomandiak, S., McAllister, N., Aravamudhan, P., Boehme, K.W., Hu, F., Samsom, J.N., Reinecker, H.-C., Kupfer, S.S., Guandalini, S., Semrad, C.E., Abadie, V., Khosla, C., Barreiro, L.B., Xavier, R.J., Ng, A., Dermody, T.S., Jabri, B. 2017. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. Science 356, 44–50. http://dx.doi.org/10.1126/science.aah5298.

Brochu-Lafontaine, V., Lemay, G., 2012. Addition of exogenous polypeptides on the mammalian reovirus outer capsid using reverse genetics. J. Virol. Methods 179, 342–350. http://dx.doi.org/10.1016/j.jviromet.2011.

Chakrabarty, R., Tran, H., Selvaggi, G., Hagerman, A., Thompson, B., Coffey, M., 2015. The oncolytic virus, pelareorep, as a novel anticancer agent: a review. Invest. New Drugs 33, 761–774. http://dx.doi.org/10.1007/s10637-015-0216-8.

Chew, T., Noyce, R., Collins, S.E., Hancock, M.H., Mossman, K.L. 2009. Characterization of the interferon regulatory factor 3-mediated antiviral response in a cell line deficient for IFN

production. Mol. Immunol. 46, 393–399. http://dx.doi.org/10.1016/j.molimm.2008.10.010.

Clements, D., Helson, E., Gujar, S.A., Lee, P.W.K., 2014. Reovirus in cancer therapy: an evidence-based review. Oncolytic Virother. 3, 69–82. http://dx.doi.org/10.2147/OV.S51321.

Danis, C., Lemay, G., 1993. Protein synthesis in different cell lines infected with orthoreovirus serotype 3: inhibition of host-cell protein synthesis correlates with accelerated viral multiplication and cell killing. Biochem. Cell Biol. 71, 81–85. http://dx.doi.org/10.1139/o93-012.

Demidenko, A.A., Blattman, J.N., Blattman, N.N., Greenberg, P.D., Nibert, M.L., 2013. Engineering recombinant reoviruses with tandem repeats and a tetravirus 2A-like element for exogenous polypeptide expression. Proc. Natl. Acad. Sci. U.S.A. 110, E1867–76. http://dx.doi.org/10.1073/pnas.1220107110.

Desmyter, J., Melnick, J.L., Rawls, W.E. 1968. Defectiveness of interferon production and of rubella virus interference in a line of African green monkey kidney cells (Vero). J. Virol. 2, 955–961.

Dionne, K.R., Galvin, J.M., Schittone, S.A., Clarke, P., Tyler, K.L., 2011. Type I interferon signaling limits reoviral tropism within the brain and prevents lethal systemic infection. J. Neurovirol. 17, 314–326. http://dx.doi.org/10.1007/s13365-011-0038-1.

Du, Y., Xin, L., Shi, Y., Zhang, T.-H., Wu, N.C., Dai, L., Gong, D., Brar, G., Shu, S., Luo, J., Reiley, W., Tseng, Y.-W., Bai, H., Wu, T.-T., Wang, J., Shu, Y., Sun, R., 2018. Genome-wide identification of interferon-sensitive mutations enables influenza vaccine design. Science 359, 290–296. http://dx.doi.org/10.1126/science.aan8806.

Eaton, H.E., Kobayashi, T., Dermody, T.S., Johnston, R.N., Jais, P.H., Shmulevitz, M., 2017.

African swine fever virus NP868R capping enzyme promotes reovirus rescue during reverse genetics by promoting reovirus protein expression, virion assembly, and RNA incorporation into infectious virions. J. Virol. 91, e02416–16–54. http://dx.doi.org/10.1128/JVI.02416-16.

Ebrahimi, S., Ghorbani, E., Khazaei, M., Avan, A., Ryzhikov, M., Azadmanesh, K., Hassanian, S.M., 2017. Interferon-mediated tumor resistance to oncolytic virotherapy. J. Cell. Biochem. 118, 1994–1999. http://dx.doi.org/10.1002/jcb.25917.

Emeny, J.M., Morgan, M.J. 1979. Regulation of the interferon system - evidence that Vero cells have a genetic defect in interferon production. J. Gen. Virol. 43, 247–252. http://dx.doi.org/10.1099/0022-1317-43-1-247.

Fensterl, V., Chattopadhyay, S., Sen, G.C., 2015. No love lost between viruses and interferons. Annu. Rev. Virol. 2, 549–572. http://dx.doi.org/10.1146/annurev-virology-100114-055249.

García-Sastre, A., 2017. Ten strategies of interferon evasion by viruses. Cell Host Microbe 22, 176–184. http://dx.doi.org/10.1016/j.chom.2017.07.012.

Garijo, R., Cuevas, J.M., Briz, Á., Sanjuán, R., 2016. Constrained evolvability of interferon suppression in an RNA virus. Sci. Rep., 1–10. http://dx.doi.org/10.1038/srep24722.

Goody, R.J., Beckham, J.D., Rubtsova, K., Tyler, K.L., 2007. JAK-STAT signaling pathways are activated in the brain following reovirus infection. J. Neurovirol. 13, 373–383. http://dx.doi.org/10.1080/13550280701344983.

Hernández-Alonso, P., Garijo, R., Cuevas, J.M., Sanjuán, R., 2015. Experimental evolution of an RNA virus in cells with innate immunity defects. Virus Evol. 1, vev008–7. http://dx.doi.org/10.1093/ve/ve008.

Hoyt, C.C., Bouchard, R.J., Tyler, K.L., 2004. Novel nuclear herniations induced by nuclear localization of a viral protein. J. Virol. 78, 6360–6369. http://dx.doi.org/10.1128/JVI.78.12.6360-6369.2004.

Ilkow, C.S., Swift, S.L., Bell, J.C., Diallo, J.-S., 2014. From scourge to cure: tumour-selective viral pathogenesis as a new strategy against cancer. PLoS Pathog. 10, e1003836. http://dx.doi.org/10.1371/journal.ppat.1003836.

Irvin, S.C., Zurney, J., Ooms, L.S., Chappell, J.D., Dermody, T.S., Sherry, B., 2012. A single-amino-acid polymorphism in reovirus protein μ2 determines repression of interferon signaling and modulates myocarditis. J. Virol. 86, 2302–2311. http://dx.doi.org/10.1128/JVI.06236-11.

Jabre, R., Sandekian, V., Lemay, G., 2013. Amino acid substitutions in σ1 and μ1 outer capsid proteins are selected during mammalian reovirus adaptation to Vero cells. Virus Res. 176, 188–198. http://dx.doi.org/10.1016/j.virusres.2013.06.007.

Jacobs, B.L., Langland, J.O., 1998. Reovirus σ3 protein: dsRNA binding and inhibition of RNA-activated protein kinase. Curr. Top. Microbiol. Immunol. 233, 185–196.

Katsoulidis, E., Kaur, S., Platanias, L.C., 2010. Deregulation of interferon signaling in malignant cells. Pharmaceuticals, 3, 406–418. http://dx.doi.org/10.3390/ph3020406.

Kaufman, H.L., Kohlhapp, F.J., Zloza, A., 2015. Oncolytic viruses: a new class of immunotherapy drugs. Nat. Rev. Drug Discov. 14, 642–662. http://dx.doi.org/10.1038/nrd4663.

Kemp, V., Hoeben, R., van den Wollenberg, D., 2016. Exploring reovirus plasticity for improving its use as oncolytic virus. Viruses 8, 4–16. http://dx.doi.org/10.3390/v8010004.

Kim, M., Egan, C., Alain, T., Urbanski, S.J., Lee, P.W., Forsyth, P.A., Johnston, R.N., 2007. Acquired resistance to reoviral oncolysis in Ras-transformed fibrosarcoma cells. Oncogene 26, 4124–4134. http://dx.doi.org/10.1038/sj.onc.1210189.

Kobayashi, T., Antar, A.A., Boehme, K.W., Danthi, P., Eby, E.A., Guglielmi, K.M., Holm, G.H., Johnson, E.M., Maginnis, M.S., Naik, S., Skelton, W.B., Wetzel, J.D., Wilson, G.J., Chappell, J.D., Dermody, T.S., 2007. A plasmid-based reverse genetics system for animal double-stranded RNA viruses. Cell Host Microbe 1, 147–157. http://dx.doi.org/10.1016/j.chom.2007.03.003.

Kobayashi, T., Ooms, L.S., Ikizler, M., Chappell, J.D., Dermody, T.S., 2010. An improved reverse genetics system for mammalian orthoreoviruses. Virology 398, 194–200. http://dx.doi.org/10.1016/j.virol.2009.11.037.

Kohl, C., Kurth, A. 2015. Bat Reoviruses. In: Wang, L.-F., Cowled, C. (Eds.), Bats and viruses: a new frontier of emerging infectious diseases. John Wiley & Sons, Inc., Hoboken, New Jersey, pp. 203–215.

Lanoie, D., Lemay, G. 2018. Multiple proteins differing between laboratory stocks of mammalian orthoreoviruses affect both virus sensitivity to interferon and induction of interferon production during infection. Virus Res. 247, 40–46. http://dx.10.1016/j.virusres.2018.01.009.

López, S., Sánchez-Tacuba, L., Moreno, J., Arias, C.F., 2015. Rotavirus strategies against the innate antiviral system. Ann. Rev. Virol. 3, 591–609. http://dx.doi.org/10.1146/annurev-virology-110615-042152.

Maitra, R., Ghalib, M.H., Goel, S., 2012. Reovirus: a targeted therapeutic – progress and potential. Mol. Cancer Res. 10, 1514–1525. http://dx.doi.org/10.1158/1541-7786.MCR-12-0157.

Matveeva, O.V., Chumakov, P.M., 2018. Defects in interferon pathways as potential biomarkers of sensitivity to oncolytic viruses. Rev. Med. Virol. 203, e2008–13. http://dx.doi.org/10.1002/rmv.2008.

Mendez, I.I., Hermann, L.L., Hazelton, P.R., Coombs, K.M., 2000. A comparative analysis of freon substitutes in the purification of reovirus and calicivirus. J. Virol. Methods 90, 59–67. http://dx.doi.org/10.1016/S0166-0934(00)00217-2.

Miest, T.S., Cattaneo, R., 2014. New viruses for cancer therapy: meeting clinical needs. Nat. Rev. Microbiol. 12, 23–34. http://dx.doi.org/10.1038/nrmicro3140.

Mohamed, A., Johnston, R., Shmulevitz, M., 2015. Potential for improving potency and specificity of reovirus oncolysis with next-generation reovirus variants. Viruses 7, 6251–6278. http://dx.doi.org/10.3390/v7122936.

Naik, S., Russell, S.J., 2009. Engineering oncolytic viruses to exploit tumor specific defects in innate immune signaling pathways. Expert Opin. Biol. Ther. 9, 1163–1176. http://dx.doi.org/10.1517/14712590903170653.

Nygaard, R.M., Lahti, L., Boehme, K.W., Ikizler, M., Doyle, J.D., Dermody, T.S., Schiff, L.A., 2013. Genetic determinants of reovirus pathogenesis in a murine model of respiratory infection. J. Virol. 87, 9279–9289. http://dx.doi.org/10.1128/JVI.00182-13.

Park, S., Choi, J., Kang, J., Choi, S., Hwang, S., Kim, J., Ahn, B., 2006. Attenuated expression of interferon-induced protein kinase PKR in a simian cell devoid of type I interferons. Mol. Cells 21, 21–28.

Perez-Cidoncha, M., Killip, M.J., Oliveros, J.C., Asensio, V.J., Fernandez, Y., Bengoechea, J.A., Randall, R.E., Ortín, J., 2014. An unbiased genetic screen reveals the polygenic nature of the influenza virus anti-interferon response. J. Virol. 88, 4632–4646. http://dx.doi.org/10.1128/JVI.00014-14.

Phillips, M.B., Stuart, J.D., Simon, E.J., Boehme, K.W., 2018. Non-structural protein σ1s is required for optimal reovirus protein expression. J. Virol. 92, e02259–17. http://dx.doi.org/10.1128/JVI.02259-17.

Pikor, L.A., Bell, J.C., Diallo, J.-S., 2015. Oncolytic viruses: exploiting cancer's deal with the devil. Trends Cancer 1, 266–277. http://dx.doi.org/10.1016/jtrecan.2015.10.004.

Poggioli, G.J., Keefer, C., Connolly, J.L., Dermody, T.S., Tyler, K.L., 2000. Reovirus-induced G(2)/M cell cycle arrest requires σ1s and occurs in the absence of apoptosis. J. Virol. 74, 9562–9570.

Poggioli, G.J., Dermody, T.S., Tyler, K.L., 2001. Reovirus-induced σ1s-dependent G2/M phase cell cycle arrest is associated with inhibition of p34cdc2. J. Virol. 75, 7429–7434. http://dx.doi.org/10.1128/JVI.75.16.7429-7434.2001.

Randall, R.E., Goodbourn, S. 2008. Interferons and viruses: an interplay between induction, signaling, antiviral responses and virus countermeasures. J. Gen. Virol. 89, 1–47. http://dx.doi.org/10.1099/vir.0.83891-0.

Rodgers, S.E., Connolly, J.L., Chappell, J.D., Dermody, T.S., 1998. Reovirus growth in cell culture does not require the full complement of viral proteins: identification of a σ1s-null mutant. Journal of Virology, 72, 8597–8604. http://dx.doi.org/10.1089/jir.2009.0072.

Roner, M.R., Joklik, W.K., 2001. Reovirus reverse genetics: Incorporation of the CAT gene into the reovirus genome. Proc. Natl. Acad. Sci. U.S.A. 98, 8036–8041. http://dx.doi.org/10.1073/pnas.131203198.

Rouault, É., Lemay, G., 2003. Incorporation of epitope-tagged viral σ3 proteins to reovirus virions. Can. J. Microbiol. 49, 407–417. http://dx.doi.org/10.1139/w03-043.

Rudd, P., Lemay, G., 2005. Correlation between interferon sensitivity of reovirus isolates and ability to discriminate between normal and Ras-transformed cells. J. Gen. Virol. 86, 1489–1497. http://dx.doi.org/10.1099/vir.0.80628-0.

Samuel, C.E., 1998. Reoviruses and the interferon system. Curr. Top. Microbiol. Immunol. 233, 125–145.

Sandekian, V., Lim, D., Prud'homme, P., Lemay, G., 2013. Transient high level mammalian reovirus replication in a bat epithelial cell line occurs without cytopathic effect. Virus Res. 173, 327–335. http://dx.doi.org/10.1016/j.virusres.2013.01.010.

Sandekian, V., Lemay, G., 2015a. A single amino acid substitution in the mRNA capping enzyme λ2 of a mammalian orthoreovirus mutant increases interferon sensitivity. Virology, 483, 229–235. http://dx.doi.org/10.1016/j.virol.2015.05.020.

Sandekian, V., Lemay, G., 2015b. Amino acids substitutions in σ1 and μ1 outer capsid proteins of a Vero cell-adapted mammalian orthoreovirus are required for optimal virus binding and disassembly. Virus Res. 196, 20–29. http://dx.doi.org/10.1016/j.virusres.2014.

Sanjuán, R., Grdzelishvili, V.Z., 2015. Evolution of oncolytic viruses. Curr. Opin. Virol. 13, 1–5. http://dx.doi.org/10.1016/j.coviro.2015.01.014.

Schiff, L.A., 2008. Structure and functions of the orthoreovirus σ3 protein. In: Patton, J.T. (Ed.), Segmented Double-Stranded RNA Viruses: Structure and Molecular Biology. Caister Academic Press, Norfolk, UK, pp. 173–188.

Schulz, K.S., Mossman, K.L., 2016. Viral evasion strategies in type I IFN signaling – A summary of recent developments. Front. Immunol. 7 459–10. http://dx.doi.org/10.3389/fimmu.2016.00498.

Sherry, B., Torres, J., Blum, M.A., 1998. Reovirus induction of and sensitivity to beta interferon in cardiac myocyte cultures correlate with induction of myocarditis and are determined by viral core proteins. J. Virol. 72, 1314–1323.

Sherry, B., 2009. Rotavirus and reovirus modulation of the interferon response. J. Interferon Cytokine Res. 29, 559–567. http://dx.doi.org/10.1089/jir.2009.0072.

Sherry, B., Patton, J.T., Dermody, T.S., 2009. Innate immune responses elicited by reovirus and rotavirus. In: Brasier, A.R., Garcia-Sastre, A., Lemon, S.M., (Eds.), Cellular signaling and innate immune responses to RNA virus infections. ASM Press, Washington, D.C. pp. 403–422.

Shmulevitz, M., Pan, L.-Z., Garant, K., Pan, D., Lee, P.W.K., 2010. Oncogenic Ras promotes reovirus spread by suppressing IFN-β production through negative regulation of RIG-I signaling. Cancer Res. 70, 4912–4921. http://dx.doi.org/10.1158/0008-5472.CAN-09-4676.

Stanifer, M.L., Kischnick, C., Rippert, A., Albrecht, D., Boulant, S., 2017. Reovirus inhibits interferon production by sequestering IRF3 into viral factories. Sci. Rep. 7, 10873. http://dx.doi.org/10.1038/s41598-017-11469-6.

Stebbing, R.E., Irvin, S.C., Rivera-Serrano, E.E., Boehme, K.W., Ikizler, M., Yoder, J.A., Dermody, T.S., Sherry, B., Lyles, D.S., 2014. An ITAM in a nonenveloped virus regulates activation of NF-κB, induction of beta interferon, and viral spread. J. Virol. 88,2572–2583. http://dx.doi.org/10.1128/JVI.02573-13.

Strong, J.E., Coffey, M.C., Tang, D., Sabinin, P., Lee, P.W., 1998. The molecular basis of viral oncolysis: usurpation of the Ras signaling pathway by reovirus. EMBO J. 17, 3351–3362. http://dx.doi.org/10.1093/emboj/17.12.3351.

Stuart, J.D., Phillips, M.B., Boehme, K.W., 2017. Reverse genetics for mammalian orthoreovirus. Methods Mol. Biol. 1602, 1–10. http://dx.doi.org/10.1007/978-1-4939-6964-7_1.

Thimmasandra Narayanappa, A., Sooryanarain, H., Deventhiran, J., Cao, D., Ammayappan Venkatachalam, B., Kambiranda, D., LeRoith, T., Heffron, C.L., Lindstrom, N., Hall, K., Jobst, P., Sexton, C., Meng, X.J., Elankumaran, S. 2015. A novel pathogenic mammalian orthoreovirus from diarrheic pigs and swine blood meal in the United States. mBio 6, e00593—15–11. http://dx.doi.org/10.1128/mBio.00593-15.

Turnbull, S., West, E., Scott, K., Appleton, E., Melcher, A., Ralph, C., 2015. Evidence for oncolytic virotherapy: where have we got to and where are we going? Viruses 7, 6291–6312. http://dx.doi.org/10.3390/v7122938.

Tyler, K.L., Leser, J.S., Phang, T.L., Clarke, P., 2010. Gene expression in the brain during reovirus encephalitis. J. Neurovirol. 16, 56–71. http://dx.doi.org/10.3109/13550280903586394.

van den Wollenberg, D.J.M., Dautzenberg, I.J.C., Ros, W., Lipińska, A.D., van den Hengel, S.K., Hoeben, R.C., 2015. Replicating reoviruses with a transgene replacing the codons for the head domain of the viral spike. Gene Ther. 22, 267–279. http://dx.doi.org/10.1038/gt.2014.126.

Weber-Gerlach, M., Weber, F., 2016. To conquer the host, Influenza virus is packing it in: interferon-antagonistic strategies beyond NS1. J. Virol. 90, 8389–8394. http://dx.doi.org/10.1128/JVI.00041-16.

Wu, A.G., Pruijssers, A.J., Brown, J.J., Stencel-Baerenwald, J.E., Sutherland, D.M., Iskarpatyoti, J.A., Dermody, T.S., 2018. Age-dependent susceptibility to reovirus encephalitis in mice

is influenced by maturation of the type-I interferon response. Pediatr. Res. 83, 1057–1066. http://dx.doi.org/10.1038/pr.2018.13.

Yang, X.-L., Tan, B., Wang, B., Li, W., Wang, N., Luo, C.-M., Wang, M.-N., Zhang, W., Li, B., Peng, C., Ge, X.-Y., Zhang, L.-B., Shi, Z.-L. 2015. Isolation and identification of bat viruses closely related to human, porcine and mink orthoreoviruses. J. Gen. Virol. 96, 3525–3531. http://dx.doi.org/10.1099/jgv.0.000314.

Zurney, J., Howard, K.E., Sherry, B., 2007. Basal expression levels of IFNAR and Jak-STAT components are determinants of cell-type-specific differences in cardiac antiviral responses. J. Virol. 81, 13668–13680. http://dx.doi.org/10.1128/JVI.01172-07.

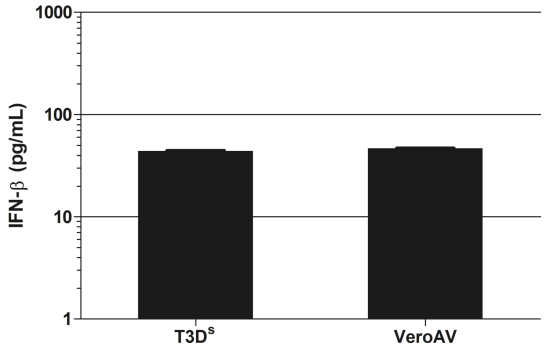
Zurney, J., Kobayashi, T., Holm, G.H., Dermody, T.S., Sherry, B., 2009. Reovirus μ2 protein inhibits interferon signaling through a novel mechanism involving nuclear accumulation of interferon regulatory factor 9. J. Virol. 83, 2178–2187. http://dx.doi.org/10.1128/JVI.01787-08.

Table 1

Protein(gene)	Wild-type ($T3D^S$)	VeroAV	Amino acid position
μ2(M1)	G	R	389
μ1(M2)	E	G	89
	A	V	114
μNS(M3)	D	N	706
σ1(S1)	Q	P	78
	N	K	198
σ1s(S1)	N	H	59

Amino acids differences between wild-type reovirus type 3 Dearing laboratory stock ($T3D^S$) and derived Vero-cell-adapted virus (VeroAV)

Fig. 1. Comparison of interferon induction by the two rescued viruses. L929 cells were infected at a MOI of 20 $TCID_{50}$ units per cell with either the rescued parental wild-type $T3D^S$ or the rescued VeroAV. Infected cells media were recovered at 15-hour post-infection and directly used in commercial ELISA assays, as described in Materials and methods. Quantitation of secreted β-interferon or α-interferons are presented, as indicated. Results are shown as the average of two independent experiments with error bars representing the standard error of the mean. The differences between $T3D^S$ and VeroAV were not statistically significant.


1000
100
10
1
IFN-β (pg/mL)
T3D^s
VeroAV

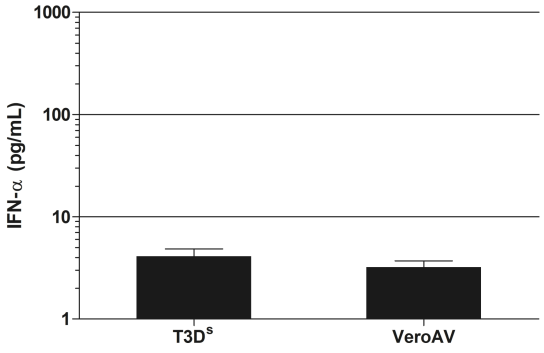

1000
100
10
1
IFN-α (pg/mL)
T3D^s
VeroAV

Fig. 2. Interferon sensitivity of $T3D^S$ harboring VeroAV genes. VeroAV genes were separately introduced in the $T3D^S$ background, except for the S1 gene that was introduced in combination with M2. Also, VeroAV S1 gene harboring either the two mutations corresponding to Q78P or N198K substitutions in σ1 were separately introduced in the $T3D^S$ background in combination with VeroAV M2 gene. Virus titers in the absence or presence of 200 IU/ml of ß-interferon were compared, as described in the text. Fold reductions in titers due to interferon presence are presented on a logarithmic scale. Error bars indicate the highest value obtained in two or more experiments. The two viruses that are not significantly different from VeroAV, using Student's t-test are indicated ($p > 0.2$; n.s.), while others are significantly different ($p < 0.01$).

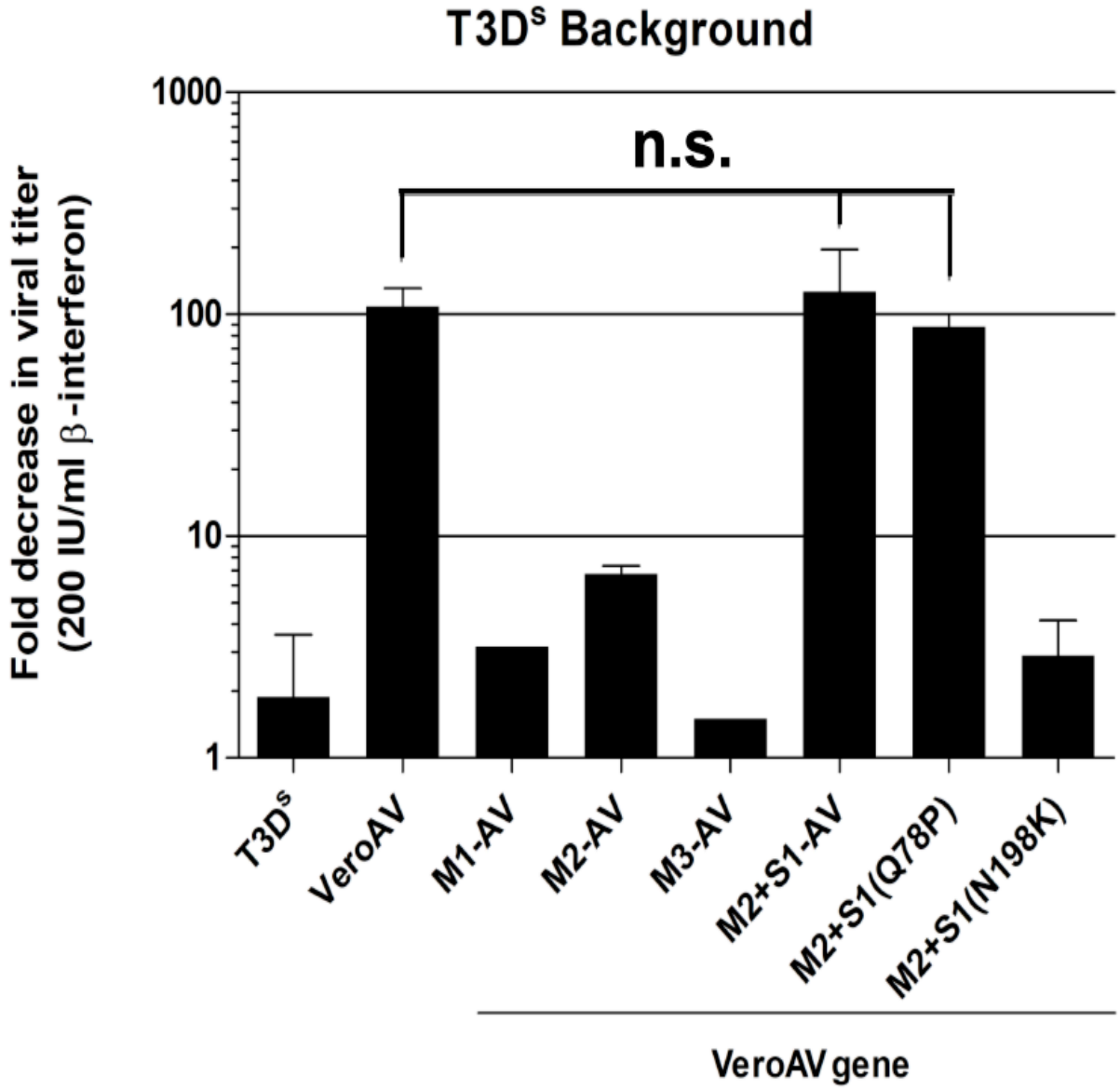
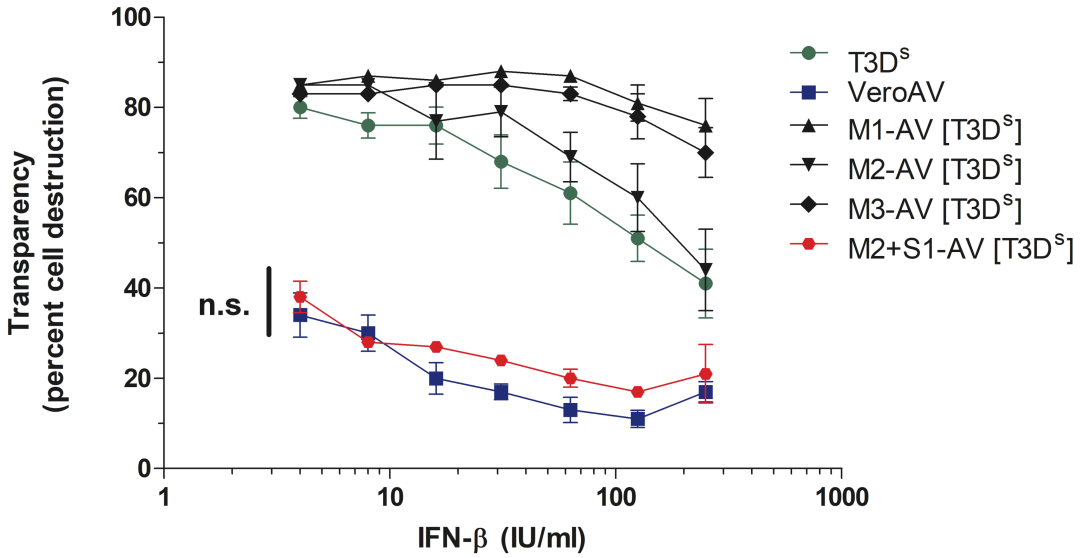
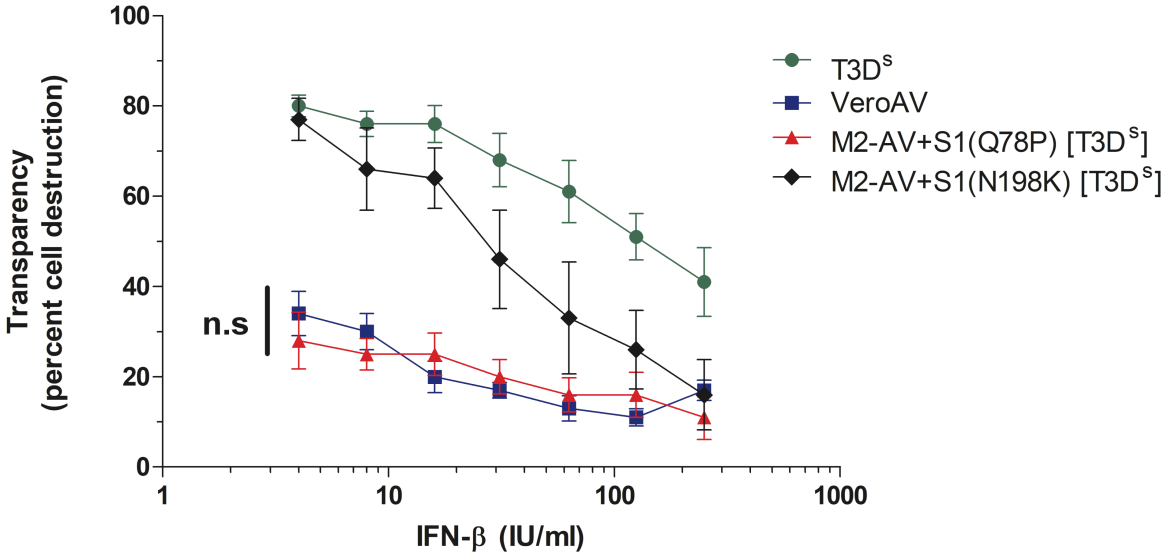

T3D^s Background
n.s.
Fold decrease in viral titer
(200 IU/ml β-interferon)
1000
100
10
1
T3D^s
VeroAV
M1-AV
M2-AV
M3-AV
M2+S1-AV
M2+S1(Q78P)
M2+S1(N198K)
VeroAV gene

Fig. 3. Sensitivity of reassortant viruses to different interferon concentrations. The same viruses as in Fig. 2 were examined by the interferon dilution assay, as described in Materials and methods. The percentage of cell destruction, as determined by optical density of methylene blue stain compared to mock-infected cells, is presented, as described in Materials and methods. On the upper panel the two parental viruses are compared with viruses harboring a single gene reassortment or the M2-S1 combination while the lower panel presents the two single substitution mutants in S1. Result are presented as the average with error bars representing standard error of the mean (n=5 for $T3D^S$ and VeroAV; n=2 for other viruses in the upper panel and n=3 for other viruses in the bottom panel). Using student's t-test to compare results at all different interferon concentrations, only two viruses M2+S1-AV [$T3D^S$] and M2-AV+S1(Q78P) [$T3D^S$] were nonsignificantly different from VeroAV ($p>0.05$ at all concentrations), as indicated (n.s.).


Transparency
(percent cell destruction)
100
80
60
40
20
0
n.s.
1
10
100
1000
IFN-β (IU/ml)
T3D^s
VeroAV
M1-AV [T3D^s]
M2-AV [T3D^s]
M3-AV [T3D^s]
M2+S1-AV [T3D^s]


Transparency
(percent cell destruction)
100
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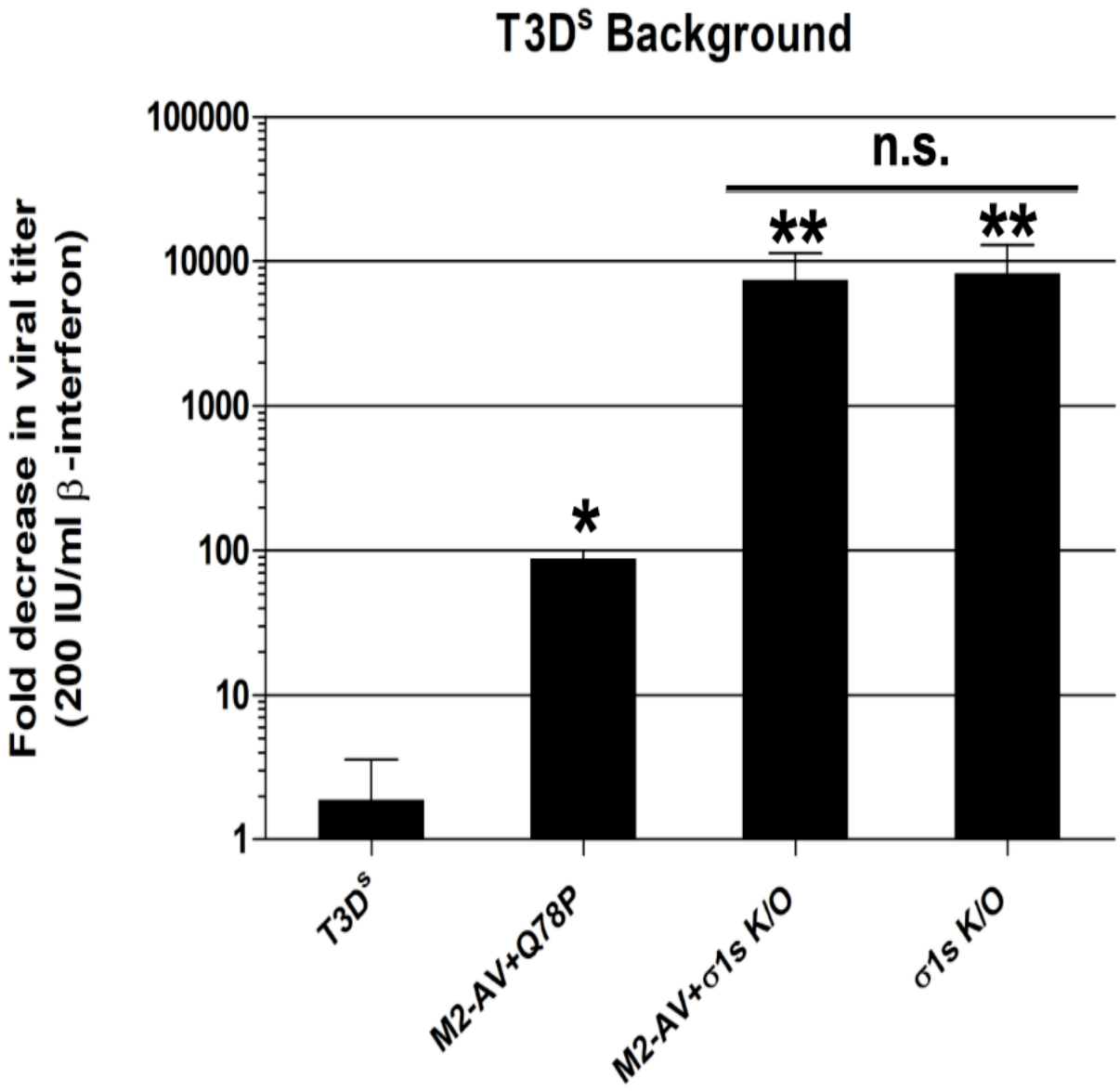
60
40
20
0
n.s
1
10
100
1000
IFN-β (IU/ml)
T3D^s
VeroAV
M2-AV+S1(Q78P) [T3D^s]
M2-AV+S1(N198K) [T3D^s]

Fig. 4. Interferon sensitivity of the σ1s-null (knockout) virus. Viruses knockout (K/O) for the σ1s protein (in the $T3D^S$ S1 gene) in the presence or absence of the VeroAV M2 gene, as described in the text, were compared with the Q78P mutant virus. Virus titers in the absence or presence of 200 IU/ml of ß- interferon were compared, as described in the text. Fold reductions in titers due to interferon presence are presented on a logarithmic scale. Error bars indicate the highest value obtained in at least two replicate experiments. The difference between $T3D^S$ and the mutant was statistically significant at $p < 0.0001$ (*) while the difference between the mutant and either of the two K/O viruses was significant at $p < 0.05$ (**) using Student's t-test; there was no significant difference (n.s.) between the two K/O viruses ($p > 0.5$).


T3D^s Background
Fold decrease in viral titer
(200 IU/ml β-interferon)
100000
10000
1000
100
10
1
n.s.
**
**
*
T3D^s
M2-AV+Q78P
M2-AV+σ1s K/O
σ1s K/O