Lymphatic type-1 interferon responses are critical for control of systemic reovirus dissemination

Matthew B. Phillips^{1,2}, Marcelle Dina Zita¹, Morgan A. Howells^{1,2}, Tiffany Weinkopff^{1,2},
and Karl W. Boehme^{1,2,*}

¹Department of Microbiology and Immunology and ²Center for Microbial Pathogenesis
and Host Inflammatory Responses, University of Arkansas for Medical Sciences, Little
Rock, AR

* Correspondence to Karl W. Boehme

Mailing address: 4301 W. Markham Dr. #511, Little Rock, AR 72205

Phone: (501) 686-5189

Email: kwboehme@uams.edu

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ABSTRACT

Mammalian orthoreovirus (reovirus) spreads from the site of infection to every organ system in the body via the blood. However, mechanisms that underlie reovirus hematogenous spread remain undefined. Non-structural protein $\sigma 1s$ is a critical determinant of reovirus bloodstream dissemination that is required for efficient viral replication in many types of cultured cells. Here, we used the specificity of the $\sigma 1s$ protein for promoting hematogenous spread as a platform to uncover a role for lymphatic type-1 interferon (IFN-1) responses in limiting reovirus systemic dissemination. We found that replication of a $\sigma 1s$ -deficient reovirus was restored to wild-type levels in cells with defective type-1 interferon α -receptor (IFNAR1) signaling. *In vivo*, reovirus spreads systemically following oral inoculation of neonatal mice, whereas the $\sigma 1s$ -null virus remains localized to the intestine. We found that $\sigma 1s$ enables reovirus spread in the presence of a functional IFN-1 response, as the $\sigma 1s$ -deficient reovirus disseminated comparably to wild-type virus in IFNAR1^{-/-} mice. Lymphatics are hypothesized to mediate reovirus spread from the intestine to the bloodstream. IFNAR1 deletion from cells expressing lymphatic vessel endothelium receptor-1 (LYVE-1), a marker for lymphatic endothelial cells, enabled the $\sigma 1s$ -deficient reovirus to disseminate systemically. Together, our findings indicate that IFN-1 responses in lymphatics limit reovirus dissemination. Our data further suggest that the lymphatics are an important conduit for reovirus hematogenous spread.

IMPORTANCE

Type-1 interferon (IFN-1) responses are a critical component of the host response to viral infections. However, the contribution of specific cell and tissue types to control of viral infections is not known. Here, we found that reoviruses lacking nonstructural protein were more sensitive to IFN-1 responses than wild-type reovirus, indicating that $\sigma 1s$ is important for reovirus resistance to IFN-1 responses. The $\sigma 1s$ protein also is a key determinant of reovirus systemic spread. We used tissue-specific IFNAR1 deletion in combination with the IFN-1-sensitive $\sigma 1s$ -null reovirus as a tool to identify a role for lymphatics in reovirus dissemination. We used Cre-lox technology to delete type-1 interferon α -receptor (IFNAR1) in lymphatic cells and found that the IFN-1-sensitive $\sigma 1s$ -deficient reovirus disseminated in mice with lymphatic endothelial cells-specific deletion of IFNAR1. Together, our results indicate that IFN-1 responses in lymphatics are critical for controlling reovirus systemic spread.

INTRODUCTION

Systemic dissemination is a fundamental step in viral pathogenesis. To spread within the host, viruses must replicate in multiple cell and tissue types and overcome a variety of physical and physiological barriers, including host antiviral defenses.

Mammalian orthoreovirus (reovirus) is a member of the *Reoviridae*, a family of non-enveloped, dsRNA viruses that infects via respiratory or enteric routes and subsequently traffics to secondary organs and tissues, including the central nervous system (CNS) (1, 2). Reoviruses primarily disseminate via the blood, although serotype 3 (T3) reoviruses also spread by neural routes (3). In the intestine, reovirus infects intestinal epithelial cells (IECs) and cells in the Peyer's patch (PP) (4-6). Reovirus is hypothesized to traffic through the mesenteric lymph node (MLN) to the blood via the lymphatics (7). However, the functional route of reovirus systemic spread is not defined.

Reovirus dissemination is influenced by a combination of host and viral factors (8). A key host determinant of reovirus spread is junctional adhesion molecule-A (JAM-A), a cell surface receptor for reovirus (9, 10). JAM-A is a tight junction protein that promotes polarization and barrier formation by epithelial and endothelial cells (11, 12). JAM-A also is expressed on monocytes, lymphocytes, dendritic cells, and platelets where it aids in cell migration and extravasation (11, 13, 14). Even though JAM-A is dispensable for reovirus replication in the intestine, it is required for hematogenous spread (10). JAM-A on endothelial cells is required for establishment of viremia, as well as egress of reovirus from the bloodstream into organs (15). A viral factor required for reovirus systemic spread is non-structural protein $\sigma 1s$ (16). Similar to JAM-A, $\sigma 1s$ is dispensable for reovirus replication in the intestine, but is essential for trafficking to the

bloodstream (17, 18). The 14 kDa $\sigma 1s$ protein is encoded by the S1 gene segment, which also encodes the attachment protein $\sigma 1$ (7). Although $\sigma 1s$ is not required for reovirus to traffic from the PP to the MLN, $\sigma 1s$ is necessary for the increase in viral MLN titers observed following oral infection (17). This result suggests that $\sigma 1s$ is necessary for efficient reovirus replication in the MLN. This data further indicates that $\sigma 1s$ is required for viral spread from the PP through intestinal lymphatics to the MLN and ultimately the bloodstream, thereby allowing the establishment of viremia and dissemination to sites of secondary replication. In culture, $\sigma 1s$ facilitates reovirus replication in numerous cell lines, including SV40-immortalized endothelial cells (SVECs) and murine embryonic fibroblasts (MEFs) (16, 19-21). In these cell lines, $\sigma 1s$ functions as a replication accessory factor that promotes reovirus protein synthesis (21). Therefore, $\sigma 1s$ may promote efficient viral replication in cells that are required for reovirus dissemination.

Type-I interferons (IFN-1) are critical for host control of viral infections (22). IFN-1 (IFN- α/β) is produced in responses viral infection and signals in an autocrine or paracrine manner to induce expression of hundreds of IFN stimulated genes (ISGs) that function to limit viral replication through a variety of mechanisms (23-25). Adult mice are normally refractory to reovirus disease. However, reovirus infection is lethal in adult mice that lack interferon- α receptor subunit 1 (IFNAR1) and cannot respond to IFN-1 (26). In IFNAR1^{-/-} mice, IFNAR1 expression on hematopoietic cell is required for protection from reovirus (26). Neonatal mice also succumb more rapidly to reovirus and have higher viral loads than wild-type mice (27, 28). Although IFN-1 is an important host

response against reoviruses *in vivo*, the cell types responsible for IFN-1-mediated protection against reoviruses are not defined.

Here, we used tissue-specific IFNAR1 deletion in combination with the IFN-1-sensitive reovirus as a tool to identify a role for lymphatics in reovirus dissemination. We found that $\sigma 1s$ is a viral determinant of reovirus resistance to IFN-1 responses in cultured cells and *in vivo*, as $\sigma 1s$ -deficient reovirus disseminates efficiently in IFNAR1^{-/-} mice. Using Cre-lox technology we found that the IFN-1-sensitive $\sigma 1s$ -deficient reovirus disseminated in mice with lymphatic endothelial cells-specific deletion of IFNAR1. Together, our results indicate that IFN-1 responses in lymphatics are a critical barrier that reovirus must overcome to spread systemically.

RESULTS

$\sigma 1s$ facilitates reovirus replication in the presence of IFN-1 responses. To determine the relationship between $\sigma 1s$ and IFN-1 responses, we tested whether $\sigma 1s$ promotes reovirus replication in cells with an intact IFN-1 response. Murine SV40-immortalized endothelial cells (SVEC 4-10; SVECs), a mouse lymphatic endothelial cell line, were infected with T1L or T1L $\sigma 1s$ -null in the presence of isotype control or anti-IFNAR1 antibodies and assessed viral spread in culture over a 7 day period (Fig 1A). T1L produced large foci and spread throughout the culture in the presence of the control antibody. In contrast, T1L $\sigma 1s$ -null was limited to individual cells with no multicellular foci apparent. Treatment with anti-IFNAR1 antibodies led to T1L $\sigma 1s$ -null spread and production of numerous large infected foci. To quantitatively assess the effect of IFN-1 responses on viral replication, we measured viral yields from wild-type and SVECs in

which *IFNAR1* was deleted using CRISPR-Cas9 editing (IFNAR1-CRISPR SVECs). Consistent with our previous work (29), T1L produced approximately 10-fold more virus than T1L σ 1s-null in wild-type SVECs. In contrast, T1L and T1L σ 1s-null replicated to equivalent levels in IFNAR1-CRISPR SVECs (Fig 1B). Similar results were obtained in *IFNAR1*^{+/+} and *IFNAR1*^{-/-} MEFs (Fig 1C). T1L replicated to significantly higher levels than T1L σ 1s-null in *IFNAR1*^{+/+} MEFs, but T1L and T1L σ 1s-null produced comparable yields in *IFNAR1*^{-/-} MEFs. These results indicate that σ 1s facilitates efficient reovirus replication in the presence of IFN-1 responses.

To determine whether σ 1s enhances reovirus sensitivity to IFN-1, replication of T1L and T1L σ 1s-null was measured in L929 cells treated with recombinant IFN β prior to infection (Fig 1D) (30). Unlike SVECs or MEFs, σ 1s is not required for reovirus replication in L929 cells and allows assessment of the relationship between σ 1s and IFN-1 independent of σ 1s effects on viral replication (21, 31). Consistent with previous work, T1L and T1L σ 1s-null replicated equivalently in untreated L929 cells. Whereas T1L replication was modestly impaired by IFN β , the reduction in T1L σ 1s-null yields were significantly more pronounced. Together, these findings indicate that σ 1s enhances reovirus resistance to IFN-1.

The σ 1s protein enhances reovirus replication in SVECs and MEFs by promoting viral protein production (29). Inhibition of viral protein synthesis is a key mechanism by which IFN-1 responses combat viral infections (22). To determine whether σ 1s allows reovirus to overcome IFN-1-mediated protein synthesis inhibition, viral protein production was assessed in wild-type and IFNAR1-CRISPR SVECs (Fig 2A) and *IFNAR1*^{+/+} and *IFNAR1*^{-/-} MEFs (Fig 2B). Consistent with previous results (21), T1L

produced more viral protein than T1L $\sigma 1s$ -null in wild-type SVECs and MEFs. Although both viruses produced more protein in IFNAR1-CRISPR SVECs and *IFNAR1*^{-/-} MEFs than in wild-type cells, protein expression by T1L $\sigma 1s$ -null remained substantially lower than T1L in both cell types. Together, these data indicate that $\sigma 1s$ does not directly counteract the inhibition of reovirus protein synthesis caused by IFN-1.

We next quantified viral RNA to determine the effect of IFN-1 responses on viral RNA synthesis by T1L and T1L $\sigma 1s$ -null. Consistent with previous results (21), viral S4 mRNA (Fig 2C) and negative-sense RNA (Fig 2D) levels were similar between T1L and T1L $\sigma 1s$ -null in wild-type SVECs. T1L and T1L $\sigma 1s$ -null produced approximately four-fold more S4 mRNA and negative-sense RNA in IFNAR1-CRISPR SVECs compared to control cells. Like control cells, S4 mRNA and negative-sense RNA levels were comparable between T1L and T1L $\sigma 1s$ -null. These data are consistent with previous findings that $\sigma 1s$ is dispensable for reovirus RNA synthesis (29). These results indicate that while IFN-1 responses limit reovirus RNA synthesis, $\sigma 1s$ does not specifically modulate antiviral responses that prevent viral RNA accumulation. These results further suggest that T1L $\sigma 1s$ -null replication in cells lacking IFN-1 responses may result from enhanced viral RNA synthesis, which increases viral protein synthesis and progeny production.

The $\sigma 1s$ protein facilitates reovirus dissemination in the face of IFN-1 responses *in vivo*. Our data and published studies (32) indicate that $\sigma 1s$ contributes to reovirus IFN-1 resistance in cultured cells. To determine whether $\sigma 1s$ is required for reovirus to overcome IFN-1 responses *in vivo*, we assessed T1L and T1L $\sigma 1s$ -null spread in wild-type and *IFNAR1*^{-/-} mice (Fig 3). T1L replicated in the intestine and

spread systemically in wild-type mice. Consistent with previous findings (16), although T1L $\sigma 1s$ -null produced comparable viral titers to T1L in the intestine of wild-type mice, T1L $\sigma 1s$ -null titers in peripheral organs (brain, heart, liver, and spleen) were near or below the limit of detection. As in wild-type mice, T1L and T1L $\sigma 1s$ -null produced comparable intestinal titers in *IFNAR1*^{-/-} mice. However, equivalent titers of T1L and T1L $\sigma 1s$ -null were recovered from peripheral organs of *IFNAR1*^{-/-} mice. These data indicate that the IFN-1 response acts as a barrier to reovirus hematogenous dissemination. These findings further suggest that $\sigma 1s$ is required for reovirus spread systemically in the presence of IFN-1 responses.

The $\sigma 1s$ protein is required for efficient reovirus replication in lymphatic endothelial cells. Reovirus is hypothesized to spread via the lymphatics, which are largely formed from lymphatic endothelial cells (LECs) (33). Previous work revealed that $\sigma 1s$ was required for efficient reovirus replication in SVECs, an immortalized lymphatic endothelial cell line (21). To determine whether $\sigma 1s$ is required for reovirus replication in primary LECs, we quantified T1L and T1L $\sigma 1s$ -null progeny yields produced by LECs derived from C57BL/6 mice (Fig 4A). We found that T1L generated significantly higher progeny yields than T1L $\sigma 1s$ -null in primary LECs at both MOIs tested. In cells where $\sigma 1s$ is required for reovirus replication, $\sigma 1s$ also mediates optimal viral protein production (21). In primary LECs, we observed that differential replication of wild-type and $\sigma 1s$ -deficient viruses correlated with differences in viral protein production, as T1L produced substantially more viral protein than T1L $\sigma 1s$ -null (Fig 4B). Together, these data indicate that $\sigma 1s$ promotes reovirus replication in primary LECs by enhancing viral protein production.

Lymphatics facilitate reovirus dissemination. If lymphatics function as

conduits for reovirus dissemination, we hypothesized that ablating IFN-1 responses specifically in lymphatic endothelial cells would enable dissemination of $\sigma 1$ s-deficient reovirus. To test this hypothesis, we used the IFN-1-sensitive $\sigma 1$ s-null reovirus in combination with lymphatic-specific deletion of *IFNAR1*. We first confirmed that primary LECs secrete IFN β in response to reovirus infection (Fig 4C) and produce ISGs following IFN-1 treatment (Fig 4D). To generate lymphatic-specific *IFNAR1* deletion mice, *IFNAR1^{fl/fl}* mice were crossed with *Lyve1-Cre* mice (34, 35). LYVE-1 is a receptor for hyaluronan that promotes LEC proliferation (36, 37). LYVE-1 is a commonly used marker for the lymphatic endothelium, but also is expressed on liver sinusoid, some tissue-resident macrophages, and a subset of hematopoietic stem cells (34, 38-40). T1L disseminated in the parental *IFNAR1^{fl/fl}* (Fig 5A) and *Lyve1-Cre* (Fig 5B) strains following oral inoculation, similar to results obtained with C57BL/6 mice (18). In contrast, T1L $\sigma 1$ s-null did not spread efficiently in either parental mouse strain.

The F1 progeny resulting from crossing *IFNAR1^{fl/fl}* and *Lyve1-Cre* strains were bred to *IFNAR1^{fl/fl}* mice. The resulting progeny (*Lyve1-Cre-IFNAR1^{+/fl}*, *IFNAR1^{+/fl}*, *IFNAR1^{fl/fl}* littermate controls and *Lyve1-Cre-IFNAR1^{fl/fl}* LEC *IFNAR1* deletions) were infected with T1L or T1L $\sigma 1$ s-null and at 4 days post-infection viral tissue titers were determined. Mice were genotyped by PCR at the time of harvest to determine their *Cre* and *IFNAR1* status (Fig 5C). Following oral infection, we found that T1L titers were substantially higher on day 4 than T1L $\sigma 1$ s-null in the MLNs of littermate control animals (Fig 5D). In contrast, T1L and T1L $\sigma 1$ s-null produced comparable titers in MLNs from

Lyve1-Cre-IFNAR1^{fl/fl} mice. These data indicate that IFN-1 responses in the lymphatics impair spread of σ 1s-deficient reovirus to the MLN.

We next assessed reovirus spread in mice with lymphatic *IFNAR1* deletion. In littermate control mice, T1L and T1L σ 1s-null exhibited comparable titers in the intestine, but only T1L had high titers in peripheral organs. T1L σ 1s-null titers in organs from littermate control mice were near or below the level of detection (Fig 5E). These data are consistent with observations in wild-type, *IFNAR1^{fl/fl}*, and *Lyve1-Cre* mice that σ 1s is required for efficient reovirus dissemination. In contrast, T1L and T1L σ 1s-null produced largely comparable titers in all organs of *Lyve1-Cre-IFNAR1^{fl/fl}* mice. Thus, deletion of *IFNAR1* in lymphatics enables dissemination of σ 1s-deficient reovirus. Taken together, these results indicate that lymphatic IFN-1 responses are critical for controlling reovirus dissemination.

DISCUSSION

Here, we identified a role for lymphatic IFN-1 responses in controlling hematogenous reovirus dissemination. In the intestine, reovirus is transcytosed by microfold cells (M-cells) in the gut-associated lymphoid tissue (GALT) where it infects the basolateral surface of IECs (8). Replication in IECs mediates reovirus release into the stool for dissemination to future hosts (8, 41, 42). To disseminate, reovirus is taken up by cells in the Peyer's patch (4, 10, 18) and hypothesized to traffic through the MLN via the lymphatics and then to the bloodstream (8). However, the operant route of reovirus dissemination is not defined. Our data provide support for the hypothesis that

the lymphatics function in hematogenous reovirus dissemination, as IFNAR1 deletion in LYVE-1-expressing cells allowed spread of the IFN-1-sensitive $\sigma 1s$ -deficient reovirus.

Global and conditional deletion of JAM-A revealed that endothelial cells, but not hematopoietic cells, mediate establishment of reovirus viremia and egress from the blood into tissues (10, 43). It is possible that $\sigma 1s$ promotes reovirus replication in LECs that line lymphatic vessels and lymph nodes to provide a reservoir that seeds virus for trafficking through the lymphatics to the blood. Consistent with this hypothesis, we found that $\sigma 1s$ was required for reovirus protein expression and replication in primary LECs (Fig 4). LYVE-1 is predominantly expressed on LECs, but also reported to be expressed on a small subset of fetal and adult hematopoietic stem cells, liver blood sinusoids, and adult tissue-resident macrophages (34, 38-40). Conditional expression of JAM-A on hematopoietic cells is insufficient to restore reovirus hematogenous spread in JAM-A-deficient mice, indicating that hematopoietic cells do not mediate reovirus dissemination (43). It also is unlikely that reovirus accesses liver sinusoids following oral inoculation. Finally, our studies use neonatal mice and adult tissue-resident macrophages would not be involved in dissemination.

It also is possible that loss of IFN-1 signaling in LYVE-1-positive cells increases the permeability of the lymphatic endothelium, thereby allowing reovirus to escape the lymphatic vessels. IFN-1 controls LEC expansion in response to viral infection (44) and also modulates vascular endothelial barrier function, particularly at the blood-brain barrier (45-47). Lack of IFN-1 signaling in the lymphatic endothelium could allow the $\sigma 1s$ -null virus to leak into the lymphatic vessels and traffic to the blood. Finally, loss of IFN-1 responses in the LECs could alter the transport dynamics of the lymphatics. In the

skin, IFN-1 signaling blocks fluid transport to the regional lymph node and limits poxvirus dissemination (48). If IFN-1 has similar effects on the dynamics of gut lymphatics, removing IFNAR1 from LECs could alleviate the normal interruption of lymphatic flow designed to impede viral dissemination.

The $\sigma 1s$ protein is dispensable for reovirus replication in the intestine, but is required for dissemination to peripheral organs (18). Our data suggest that the IFN-1 system restricts reovirus spread in the absence of $\sigma 1s$. However, why $\sigma 1s$ is dispensable for reovirus replication in the intestine remains an open question. One possibility is that reovirus replication in the intestine is largely controlled by interferon- λ (IFN- λ), as opposed to IFN-1 (41, 42, 49). Expression of the IFN- λ receptor, IFN- λ receptor 1 (IFNLR1), is largely restricted to the epithelial compartment, which also lacks IFNAR expression (50, 51). Consequently, IFN- λ protects IECs whereas IFN-1 functions in non-epithelial tissues (52). Like IFN-1, IFN- λ induces ISG expression, but IFN-1 induces ISG with more rapid kinetics than IFN- λ and the magnitude of the antiviral response is greater for IFN-1 than IFN- λ (53). IFN- λ limits reovirus replication in the intestine, as mice lacking IFNLR1 or IFN- $\lambda 2/3$ have elevated reovirus IEC infection and shedding (41, 42, 54). We found no difference in viral intestinal titers between wild-type and $\sigma 1s$ -deficient viruses in wild-type or IFNAR1-knockout mice. These data are consistent with IFN- λ as the primary means of controlling reovirus replication in the intestine. These findings also suggest that $\sigma 1s$ is not required for reovirus to overcome IFN- λ . It is possible that $\sigma 1s$ is more important for resisting IFN-1 than IFN- λ responses due to the lower potency of IFN- λ compared to IFN-1. However, the relationship between $\sigma 1s$ and IFN- λ remains to be explored.

Like most viruses, reovirus activates a variety of cellular mechanisms that function to impair viral protein synthesis, including PKR, which phosphorylates eIF2 α to block translation (55-57) and the OAS-RNaseL system that mediates RNA degradation (55). Reovirus must produce viral proteins in the face of host translational shutoff in order to replicate efficiently. Reovirus may even benefit from host shutoff, as viral replication is decreased in MEFs lacking PKR or expressing a constitutively active form of eIF2 α (57). Reovirus uses multiple mechanisms to evade host translational shutoff, including outer capsid protein σ 3 binding dsRNA to blunt PKR activation (58) and non-structural protein σ NS facilitating escape from host shutoff by mediating dissolution of stress granules (59). However, reovirus has other means to circumvent host shutoff responses. It is possible that σ 1s promotes reovirus protein expression by counteracting the function of one or more ISGs that block host translation (22, 60). Although σ 1s is required for reovirus replication in the presence of IFN-1 responses, σ 1s does not function as a classical IFN-1 antagonist (21), as IFN-1 secretion, IFNAR signaling, and ISG induction are comparable between wild-type and σ 1s-deficient viruses (21). We observed that viral protein expression by the σ 1s-deficient virus is only partially restored in the absence of IFNAR1 signaling. This result suggests that σ 1s promotes reovirus protein expression via an IFN-1-independent mechanism.

The σ 1s protein is required for systemic reovirus spread (17, 18). Here, we found that σ 1s is important for reovirus resistance to IFN-1 in cell culture and *in vivo*. We used the IFN-1-sensitive σ 1s-deficient reovirus in combination with tissue-specific deletion of IFNAR1 in lymphatic endothelial cells to identify a role for IFN-1 responses in lymphatics in controlling reovirus spread. Together, our findings provide new insight

into mechanisms that control reovirus dissemination and further define how reovirus spreads from mucosal sites of infection to target organs and tissues.

MATERIALS AND METHODS

Cells and viruses. Murine L929 fibroblasts were maintained in Joklik's modified Eagle medium (JMEM, Sigma) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Invitrogen), 1% L-glutamine (Invitrogen), 1% penicillin/streptomycin (Invitrogen), and 0.1% amphotericin B (Sigma). SV-40 immortalized endothelial cells (SVECs, ATCC), C57BL/6 murine embryonic fibroblasts (MEFs), IFNAR1^{-/-} MEFs, and human embryonic kidney 293 cells (HEK293T) were maintained in Dulbecco's modified Eagle medium (DMEM, Invitrogen) supplemented to contain 10% heat-inactivated FBS and 1% L-glutamine. SVECs lacking the IFN- α/β receptor (IFNAR1-CRISPR) were maintained in the same media as SVECs with 2 μ g/mL puromycin (Gibco). Primary LECs (Cell Biologics) were maintained in Endothelial Cell Medium (Cell Biologics) supplemented to contain 5% FBS, 0.1% VEGF, 0.1% heparin, 0.1% endothelial cell growth supplement (ECGS), 0.1% epidermal growth factor (EGF), 0.1% hydrocortisone, 1% L- glutamine, and 1% antibiotic-antimycotic solution.

Reoviruses were generated using plasmid-based reverse genetics as described previously (8, 16, 19, 61, 62). Purified reovirus stocks were obtained from second- or third-passage L929 cell lysates from twice-plaque-purified reovirus (63). Vertrel was used to extract reovirus particles, which were separated on a 1.2-1.4 g/cm³ CsCl density gradient and exhaustively dialyzed in virion storage buffer (150 mM NaCl, 15 mM MgCl₂, 10 mM Tris-HCl pH 7.8). Reovirus stocks were titrated by plaque assay on L929 fibroblasts (64).

CRISPR-Cas9 deletion of IFNAR1. IFNAR1 was deleted in SVECs using the

CRISPR-Cas9 system (65). The plentiCRISPRV.2 (Addgene) was digested with *BsmBI* and ligated with guide RNA sequences specific for IFNAR1, (IFNAR1+) 5'-CACCGGCTCGCTGTCTGTGGCGCGG-3' and (IFNAR1-) 5'-AAACCCGCGCCCACGACAGCGAGCC-3'. The cloned plasmids were transfected in to HEK293T cells in combination with pSPAX2 and pCMV-G plasmids using Lipofectemine 2000 (Invitrogen). Supernatants were collected at 24 and 48 h post-transfection, passed through a 0.45 µm syringe filters, and applied to SVECs in 6-well plates (~50% confluent). At 48 h post-transduction, puromycin (Invitrogen, 2 µg/mL) was added. Puromycin-selected SVECs were tested for IFNAR1 deletion by treatment with IFN-β (PBL) followed by RT-qPCR to measure ISG expression.

Viral replication assays. Monolayers of cells in 24 well plates (1x10⁵ cells/well)

were infected with T1L or T1L σ1s-null at an MOI of 1 PFU/cell at 4°C for 1 h. After adsorption, cells were washed twice with cold PBS and fresh media was added. Infected cells were freeze-thawed twice at the indicated times in the figure legends and viral titer was determined by plaque assay on L929 fibroblasts (64). Viral yields were calculated using the formula: $\log_{10}\text{yield}_{\text{tx}} = \log_{10}(\text{PFU/mL})_{\text{tx}} - \log_{10}(\text{PFU/mL})_{\text{t0}}$ where tx is the time post-infection.

Immunoblotting. Monolayers of cells in 6-well plates (1x10⁶ cells/well) were

mock infected or infected reovirus or treated with recombinant IFNβ (PBL Assay Science) as indicated in the figure legends. Whole cell lysates were collected in RIPA buffer (20 mM Tris pH 7.4, 150 mM NaCl, 1mM EDTA, 1% sodium dodecyl sulfate, 1% desoxycholate, 1/100 IGEPAL [NP-40]) at the indicated times. Total protein in each

sample was quantified using the DC protein assay (BioRad) and 25 µg protein were separated by SDS-PAGE. Proteins were transferred to nitrocellulose membrane and incubated in blocking buffer (5% milk in 1X TBS with 0.05% Tween-20 [TBS-T]) for 1 h. Membranes were incubated in blocking buffer containing reovirus-specific rabbit polyclonal anti-serum (1:2000 dilution), (need info for IFIT1 and IFIT2 antibodies) overnight at 4°C. Membranes were washed three times with TBS-T followed by incubation in blocking buffer containing horseradish peroxidase-conjugated goat anti-rabbit IgG (Jackson Immunolabs, 1:2000 dilution) for 1 h with rocking. Following three TBS-T washes, proteins were detected using SuperSignal West Chemiluminescent Substrate (ThermoFisher). Blots were stripped for re-probing by washing membranes three times with TBS followed by incubation in Restore Western Blot Stripping buffer (Thermo Scientific) for 15 min at RT. Following three washes in TBS, membranes were blocked as described above and β-actin was detected using mouse β-actin specific monoclonal antibody (Sigma, 1:10,000 dilution) and peroxidase-conjugated goat anti-mouse IgG (Jackson Immunolabs, 1:2000 dilution).

RT-qPCR. Monolayers of cells in 6 well plates (1×10^6 cells/well) were infected with T1L or T1L σ1s-null at an MOI of 10 PFU/cell. Total RNA was collected using the RNeasy Plus kit (QIAGEN). Reovirus S4 RNA was quantified using the Taq-Man Fast Virus One-Step Master Mix (Applied Biosystems) and GAPDH was detected as an endogenous control using the Pre-Developed TaqMan Assay Reagent kit for mouse GAPDH (Applied Biosystems) as described previously (21). The relative quantity (RQ) of reovirus positive- or negative-sense RNA was quantified using $t = 0$ h post-infection as the reference sample. the $\Delta\Delta C_T$ was calculated for each sample by the formula:

$\Delta\Delta C_T = (\text{Unknown } C_{T_{tx}} - \text{GAPDH } C_{T_{tx}}) - (\text{Unknown } C_{T_{t0}} - \text{GAPDH } C_{T_{t0}})$ where tx = time post-infection. The $\Delta\Delta C_T$ was then used to calculate RQ by the formula: $RQ = 2^{-\Delta\Delta C_T}$.

Mouse experiments. Animal husbandry, housing, and experiments were performed according to the guidelines of the Division of Laboratory Animal Medicine (DLAM) at UAMS. C57BL/6 (JAX stock #000664), C57BL/6 *IFNAR1*^{-/-} (JAX stock #028288), C57BL/6 *IFNAR1*^{fl/fl} (JAX stock #028256), and C57BL/6 *Lyve1-Cre* (JAX stock #012601) mice were obtained from Jackson Laboratory. *IFNAR1*^{fl/fl} and *Lyve1-Cre* mice were crossed to obtain *IFNAR1*^{+fl}/*Lyve1-Cre* heterozygous mice. *IFNAR1*^{+fl}/*LYVE1-Cre* heterozygous mice were crossed to *IFNAR1*^{fl/fl} mice for experiments, yielding litters of *IFNAR1*^{fl/fl}, *IFNAR1*^{+fl}, *IFNAR1*^{+fl}/*Lyve1-Cre*, and *IFNAR1*^{fl/fl}/*Lyve1-Cre* mice. Three-four day old mice were infected orally with 10⁴ PFU T1L or T1L σ 1s-null diluted in PBS as previously described (10, 66). At 4 days post-infection, organs were resected, homogenized, and viral titer was determined by plaque assay. Infected mice were genotyped after experiments using the KAPA HotStart Mouse Genotyping Kit (KAPA Biosystems) and primers for the floxed *IFNAR1* allele and the *Lyve1-Cre* gene from the Jackson Laboratory website.

Statistics. Differences in viral replication were determined by an unpaired Student's *t*-test. Differences in viral titer from mouse experiments were determined by Mann-Whitney test. Statistical tests were performed using Prism software (GraphPad Software, Inc.). *p* values < 0.05 were considered significant.

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FIGURE LEGENDS

Figure 1. The $\sigma 1s$ protein facilitates reovirus replication in the presence of IFN-1

responses. (A) SVECs were treated with control or anti-IFNAR1 antibodies immediately following infection with T1L or T1L $\sigma 1s$ -null at an MOI of 10 PFU/cell. At 7 days, infected cells were identified via indirect immunofluorescence using reovirus polyclonal antiserum. (B) Wild-type or IFNAR1-CRISPR SVECs or (C) wild-type (*IFNAR1^{+/+}*) or *IFNAR1^{-/-}* MEFs were infected with T1L or T1L $\sigma 1s$ -null at an MOI of 1 PFU/cell. Viral titers were determined at 0 and 24 h, and results are presented as the mean viral yield from three independent experiments. (D) L929 cells were left untreated or treated with 25 or 50 units of recombinant IFN- α/β for 6 h prior to infection with T1L or T1L $\sigma 1s$ -null at an MOI of 1 PFU/cell. Viral titers were determined at 0 and 24 h, and results are presented as the mean viral yield. Error bars represent SD. *, $p < 0.05$ as determined by Student's *t*-test.

Figure 2. The $\sigma 1s$ protein does not counteract IFN-1-mediated inhibition of

reovirus protein synthesis. (A) Wild-type or IFNAR1-CRISPR SVECs or (B) wild-type (*IFNAR1^{+/+}*) or *IFNAR1^{-/-}* MEFs were infected with T1L or T1L $\sigma 1s$ -null at an MOI of 10 PFU/cell. At 18 h, whole cell lysates were collected and separated by SDS-PAGE. Reovirus proteins were detected by western blot. β -actin was detected as a loading control. (C and D) WT or IFNAR1-CRISPR SVECs were infected with T1L or T1L $\sigma 1s$ -null at an MOI of 10 PFU/cell. At 0 or 18 h, total RNA was collected and relative expression of (C) mRNA or (D) negative-sense RNA was determined compared to 0 h. The RQ of positive- or negative-sense RNA was determined with reference to the

quantity at the 0 h. The RQ of reovirus mRNA was determined by subtracting the RQ of negative-sense viral RNA (representing genomic RNA production) from the RQ of positive-sense RNA. Data are presented as the mean log₂ RQ from three independent experiments. Error bars indicate standard deviations. *, $p < 0.05$; **, $p < 0.005$ as determined by Student's *t*-test.

Figure 3. The $\sigma 1s$ protein is required for reovirus dissemination in the presence of IFN-1 responses. Three-to-four day-old wild-type (*IFNAR1*^{+/+}) or *IFNAR1*^{-/-} neonatal mice were infected orally with 10⁴ PFU of T1L or T1L $\sigma 1s$ -null. At 4 days, the indicated organs were resected, homogenized, and viral titer was determined by plaque assay. Error bars represent SD. **, $p < 0.005$ as determined by Mann-Whitney test.

Figure 4. The $\sigma 1s$ protein facilitates reovirus protein synthesis and replication in primary lymphatic endothelial cells. (A) Primary LECs from C57BL/6 mice were infected with T1L or T1L $\sigma 1s$ -null at MOIs of 1 (left) or 10 (right) PFU/cell. At 0, 24, and 48 h, viral titers were determined by plaque assay. Results are presented as the mean viral yield from two independent experiments. Error bars represent SD. *, $p < 0.05$ as determined by Student's *t*-test. (B) Primary LECs were mock infected or infected with T1L or T1L at an MOI of 5 PFU/cell. At the indicated times, whole cell lysates were collected and separated by SDS-PAGE. Reovirus proteins were detected by western blot. β -actin was detected as a loading control. (C) Primary LECs were mock infected or infected with T1L or T1L $\sigma 1s$ -null at an MOI of 100 PFU/cell. At the indicated times, IFN β levels in the supernatants were determined by ELISA. (D) Primary LECs were

treated with 200 U IFN β or left untreated. At 6 h, whole cell lysates were collected and separated by SDS-PAGE. IFIT1 and IFIT2 were detected by western blot. β -actin was detected as a loading control.

Figure 5. IFN-1 responses in lymphatics limits reovirus dissemination. (A)

IFNAR1^{fl/fl} or (B) *Lyve1-Cre* mice were orally infected with 10⁴ PFU T1L or T1L σ 1s-null. At 4 days, the indicated organs were resected, homogenized, and viral titer was determined by plaque assay. (C) The genotype of transgenic mice was determined by performing PCR analysis of chromosomal DNA using primers specific for *IFNAR1* and *Cre*. The floxed *IFNAR1* alleles and *Cre* were differentiated based on migration of the PCR products in 1% agarose gel and stained with EtBr. (D and E) Littermate control (*IFNAR1^{fl/fl}*, *IFNAR1^{+/-}*, *Lyve1-Cre-IFNAR1^{+/-}*) or *Lyve1-Cre-IFNAR1^{fl/fl}* mice were orally infected with 10⁴ PFU T1L or T1L σ 1s-null. At 4 days, (D) MLNs or (E) the indicated organs were resected, homogenized, and viral titer was determined by plaque assay. Error bars represent SD. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0005$; ****, $p < 0.0001$ as determined Mann-Whitney test.

LITERATURE CITED

1. Sabin AB. 1959. Reoviruses. A new group of respiratory and enteric viruses formerly classified as ECHO type 10 is described. *Science* 130:1387-9
2. Dryden KA, Wang G, Yeager M, Nibert ML, Coombs KM, Furlong DB, Fields BN, Baker TS. 1993. Early steps in reovirus infection are associated with dramatic changes in supramolecular structure and protein conformation: analysis of virions and subviral particles by cryoelectron microscopy and image reconstruction. *J Cell Biol* 122:1023-41
3. Tyler KL, McPhee DA, Fields BN. 1986. Distinct pathways of viral spread in the host determined by reovirus S1 gene segment. *Science* 233:770-4
4. Wolf JL, Rubin DH, Finberg R, Kauffman RS, Sharpe AH, Trier JS, Fields BN. 1981. Intestinal M cells: a pathway for entry of reovirus into the host. *Science* 212:471-2
5. Wolf JL, Kauffman RS, Finberg R, Dambrauskas R, Fields BN, Trier JS. 1983. Determinants of reovirus interaction with the intestinal M cells and absorptive cells of murine intestine. *Gastroenterology* 85:291-300
6. Fleeton MN, Contractor N, Leon F, Wetzel JD, Dermody TS, Kelsall BL. 2004. Peyer's patch dendritic cells process viral antigen from apoptotic epithelial cells in the intestine of reovirus-infected mice. *J Exp Med* 200:235-45
<https://doi.org/10.1084/jem.20041132>.
7. Terence S. Dermody JP, Barbara Sherry. 2013. *Mammalian Orthoreovirus*, 6th ed. Lippincott Williams & Williams, Philadelphia, PA.
8. Boehme KW, Lai CM, Dermody TS. 2013. Mechanisms of reovirus bloodstream dissemination. *Adv Virus Res* 87:1-35 <https://doi.org/10.1016/B978-0-12-407698-3.00001-6>.
9. Barton ES, Forrest JC, Connolly JL, Chappell JD, Liu Y, Schnell FJ, Nusrat A, Parkos CA, Dermody TS. 2001. Junction adhesion molecule is a receptor for reovirus. *Cell* 104:441-51
10. Antar AA, Konopka JL, Campbell JA, Henry RA, Perdigoto AL, Carter BD, Pozzi A, Abel TW, Dermody TS. 2009. Junctional adhesion molecule-A is required for hematogenous dissemination of reovirus. *Cell Host Microbe* 5:59-71
<https://doi.org/10.1016/j.chom.2008.12.001>.
11. Williams LA, Martin-Padura I, Dejana E, Hogg N, Simmons DL. 1999. Identification and characterisation of human Junctional Adhesion Molecule (JAM). *Mol Immunol* 36:1175-88 [https://doi.org/10.1016/S0161-5890\(99\)00122-4](https://doi.org/10.1016/S0161-5890(99)00122-4).
12. Ebnet K. 2017. Junctional Adhesion Molecules (JAMs): Cell Adhesion Receptors With Pleiotropic Functions in Cell Physiology and Development. *Physiol Rev* 97:1529-1554 <https://doi.org/10.1152/physrev.00004.2017>.
13. Liu Y, Nusrat A, Schnell FJ, Reaves TA, Walsh S, Pochet M, Parkos CA. 2000. Human junction adhesion molecule regulates tight junction resealing in epithelia. *J Cell Sci* 113 (Pt 13):2363-74
14. Martin-Padura I, Lostaglio S, Schneemann M, Williams L, Romano M, Fruscella P, Panzeri C, Stoppacciaro A, Ruco L, Villa A, Simmons D, Dejana E. 1998. Junctional adhesion molecule, a novel member of the immunoglobulin

- superfamily that distributes at intercellular junctions and modulates monocyte transmigration. *J Cell Biol* 142:117-27 <https://doi.org/10.1083/jcb.142.1.117>.
15. Lai CM, Boehme KW, Puijssers AJ, Parekh VV, Van Kaer L, Parkos CA, Dermody TS. 2014. Endothelial JAM-A Promotes Reovirus Viremia and Bloodstream Dissemination. *J Infect Dis* doi:10.1093/infdis/jiu476 <https://doi.org/10.1093/infdis/jiu476>.
16. Boehme KW, Guglielmi KM, Dermody TS. 2009. Reovirus nonstructural protein sigma1s is required for establishment of viremia and systemic dissemination. *Proc Natl Acad Sci U S A* 106:19986-91 <https://doi.org/10.1073/pnas.0907412106>.
17. Boehme KW, Frierson JM, Konopka JL, Kobayashi T, Dermody TS. 2011. The reovirus sigma1s protein is a determinant of hematogenous but not neural virus dissemination in mice. *J Virol* 85:11781-90 <https://doi.org/10.1128/JVI.02289-10>.
18. Boehme KW, Guglielmi KM, Dermody TS. 2009. Reovirus nonstructural protein sigma1s is required for establishment of viremia and systemic dissemination. *Proc Natl Acad Sci U S A* 106:19986-91 <https://doi.org/10.1073/pnas.0907412106>.
19. Boehme KW, Frierson JM, Konopka JL, Kobayashi T, Dermody TS. 2011. The reovirus sigma1s protein is a determinant of hematogenous but not neural virus dissemination in mice. *J Virol* 85:11781-90 <https://doi.org/10.1128/JVI.02289-10>.
20. Hoyt CC, Richardson-Burns SM, Goody RJ, Robinson BA, DeBiasi RL, Tyler KL. 2005. Nonstructural protein sigma1s is a determinant of reovirus virulence and influences the kinetics and severity of apoptosis induction in the heart and central nervous system. *J Virol* 79:2743-53 <https://doi.org/10.1128/JVI.79.5.2743-2753.2005>.
21. Phillips MB, Stuart JD, Simon EJ, Boehme KW. 2018. Non-structural protein sigma1s is required for optimal reovirus protein expression. *J Virol* doi:10.1128/JVI.02259-17 <https://doi.org/10.1128/JVI.02259-17>.
22. Schneider WM, Chevillotte MD, Rice CM. 2014. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* 32:513-45 <https://doi.org/10.1146/annurev-immunol-032713-120231>.
23. Goubau D, Deddouche S, Reis e Sousa C. 2013. Cytosolic sensing of viruses. *Immunity* 38:855-69 <https://doi.org/10.1016/j.immuni.2013.05.007>.
24. Schlee M. 2013. Master sensors of pathogenic RNA - RIG-I like receptors. *Immunobiology* 218:1322-35 <https://doi.org/10.1016/j.imbio.2013.06.007>.
25. Schneider WM, Chevillotte MD, Rice CM. 2014. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* 32:513-45 <https://doi.org/10.1146/annurev-immunol-032713-120231>.
26. Johansson C, Wetzel JD, He J, Mikacenic C, Dermody TS, Kelsall BL. 2007. Type I interferons produced by hematopoietic cells protect mice against lethal infection by mammalian reovirus. *J Exp Med* 204:1349-58 <https://doi.org/10.1084/jem.20061587>.
27. Wu AG, Puijssers AJ, Brown JJ, Stencel-Baerenwald JE, Sutherland DM, Iskarpatyoti JA, Dermody TS. 2018. Age-dependent susceptibility to reovirus encephalitis in mice is influenced by maturation of the type-I interferon response. *Pediatr Res* doi:10.1038/pr.2018.13 <https://doi.org/10.1038/pr.2018.13>.

28. Luethy LN, Erickson AK, Jesudhasan PR, Ikizler M, Dermody TS, Pfeiffer JK. 2016. Comparison of three neurotropic viruses reveals differences in viral dissemination to the central nervous system. *Virology* 487:1-10
<https://doi.org/10.1016/j.virol.2015.09.019>.
29. Phillips MB, Stuart JD, Simon EJ, Boehme KW. 2018. Non-structural protein sigma1s is required for optimal reovirus protein expression. *J Virol* doi:10.1128/JVI.02259-17 <https://doi.org/10.1128/JVI.02259-17>.
30. Jacobs BL, Ferguson RE. 1991. The Lang strain of reovirus serotype 1 and the Dearing strain of reovirus serotype 3 differ in their sensitivities to beta interferon. *J Virol* 65:5102-4
31. Rodgers SE, Connolly JL, Chappell JD, Dermody TS. 1998. Reovirus growth in cell culture does not require the full complement of viral proteins: identification of a sigma1s-null mutant. *J Virol* 72:8597-604
32. Lanoie D, Cote S, Degeorges E, Lemay G. 2019. A single mutation in the mammalian orthoreovirus S1 gene is responsible for increased interferon sensitivity in a virus mutant selected in Vero cells. *Virology* 528:73-79
<https://doi.org/10.1016/j.virol.2018.12.010>.
33. Randolph GJ, Ivanov S, Zinselmeyer BH, Scallan JP. 2017. The Lymphatic System: Integral Roles in Immunity. *Annu Rev Immunol* 35:31-52
<https://doi.org/10.1146/annurev-immunol-041015-055354>.
34. Pham TH, Baluk P, Xu Y, Grigorova I, Bankovich AJ, Pappu R, Coughlin SR, McDonald DM, Schwab SR, Cyster JG. 2010. Lymphatic endothelial cell sphingosine kinase activity is required for lymphocyte egress and lymphatic patterning. *J Exp Med* 207:17-27 <https://doi.org/10.1084/jem.20091619>.
35. Prigge JR, Hoyt TR, Dobrinen E, Capecchi MR, Schmidt EE, Meissner N. 2015. Type I IFNs Act upon Hematopoietic Progenitors To Protect and Maintain Hematopoiesis during Pneumocystis Lung Infection in Mice. *J Immunol* 195:5347-57 <https://doi.org/10.4049/jimmunol.1501553>.
36. Banerji S, Ni J, Wang SX, Clasper S, Su J, Tammi R, Jones M, Jackson DG. 1999. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. *J Cell Biol* 144:789-801
37. Wu M, Du Y, Liu Y, He Y, Yang C, Wang W, Gao F. 2014. Low molecular weight hyaluronan induces lymphangiogenesis through LYVE-1-mediated signaling pathways. *PLoS One* 9:e92857 <https://doi.org/10.1371/journal.pone.0092857>.
38. Mouta Carreira C, Nasser SM, di Tomaso E, Padera TP, Boucher Y, Tomarev SI, Jain RK. 2001. LYVE-1 is not restricted to the lymph vessels: expression in normal liver blood sinusoids and down-regulation in human liver cancer and cirrhosis. *Cancer Res* 61:8079-84
39. Lee LK, Ghorbanian Y, Wang W, Wang Y, Kim YJ, Weissman IL, Inlay MA, Mikkola HKA. 2016. LYVE1 Marks the Divergence of Yolk Sac Definitive Hemogenic Endothelium from the Primitive Erythroid Lineage. *Cell Rep* 17:2286-2298 <https://doi.org/10.1016/j.celrep.2016.10.080>.
40. Schledzewski K, Falkowski M, Moldenhauer G, Metharom P, Kzhyshkowska J, Ganss R, Demory A, Falkowska-Hansen B, Kurzen H, Ugurel S, Geginat G, Arnold B, Goerdts S. 2006. Lymphatic endothelium-specific hyaluronan receptor LYVE-1 is expressed by stabilin-1+, F4/80+, CD11b+ macrophages in malignant

- tumours and wound healing tissue in vivo and in bone marrow cultures in vitro: implications for the assessment of lymphangiogenesis. *J Pathol* 209:67-77
<https://doi.org/10.1002/path.1942>.
41. Baldrige MT, Lee S, Brown JJ, McAllister N, Urbanek K, Dermody TS, Nice TJ, Virgin HW. 2017. Expression of Ifnlr1 on Intestinal Epithelial Cells Is Critical to the Antiviral Effects of Interferon Lambda against Norovirus and Reovirus. *J Virol* 91 <https://doi.org/10.1128/JVI.02079-16>.
42. Peterson ST, Kennedy EA, Bringleb PH, Taylor GM, Urbanek K, Bricker TL, Lee S, Shin H, Dermody TS, Boon ACM, Baldrige MT. 2019. Disruption of type III interferon genes Ifnl2 and Ifnl3 recapitulates loss of the type III IFN receptor in the mucosal antiviral response. *J Virol* doi:10.1128/JVI.01073-19
<https://doi.org/10.1128/JVI.01073-19>.
43. Lai CM, Boehme KW, Puijssers AJ, Parekh VV, Van Kaer L, Parkos CA, Dermody TS. 2015. Endothelial JAM-A promotes reovirus viremia and bloodstream dissemination. *J Infect Dis* 211:383-93
<https://doi.org/10.1093/infdis/jiu476>.
44. Lucas ED, Finlon JM, Burchill MA, McCarthy MK, Morrison TE, Colpitts TM, Tamburini BAJ. 2018. Type 1 IFN and PD-L1 Coordinate Lymphatic Endothelial Cell Expansion and Contraction during an Inflammatory Immune Response. *J Immunol* 201:1735-1747 <https://doi.org/10.4049/jimmunol.1800271>.
45. Kraus J, Oschmann P. 2006. The impact of interferon-beta treatment on the blood-brain barrier. *Drug Discov Today* 11:755-62
<https://doi.org/10.1016/j.drudis.2006.06.008>.
46. Minagar A, Long A, Ma T, Jackson TH, Kelley RE, Ostanin DV, Sasaki M, Warren AC, Jawahar A, Cappell B, Alexander JS. 2003. Interferon (IFN)-beta 1a and IFN-beta 1b block IFN-gamma-induced disintegration of endothelial junction integrity and barrier. *Endothelium* 10:299-307
47. Liu P, Woda M, Ennis FA, Libraty DH. 2009. Dengue virus infection differentially regulates endothelial barrier function over time through type I interferon effects. *J Infect Dis* 200:191-201 <https://doi.org/10.1086/599795>.
48. Loo CP, Nelson NA, Lane RS, Booth JL, Loprinzi Hardin SC, Thomas A, Slifka MK, Nolz JC, Lund AW. 2017. Lymphatic Vessels Balance Viral Dissemination and Immune Activation following Cutaneous Viral Infection. *Cell Rep* 20:3176-3187 <https://doi.org/10.1016/j.celrep.2017.09.006>.
49. Ingle H, Peterson ST, Baldrige MT. 2018. Distinct Effects of Type I and III Interferons on Enteric Viruses. *Viruses* 10 <https://doi.org/10.3390/v10010046>.
50. Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, Langer JA, Sheikh F, Dickensheets H, Donnelly RP. 2003. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat Immunol* 4:69-77 <https://doi.org/10.1038/ni875>.
51. Sommereyns C, Paul S, Staeheli P, Michiels T. 2008. IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. *PLoS Pathog* 4:e1000017 <https://doi.org/10.1371/journal.ppat.1000017>.
52. Lee S, Baldrige MT. 2017. Interferon-Lambda: A Potent Regulator of Intestinal Viral Infections. *Front Immunol* 8:749 <https://doi.org/10.3389/fimmu.2017.00749>.

53. Lazear HM, Schoggins JW, Diamond MS. 2019. Shared and Distinct Functions of Type I and Type III Interferons. *Immunity* 50:907-923
<https://doi.org/10.1016/j.immuni.2019.03.025>.
54. Mahlakoiv T, Hernandez P, Gronke K, Diefenbach A, Staeheli P. 2015. Leukocyte-derived IFN-alpha/beta and epithelial IFN-lambda constitute a compartmentalized mucosal defense system that restricts enteric virus infections. *PLoS Pathog* 11:e1004782 <https://doi.org/10.1371/journal.ppat.1004782>.
55. Smith JA, Schmechel SC, Williams BR, Silverman RH, Schiff LA. 2005. Involvement of the interferon-regulated antiviral proteins PKR and RNase L in reovirus-induced shutoff of cellular translation. *J Virol* 79:2240-50
<https://doi.org/10.1128/JVI.79.4.2240-2250.2005>.
56. Qin Q, Hastings C, Miller CL. 2009. Mammalian orthoreovirus particles induce and are recruited into stress granules at early times postinfection. *J Virol* 83:11090-101 <https://doi.org/10.1128/JVI.01239-09>.
57. Qin Q, Carroll K, Hastings C, Miller CL. 2011. Mammalian orthoreovirus escape from host translational shutoff correlates with stress granule disruption and is independent of eIF2alpha phosphorylation and PKR. *J Virol* 85:8798-810
<https://doi.org/10.1128/JVI.01831-10>.
58. Yue Z, Shatkin AJ. 1997. Double-stranded RNA-dependent protein kinase (PKR) is regulated by reovirus structural proteins. *Virology* 234:364-71
<https://doi.org/10.1006/viro.1997.8664>.
59. Choudhury P, Bussiere L, Miller CL. 2017. Mammalian Orthoreovirus Factories Modulate Stress Granule Protein Localization by Interaction with G3BP1. *J Virol* doi:10.1128/JVI.01298-17 <https://doi.org/10.1128/JVI.01298-17>.
60. Sharpe AH, Fields BN. 1982. Reovirus inhibition of cellular RNA and protein synthesis: role of the S4 gene. *Virology* 122:381-91
61. Boehme KW, Ikizler M, Kobayashi T, Dermody TS. 2011. Reverse genetics for mammalian reovirus. *Methods* 55:109-13
<https://doi.org/10.1016/j.ymeth.2011.07.002>.
62. Kobayashi T, Antar AA, Boehme KW, Danthi P, Eby EA, Guglielmi KM, Holm GH, Johnson EM, Maginnis MS, Naik S, Skelton WB, Wetzel JD, Wilson GJ, Chappell JD, Dermody TS. 2007. A plasmid-based reverse genetics system for animal double-stranded RNA viruses. *Cell Host Microbe* 1:147-57
<https://doi.org/10.1016/j.chom.2007.03.003>.
63. Furlong DB, Nibert ML, Fields BN. 1988. Sigma 1 protein of mammalian reoviruses extends from the surfaces of viral particles. *J Virol* 62:246-56
64. Virgin HWt, Bassel-Duby R, Fields BN, Tyler KL. 1988. Antibody protects against lethal infection with the neurally spreading reovirus type 3 (Dearing). *J Virol* 62:4594-604
65. Sanjana NE, Shalem O, Zhang F. 2014. Improved vectors and genome-wide libraries for CRISPR screening. *Nat Methods* 11:783-784
<https://doi.org/10.1038/nmeth.3047>.
66. Tyler KL, Bronson RT, Byers KB, Fields B. 1985. Molecular basis of viral neurotropism: experimental reovirus infection. *Neurology* 35:88-92

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