studies performed on such mice. Passenger mutations may also affect regulatory elements, and some differences in gene expression between wild-type and *Sarm1*$^{-/-}$ mice may be attributable to this. Differentially expressed genes will therefore next be validated in macrophages from alternatively generated *Sarm1*$^{-/-}$ mice (using CRISPR). How SARM regulates events at the *Ccl5* promoter from outside of the nucleus requires further investigation. Since SARM has recently been shown to be an NADase, we generated a SARM mutant NADase knock-in mouse in order to assess the role of the NADase activity in SARM-dependent gene induction.
Disclosure of Interest: None Declared

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POSITIVE REGULATORY ROLE OF THE E3 UBIQUITIN LIGASE TRIM65 DOWNSTREAM THE IFNB INDUCTION PATHWAY
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Introduction: Viral infection triggers a fast and effective cellular response mediated primarily by the production of interferon β (IFNβ) that induces an anti-viral state through complex signal cascades. Therefore, the regulation of its induction and subsequent IFNβ signaling needs to be tightly controlled. There is growing evidence implicating the members of Tripartite-motif (TRIM) protein family of E3 ligases as critical players in this regulation. However, the exact role, mechanism of action, and the physiological relevance of their activity in *vivo* still remain poorly investigated. Previous work in our lab revealed that an unprecedented large number of TRIMs play critical roles as enhancers in the regulation of innate immune signaling pathways.
Methods: To study the role of TRIM65 in innate immune signaling we have used luciferase assays, overexpression in A549 and 293T cells, transient knock down using siRNAs in 293T cells, TRIM65 CRISPR knock out cell lines, Western blots, RT-qPCR, PR8-GLuc antiviral assays and immunofluorescence.
Results: Our recent studies focused on TRIM65 showed that this protein possesses antiviral activity comparable to TRIM25 in a PR8-GLuc antiviral assay. We have also demonstrated that its overexpression strongly increased not only the 2CARD-RIG-I- but also the IRF3-dependent activation of the INFβ and ISRE reporters. Consequently, IFNβ, ISG54 as well as other cytokines and ISGs mRNA levels are decreased in TRIM65 knock down and knock out (KO) cells upon infection compared to infected/treated control cells. Re-constitution assays on TRIM65 KO cells reverted the inhibition of IFNβ and ISG54 confirming the phenotype. Altogether, these data indicates a stimulatory role for TRIM65 downstream the interferon induction pathway.
Since the E3 ubiquitin ligase activity of many TRIMs has been linked to their antiviral functions, we have identified IRF3 and IRF7 as TRIM65 interacting factors and putative substrates. Immunofluorescence experiments suggest that IRF3 binds TRIM65 in the nucleus and *in vitro* ubiquitination assays indicates that TRIM65 is able to ubiquitinate IRF3 in a RING-independent manner. Our current studies are focused on completely delineate the molecular mechanism by which TRIM65-mediated ubiquitination or TRIM65 E3 Ub ligase-independent function could regulate the response to viral infection.
Conclusion: A better understanding of positive regulatory networks of the IFN response will provide new knowledge that will help to design more effective therapeutics.
Disclosure of Interest: None Declared

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INTRACELLULAR LIPID DROPLET ACCUMULATION OCCURS EARLY FOLLOWING VIRAL INFECTION AND IS REQUIRED FOR AN EFFICIENT INTERFERON RESPONSE
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Introduction: Lipid droplets (LDs) are increasingly becoming recognized as critical organelles in signaling events, transient protein sequestration and inter-organelle interactions. However, the role LDs play in anti-viral innate immune pathways remains largely unknown. This study sought to investigate whether LDs play a role in eliciting a robust cellular host response to viral infection.
Methods: We utilised a range of epithelial and non-epithelial cell types, including both primary murine astrocytes, embryonic fibroblasts and primary human blood derived monocytes/macrophages in a microscopy screen to visualize the dynamic induction of LDs following viral infection. ImageJ analysis of confocal images were used to determine the size and number of LDs in all cells. Both RNA (zika and influenza virus) and DNA (herpes simplex virus 1, HSV-1) viral replication was analyzed using RT-qPCR, plaque assays and confocal microscopy. Interferon (IFN) levels were assessed utilizing RT-qPCR and ELISA assays. The use of oleic acid treatment, as well as a chemical inhibitor of PLIN2 (ADRP), AACOCF3, enabled the assessment of LD biogenesis on viral infection. Both AG-1478, a kinase inhibitor of EGFR and EGFR$^{-/-}$ cell lines were utilized to determine EGFR's