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Influenza A Virus TRIMs the Type I Interferon Response

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The virulence of many pathogenic viruses depends on suppression of the innate type I interferon defense. For influenza viruses, a unique strategy has now been unraveled, as the viral nonstructural protein 1 was shown to inhibit activation of the pathogen recognition receptor RIG-I by binding the ubiquitin ligase TRIM25.

Induction of type I interferon (IFN) confers an antiviral state to cells and limits the replication and spread of viral pathogens, including human and swine influenza viruses (Garcia-Sastre and Biron, 2006). Many different cell types are capable of producing type I IFN, allowing them to respond immediately to an invading virus and prepare neighboring cells for the imminent pathogen attack. Secreted IFN-α and IFN-β bind to a common IFN-α/-β receptor (IFNAR), which, by signaling through the JAK-STAT pathway, leads to the activation of the trimeric transcription factor ISGF3 that in turn induces a multitude of latent host genes. Many of those IFN-induced gene products, such as the Mx protein or p56, have strong antiviral activities by themselves. Others, like the 2′-5′-oligoadenylate synthetases (OAS) and the protein kinase PKR, require the additional cofactor dsRNA for activation after virus infection. In addition, type I IFNs also activate various immune cells, such as dendritic cells, and therefore have additional importance for the initiation of adaptive immune responses (Fernandez-Sesma, 2007; Garcia-Sastre and Biron, 2006).

For decades, it was believed that long dsRNAs formed during viral replication were responsible for type I IFN induction, as this reaction could be mimicked by transfection of synthetic dsRNA. However, the specific receptor proteins for viral nucleic acids leading to IFN induction remained unknown, and there was also little experimental evidence for the production of dsRNA in cells infected with some groups of viruses, including influenza viruses.

However, our understanding of these processes was substantially advanced since the recent identification of a receptor-signaling system that is essential for antiviral responses to RNA viruses (Figure 1). It became evident that the RNA helicases MDA5 and RIG-I are intracellular receptors for dsRNA and/or single-stranded RNAs carrying 5′-triphosphate groups. Engagement of these receptors triggers a signaling module that leads, via interaction with the mitochondrial IFN-β promoter stimulator 1 protein (IPS-1, also known as MAVS/VISA/Cardif), to the activation of type I IFN genes by transcription factors, most importantly the IFN regulatory factors IRF-3 and IRF-7. These factors are activated through phosphorylation by the Iκ-B kinase family members TBK-1 or IKK-ε (reviewed by Wolff et al., 2008). Just recently, the picture was completed by the finding that interaction of RIG-I with its downstream effectors requires polyubiquitination within its second caspase recruitment domain (CARD), induced by the tripartite motif protein 25 (TRIM25), an E3 ubiquitin ligase (Gack et al., 2007) (Figure 1).

Given this powerful antiviral defense signaling program, it is no surprise that during coevolution with their hosts, probably all natural viruses have evolved gene products that interfere with the IFN-α/-β system at the induction or effector level (Garcia-Sastre and Biron, 2006). Influenza virus was the virus of choice that led to the discovery of IFN more than 50 years ago by Isaacs and Lindenmann (Isaacs and Lindenmann, 1957). Studies with this virus also provided the first indications of viral IFN counteraction. While Isaacs and Lindenmann observed induction of an "interfering" activity with heat-inactivated influenza virus, this was not seen with the replication competent strain, providing the first evidence that live influenza virus actually produces suppressive factors. However, it took many years in virus research before it was realized that the viral nonstructural protein 1 (NS1) is the main weapon of influenza virus to antagonize IFN expression, both at the level of induction and mRNA processing (Figure 1) (reviewed by Hale et al., 2008). The paramount importance of the NS1 protein for viral virulence was first illustrated by the apathogenic phenotype of an engineered NS1-deletion virus in wild-type hosts, which retained virulence in STAT1$^{-/-}$ mice (Garcia-Sastre et al., 1998). In the following years, it was well established by several laboratories that the NS1 proteins of both influenza A and B viruses antagonize antiviral signaling events initiated by the RIG-I helicase, the major pathogen recognition receptor for influenza viruses (Kato et al., 2006). However, the exact mode of action remained unclear. It was widely believed that the dsRNA-binding NS1 proteins inhibit antiviral reactions by sequestration of suspected dsRNA produced during viral replication. However, little dsRNA was detected in influenza virus-infected cells, and mutational inactivation of the dsRNA binding domain of the NS1 protein only partially eliminated IFN inhibition (reviewed in Wolff et al., 2008).

This puzzle has now been resolved by the work of Gack and colleagues, presented in this issue of *Cell Host & Microbe*. The human TRIM25 protein was identified as a crucial binding target of the NS1 protein (Figure 1). The NS1-TRIM25 interaction inhibits ubiquitination of RIG-I and further downstream antiviral signaling events, including binding to IPS-1 (Gack et al., 2009). The mutational perturbation

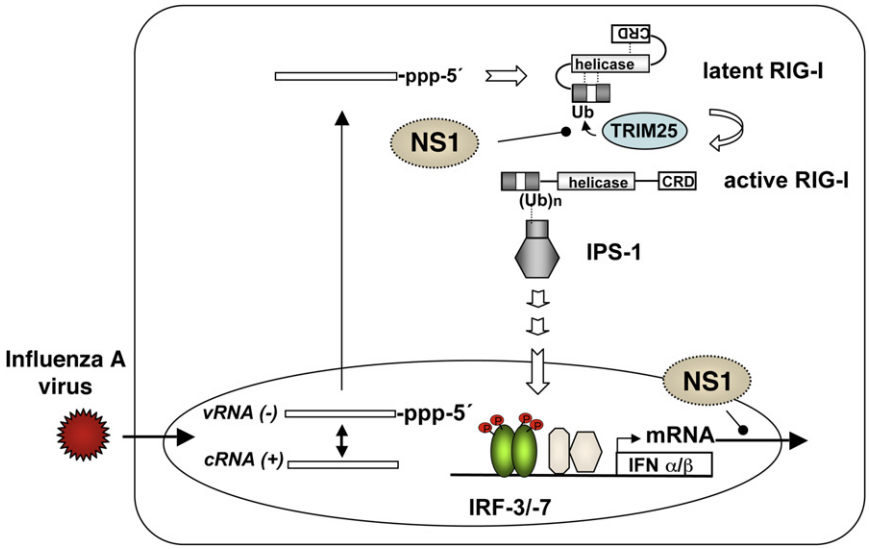

Figure 1. Influenza A Virus Inhibits the RIG-I Signaling Module by Binding of the Viral NS1 Protein to TRIM25

The viral genome is replicated in the nucleus of the infected cell. Late in infection, the viral gene segments carrying a 5′-triphosphate group are exported to the cytoplasm, where they are recognized by the RNA helicase RIG-I, inducing a conformational change. Subsequently, RIG-I binds to the mitochondrial IPS-1, which requires ubiquitination of RIG-I in the second CARD by the ubiquitin ligase TRIM25. This interaction triggers subsequent signaling for the activation of the transcription factors IRF-3/-7 that induce type I IFN genes. As shown by Gack et al., 2009, the viral NS1 protein forms a complex with TRIM25, which was shown to reduce ubiquitination of RIG-I and its downstream signaling for IFN induction. NS1 proteins of some influenza A viruses also inhibit the export of cellular poly(A)-RNA to the cytoplasm, including transcripts of antiviral genes.

of NS1 binding to TRIM25 in a recombinant influenza A virus led to a strong increase in IFN induction in vitro and concomitant loss of virulence in vivo. Remarkably, NS1 proteins from human, avian, and porcine influenza viruses interacted with TRIM25, indicating that the suppressive activity on RIG-I signaling is conserved in influenza A virus strains of different host backgrounds. This mechanism of action of the NS1 protein is a mode of IFN suppression that has not been observed for other viruses before.

While this is an important step to improve our understanding of how the NS1 manipulates the cell for efficient replication, the complete picture of NS1 action has yet to be unveiled. Different recent studies have identified a variety of other cellular interaction partners of NS1 besides TRIM25, including PKR, PI3K, Crkl/CrkL, CPSF, and many others (Hale et al., 2008). It has yet to be determined when and where binding of NS1 to these proteins occurs and to what extent the interaction with the different proteins contributes to efficient viral replication.

Knowledge of IFN suppressive mechanisms of the influenza virus NS1 protein is not only interesting from an academic point of view but is also important with regard to translation into practical approaches for the control of these dangerous pathogens. To this end, novel concepts for live attenuated vaccines based on NS1-engineered influenza virus have been successfully explored in animal models and were found promising for further clinical development (reviewed in Hale et al., 2008). In addition, a recent study suggested that the NS1 protein might be amenable to a novel antiviral concept that strengthens the innate antiviral defense. Screenings of a small molecule library led to the identification of three compounds that were able to inhibit NS1 activities in the suppression of IFN induction and reduced viral propagation (Basu et al., 2009). The recent appearance of the novel influenza A (H1N1) reassortant virus in the Mexican population and its unprecedented rapid worldwide spread highlight that such innovative antiviral approaches are highly needed for strengthening our arsenal against these malicious pathogens.

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