Influenza Antiviral Subversion: Now the Host Is in on the Act

Respiratory infections, whether novel zoonotic events like the current coronavirus disease (COVID-19) pandemic or recurrent seasonal epidemics like influenza, remain as intractable global health problems, and elucidation of the underlying innate immune mechanisms that drive antiviral immunity is urgently needed. Uniformly, pathogenic viruses encode mechanisms to subvert antiviral defenses, particularly those conferred by IFN, thus supporting the notion that IFN serves as an important antiviral defense (1–4). More recently, pathogen pattern recognition mechanisms such as TLR7 (Toll-like receptor 7) and TLR9 that recognize viral RNA in endosomes and RNA helicases such as RIG-I (retinoic induced gene) and MDA5 (melanoma differentiation-associated antigen 5) that sense cytoplasmic single- or double-stranded RNA (a unique feature of RNA virus replication and transcription) have been elucidated (5, 6). Unsurprisingly, deeper investigation has revealed virally encoded mechanisms of evading pathogen-associated molecular pattern (PAMP)-driven antiviral immunity through uncoupling of the signaling cascades stimulated by these PAMPs (7). Much research has focused on the viral-encoded mechanisms, yet little attention has focused on how host mechanisms may be recruited to usurp antiviral defense. In this issue of the *Journal*, Ouyang and colleagues (pp. 30–40) identify a host factor, NMI (N-Myc and STAT interactor), that is recruited by the influenza virus to reduce IFN type I antiviral signaling (8). Here, the authors show that NMI can enhance proteosomal degradation of IRF7 (IFN regulatory factor 7) via recruitment of the E3 ligase TRIM21 (tripartite motif-containing 21), leading to a decrease in type I IFN expression (Figure 1). The work is novel in that for the first time, a host-regulatory mechanism is used for the nefarious reduction in host clearance of the influenza virus. Much work remains to be done to fully uncover the importance of these findings; however, the study indicates that viral regulation of antiviral defense is not limited to virally encoded mechanisms.

Major advances in knowledge have been achieved during the past 20 years regarding viral host defense in mucosal, epithelial, and hematopoietic lineage tissues and cells. First, the elucidation of *Toll* proteins, particularly the TLR7/9 proteins (9), and subsequently the RNA helicase RIG-I–like receptors (RLRs) RIG-I and MDA5 (10), provide a starting point in antiviral defense. Although these sensors ultimately use distinct signaling cascades, such as Myd88 or TOLLIP by TLRs or MAVS (mitochondrial antiviral signaling protein) by RLRs, the cascades culminate in activating transactivating transcription factors in the IRF family, including IRF3 and IRF7, thus initiating IFN type I α or β gene expression (11). Subsequent induction and release of IFN α or β proteins from infected cells triggers antiviral defenses through ISGs (IFN-stimulated genes) in surrounding cells and tissues to mount a preemptive antiviral state in neighboring cells and tissues. Furthermore, IFNs represent a molecular bridge to the adaptive immune system, facilitating T and B lymphocyte maturation and playing a critical role in mounting cell-mediated and humoral immunity (12).

The role of NMI in normal cell homeostasis is largely unknown, although a role in inflammation involving the release of proinflammatory cytokines has been reported (13). In this issue, Ouyang and colleagues show that leukocytes from natural influenza infection in humans and experimental influenza infection in the lungs of mice produce elevated levels of NMI. Moreover, $NMI^{-/-}$ mice exhibited some protection against flu infection, including decreased lung viral burden and reduced weight loss, as well as increased survival in a lethal challenge model. These changes were concurrent with increased type I IFN and ISG expression, suggesting that NMI negatively regulates the induction of type I IFN transcriptional programs. Subsequent studies used molecular approaches to show direct interaction of NMI with IRF7. Moreover, NMI promotes activation of the E3 ligase TRIM21, which leads to ubiquitination and proteosomal degradation of IRF7, a well-recognized means by which virally encoded mediators subvert antiviral defenses. The finding that host proteins may contribute to this subversion of antiviral mechanisms adds yet another wrinkle to the complex biology of the subcellular environment during viral infection. Although the effects of *NMI* ablation in mice is somewhat mild, other mechanisms, most notably the viral NS1 (nonstructural 1) protein regulation of antiviral defenses, are still likely functional and likely still active in this experimental model (3).

Despite the clear role of NMI as a host regulator of IRF7, some caution is warranted. The studies in $NMI^{-/-}$ mice did not determine the role of NMI in either the lung epithelium or hematopoietic lineage cells in the lung. As such, it remains to be determined whether the biology identified here imparts its effects on antiviral defense in the infected epithelial cell or on the regulation of the innate immune cells involved in adaptive immunity. The heavy reliance on transfected cells for addressing the interactions between NMI, IRF7, and E3 ligase TRIM25 may not be representative of what occurs with physiological protein levels. Importantly, several lines of evidence indicate that murine and human antiviral defenses may be distinct. Influenza A virus (IAV) NS1 can bind to the RIG-1 CARD domain directly and facilitate TRIM25 ubiquitination and proteosomal degradation of RIG-I (14). However, NS1 binding to TRIM25 is species specific, with human culture-isolated IAV binding to human TRIM25 but not avian TRIM25, and avian-cultured IAV binding to avian TRIM25 but not to human. Neither human- nor avian-derived NS1 bind to murine TRIM25 (15). This, however, is advantageous, as it would eliminate the role of NS1-activated TRIM25-mediated proteosomal degradation of RIG-I/CARD in mice, thus reducing antiviral defenses that would obfuscate the role of NMI. It should be noted here that NMI-mediated regulation should also be investigated across multiple lineages of influenza species (e.g., A and B) and

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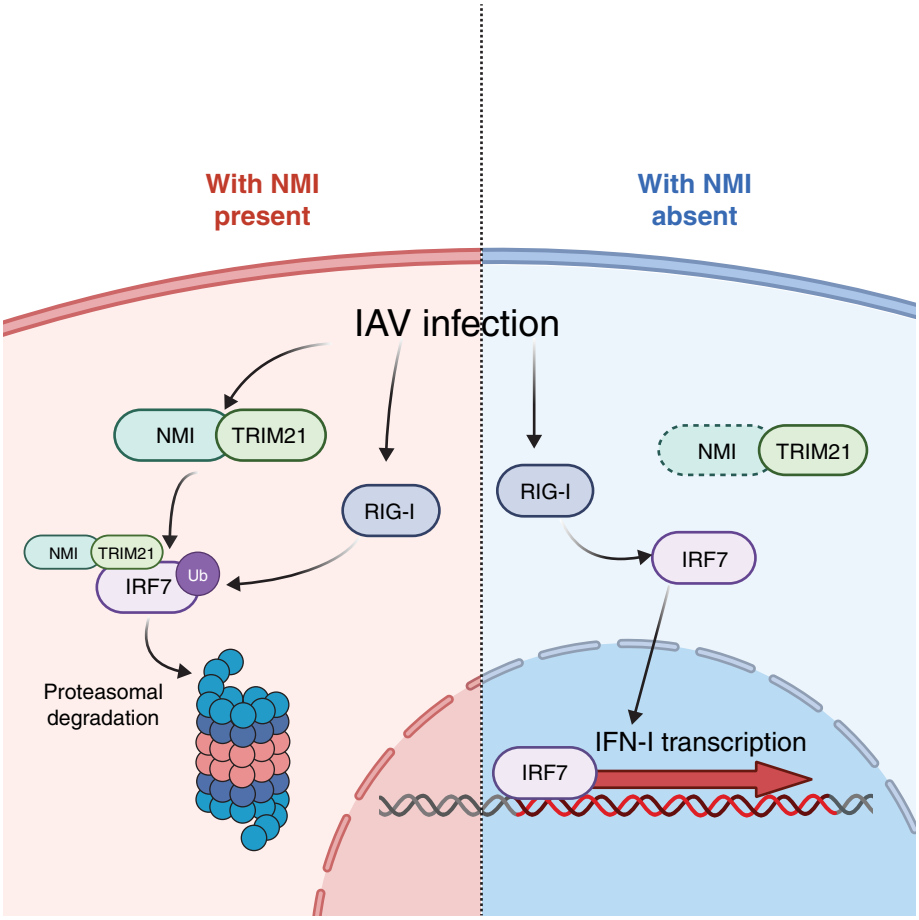

Figure 1. Overview of the role of NMI (N-Myc and STAT interactor) in subverting antiviral defense to influenza virus (created with BioRender.com). IAV = influenza A virus; IRF7 = IFN regulatory factor 7; RIG-I = retinoic acid-inducible gene-I; TRIM21 = tripartite motif 21; Ub = ubiquitin.

strains (e.g., H1N1 or H3N2). In particular, the mouse-adapted A/Puerto Rico/8/1934 strain used here encodes an additional protein in the PB1 gene, called PB1-F2, an important virulence factor affecting lethality in mice, and it is unclear how this might contribute to the biology elucidated here (16, 17). Despite these limitations, the finding of a host cellular factor that regulates antiviral defense is important and should be addressed in any future studies of type I IFN signaling after influenza.

In summary, the regulation of antiviral signaling and mediators that control activation remain complex; however, further elucidation of binding partners and activators/inhibitors will be required before a full understanding is obtained and interventional strategies can be investigated. In light of the role of subversion of IFN type I signaling encoded by viruses of distant phylogeny, targeting mechanisms of antiviral immunity could lead to the identification of a broad-spectrum antiviral agent that could be used to combat both endemic and emergent viruses such as influenza and coronaviruses. ■

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Erin N. Yepsen, B.S.
Kevin S. Harrod, Ph.D.
Department of Anesthesiology and Perioperative Medicine
University of Alabama at Birmingham
Birmingham, Alabama

ORCID ID: 0000-0003-0780-9470 (K.S.H.)

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