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Previews

Upping the ante on mammalian antiviral RNA interference

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SARS-CoV-2 has mutually illuminated our collective knowledge and knowledge gaps, particularly in antiviral defense and therapeutic strategies. A recent study in *Science* (Poirier et al., 2021) uncovers an ancient antiviral mechanism that mammals utilize to suppress viruses, including SARS-CoV-2 and Zika virus, that could have broad implications for therapeutic strategies.

Early antiviral defense in mammals centers on the detection of foreign RNA or DNA virus moieties by cytosolic or endosomal pattern recognition receptors (PRRs) (Lind et al., 2021) and the subsequent production of type I or III interferons (IFNs) and other cytokines such as IL-6 and IL-1β. IFNs trigger the transcription of hundreds of IFN stimulated genes (ISGs) that aim to eliminate the virus or the cell that it infects, although the precise function of most ISGs still elude us. Unlike in invertebrates, the innate immune response to viruses in mammals is also essential for then triggering critical adaptive immune memory.

However, not every mammalian cell executes this cytokine-based mode of innate antiviral defense equally. Owing to differences in cell-type-specific chromatin organization and tailored regulatory mechanisms to prevent autoinflammation and autoimmunity (Lind et al., 2021), the expression profile of PRRs and their signal transduction components, as well as accessibility at IFN gene promoters and enhancers, varies widely across cell types (Daman and Josefowicz, 2021). The exemplary IFN-inept cell type is embryonic stem cells (ESCs), whereas plasmacytoid dendritic cells and macrophages are chief IFN producers *in vivo*, although this is highly dependent on the infecting virus type. Furthermore, as we are witnessing in unprecedented real-time human experiments of COVID-19 patients, the IFN-mediated antiviral response depends on the tropism of the viral pathogen, which cell type responds accordingly, the ability of the virus and its variants to suppress host defense components, and a myriad of host-associated factors (e.g., genetic variations of innate immune components or inhibitory IFN-specific autoantibodies). Thus, other innate antiviral mechanisms may collaborate and/or compensate in mammals to enable robust defense in the interminable host arms race.

One alternative to the IFN-centric protein response has been suggested to be the ancient RNA-based defense strategy of RNA interference (RNAi). With this strategy, used dominantly in invertebrates, viral RNA is sensed by Dicer, a related helicase to mammalian PRRs retinoic-acid-inducible gene I (RIG-I) and melanoma-differentiation-associated protein 5 (MDA5) (Figure 1), that instead "dices" viral RNAs into ~22-nucleotide short interfering (si)RNAs that bind Argonaute (AGO) proteins for complementary virus silencing (Guo et al., 2019). However, in mammals, the highly conserved RNAi effector proteins Dicer and AGOs also mediate cellular micro (mi)RNA regulation of host messenger (m)RNAs, as well as RNAi responses driven by exogenously introduced siRNAs or shRNAs—the basis of Nobel Prizes and two recently FDA-approved drugs. For this reason, and due to the fact that both viruses (Guo et al., 2019; Li et al., 2016; Qiu et al., 2017) and host IFN components extensively suppress antiviral RNAi (Maillard et al., 2016), the utility of RNAi for antiviral defense in mammals has been persistently questioned (Cullen et al., 2013).

Central to this debate is the predicament that the antiviral roles of mammalian RNAi components, including Dicer and AGO2, have been notoriously difficult to cleanly decipher, since their deletion in mice and cells results in embryonic lethality and significantly disrupted miRNA populations. Unfortunately, this has also led to the study of mammalian antiviral RNAi in immortalized cells with limited physiological relevance. Moreover, RNAs of viral origin with canonical features of siRNAs, the molecular marker of active RNAi, have been challenging to cleanly detect in virus-infected mammalian cells. However, solutions to these experimental conundrums were recently provided, and evidence for antiviral RNAi in mammalian cells was demonstrated (Adiliaghdam et al., 2020; Guo et al., 2019; Li et al., 2016; Qiu et al., 2017). Another major sticking point is that mammalian Dicer is inefficient at processing long dsRNAs (Kennedy et al., 2015). Therefore, it has been difficult to fathom how a single Dicer protein—other organisms that utilize antiviral RNAi in concert with cellular miRNA regulation possess multiple Dicers—could lead to a robust and meaningful antiviral response in mammals.

Poirier et al. (2021) have illuminated mechanisms and utilization of antiviral RNAi in mammals. Here they identified an alternatively spliced isoform of Dicer in mammals, named antiviral Dicer (aviD), that possesses enhanced activity toward viruses. This Dicer isoform lacks exons 7 and 8, which code for the central Hel2i domain of the N-terminal helicase segment (Figure 1)—normally a negative regulator of Dicer's ability to process double-stranded (ds)RNA (Kennedy et al., 2015). aviD abundance is ~10-fold lower than full-length Dicer and is found preferentially in murine and human stem cells,

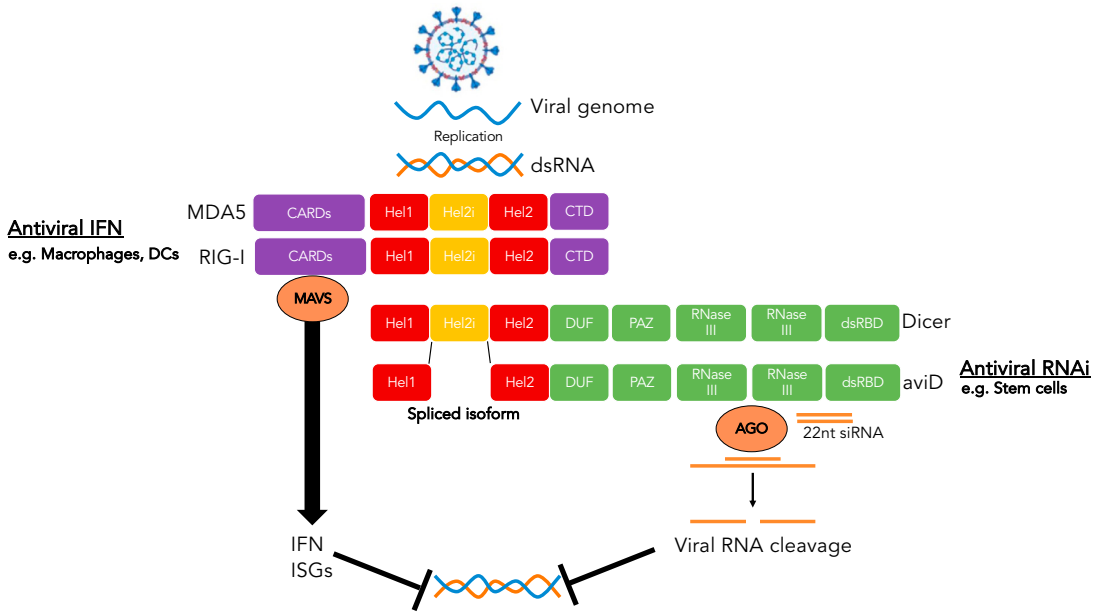

Figure 1. Cell-type-specific IFN and RNAi antiviral defense strategies triggered by viral dsRNA
Abbreviations: MDA5, melanoma-differentiation-associated protein 5; RIG-I, retinoic-acid-inducible gene I; MAVS, mitochondrial antiviral-signalling protein; RNAi, RNA interference; AGO, Argonaute; nt, nucleotide; dsRNA, double-stranded RNA; siRNA, short interfering RNA; aviD, antiviral Dicer; IFN, inteferon (type I and III); ISGs, inteferon-stimulated genes; DCs, dendritic cells; RNA, ribonucleic acid.

although it was detected in mature cells and tissues. In an *in vitro* dicing assay, aviD was ~2-fold more effective than Dicer at dicing dsRNA substrates into canonical siRNAs but interestingly had no advantage in miRNA processing. Furthermore, through mechanisms still to be elucidated, aviD was more resistant to LGP2, an ISG product known to inhibit dsRNA cleavage by full-length Dicer. aviD ($Dicer^{-/-}aviD^{+/+}$)-expressing HEK29 3T cells infected with RNA viruses (Sindbis virus [SINV], Zika virus [ZIKV], or severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) suppressed virus levels more effectively than infected Dicer ($Dicer^{+/+}aviD^{-/-}$)-expressing cells. Moreover, this antiviral activity of aviD was RNAi-dependent; $Dicer^{-/-}aviD^{+/+}$ cells had normal miRNA levels, aviD had little effect on virus with diminished AGO2, a version of aviD that was catalytically deficient in dsRNA cleavage was unable to control virus levels, and aviD had no activity toward two DNA viruses (vaccinia virus and herpes simplex virus 1). Importantly, the authors convincingly demonstrated the physiological significance of aviD antiviral function using brain organoids generated from $Dicer^{+/+}aviD^{-/-}$ stem cells. aviD-deficient/Dicer-sufficient brain organoids displayed significantly enhanced virus levels when infected with either ZIKV or SARS-CoV-2 than their Dicer-deficient/aviD-sufficient counterparts; this was correlated with loss of virus-derived siRNA production in the absence of aviD. Certainly, future work with aviD-deficient or aviD-transgenic animal models will be beneficial in solidifying aviD's role in antiviral defense *in vivo* and its potential use as an antiviral therapeutic.

Many components of our first line of antiviral defense in mammals originated early in evolution. Viperins, dominant antiviral ISGs in mammals, are found in bacteria, and a functional cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) DNA sensing pathway was recently revealed in *Drosophila* and bacteria. This elegantly controlled and executed study by Reis e Sousa and colleagues provides a key piece of data to an area that has been controversial: whether ancient antiviral RNAi is conserved in mammals. The work uncovers the use of an alternative isoform of the evolutionary conserved Dicer in mammals that has a greater capacity to dice dsRNA substrates into siRNA mediators and has a superior impact on virus infections than the full-length counterpart. ~95% of human genes undergo alternative splicing, which is essential to create protein diversity, often in a tissue-specific manner. It is interesting to speculate how IFN mechanisms, as well as other evolutionary pressures, may regulate the alternative splicing of Dicer to aviD for virus-centric functions when such alternative antiviral strategies are needed, especially in stem cells or other cells with a compromised antiviral IFN response. The need to understand how we humans mount a defense against viruses, and in turn how viruses counteract these strategies, has never felt more pressing. Undoubtedly, continuing the comprehensive, unblinkered, and unbiased study of life-virus interactions, with the ultimate goal of harnessing host antiviral strategies for therapeutic benefit, remains paramount.

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Changing the locks on intestinal signaling

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Endogenous peptides and structurally similar bacterial heat-stable enterotoxins (ST) bind guanylate cyclase-C (GC-C), resulting in fluid homeostasis or diarrhea, respectively. In this issue of *Cell Host & Microbe*, Carey et al., show how bats have evolutionarily maintained homeostatic signaling while avoiding pathogenic effects of ST.

Mammals have highly conserved mechanisms for maintaining fluid homeostasis in the intestine. Guanylate cyclase-C (GC-C), a protein dimer localized to the membrane of intestinal enterocytes, plays a central role by serving as a receptor for small cysteine-rich peptides produced by intestinal epithelia, guanylin, and uroguanylin (Figure 1A). Binding of these ligands to the receptor domain of GC-C stimulates the conversion of GTP to cGMP by the intracellular catalytic domain. The resulting increases in cGMP activate cGMP-dependent protein kinase II (PKG), which in turn modulates the activity of cellular ion channels. This includes the sodium-hydrogen ion exchanger NHE3 and the CFTR chloride channel, and it results in net export of salt and water into the intestinal lumen. These peptide hormones, in concert with their GC-C receptor, act in a paracrine fashion to maintain critical fluid efflux into the intestinal lumen.

Heat-stable enterotoxins (ST) are cysteine-rich short (18–19 amino acid) peptides secreted by Gram-negative enteric pathogens, particularly by enterotoxigenic *Escherichia coli* (ETEC). They share sequence homology and overall structure with the endogenous peptide hormones, and they bind GC-C with high affinity (Schulz et al., 1990), resulting in robust stimulation of the cGMP-PKG axis and pronounced secretory diarrhea (Figure 1B). Mutations in GC-C which preserve binding of peptide hormones at the expense of ST could mitigate the diarrheal effects of the toxins and offer a selective advantage.

Interestingly, in studies reported here by Carey, et al. (Carey et al., 2021), phylogenetic analysis of primate and bat GC-C sequences provides evidence for positive selection of sites confined to the ligand binding domain of the receptor, which are potentially selected by pressure from ST. Of note, variation in the ligand-binding domain was particularly intense in bats. These mutations in GC-C conferred a differential ability to respond to ST peptides, mitigating the impact of some while enhancing signaling by others. Remarkably, the investigators discovered that some species of bats had developed compensatory mutations in uroguanylin, offsetting the variation in GC-C and permitting continued paracrine signaling through the cGMP-PKG axis (Figure 1C). In effect, bats have evolved to change the lock to bar toxin engagement while keeping the new keys to themselves.

Although it remains unclear precisely which toxins, or which pathogens, might be driving the evolutionary changes to GC-C in bats and their corresponding endogenous peptides, the studies illustrate the potential for extraordinary co-evolution under microbial selection. An open question is whether it is actually ST-induced diarrhea per se that has driven the selection. Activation of cGMP production in the host is likely to have a multitude of effects that extend beyond modulation of ion channels to export NaCl and water. In fact, signaling through