RESEARCH HIGHLIGHT

ZBP1 inflames the SARS-CoV-2-infected lung

Chaoran Yin[1] and Siddharth Balachandran[1]✉

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The innate immune sensor Z-form nucleic acid binding protein 1 (ZBP1) detects Z-RNAs and induces Receptor Interacting Protein Kinase 3 (RIPK3)-driven pathways of cell death and inflammation during acute virus infections. In a recent paper published in *Cell Research*, Li et al. report that SARS-CoV-2 infections generate Z-RNAs and activate ZBP1/RIPK3-mediated necroptosis and inflammation, leading to lung injury in a mouse model.

Acute RNA virus infections typically result in the death of the infected cell. Programmed death pathways, e.g., apoptosis and necroptosis, are often responsible for such cell death. These pathways are important to limit virus spread (by destroying the infected cell before it becomes a virus factory) and to supply viral antigens and other immunogenic stimuli essential for the instigation of effective adaptive immune responses.[1] Cell death can also drive pathology, particularly if unchecked or necrotic in nature. Such pathology may underlie the 'cytokine storm' and allied lung damage seen during severe disease caused by respiratory viruses like influenza viruses and SARS-family coronaviruses.[2] Indeed, aberrant cell death is implicated in the pathogenesis of COVID-19,[2] but the underlying mechanisms by which such cell death is activated, and the contribution of misfiring programmed cell death pathways to this disease are still not clear.

In a recent study of *Cell Research*, Li et al.[3] provide new insights into how SARS-CoV-2 activates pathways of programmed cell death and consequent necro-inflammation, and how these pathways may contribute to the rampant lung inflammation seen in severe cases of COVID-19. Previously, the same group had reported that lung sections from fatal cases of COVID-19 manifested signs of both apoptosis and necroptosis, accompanied by a massive influx of inflammatory cells, necrotic cell debris, and fibrotic lung damage.[4] Caspase 8 was implicated in provoking apoptosis and inflammation,[4] but the upstream signals responsible for engendering these outcomes, and the ligands generated by the virus that activated these signals, were not known. Li et al. now show that Z-form nucleic acid binding protein 1 (ZBP1)-initiated signaling may underlie both cell death and lung inflammation, and that virus-generated Z-RNAs may serve as ZBP1 ligands to instigate necro-inflammatory signaling.

The first demonstration that ZBP1 was a necroptosis-activating innate immune sensor came from studies employing murine cytomegalovirus, a herpesvirus with a DNA genome.[5] More recent work has shown that ZBP1 also senses RNA viruses (e.g., influenza A and B viruses) and initiates necroptosis in cells infected with these viruses.[6] In the latter case, the virus-generated ligands are Z-RNAs. Once ZBP1 detects Z-form nucleic acids (i.e., either Z-DNA or Z-RNA), it associates with Receptor Interacting Protein Kinase 3 (RIPK3) via homotypic interactions between the RIP Homology Interaction Motifs (RHIMs) found in both proteins. RIPK3 then phosphorylates Mixed Lineage Kinase Like protein (MLKL), triggering necroptosis. Notably, RIPK3, via FADD and caspase 8, can also activate apoptosis and, via RIPK1-NF-κB signaling, promote inflammatory gene expression (Fig. 1).[7]

In severe influenza, ZBP1-initiated necroptosis and likely cell death-independent inflammatory gene expression, provoke lung inflammation.[8] Here, Li et al.[3] extend these observations to COVID-19 by implicating ZBP1 in SARS-CoV-2-initiated lung damage. The authors first observed that in the human lung epithelium-derived cell line Calu-3, SARS-CoV-2 infection caused up-regulation of ZBP1 expression and triggered cell death, including necroptosis. Such necroptosis was at least partially dependent on ZBP1, because depleting ZBP1 in Calu-3 cells reduced SARS-CoV-2-triggered phosphorylation of MLKL and the extent of cell death. Eliminating ZBP1 expression also reduced the release of inflammatory cytokines, implicating ZBP1 as an important driver of necro-inflammatory signaling during SARS-CoV-2 infections.

Next, Li et al. sought to understand how ZBP1 was activated in SARS-CoV-2-infected cells. Using immunofluorescence approaches, Li et al. discovered that SARS-CoV-2 infection induced production of Z-RNAs, and that these Z-RNAs co-localized with ZBP1. They observed that RNAs mapped to ORF1a and ORF1b in the SARS-CoV-2 genome were enriched in the pool of RNA species immunoprecipitated by an anti-Z-NA antibody, suggesting that ORF1a and ORF1b RNAs are potential Z-RNA-forming ligands for ZBP1.

Moving into an in vivo setting, the authors employed a mouse model in which prior intranasal administration of an adenovirus encoding human ACE2 (Ad5-hACE2) allows SARS-CoV-2 infection of mouse lung epithelial cells. In this model, SARS-CoV-2-infected *Zbp1*$^{-/-}$ mice showed no major differences in lung viral load, but manifested lower levels of inflammatory cytokines and chemokines, compared to controls. *Zbp1*$^{-/-}$ mice also displayed a lower extent of immune cell infiltration and less alveolar damage than wild-type mice. Consistent with these findings, *Ripk3*$^{-/-}$ mice also showed reduced expression of pro-inflammatory cytokines and chemokines, but neither loss of MLKL nor pharmacological blockade of RIPK3 kinase activity, both of which selectively nullify necroptosis downstream of RIPK3, affected the induction of pro-inflammatory cytokines and chemokines by SARS-CoV-2 either in cellulo or in vivo. Further, whereas *Ripk3*$^{-/-}$ mice displayed significantly reduced immune cell infiltration and histological signs of lung injury compared to wild-type mice, *Mlkl*$^{-/-}$ mice did not. Thus, ZBP1/RIPK3-initiated MLKL activation mediates

[1]Fox Chase Cancer Center, Philadelphia, PA, USA. ✉email: Siddharth.balachandran@fccc.edu

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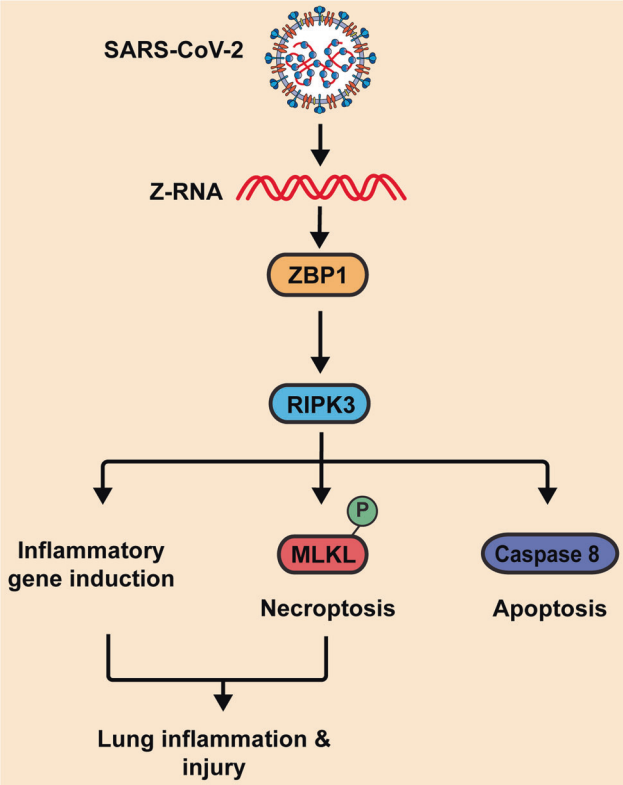

Fig. 1 SARS-CoV-2-initiated pathways of ZBP1-dependent lung inflammation. SARS-CoV-2 infection produces Z-RNAs, which are sensed by the host protein ZBP1. ZBP1 activates RIPK3, which induces cell death via parallel pathways of necroptosis driven by MLKL, and apoptosis mediated by caspase 8. RIPK3 can also induce expression of inflammatory genes in a necroptosis-independent manner. ZBP1/RIPK3-driven necroptosis and inflammatory gene expression may contribute to lung inflammation and injury in COVID-19.

necroptosis signaling, but an MLKL-independent signaling appears responsible for ZBP1/RIPK3-mediated induction of most inflammatory cytokines and chemokines in SARS-CoV-2-infected cells. How this signal is propagated is currently mysterious.

Overall, this paper sheds new light on the pathogenesis of COVID-19 by showing that (1) SARS-CoV-2 infection generates Z-RNAs, which may serve as ZBP1 ligands in infected cells; (2) ZBP1/RIPK3-initiated signaling is important for promoting SARS2-CoV-2 infection-mediated lung inflammation and injury in mice; and (3) most of these effects are driven by necroptosis-independent inflammatory signaling downstream of RIPK3. It will be very interesting to see how SARS-CoV-2 RNAs adopt the Z-conformation, and which of these are bona fide ZBP1 ligands. Also currently unclear is how RIPK3 induces MLKL-independent inflammatory gene expression. One attractive possibility here is that RIPK3 associates with RIPK1 and activates an NF-κB-mediated inflammatory gene expression program, as has been seen in other settings.[7]

These findings also raise the interesting question of how bats, which appear to encode ZBP1, RIPK3 and MLKL,[9] can harbor SARS-family coronaviruses with few apparently ill effects. In this regard, it is noteworthy that the SARS-CoV-2 nonstructural protein Nsp13 may harbor a putative RHIM.[10] Whether Nsp13 regulates ZBP1-initiated inflammation by interfering with RHIM-based interactions between ZBP1 and RIPK3 remains to be explored. Finally, it will be of great clinical interest to see whether any of the signaling nodes identified in this study are potentially druggable, positioning them as new therapeutic entry points for COVID-19.

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ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Siddharth Balachandran.