Having identified the *S.* Typhimurium effector protein that mediates class II MHC ubiquitination and down-modulation, the present study by Bayer-Santos et al. (2016) answers an important and long-standing question in the field. The study provides mechanistic insights into how *S.* Typhimurium limits T cell responses, which in turn has significant implications for advancing knowledge of the pathogenesis of and host response to bacterial infection, and the cell biology of antigen presentation.

ACKNOWLEDGMENTS

We thank Drs. James Bliska and Michael Starnbach for constructive feedback on the manuscript and apologize to our colleagues whose work could not be cited due to space limitations. Research in the laboratory of A.W.M.v.d.V. is supported by NIH grant R01AI101221.

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Getting a gRIP on Flu by Casting the DAI

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http://dx.doi.org/10.1016/j.chom.2016.10.013

Influenza A initiates host cell death through unknown mechanisms. Thapa et al. (2016) in this issue of *Cell Host & Microbe*, along with recent work by Kuriakose et al. (2016), indicate that this virus provokes divergent modes of cell death, including apoptosis and necroptosis, via the nucleic acid sensor, DAI.

When Julius Caesar debated whether to cross the Rubicon River in northern Italy in 49 BC to march on Rome, he knew that the potential outcomes were starkly different: victory and even greater power, or defeat and certain death. When Caesar finally decided to cross the Rubicon, thereby invading Roman territory, he uttered the immortal words "now the die is cast." As in all games of chance, casting the die can have multiple outcomes, not all of which are favorable. In this issue of *Cell Host and Microbe*, Balachandran and colleagues report the outcome of an entirely different invasion—that of influenza A-mediated infection, which can have fatal consequences depending on how a different type of DAI is cast (Thapa et al., 2016). This study suggests that the cytotoxic effects of flu infection are influenced by formation of three different complexes between the host nucleic acid sensor, DAI (also known as ZBP1 and DLM-1), and members of the RIP kinase (RIPK) family, which can lead either to rapid apoptosis, rapid necroptosis, or delayed apoptosis (Figure 1). Deletion of DAI from host cells reduced the direct cytopathic effects of influenza A infection but led to prolongation of virus replication and increased mortality (Thapa et al., 2016). These data argue that DAI is a critical determinant of the host response to influenza A, serving to limit viral replication by initiating death of host cells via redundant pathways involving RIPK3 and RIPK1.

RIPK1, RIPK3, and MLKL have emerged in recent years as central players in a regulated form of necrosis, called necroptosis (Vercammen et al., 1998; Vandenabeele et al., 2010). Although necrosis is typically unprogrammed, studies over the past decade have revealed that a regulated form of necrosis can occur upon interference with proteases that play a critical role in certain pathways leading to apoptosis (He et al., 2009). Specifically, caspase-8 and its upstream adaptor FADD play central roles in apoptosis instigated by certain TNF family cytokines (TNF, FasL, TRAIL). However, if caspase-8 activation in response to TNF, FasL, or TRAIL is inhibited (through either virally encoded or synthetic caspase inhibitors) then cell death is typically blocked (Kearney et al., 2015)—or at least that's what happens in cells that do not express RIPK3. In cells that do express this latter kinase, interference with caspase-8 activation instead leads to the assembly of what has been dubbed the necrosome, a

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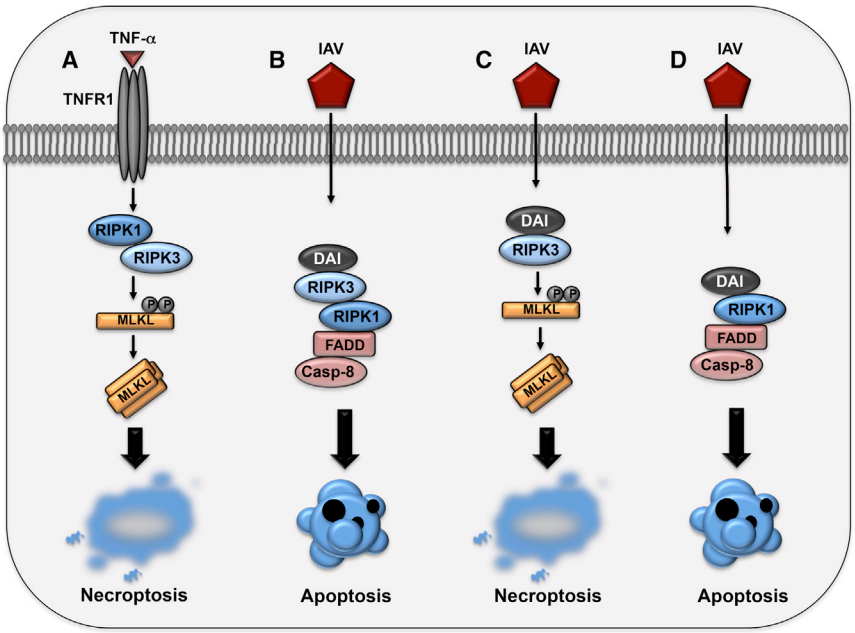

Figure 1. DAI-Dependent RIPK1 and RIPK3-Dependent Cell Death Pathways

(A) In the context of caspase inhibition, TNF signaling can promote necroptosis via RIPK1/RIPK3-dependent MLKL phosphorylation, resulting in oligomerization of the latter and rapid membrane permeabilization.

(B–D) Influenza A virus (IAV) infection can result in the assembly of three distinct DAI-containing complexes, resulting in either RIPK3/RIPK1-dependent caspase-8 activation and apoptosis (B), RIPK3/MLKL-dependent necroptosis (C), or RIPK1/caspase-8-dependent apoptosis (D).

complex that comprises, at a minimum, RIPK3 and MLKL but can also include the related kinase RIPK1 (He et al., 2009; Wang et al., 2014). In this scenario, RIPK3 phosphorylates MLKL, leading the latter to form oligomeric pores in membranes resulting in rapid cell lysis (Figure 1A). Because necroptosis typically only occurs where apoptosis-associated caspase activation is blocked, this mode of cell death may act as a back-up in situations where viruses or other pathogens enter cells and attempt to block apoptosis.

Influenza A virus (IAV) is a highly infectious RNA virus that is associated with considerable human morbidity and mortality annually. IAV infection typically leads to rapid cell death in cells of the lung epithelium, which most likely serves as an important host response to this virus by limiting viral replication and facilitating viral clearance. Despite intensive study, how IAV triggers death of its host cells has remained enigmatic. However, recent reports suggest that IAV infection can lead to at least three alternative modes of regulated cell death, two of which are critically dependent on RIPK3 (Nogusa et al., 2016; Thapa et al., 2016). Nogusa et al. (2016) demonstrated that RIPK3 plays an instrumental role in IAV-associated cell death in fibroblasts and the lung epithelium, by promoting a RIPK3-RIPK1-FADD-caspase-8-dependent pathway to apoptosis (Figure 1B), as well as a RIPK3-MLKL-dependent pathway to necroptosis (Figure 1C). Interference with either cell death pathway, through deletion of the genes encoding MLKL or FADD, failed to block cell death in response to IAV, suggesting redundancy between these pathways (Nogusa et al., 2016). In contrast, combined deletion of *fadd* and *mlkl* profoundly protected cells from the cytopathic effects of this virus in vitro (Nogusa et al., 2016). However, although much of the cell death associated with IAV infection was RIPK3 dependent (Figure 1B and 1C), deletion of *ripk3* did not protect from IAV-associated cell death to the same degree as loss of both *fadd* and *mlkl*, or indeed as compared with combined loss of *ripk1* and *ripk3* (Nogusa et al., 2016). This suggested that IAV also promotes a RIPK3-independent route to apoptosis through initiating assembly of a RIPK1-FADD-caspase-8 complex (Figure 1D).

Consistent with their in vitro observations, previous studies from Balachandran and colleagues found that RIPK3 plays an important role in clearance of IAV in vivo (Nogusa et al., 2016). Mice deficient in *ripk3* exhibit difficulty in clearing IAV infections, exhibiting greatly elevated IAV titers in lung tissues and exhibiting 2-fold increased mortality in response to IAV infection compared with wild-type animals (Nogusa et al., 2016). Although previous implications of this observation would have almost certainly led to the interpretation that RIPK3-dependent necroptosis is involved in IAV clearance (Figure 1C), infection of *mlkl*$^{-/-}$ mice did not lead to increased susceptibility to IAV infection (Nogusa et al., 2016). Indeed, *mlkl*$^{-/-}$ animals displayed no impairment in clearing IAV, again arguing that the role of RIPK3 in this context extends beyond its ability to trigger necroptosis (Figure 1B and 1C). Furthermore, mice doubly deficient in *fadd* (i.e., defective in caspase-8-dependent apoptosis) and *mlkl* (defective in necroptosis) were even more vulnerable to the detrimental effects of IAV infection, due to defective IAV clearance (Nogusa et al., 2016). Taken together with the in vitro observations of Nogusa et al. (2016), these data imply that RIPK3 can trigger two parallel pathways to cell death upon IAV infection, one leading to assembly of a RIPK3-RIPK1-FADD-caspase-8 complex that promotes apoptosis (Figure 1B) and the other involving the assembly of a RIPK3-MLKL complex that promotes necroptosis (Figure 1C). While neither pathway alone is essential for IAV clearance, elimination of both pathways through loss of RIPK3 results in a major deficiency in viral clearance. However, two questions arise from these studies. How is RIPK3 activated upon IAV infection, and why are *mlkl*$^{-/-}$/*fadd*$^{-/-}$ mice more vulnerable to IAV-initiated lethality than *ripk3*$^{-/-}$ animals?

Now, studies from two laboratories resolve these issues, implicating DAI as the critical instigator of RIPK3- and RIPK1-dependent cell death in the context of IAV infection (Thapa et al., 2016; Kuriakose et al., 2016). However, although the conclusions of these studies are broadly similar, there are important differences in terms of the specific role

that DAI plays in this context, as well as the functional consequences of DAI loss for the outcome of IAV infection. DAI contains two nucleic acid binding domains toward its N terminus and, along with RIPK1, RIPK3, and TRIF, is one of only four known vertebrate proteins with a RIP homology interaction motif (RHIM; Rebsamen et al., 2009). Thapa et al. (2016) suggest that DAI can act as a direct sensor for influenza A RNA via its Zα2 domain, leading to DAI oligomerization and downstream recruitment of RIPK3 through RHIM-RHIM interactions. Assembly of the DAI-RIPK3 complex can then lead to either RIPK3/RIPK1/FADD/caspase-8-dependent apoptosis (Figure 1B) or RIPK3/MLKL-dependent necroptosis (Figure 1C). However, in the absence of RIPK3, DAI can also promote a slower route to apoptosis via RIPK1-mediated recruitment of FADD and caspase-8 (Figure 1D). Consistent with these observations, Thapa et al. find that cells lacking DAI were remarkably resistant to flu-mediated cell lysis in vitro, and *dai/zbp1* null mice were rendered highly susceptible to IAV-associated lethality due to accumulation of greatly elevated viral titers in these animals (Thapa et al., 2016).

Although Kuriakose et al. (2016) also find that DAI is a critical effector of cell death associated with IAV infection, these authors suggest that the C-terminal region of DAI acts as a sensor for two IAV proteins, nucleoprotein (NP) and the RNAase subunit PB1, which is at odds with observations by Thapa et al. (2016) that the C-terminal domain of DAI is dispensible for cell death in response to IAV. Both groups agree on the importance of RIPK3 in regulating DAI-initiated cell death in this context, as well as the functional redundancy between RIPK3/FADD/caspase-8-dependent apoptosis and RIPK3/MLKL-dependent necroptosis in this setting. However, there is surprising disagreement between the studies with respect to the consequences of DAI loss for the outcome of IAV infection in vivo. While Kuriakose et al. (2016) observed decreased lethality in IAV-infected *dai/zbp-1* null mice, despite these animals manifesting higher viral titers in the lung and slower recovery times than controls, Thapa et al. (2016) observed increased lethality in the same animals in response to the same IAV strain. The reason(s) underlying this difference in survival outcomes is currently unclear. On the one hand, it is noteworthy that the increased vulnerability to IAV infection displayed by *dai/zbp1*$^{-/-}$ animals, as reported by Thapa et al. (2016), is consistent with their prior report that *mlkl*$^{-/-}$/*fadd*$^{-/-}$ and *ripk3*$^{-/-}$ animals also exhibit elevated viral titers and increased IAV-induced lethality (Nogusa et al., 2016). On the other hand, Saleh and colleagues have previously reported that *ripk3*$^{-/-}$ animals do not exhibit any significant vulnerability to IAV-associated mortality (Rodrigue-Gervais et al., 2014). So, the jury is still out on whether less cell death in response to IAV infection is a good or bad thing.

Notwithstanding the differences outlined above, it appears clear that DAI plays a critical role in sensing IAV infection and coupling this to the initiation of redundant RIPK3- and RIPK1-dependent cell death pathways that can influence viral clearance by depriving IAV of its replicative niche. Whether cell death in this context is protective or detrimental most likely depends on the degree of cell death invoked. Too much, and the barrier integrity of the lung is compromised, leading to increased mortality (Rodrigue-Gervais et al., 2014); too little, and viral replication can continue relatively unabated, which can lead to increased viral spread, excessive inflammation, and eventual destruction of virally infected cells. When it comes to viral sensing and cell death outcomes, there is clearly more than one way to roll the DAI.

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