Immunity Preview:

Orzalli et al., 2021. Immunity. *Virus-mediated inactivation of anti-apoptotic Bcl-2 family members promotes Gasdermin E- dependent pyroptosis in barrier epithelial cells*

Guard proteins keep watch at epithelial walls

Alejandra Zárate-Potes[1] & Katja Dierking[2]*

[1]Division of Biomedical and Life Sciences, Lancaster University, Lancaster LA1 4YE, United Kingdom. Email: a.zarate-potes@lancaster.ac.uk Twitter: @PotesZarate
[2]Department of Evolutionary Ecology and Genetics, Christian-Albrechts-Universität zu Kiel, 24118 Kiel, Germany. Email: kdierking@zoologie.uni-kiel.de
*Correspondence: kdierking@zoologie.uni-kiel.de

Summary

Cells can detect pathogens through guard proteins that sense disturbances in core cellular processes, but the exact mechanisms often remain elusive. In this issue of Immunity Orzalli et al., (Orzalli et all)2021 identify Bcl-2 family members as guard proteins that detect virus-induced translational inhibition and induce pyroptosis in human keratinocytes.

Preview

Animal cells sense pathogen invasion by multiple mechanisms: Pattern recognition receptors (PRRs) directly bind to microbe-associated molecular patterns (MAMPs) and to endogenous, i.e. host-derived, damage-associated molecular patterns (DAMPs) that are released to the extracellular space as a result of tissue damage during microbial infection. Guard proteins detect pathogen-induced changes in host cellular homeostasis. Here, molecular perturbations that result from a pathogen effector's virulence function, such as inhibition of protein translation, modifications of the actin cytoskeleton, mitochondrial dysfunction, or changes in ion concentrations, are recognized by host factors (guard proteins) that surveil these core cellular processes. This mechanism of pathogen sensing is termed effector-triggered immunity (ETI) (Boyer et al., 2011; Lopes Fischer et al., 2020). While ETI is a well-described pathogen sensing mechanism in plants (Lopes Fischer et al., 2020), our understanding of the role of guard proteins and the processes that they guard in metazoans is still emerging. Mechanisms of ETI have been elucidated in invertebrate model systems such as *Caenorhabditis elegans*. In mammals, ETI mechanisms have so far been best described in the context of bacterial infections in professional immune cells, while sensors of viral replication at barrier surfaces such as epithelia of the mammalian intestine, lung, or skin have only recently been reported. For example, the nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domains-containing protein 1 (NLRP1) senses the enzymatic activity of the 3C protease of human rhinovirus (HRV) in airway epithelia (Robinson et al., 2020). Cleavage of NLRP1 by the viral protease leads to inflammasome activation resulting in engagement of caspase 1, which in turn activates the pore-forming protein Gasdermin D (GSDMD), leading to the release of inflammatory cytokines through an inflammatory form of lytic cell death, called pyroptosis (Robinson et al., 2020). Now, the study presented by Orzalli

et al., 2021 in this issue of Immunity extends the repertoire of known viral sensors in barrier surfaces to members of the Bcl-2 family that surveil protein translation in human skin epithelial cells (keratinocytes).

Orzalli et al., 2021 used two experimental *in vitro* models for viral infection of skin epithelial cells to explore the role of guard proteins in antiviral responses at barrier surfaces: Primary human keratinocytes and human skin equivalents (HSE), which are *in vitro* multi-layered tissues formed by keratinocytes in an air-liquid interface on a collagen matrix containing dermal fibroblasts. Using this system, the authors observed that infection of keratinocytes and HSE with Vesicular Stomatitis Virus (VSV) led to release of interleukin-1 (IL-1) family cytokines from dead or dying keratinocytes undergoing lysis. Since pyroptosis and release of IL-1 family cytokines is a hallmark of guard protein-driven inflammatory responses, the authors further investigated the mechanism of VSV-induced pyroptosis. Caspases are cysteine proteases with well-established functions in apoptosis or cytokine processing and proinflammatory cell death. CASP3 is thought to mainly function in apoptosis, while CASP1 plays an important role in pro-inflammatory immune response activation downstream of NLR-inflammosome pathways. Interestingly, Orzalli et al found accumulation of cleaved CASP3, but not CASP1 or CASP8, in VSV-infected cell lysates. This suggested a role of CASP3 in VSV infection-induced cell death. Indeed, CASP3-deficient keratinocytes did not undergo lysis. Gasdermin E (GSDME) is a known substrate of CASP3. While GSDMD mediates pyroptosis downstream of the NLR-inflammasome pathways (Lopes Fischer et al., 2020; Shi et al., 2017), GSDME mediates cell lysis induced by chemotherapies (Zhang et al., 2020) and had not been implicated in response to infection before this work. Orzalli et al., 2021 revealed that VSV infection resulted in CASP3-dependent activation of GSDME and that VSV-induced cell lysis and IL-1α release is GSDME dependent. This illustrates that CASP3-dependent cleavage of GSDME and IL-1α release in VSV-infected keratinocytes is distinct from canonical pyroptotic death, which is mediated by GSDMD downstream of NLR-inflammasome pathways.

If VSV-induced pyroptosis is independent of GSDMD and NLR-inflammasomes, which proteins serve as guards of core cellular processes in epithelial cells? Orzalli et al., 2021 approach this question considering that VSV, as many other viruses, is known to inhibit host protein translation to suppress immune responses and promote viral gene expression (Stern-Ginossar et al., 2019). They thus focused their investigation on potential mechanisms of host translation surveillance. They had the intriguing idea that guard proteins, which can perceive translational activity and at the same time regulate cell survival, would directly link perception of viral-mediated protein translation inhibition to the induction of cell death. The Bcl-2 family of proteins promote cell survival primarily by inhibiting mitochondrial membrane pore-forming proteins (Singh et al., 2019). Importantly, the Bcl-2 family member Mcl-1 has a short half-life and must be constantly replenished by protein translation, making it a good sensor of translational activity (Orzalli et al., 2021; Singh et al., 2019). Indeed, VSV infection and chemical inhibition of translation led to a reduction of Mcl-1 levels in keratinocytes, and this correlated with GSDME cleavage. Importantly, a viral mutant unable to inhibit host protein synthesis was incapable of reducing Mcl-1 protein, further supporting the idea that Mcl-1 acts as sensor of translational activity. Of note, knock-down of Mcl-1 was not sufficient to promote GSDME cleavage and lysis of keratinocytes. However, as coincident inhibition of multiple Bcl-2 family members is sometimes necessary to initiate cell death (Singh et al., 2019) , Orzalli et

al. explored the involvement of other Bcl-2 members in VSV-induced pyroptosis (Orzalli et al., 2021). They revealed that the Bcl2-associated agonist of cell death (BAD) was dephosphorylated by VSV infection and by chemical inhibition of translation. As dephosphorylation of BAD promotes association with and inhibition of the Bcl2 member Bcl-xL (Singh et al., 2019), the results indicated an involvement of Bcl-xL in sensing translational activity and inhibiting pyroptosis. Indeed, the combined loss of Mcl-1 and inactivation of Bcl-xL (by constitutive activation of BAD) was sufficient to induce pyroptosis in keratinocytes and their overexpression was enough to prevent VSV-induced pyroptosis (Orzalli et al., 2021). These data suggest that Mcl-1 and Bcl-xL are sensors of translational activity and that their simultaneous inactivation following VSV infection promotes GSDME-dependent pyroptosis.

Since disruption of translation is a common strategy of viruses (Stern-Ginossar et al., 2019) Orzalli et al. then asked whether other viruses also induce CASP3-dependent GSDME cleavage (Orzalli et al., 2021). They investigated Herpes Simplex Virus 1 (HSV-1), which infects keratinocytes before establishing latency in sensory neurons (Stern-Ginossar et al., 2019). Intriguingly, HSV-1 infection resulted in loss of Mcl-1 but not in cleavage of GSDME or cell lysis, whereas HSV-1 mutants that lack the immediate early protein ICP27 did drive GSDME cleavage and host cell lysis. How ICP27 inhibits HSV-1 induced pyroptosis remains elusive, but these results suggest that HSV-1 may use ICP27 to manipulate the surveillance by Bcl-2 guard proteins and promote host cell survival.

Finally, Orzalli et al., 1 explored if VSV-induced pyroptosis plays a role in antiviral host defense. They did not observe any increase in virus production in keratinocytes overexpressing Bcl-xL, suggesting that the pathway is not intrinsically antiviral. However, overexpressing Bcl-xL in keratinocytes within the HSE organotypic model of human skin reduced IL-1α release and accumulation of virus in the supernatant. This suggests that keratinocytes in these multicellular organoids signal IL-1 to fibroblasts to induce an antiviral response and that pyroptosis can restrict virus replication in HSEs.

The study presented by (Orzalli et al., 2021) suggests that the pro-survival Bcl-2 family members Mcl-1 and Bcl-xL can act as guard proteins that surveil protein translation in keratinocytes. When protein translation is inhibited by viral infection or chemical inhibition, Mcl-1 protein abundance is reduced and Bcl-xL is inactivated (through dephosphorylation of BAD), leading to caspase-3 activation, GSDME cleavage and pyroptosis as an antiviral defense mechanism (Figure 1). Thus, perception of viral-mediated protein translation inhibition is directly linked to the induction of pyroptosis. This study implicates GSDME in antiviral pyroptosis, which was previously known to drive antitumoral and chemically induced cell death (Shi et al., 2017; Zhang et al., 2020). Importantly, it also highlights the fundamental role of translational surveillance as mechanism of effector-triggered immunity in epithelial cells. Interestingly, in the nematode *C. elegans*, which lacks professional immune cells and exclusively relies on epithelial cells to mount defense responses against pathogens, the sensing of pathogen-mediated disruption of cellular activities such as translation inhibition seems to be the principal pathogen detection mechanism. *C. elegans* intestinal epithelial cells sense microbial toxin-induced inhibition of protein translation, and subsequently activate anti-bacterial defense responses (Dunbar et al., 2012; McEwan et al., 2012). This implies that detection of translation inhibition is an evolutionary conserved ETI mechanism in epithelial cells. It will clearly be interesting to further investigate how protein translation and other

cellular processes are monitored in epithelial cells as part of pathogen defenses against both bacterial and viral pathogens. In mammals, the pathogen sensor functions of barrier epithelia often remain in the shadow of the sensor functions of professional immune cells. The study of Orzalli et al., 2021 now puts the spotlight on this first line of defense, where the Bcl-2 watch guards the epithelial wall from the threat of viral infection.

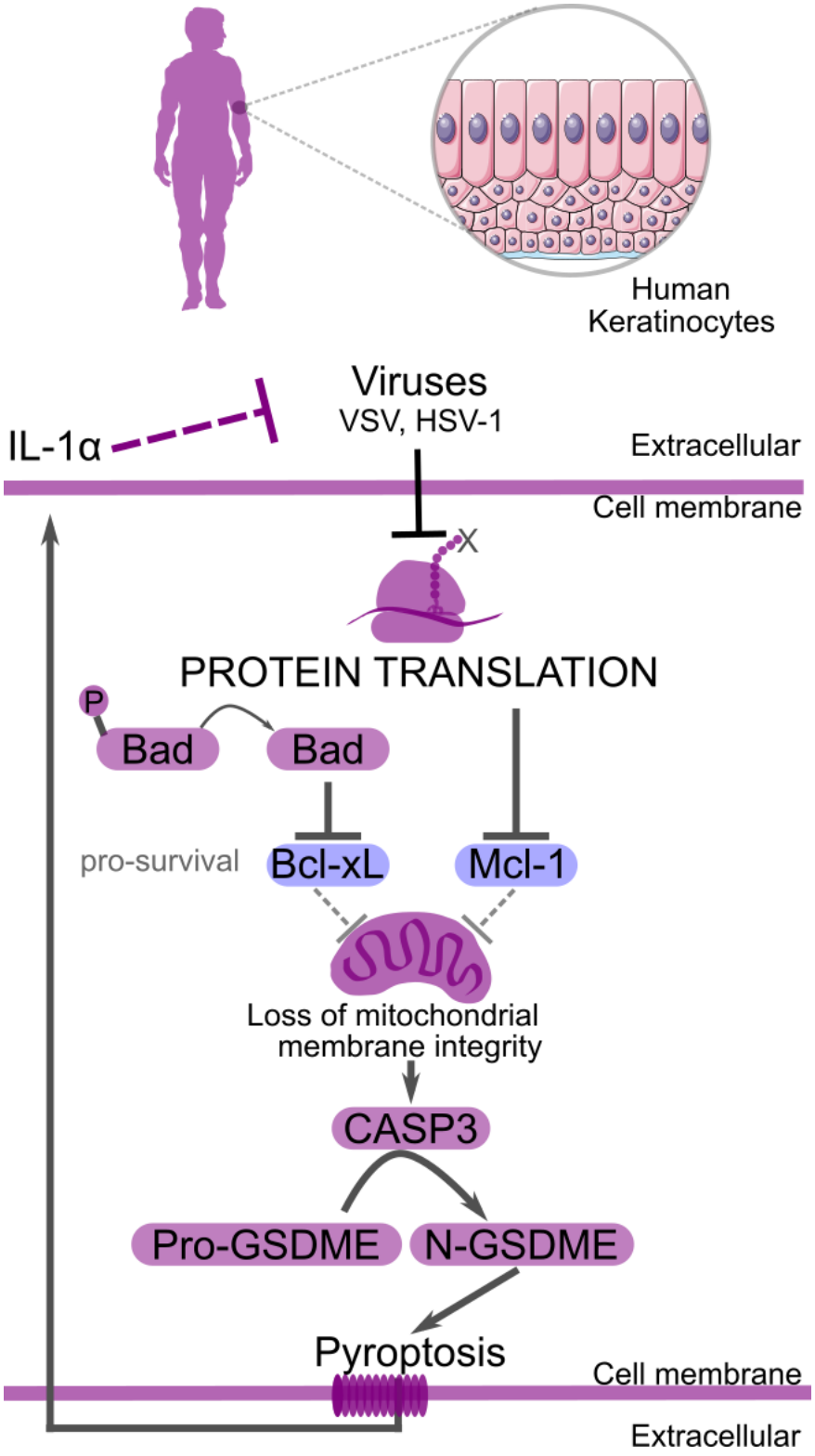

Figure 1 In humans and *C. elegans* guard proteins detect pathogen-induced inhibition of translation and activate immune responses in epithelial cells
In human epithelial cells (keratinocytes) infection with Vesicular Stomatitis Virus (VSV) and Herpes Simplex Virus 1 (HSV-1) leads to inhibition of protein translation, resulting in rapid depletion of the pro-survival Bcl-2 family member Mcl-1. Additionally, infection with VSV leads to dephosphorylation and thus activation of Bcl2-associated agonist of cell death (BAD), which in turn inhibits BCL-2 family member Bcl-xL. Joint depletion of Mcl-1 and inactivation of Bcl-xL lead to loss of mitochondrial membrane integrity and activation of Caspase 3 (CASP3), which in turn leads to activation of Gasdermin E (GSDME)-mediated inflammatory lytic cell death (pyroptosis) and the release of Interleukin 1α (IL-1α) (Orzalli et al., 2021). Thus, perception of viral-mediated protein translation inhibition is directly linked to the induction of pyroptosis. In intestinal epithelial cells of the nematode *Caenorhabditis elegans*, the *Pseudomonas aeruginosa* PA14 virulence factor Exotoxin A inhibits protein translation, which is detected by decrease in translation of an upstream Open Reading Frame (uORF) to the gene encoding for the immune-related bZIP transcription factor *zip-2*, triggering activation of defense gene expression (Dunbar et al., 2012; McEwan et al., 2012). Inhibition of translation additionally activates immune responses through the p38 Mitogen-Activated Protein Kinase (p38 MAPK) pathway and G-protein coupled mammalian follicle stimulation hormone receptor homolog (FSHR-1) through unknown mechanisms (Dunbar et al., 2012; McEwan et al., 2012). These findings highlight the importance of surveillance of protein translation by guard proteins at barrier epithelia, allowing rapid activation of defense responses.

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