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Programmed Cell Death in the Evolutionary Race against Bacterial Virulence Factors

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Innate immune sensors can recognize when host cells are irrevocably compromised by pathogens, and in response can trigger programmed cell death (pyroptosis, apoptosis, and necroptosis). Innate sensors can directly bind microbial ligands; for example, NAIP/NLRC4 detects flagellin/rod/needle, whereas caspase-11 detects lipopolysaccharide. Other sensors are guards that monitor normal function of cellular proteins; for instance, pyrin monitors Rho GTPases, whereas caspase-8 and receptor-interacting protein kinase (RIPK)3 guards RIPK1 transcriptional signaling. Some proteins that need to be guarded can be duplicated as decoy domains, as seen in the integrated decoy domains within NLRP1 that watch for microbial attack. Here, we discuss the evolutionary battle between pathogens and host innate immune sensors/guards, illustrated by the Red Queen hypothesis. We discuss in depth four pathogens, and how they either fail in this evolutionary race (*Chromobacterium violaceum*, *Burkholderia thailandensis*), or how the evolutionary race generates increasingly complex virulence factors and host innate immune signaling pathways (*Yersinia* species, and enteropathogenic *Escherichia coli* [EPEC]).

The innate immune system can combat intracellular bacteria by inducing programmed cell death, which eliminates the infected cell niche. Killing host cells can also be useful in the innate immune response in cases in which a cell has been irrevocably reprogrammed to act in the benefit of the pathogen. Programmed cell death can be initiated by a variety of interconnected pathways, resulting in pyroptosis, apoptosis, or necroptosis. There are notable recent advances in our understanding of how programmed cell death either restricts bacterial pathogens, or fails to do so as certain pathogens have evolved to evade this defense. The evolutionary race between pathogen virulence and host defense is vital in determining whether a bacterial pathogen can cause disease, or is readily cleared.

Direct sensors, guards, and decoys initiate specific forms of programmed cell death, including pyroptosis, necroptosis, and apoptosis. Pyroptosis and necroptosis result in membrane rupture that releases soluble cytosolic contents to the extracellular space, whereas apoptosis converts a cell into apoptotic bodies that retain cellular contents within membranes. Cell death may lead to enhanced clearance of the pathogen; however, inappropriate or excessive cell death

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can be extremely detrimental to the host. These tightly regulated cell death mechanisms are often triggered by the activation of specific caspase proteases. Pyroptosis results from activation of caspase-1 or murine caspase-11/human caspase-4, -5 (Jorgensen et al. 2017). Apoptosis ensues when apoptotic initiators (caspase-8, -9) activate apoptotic effectors (caspase-3, -6, -7) (Taylor et al. 2008). Lastly, necroptosis occurs when RIPK3 phosphorylates the pseudokinase MLKL (Dondelinger et al. 2016a; Vanden Berghe et al. 2016). Many of these signaling pathways use death fold family domains to drive homotypic interactions, including death domains (DDs), death effector domains (DEDs), caspase activation and recruitment domains (CARDs), and pyrin domains (PYDs) (Weber and Vincenz 2001).

RED QUEEN HYPOTHESIS

The evolutionary biologist Leigh Van Valen proposed that each species must constantly evolve to avoid extinction in the face of competitors who are also constantly evolving. To illustrate this race, Van Valen drew upon the imagery in Lewis Carol's book *Through the Looking Glass*, in which the protagonist, Alice, engages in a footrace with the Red Queen. Alice soon finds that they have been running, but have stayed in the same place. The Red Queen informs Alice "Now, here, you see, it takes all the running you can do, to keep in the same place." Van Valen's Red Queen hypothesis proposes that organisms must constantly evolve to maintain their place in an environment where competitors are also constantly evolving (Van Valen 1973).

The Red Queen hypothesis also applies to host–pathogen interactions; as hosts evolve defenses against infection, pathogens must evolve virulence factors to overcome those defenses. This constant evolutionary race by both competitors ensures that neither the host nor pathogen go extinct. In the perpetual drive to outrun each other, hosts evolve increasingly complex defense mechanisms while, simultaneously, pathogens evolve equally complex virulence factors. Typically, pathogens are able to keep up with the evolutionary challenge in the race with the innate immune system. However, most pathogens lose the race against the adaptive immune system, which eventually resolves the infection, but not before the pathogen transmits to a new host. Thus, the host (which we herein personify as Alice) constantly evolves new immunologic defenses. Meanwhile, pathogens (which we herein personify as Red Queens), must constantly evolve novel virulence factors.

INNATE IMMUNE SENSORS: DIRECT SENSORS, GUARDS, AND DECOYS

Programmed cell death can be initiated by a variety of sensors in the innate immune system. Notable among these are the nucleotide-binding domain, leucine-rich repeat (NLR) superfamily of cytosolic sensors, which cause cell death in eukaryotes ranging from plants to animals. Jones, Vance, and Dangl proposed that NLRs fall into three categories: direct sensors, guards, and decoys (Jones et al. 2016). These concepts not only apply to NLRs, but are also broadly applicable to many pathways in the innate immune system (Fig. 1).

"Direct sensors" bind microbial ligands. For example, TLR4 and its coreceptor MD2 directly bind lipopolysaccharide (LPS) in the extracellular space, triggering a transcriptional response. In the cytosol, caspase-11 is the direct sensor for LPS, triggering pyroptotic cell death. Similarly, extracellular flagellin detection by TLR5 drives a transcriptional response, whereas cytosolic flagellin detection by NAIP/NLRC4 triggers pyroptosis.

"Guards" monitor a cellular protein(s) for evidence of attack by virulence factors, and activate innate immune signaling in response. Guards can directly bind to the guarded protein, but we propose they may also monitor the enzymatic activity of the guarded protein. If the guarded protein is functioning normally, guards do not respond. However, if a guarded protein is attacked by a pathogen virulence factor, then the guard responds by activating and inducing a new response, often programmed cell death. Note that guards do not prevent the guarded protein from being attacked; rather they sound the alarm when such an attack occurs. More

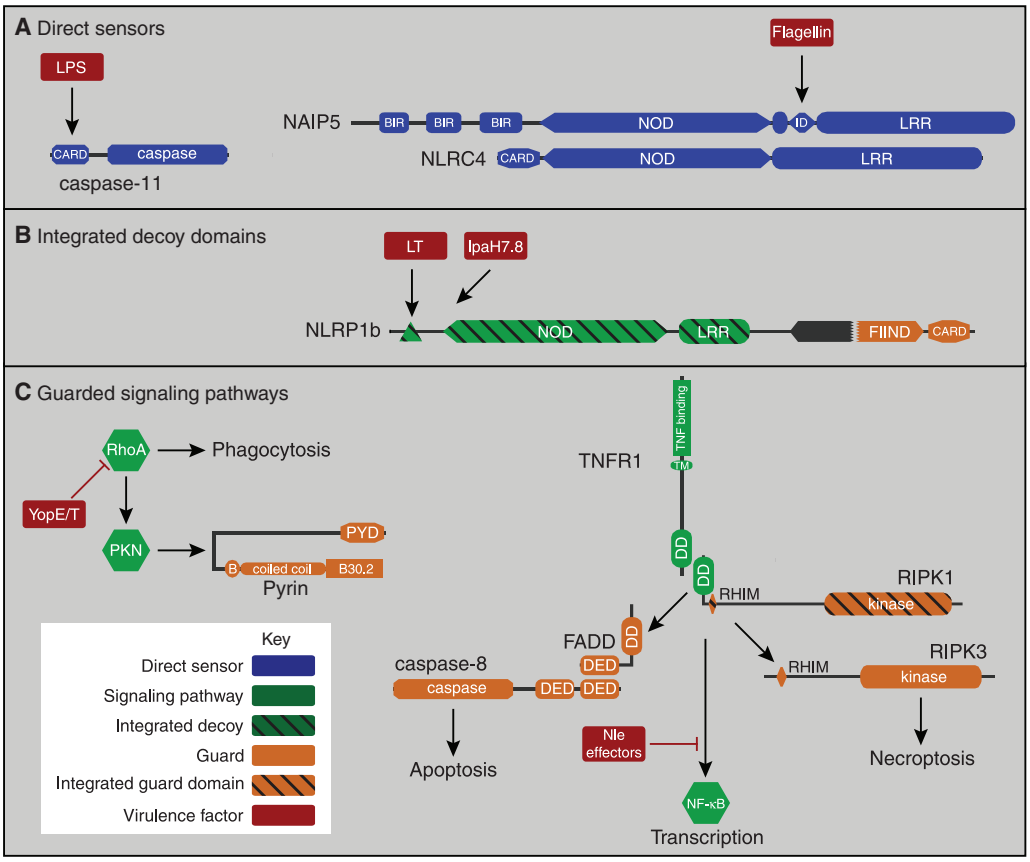

Figure 1. Innate immune surveillance: direct sensors, integrated decoy domains, and guarded signaling pathways. (*A*) Direct sensors include both caspase-11 and NAIP5/NLRC4. Caspase-11 activates when it directly binds to bacterial lipopolysaccharide (LPS) via its caspase activation and recruitment domain (CARD). NAIP5 directly binds bacterial flagellin; the NAIP5-flagellin complex then oligomerizes with NLRC4 molecules to form a caspase-1 activating inflammasome. (*B*) Integrated decoy domains are similar to domains of other signaling pathways, acting as lures for bacterial virulence factors. Once the decoy domain is attacked, this triggers signaling by the innate immune guard domains within the same protein. For example, the anthrax lethal toxin (LT) intends to target host mitogen-activated protein kinase (MAPK) but also cleaves the amino-terminal integrated decoy domain of NLRP1. IpaH7.8 is an E3 ubiquitinase that presumably intends to target another host protein, but also targets the integrated decoy domains of NLRP1 for degradation. Either of these attack events cause the NLRP1 signaling guard domains (the FIIND-CARD fragment) to form an active inflammasome. (*C*) Guards can watch a protein or a specific signaling pathway. Pyrin monitors RhoA signaling to protein kinase (PKN). When the *Yersinia* effectors YopE and T attack RhoA, pyrin detects the loss of PKN activation and in response forms an active inflammasome. Integrated guard domains can also be added within normal signaling pathways; for example, receptor-interacting protein kinase (RIPK)1 has two guard functions that survey the tumor necrosis factor (TNF) transcriptional response, one of which uses an integrated guard domain. When bacterial virulence factors, such as Nle effectors, attack the TNF transcriptional response, the first guard pathway is triggered by exposed death domains (DDs) within RIPK1, which are normally occupied by interacting with the TNF receptor signaling complex. When these DDs are abnormally exposed, they are detected by the DD containing guard adaptor Fas-associated death domain protein (FADD), which in turn signals to the guard caspase-8 to initiate apoptosis. Additionally, RIPK1 also encodes a RHIM and a kinase domain that can be considered integrated guard domains. These domains activate RIPK3, but only when both the transcriptional response and the apoptosis guard pathways are inhibited.

generally applied, the guard concept encompasses sensors that monitor whether signaling pathways are functioning normally. In this context, pyrin is a guard for Rho GTPases, and caspase-8/RIPK3 are guards for the tumor necrosis factor (TNF) signaling pathway. Extending the guard concept further, a guard function may be added to the protein that needs to be guarded within the same open reading frame (an "integrated guard"). Integrated guard domains are added to normal signaling proteins and are not strictly required for their normal function. The kinase domain of RIPK1 may be an example of an integrated guard domain whereby RIPK1 monitors its own scaffolding function.

"Decoys" are duplicates of the protein that needs to be monitored—the guard watches the decoy. Decoys act like lures, tempting virulence factors to attack them, triggering cell death in response. The decoy and guard can also be combined into a single protein; "integrated decoy domains" are decoys that are encoded in the same open reading frame as the guard domains. NLRP1 may be an example of an integrated decoy that monitors for attack on other NLR proteins.

PYROPTOSIS

Caspase-1 is activated by a variety of inflammasome sensors that act as direct sensors (NAIP/NLRC4, AIM2), guards (pyrin), or decoys (NLRP1), or whose mode of sensing remains to be elucidated (NLRP3). Inflammasomes can signal directly to caspase-1 if they contain a CARD (NLRC4, NLRP1), or indirectly via the ASC adaptor if they signal via a PYD (AIM2, pyrin, NLRP3). In contrast, caspase-11 is a combined direct sensor and protease that activates itself when it binds to LPS.

Either activated caspase-1 or caspase-11 can independently cleave and activate gasdermin D (Kayagaki et al. 2015; Shi et al. 2015) (for a gasdermin review, see Kovacs and Miao 2017). Cleavage of gasdermin D separates the amino-terminal pore-forming domain from the carboxy-terminal regulatory domain. Twenty-seven gasdermin pore-forming domains oligomerize to form an 18-nm pore that is large enough to allow the egress of all small molecules as well as small proteins (Ruan et al. 2018). In addition to gasdermin D, caspase-1 (but not caspase-11[Ramirez et al. 2018]) also cleaves IL-1β (4.5 nm) and IL-18 (5.0 nm), which will easily pass through the gasdermin pore. Sodium, and thereby water, enter the cell, increasing the cytosolic turgor pressure until the membrane ruptures. We define this rupture event as pyroptosis.

Pyroptosis is often described as lytic programmed cell death, which often conjures an image of a fully dispersed cell. Indeed, the membrane rupture is large enough to allow all soluble cytosolic contents to immediately escape from the cell. However, the torn plasma membrane remains otherwise intact, and retains the organelles, cytoskeleton, nucleus, and intracellular bacteria. These bacteria remain viable, but trapped within the remains of the pyroptotic cell. We termed the corpse of the pyroptotic cell as a pore-induced intracellular trap (PIT); as apoptosis converts cells into apoptotic bodies, pyroptosis converts cells into PITs. PITs simultaneously attract neutrophils, which efferocytose both the PIT and its trapped bacteria (Jorgensen et al. 2016a,b). The neutrophil then generates reactive oxygen species and kills the detained pathogen (Miao et al. 2010a).

NAIP–NLRC4 Defending against *Chromobacterium violaceum*

Perhaps the best understood inflammasome is NAIP/NLRC4, which detects the activity of bacterial type III secretion systems (T3SS). T3SS are syringe-like mechanisms that inject effector proteins into the cytosol of host cells. These effector proteins reprogram the host cell to the benefit of the pathogen. However, T3SS also aberrantly translocates flagellin, rod, and needle proteins into the host cytosol. Flagellin binds to mouse NAIP5 or NAIP6, T3SS rod proteins bind to NAIP2, whereas the T3SS needle binds to NAIP1 (Vance 2015). The structural basis for detection and activation have recently been delineated (Hu et al. 2015; Zhang et al. 2015; Tenthorey et al. 2017; Yang et al. 2018). For example, a single flagellin protein molecule binds to a single NAIP5, triggering a conformational change that exposes a polymerization interface on

NAIP5. This interface recruits NLRC4, causing a similar conformational change that recruits another NLRC4, and so on until 10 NLRC4s have been oligomerized. This creates a hub-like structure composed of one NAIP and 10 NLRC4 proteins that is termed an inflammasome. The CARD domains of the 10 NLRC4 proteins are clustered in the middle of the inflammasome hub, and initiate caspase-1 polymerization. Thus, it is possible that a single molecule of flagellin, rod, or needle protein can result in pyroptosis. This seems to be an incredibly decisive system to destroy cells that have been compromised by T3SS injection.

One would expect that the existence of NLRC4 would make it very difficult for bacterial pathogens to use a T3SS. Yet, approximately half of all Gram-negative bacterial pathogens use T3SS. The Red Queen's race may have selected for pathogens that uniformly evade or inhibit NLRC4. For example, flagellin repression strategies are common and at least one case of an NLRC4-evasive T3SS has been described (Miao et al. 2010a,b). Remarkably, engineering bacteria to reverse flagellin repression generates stains that are eliminated by NLRC4 with exquisite sensitivity.

At least one bacterium, *C. violaceum,* appears to have no ability to evade NLRC4. *C. violaceum* is a ubiquitous environmental pathogen that encodes a T3SS, but whose natural host is unknown (Batista and da Silva Neto 2017). It infects people with significant immunologic defects, primarily those with chronic granulomatous disease (Batista and da Silva Neto 2017). Inflammasome responses clear *C. violaceum* infection in mouse models. Pyroptosis is required for splenic clearance, in which macrophages are most likely infected. In contrast, in the liver, inflammasome-driven IL-18 primes a natural killer (NK) cell response in which perforin-mediated cytotoxicity clears the hepatocyte niche, presumably by triggering apoptosis. Just as normal people are resistant to *C. violaceum* infection, wild-type (WT) mice resist high dose challenge (1,000,000 CFUs). However, *Nlrc4*$^{-/-}$ mice succumb to low-dose challenge (100 CFUs) (Maltez et al. 2015). We estimate that this represents a >50,000-fold change in the effective 100% lethal dose (LD_{100}; Table 1) when comparing WT to inflammasome-deficient mice (Maltez and Miao 2016). The remarkable change in the effective lethal dose is a phenotype that is nearly unheard of in the inflammasome literature, equaled only by *B. thailandensis* (discussed in the next section).

Caspase-11 Defending against *Burkholderia thailandensis*

Caspase-11 is the most direct pathway to programmed cell death, in that it requires just two proteins: caspase-11 and gasdermin D. Caspase-11 itself serves both as a sensor and as a catalytic protease. The CARD domain of caspase-11 directly binds to LPS in the cytosol, which triggers CARD-oligomerization and activation of the protease (Hagar et al. 2013; Kayagaki et al. 2013; Shi et al. 2014). Active caspase-11 then cleaves gasdermin D and induces pyroptosis (Kayagaki et al. 2015; Shi et al. 2015).

By sensing cytosolic LPS, caspase-11 discriminates cytosol-invasive bacteria from vacuolar or extracellular bacteria (Aachoui et al. 2013). Caspase-11 efficiently detects *B. thailandensis*, a cytosol invasive bacterium, in vivo and is incredibly effective at clearing the bacterium. This defense pathway is so effective that a systemic challenge of 20,000,000 CFUs are cleared within 1 day. In contrast, *Casp11*$^{-/-}$ mice succumb following challenge with as few as 100 CFUs (Aachoui et al. 2015). This is the strongest in vivo effect for caspase-11 against any infectious challenge (Maltez and Miao 2016). We estimate a 1,000,000-fold change in the effective LD_{100} (Table 1). It is remarkable that the strongest phenotypes for caspase-1 and caspase-11 in defense against infection arise from two ubiquitous environmental soil microbes, which only infect humans under extraordinary settings of host compromise such as patients with chronic granulomatous disease or after near drowning (Macher et al. 1982; Glass et al. 2006). This suggests that caspase-1/11 provide near permanent evolutionary victories over ubiquitous environmental pathogens, which are running the Red Queen's race against other hosts that lack caspase-1/11. (This hypothesis is de-

Table 1. Inflammasome and necroptosis survival studies

Pathogen	Mutation	Dose	Route	Time to death (median days) WT	Time to death (median days) KO	% Survival WT	% Survival KO	Δ lethal dose[a]	Micro-biota ctrl	Background	KO used in paper to get results shown in Time and Survival columns (and other KO with similar results)	References
BACTERIA versus INFLAMMASOMES												
Acinetobacter baumanii		5×10^8	in	1	2	88%	25%	>2	ns	C57BL/6	*Casp11*$^{-/-}$	Wang et al. 2017a
Bacillus anthracis Ames 35—vegetative		10^5	sc	∞	4	100%	0%	>5	ns	C57BL/6NTac	"WT" are Nlrp1$^{S/S}$, KO are Nlrp1$^{R/R}$	Moayeri et al. 2010
—vegetative		10^5	iv	∞	4	100%	0%	>5	ns	C57BL/6NTac	"WT" are Nlrp1$^{S/S}$, KO are Nlrp1$^{R/R}$	Moayeri et al. 2010
—spores		10^7	sc	∞	4.5	100%	20%	>2	ns	C57BL/6NTac	"WT" are Nlrp1$^{S/S}$, KO are Nlrp1$^{R/R}$	Moayeri et al. 2010
—spores		2×10^7	sc	∞	1	100%	0%	>5	L	BALB/c	All Nlrp1S/S; *Casp1/11*$^{+/+}$ or *Casp1/11*$^{-/-}$	Moayeri et al. 2010
Bacillus anthracis Ames —spores		4×10^2	ip	3	3	50%	25%	>1	L	C57BL/6	"WT" are Nlrp1b^{129Tg}; KO are no Tg	Terra et al. 2010
Bacillus anthracis Sterne —spores		2.5×10^7	ip	∞	4	100%	0%	>5	L	C57BL/6	"WT" are Nlrp1b^{129Tg}; KO are no Tg	Terra et al. 2010
Bacillus anthracis Stern —spores		10^6	ip	∞	3	100%	0%	>5	ns	C3H/HeJCr	*Casp1/11*$^{-/-}$	Kang et al. 2008
Burkholderia cepacia		10^6	ip	∞	∞	100%	100%	1	N	C57BL/6	*Casp1/11*$^{-/-}$	Maltez et al. 2015
Burkholderia cenocepacia		2×10^8	ip	∞	4	100%	17%	>2	ns	C57BL/6	*Mefv*$^{-/-}$	Aubert et al. 2016
Burkholderia pseudomallei		10^2	in	4	4	65%	0%	>5	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Nlrc4*$^{-/-}$, *Asc*$^{-/-}$)	Ceballos-Olvera et al. 2011
		25	in	∞	4	100%	0%	>5	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Nlrc4*$^{-/-}$, *Asc*$^{-/-}$)	Ceballos-Olvera et al. 2011
		10^2	in	4	5	65%	20%	>1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Ceballos-Olvera et al. 2011
		10^2	in	∞	2–3	100%	0%	>5	ns	C57BL/6	*Casp1/11*$^{-/-}$	Breitbach et al. 2009
		10^2	in	∞	3.5	100%	0%	>5	N	C57BL/6	*Casp1/11*$^{-/-}$ (*Casp11*$^{-/-}$)	Aachoui et al. 2013

Continued

Table 1. *Continued*

Pathogen	Mutation	Dose	Route	Time to death (median days)		% Survival		Δ lethal dose[a]	Micro-biota ctrl	Background	KO used in paper to get results shown in Time and Survival columns (and other KO with similar results)	References
				WT	KO	WT	KO					
Burkholderia thailandensis (unpassaged)		2×10^7	ip	∞	16	100%	13%	>20	N	C57BL/6	*Casp1/11*$^{-/-}$	Aachoui et al. 2013, 2015
		2×10^6	ip	∞	14	100%	20%	>20	N	C57BL/6	*Casp1/11*$^{-/-}$	Aachoui et al. 2013
		2×10^7	ip	∞	∞	100%	100%	1	N	C57BL/6	*Nlrc4*$^{-/-}$	Aachoui et al. 2013
		2×10^6	ip	∞	∞	100%	100%	1	N	C57BL/6	*Nlrc4*$^{-/-}$*Asc*$^{-/-}$	Aachoui et al. 2013
		10^4	in	∞	5	100%	0%	>5	N	C57BL/6	*Casp1/11*$^{-/-}$	Aachoui et al. 2013
		10^4	in	∞	∞	100%	100%	1	N	C57BL/6	*Nlrc4*$^{-/-}$*Asc*$^{-/-}$ (*Nlrc4*$^{-/-}$, *Asc*$^{-/-}$, *Nlrp3*$^{-/-}$)	Aachoui et al. 2013
Burkholderia thailandensis (one mouse passage)		2×10^7	ip	∞	1	100%	0%	>1,000,000	N	C57BL/6	*Casp1/11*$^{-/-}$ (*Casp11*$^{-/-}$)	Aachoui et al. 2013
		2×10^7	ip	∞	1	100%	0%	>1,000,000	N	C57BL/6	*Casp1/11*$^{-/-}$ (*Casp11*$^{-/-}$)	Aachoui et al. 2015; Maltez et al. 2015
		10^6	ip	∞	2	100%	0%	>1,000,000	N	C57BL/6	*Casp1/11*$^{-/-}$	Aachoui et al. 2015
		10^5	ip	∞	2	100%	0%	>1,000,000	N	C57BL/6	*Casp1/11*$^{-/-}$	Aachoui et al. 2015
		10^4	ip	∞	2	100%	0%	>1,000,000	N	C57BL/6	*Casp1/11*$^{-/-}$ (*Casp11*$^{-/-}$)	Aachoui et al. 2015; Maltez et al. 2015
		10^3	ip	∞	3	100%	0%	>1,000,000	N	C57BL/6	*Casp1/11*$^{-/-}$	Aachoui et al. 2015
		10^2	ip	∞	3	100%	0%	>1,000,000	N	C57BL/6	*Casp1/11*$^{-/-}$ (*Casp11*$^{-/-}$)	Aachoui et al. 2015
		2×10^7	ip	∞	2	100%	0%	>5	N	C57BL/6	*Nlrc4*$^{-/-}$*Asc*$^{-/-}$ (*Nlrc4*$^{-/-}$*Nlrp3*$^{-/-}$)	Aachoui et al. 2015
		10^4	ip	∞	∞	100%	100%	>5	N	C57BL/6	*Nlrc4*$^{-/-}$*Asc*$^{-/-}$	Aachoui et al. 2015
		10^2	ip	∞	∞	100%	100%	>5	N	C57BL/6	*Nlrc4*$^{-/-}$*Asc*$^{-/-}$	Aachoui et al. 2015
		2×10^7	ip	∞	∞	100%	100%	1	N	C57BL/6	*Nlrc4*$^{-/-}$ (*Nlrp3*$^{-/-}$, *Nlrc3*$^{-/-}$, *Nlrc5*$^{-/-}$, *Nlrp12*$^{-/-}$)	Aachoui et al. 2015
Chromobacterium violaceum		10^6	ip	∞	n.d.	100%	n.d.	>50,000	N	C57BL/6	*Casp1/11*$^{-/-}$ (*Nlrc4*$^{-/-}$, *Nlrc4*$^{-/-}$*Asc*$^{-/-}$)	Maltez et al. 2015
		10^4	ip	∞	3	100%	0%	>50,000	N	C57BL/6		Maltez et al. 2015

Continued

Table 1. *Continued*

Pathogen	Mutation	Dose	Route	Time to death (median days)		% Survival		Δ lethal dose[a]	Micro-biota ctrl	Background	KO used in paper to get results shown in Time and Survival columns (and other KO with similar results)	References
											Casp1/11$^{-/-}$ (*Nlrc4*$^{-/-}$, *Nlrc4*$^{-/-}$*Asc*$^{-/-}$)	
		10^2	ip	∞	4	100%	0%	>50,000	N	C57BL/6	*Casp1/11*$^{-/-}$ (*Nlrc4*$^{-/-}$, *Nlrc4*$^{-/-}$*Asc*$^{-/-}$)	Maltez et al. 2015
Ehrlichia (Ixodes ovatus ehrlichia)		~10^3	ip	9	9	100%	100%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Nlrp3*$^{-/-}$)	Yang et al. 2015
Escherichia coli O18:K1:H7		Range	ip	ns	ns	ns	ns	4	L	CeH/HeJ	*Casp1/11*$^{-/-}$	Joshi et al. 2002
Francisella tularensis subsp. *novicida*		1.5×10^5	sc	4	3	65%	0%	>2	L	C57BL/6	*Aim2*$^{-/-}$	Fernandes-Alnemri et al. 2010
		1.5×10^5	sc	6	4	30%	0%	″	N, (L)	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$)	Mariathasan et al. 2005
		1.5×10^5	sc	5	4	22%	0%	<1	L	C57BL/6	*Nlrc4*$^{-/-}$	Mariathasan et al. 2005
		5×10^3	sc	3	2.5	75%	0%	″	ns	ns	*Casp1/11*$^{-/-}$	Meunier et al. 2015
		7.5×10^4	sc	4	4	65%	0%	″	ns	ns	*Casp1/11*$^{-/-}$ (*Aim2*$^{-/-}$)	Man et al. 2015
Francisella turlarensis subsp. *Holartica*	LVS	2×10^4	in	7	7	0%	0%	1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Duffy et al. 2016
Francisella philomiragia		10^6	ip	∞	∞	100%	100%	1	N	C57BL/6	*Casp1/11*$^{-/-}$	Maltez et al. 2015
Klebsiella pneumoniae		7.4×10^4	it	2.1	1.9	15%	0%	>1	ns	C57BL/6	*Nlrp3*$^{-/-}$ (*Asc*$^{-/-}$)	Willingham et al. 2009
		7.4×10^4	it	2	2.5	0%	0%	1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Willingham et al. 2009
		10^3	in	5	5	75%	40%	>1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Cai et al. 2012
		10^4	in	4	6	50%	15%	>1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Cai et al. 2012
Listeria monocytogenes		10^6	iv	5	3–4	35%–65%	0%	>2	ns	C57BL/6J	*Casp1/11*$^{-/-}$	Tsuji et al. 2004
		10^5	iv	5	6	0%	50%	<2	N	C57BL/6	*Casp11*$^{-/-}$ (*Nlrp6*$^{-/-}$)	Hara et al. 2018

Continued

Table 1. *Continued*

Pathogen	Mutation	Dose	Route	Time to death (median days)		% Survival		Δ lethal dose[a]	Micro-biota ctrl	Background	KO used in paper to get results shown in Time and Survival columns (and other KO with similar results)	References
				WT	KO	WT	KO					
Mycobacterium tuberculosis		250–350	in	200	148	0%	0%	1	ns	C57BL/6	*Asc*$^{-/-}$	Mcelvania-Tekippe et al. 2010
		250–350	in	170	170	0%	0%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Nlrp3*$^{-/-}$, *Nlrc4*$^{-/-}$)	Mcelvania-Tekippe et al. 2010
		50–100	in	200	110	90%	0%	>2	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$)	Mayer-Barber et al. 2010
		10^6	it	∞	6	0%	0%	>5	L	C57BL/6	*Aim2*$^{-/-}$	Saiga et al. 2012
Pseudomonas aeruginosa		2×10^7	in	1.5	1.7	20%	65%	<1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Faure et al. 2014
	Δ*popBD*	10^8	in	2.5	1.5	44%	0%	>1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Faure et al. 2014
		7×10^5	it	3	∞	90%	100%	<1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Tolle et al. 2015
		3×10^7	in	∞	4	100%	0%	>5	ns	C57BL/6	*Nlrc4*$^{-/-}$	Iannitti et al. 2016
		3×10^7	in	∞	∞	100%	100%	1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Iannitti et al. 2016
		3.5×10^5	in	2.0	1.8	12%	4%	>1	ns	C57BL/6	*Casp1/11*$^{-/-}$	Hughes et al. 2018
		3.5×10^5	in	2.0	2.9	12%	46%	<1	ns	C57BL/6	*Asc*$^{-/-}$	Hughes et al. 2018
Salmonella enterica sv. Typhimurium		100	ip	4.5	5.5	0%	0%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$	Monack et al. 2000
		10^5	po	9	6	0%	0%	1	L	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$)	Lara-Tejero et al. 2006
		10^5	po	9	9	0%	0%	1	L	C57BL/6	*Nlrp3*$^{-/-}$	Lara-Tejero et al. 2006
		10^6	po	19	11	30%	20%	>1	L	*Nramp1*$^{+/+}$	*Casp1/11*$^{-/-}$	Lara-Tejero et al. 2006
		10^8	po	8	5.5	0%	0%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$	Raupach et al. 2006
		2×10^{10}	po	∞	20	100%	22%	>2	ns	129Sv/J	*Casp1/11*$^{-/-}$	Raupach et al. 2006
		10^8	ip	3	8	0%	0%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$	Raupach et al. 2006
		$1–5 \times 10^5$	po+s	6	6	0%	0%	1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Franchi et al. 2012
		10^5	ip	4	5	0%	0%	1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Franchi et al. 2012

Continued

Table 1. *Continued*

Pathogen	Mutation	Dose	Route	Time to death (median days)		% Survival		Δ lethal dose[a]	Micro-biota ctrl	Background	KO used in paper to get results shown in Time and Survival columns (and other KO with similar results)	References
				WT	KO	WT	KO					
		$1–5 \times 10^5$	po	8	7	0%	0%	1	ns	BALB/c	*Nlrc4*$^{-/-}$	Franchi et al. 2012
		$1–5 \times 10^5$	po+s	6	4	0%	0%	1	ns	BALB/c	*Nlrc4*$^{-/-}$	Franchi et al. 2012
		10^5	ip	4	5	0%	0%	1	ns	BALB/c	*Nlrc4*$^{-/-}$	Franchi et al. 2012
		10^3	ip	6	5	0%	0%	1	N	C57BL/6	*Nlrc4*$^{-/-}$ (*Naip2*$^{-/-}$, *Naip5*$^{-/-}$)	Zhao et al. 2016
Salmonella enterica sv. Typhimurium	*FliC*^{ON}	10^2	ip	∞	7	100%	0%	>5	N	C57BL/6	*Nlrc4*$^{-/-}$	Miao et al. 2010a
	FliC^{ON}	10^3	ip	∞	6	100%	0%	>5	ns	C57BL/6	*Nlrc4*$^{-/-}$ (*Naip5*$^{-/-}$)	Zhao et al. 2016
	FliC^{ON}	10^3	ip	∞	∞	100%	100%	1	ns	C57BL/6	*Naip2*$^{-/-}$	Zhao et al. 2016
	PrgJ^{ON}	10^3	ip	∞	5	100%	0%	>5	ns	C57BL/6	*Nlrc4*$^{-/-}$ (*Naip2*$^{-/-}$)	Zhao et al. 2016
	PrgJ^{ON}	10^3	ip	∞	∞	100%	100%	1	ns	C57BL/6	*Naip5*$^{-/-}$	Zhao et al. 2016
Shigella flexneri		2×10^8	in	0.8	1.9	75%	20%	>2	ns	C57BL/6	*Casp1/11*$^{-/-}$	Sansonetti et al. 2000
Staphylococcus aureus		10^4	ic	0.8	1	60%	25%	>1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$, *Aim2*$^{-/-}$)	Hanamsagar et al. 2014
		10^4	ic	5	5	95%	70%	>1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Hanamsagar et al. 2014
		10^8	ro	2	1	84%	40%	>1	ns	C57BL/6	*Casp1/11*$^{-/-}$	Kitur et al. 2016
		2×10^8	iv	0.5	1.5	0%	60%	>2	N	C57BL/6	*Casp11*$^{-/-}$ (*Nlrp6*$^{-/-}$)	Hara et al. 2018
Streptococcus agalactiae (group B)		10^5	ip	∞	1	100%	40%	>2	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$, *Nlrp3*$^{-/-}$)	Costa et al. 2012
Streptococcus pneumoniae		10^5	in	3	2.5	87%	90%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$	Albiger et al. 2007
		10^3	in	6	5	90%	50%	>1	ns	C57BL/6	*Nlrp3*$^{-/-}$, *Aim2*$^{-/-}$	Rodriguez et al. 2019
Vibrio vulnificus		1.5×10^4	ip	∞	1	100%	60%	>1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$, *Nlrp3*$^{-/-}$)	Toma et al. 2010
Yersinia pestis		10^4	in	3	3	0%	0%	1	N	C57BL/6	*Casp1/11*$^{-/-}$	Sivaraman et al. 2015
Yersinia pestis	*ΔyopJM*	2×10^2	sc	7	6	72%	25%	>1	ns	C57BL/6	*Casp1/11*$^{-/-}$	Ratner et al. 2016
Yersinia pestis		10^1	sc	7	5	0%	0%	1	ns	C57BL/6	*Nlrp12*$^{-/-}$	Vladimer et al. 2012

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Table 1. *Continued*

Pathogen	Mutation	Dose	Route	Time to death (median days)		% Survival		Δ lethal dose[a]	Micro-biota ctrl	Background	KO used in paper to get results shown in Time and Survival columns (and other KO with similar results)	References
				WT	KO	WT	KO					
Yersinia pestis	*pYtbLpxL*	5×10^2	sc	∞	8	100%	20%	>2	ns	C57BL/6	*Nlrp12*$^{-/-}$ (*Nlrp3*$^{-/-}$)	Vladimer et al. 2012
Yersinia pseudotuberculosis		10^3	ip	6	4	0%	0%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$	LaRock and Cookson 2012
Yersinia pseudotuberculosis	*ΔyopM*	10^3	ip	∞	6	100%	0%	>5	ns	C57BL/6	*Casp1/11*$^{-/-}$	LaRock and Cookson 2012
Yersinia pseudotuberculosis		2×10^3	iv	7	7	0%	0%	1	ns	C57BL/6	*Mefv*$^{-/-}$	Chung et al. 2016
Yersinia pseudotuberculosis	*ΔyopM*	2×10^3	iv	13	7	89%	0%	>2	ns	C57BL/6	*Mefv*$^{-/-}$	Chung et al. 2016
Yersinia pseudotuberculosis	Comp. mutant	1×10^9	po	5	5	0%	0%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$	Zheng et al. 2012
VIRUSES versus INFLAMMASOMES												
Encephalomyocarditis virus		2xLD50	ip	5	5	10%	15%	>1	N	C57BL/6	*Casp1/11*$^{-/-}$	Rajan et al. 2011
Influenza A virus		6×10^4	in	8	7	65%	40%	>1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$, *Nlrp3*$^{-/-}$)	Allen et al. 2009
		6×10^4	in	8	7	68%	71%	1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Allen et al. 2009
		8×10^3	in	11	10	65%	35%	>1	ns	C57BL/6	*Casp1/11*$^{-/-}$, (*Nlrp3*$^{-/-}$)	Thomas et al. 2009
		8×10^3	in	12	9	83%	85	1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Thomas et al. 2009
		10^1	in	∞	11	100%	0%	>5	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$)	Ichinohe et al. 2009
		10^1	in	∞	∞	100%	100%	1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Ichinohe et al. 2009
		1.25×10^2	in	∞	∞	100%	100%	1	ns	C57BL/6	*Nlrp3*$^{-/-}$ (*Aim2*$^{-/-}$)	Rodriguez et al. 2019
Influenza A virus H7N9		5×10^4	in	9	9	18%	64%	<1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Nlrp3*$^{-/-}$, *Asc*$^{-/-}$)	Ren et al. 2017
Herpes simplex virus-1		1.5×10^6	scr	11	12.5	71%	73%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$	Milora et al. 2014
Murine hepatitis virus A59		5×10^3	ic	10	10	90%	35%	>2	ns	C57BL/6	*Casp1/11*$^{-/-}$	Zalinger et al. 2017
Murine hepatitis virus-3		10^2	ip	4	5	0%	28%	<1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Nlrp3*$^{-/-}$)	Guo et al. 2015

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Table 1. *Continued*

Pathogen	Mutation	Dose	Route	Time to death (median days) WT	Time to death (median days) KO	% Survival WT	% Survival KO	Δ lethal dose[a]	Micro-biota ctrl	Background	KO used in paper to get results shown in Time and Survival columns (and other KO with similar results)	References
Murine norovirus		10^2	po	6	7	0%	0%	1		C57BL/6 *Stat1*$^{-/-}$	*Nlrp3*$^{-/-}$ (*Gsdmd*$^{-/-}$)	Dubois et al. 2019
Vaccinia		3×10^6	iv	6	7	0%	0%	1	ns	C57BL/6	*Casp1*$^{-/-}$ (*Asc*$^{-/-}$)	Wang et al. 2017b
Vesicular stomatitis virus		2×10^5	in	7	7	40%	20%	>1	N	C57BL/6	*Casp1/11*$^{-/-}$	Rajan et al. 2011
		2×10^7	iv	5	5	0%	0%	1	ns	C57BL/6	*Casp1*$^{-/-}$ (*Asc*$^{-/-}$)	Wang et al. 2017b
West Nile virus		10^2	sc	11	11	81%	50%	>1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Nlrp3*$^{-/-}$)	Ramos et al. 2012
		10^2	sc	11	12	75%	75%	1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Ramos et al. 2012
		10^2	sc	11	10	42%	11%	>1	ns	C57BL/6	*Asc*$^{-/-}$	Kumar et al. 2013
		10^1	sc	11	11	89%	58%	>1	ns	C57BL/6	*Asc*$^{-/-}$	Kumar et al. 2013
FUNGI versus INFLAMMASOMES												
Aspergillus fumigatus		2×10^7	in	∞	∞	100%	100%	1	ns	C57BL/6	*Aim2*$^{-/-}$*Nlrp3*$^{-/-}$	Man et al. 2017
		2×10^7	in	∞	∞	100%	100%	1	ns	C57BL/6	*Nlrp3*$^{-/-}$, (*Nlrc4*$^{-/-}$)	Iannitti et al. 2016
Aspergillus fumigatus (imm. suppressed)		10^5	in	6	4	70%	0%	>2	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Aim2*$^{-/-}$*Nlrp3*$^{-/-}$, *Asc*$^{-/-}$)	Man et al. 2017
		10^5	in	6	5	70%	50%	>1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Man et al. 2017
		10^5	in	6	5.5	70%	67%	>1	ns	C57BL/6	*Aim2*$^{-/-}$	Man et al. 2017
		5×10^5	in	5	4	43%	0%	>1	ns	C57BL/6	*Casp1*$^{-/-}$ (*Casp1/11*$^{-/-}$)	Karki et al. 2015
		5×10^5	in	5	5	43%	0%	>1	ns	C57BL/6	*Casp11*$^{-/-}$	Karki et al. 2015
Candida albicans		10^5	iv	9	5	40%	0%	>1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Gross et al. 2009
		2×10^5	iv	18	17	83%	50%	>1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$)	Van De Veerdonk et al. 2011
		5×10^6	ts	N/A	3	100%	60%	>1	ns	C57BL/6	*Nlrc4*$^{-/-}$	Tomalka et al. 2011
		ns	ts	∞	5	97%	60%	>1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$)	Hise et al. 2009
Paracoccidioides brasiliensis		2×10^6	iv	98	93	73%	0%	>2	ns	C57BL/6	*Casp1/11*$^{-/-}$	Ketelut-Carneiro et al. 2015
		2×10^6	iv	98	93	73%	30%	>1	ns	C57BL/6	*Asc*$^{-/-}$	Ketelut-Carneiro et al. 2015
		10^6	it	85	50	54%	0%	>2	ns	C57BL/6	*Casp1/11*$^{-/-}$	Feriotti et al. 2017
		10^6	it	85	120	54%	0%	>2	ns	C57BL/6	*Asc*$^{-/-}$ (*Nlrp3*$^{-/-}$)	Feriotti et al. 2017

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Table 1. *Continued*

Pathogen	Mutation	Dose	Route	Time to death (median days) WT	Time to death (median days) KO	% Survival WT	% Survival KO	Δ lethal dose[a]	Micro-biota ctrl	Background	KO used in paper to get results shown in Time and Survival columns (and other KO with similar results)	References
PARASITES versus INFLAMMASOMES												
Plasmodium berghei sporozoites		10	iv	9	12	45%	73%	<1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Dostert et al. 2009
Plasmodium berghei sporozoites		10^4	iv	6.5	6.5	0%	0%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$	Kordes et al. 2011
Plasmodium berghei iRBCs		10^4	iv	6.5	6.5	0%	0%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$	Kordes et al. 2011
Plasmodium berghei iRBCs		10^6	ip	6	6	0%	0%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$)	Reimer et al. 2010
		10^6	ip	6	8	0%	0%	1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Reimer et al. 2010
Plasmodium chabaudi adami		5×10^4	ip	11	12	0%	0%	1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Shio et al. 2009
Toxoplasma gondii tachyzoites		10^4	ip	10	9	75%	10%	>2	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Asc*$^{-/-}$, *Nlrp3*$^{-/-}$, *Nlrp1abc*$^{-/-}$)	Gorfu et al. 2014
		10^3	ip	13	13	50%	30%	>1	ns	C57BL/6	*Asc*$^{-/-}$	Coutermarsh-Ott et al. 2016
		10^3	ip	13	∞	50%	100%	<2	ns	C57BL/6	*Casp11*$^{-/-}$	Coutermarsh-Ott et al. 2016
Trypanosoma cruzi trypomastigotes		10^3	ip	28	22	90%	0%	>2	ns	C57BL/6	*Asc*$^{-/-}$	Silva et al. 2013
		10^3	ip	28	28	90%	17%	>2	ns	C57BL/6	*Casp1/11*$^{-/-}$	Silva et al. 2013
		10^3	ip	28	28	90%	67%	>1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Silva et al. 2013
		10^3	sc	22	28	85%	92%	1	ns	C57BL/6	*Casp1/11*$^{-/-}$ (*Nlrp3*$^{-/-}$)	Gonçalves et al. 2013
		10^3	ip	∞	26	100%	50%	>2	ns	C57BL/6	*Casp1/11*$^{-/-}$	Paroli et al. 2018
		10^3	ip	∞	∞	100%	100%	1	ns	C57BL/6	*Nlrp3*$^{-/-}$	Paroli et al. 2018

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Table 1. *Continued*

Pathogen	Mutation	Dose	Route	Time to death (median days)		% Survival		Δ lethal dose[a]	Micro-biota ctrl	Background	KO used in paper to get results shown in Time and Survival columns (and other KO with similar results)	References
				WT	KO	WT	KO					
BACTERIA versus NECROPTOSIS												
Salmonella enterica sv. Typhimurium		10^2	iv	11	10	0%	0%	1	ns	C57BL/6	*Ripk3*$^{-/-}$	Robinson et al. 2012
		10^8	po	10	7	0%	0%	1	ns	C57BL/6	*Mlkl*$^{-/-}$	Yu et al. 2018
Staphylococcus aureus		10^8	ro	2	4	84%	88%	1	ns	C57BL/6	*Ripk3*$^{-/-}$	Kitur et al. 2016
		10^8	ro	2	3	84%	44%	>1	ns	C57BL/6	*Mlkl*$^{-/-}$	Kitur et al. 2016
Yersinia pestis	*pEcLpxL*	5×10^2	sc	∞	11	100%	83%	>1	ns	C57BL/6	*Ripk3*$^{-/-}$ (BMT)	Weng et al. 2014
		2×10^5	fp	5	7	36%	70%	<1	ns	C57BL/6	*Ripk1*$^{D138N/D138N}$	Arifuzzaman et al. 2018
Yersinia pseudotuberculosis		9×10^7	po	>6	>6	100%	100%	1	ns	C57BL/6	*Ripk3*$^{-/-}$ (BMT)	Philip et al. 2014
VIRUSES versus NECROPTOSIS												
Influenza A		13 HAU	in	11	13	78%	62%	1	ns	C57BL/6	*Ripk3*$^{-/-}$	Rodrigue-Gervais et al. 2014
		4×10^3	in	11	11	71%	38%	>1	ns	C57BL/6	*Ripk3*$^{-/-}$	Nogusa et al. 2016
		4×10^3	in	10	11	74%	81%	1	ns	C57BL/6	*Mlkl*$^{-/-}$	Nogusa et al. 2016
		10^3	in	8.5	∞	67%	100%	<1	ns	C57BL/6	*Zbp1*$^{-/-}$	Kuriakose et al. 2016
		10^3	in	∞	10	100%	22%	>2	L	C57BL/6	*Zbp1*$^{-/-}$	Thapa et al. 2016
Herpes simplex virus-1		2×10^7	ip	∞	6	100%	58%	>2	ns	ns	*Ripk3*$^{-/-}$	Wang et al. 2014
		10^7	iv	∞	5	100%	50%	>2	ns	ns	*Ripk3*$^{-/-}$	Huang et al. 2015
Herpes simplex virus-1	*ΔICP6*	10^7	iv	3.5	3.5	38%	56%	1	ns	ns	*Ripk3*$^{-/-}$	Huang et al. 2015
Vaccinia		2×10^6	ip	9	7	86%	0%	>2	ns	C57BL/6	*Ripk3*$^{-/-}$	Cho et al. 2009
West Nile virus		10^2	sc	11	11	56%	0%	>2	ns	C57BL/6	*Ripk3*$^{-/-}$	Daniels et al. 2017
		10^2	sc	11	12	40%	46%	1	ns	C57BL/6	*Mlkl*$^{-/-}$	Daniels et al. 2017
		10^2	sc	11	11	56%	0%	>2	ns	C57BL/6	*Ripk1*$^{KD/KD}$	Daniels et al. 2017
		10^1	ic	9	7	0%	0%	0	ns	C57BL/6	*Ripk3*$^{-/-}$	Daniels et al. 2017
Zika virus		10^3	ic	7	6.5	73%	0%	>2	ns	C57BL/6N	*Ripk3*$^{-/-}$	Daniels et al. 2019
		10^3	ic	7.5	7	60%	0%	>2	ns	C57BL/6J	*Ripk1*$^{KD/KD}$	Daniels et al. 2019

Continued

Table 1. *Continued*

Pathogen	Mutation	Dose	Route	Time to death (median days)		% Survival		Δ lethal dose[a]	Micro-biota ctrl	Background	KO used in paper to get results shown in Time and Survival columns (and other KO with similar results)	References
				WT	KO	WT	KO					
		10^3	ic	7.5	7.5	60%	70%	1	ns	C57BL/6J	*Mlkl*$^{-/-}$	Daniels et al. 2019
		10^3	ic	6.5	7	82%	7%	>2	L	C57BL/6	*Ripk3*$^{fl/fl}$ CamKIIα-Cre^{+}	Daniels et al. 2019
		10^3	ic	7.5	8	80%	20%	>2	ns	C57BL/6J	*Zbp1*$^{-/-}$	Daniels et al. 2019

The investigators intend to continue to update this table and thus would appreciate notification of omissions or errors. This table includes experiments performed with mice defective for necroptosis genes, but does not include apoptosis knockout (KO) mice such as *Casp8*$^{-/-}$ mice.

BMT, KO mice instead are wild-type (WT) mice that received knockout bone marrow; iv, intravenous; sc, subcutaneous; scr, scratch to flank skin; po, per oral; po+s, per oral pretreated with streptomycin; in, intranasal; ro, retroorbital intravenous; it, intratracheal; ic, intracranial; fp, footpad; ts, tongue scratch; L, littermate; CO; cohoused; N; not littermate and not cohoused; ns, not stated.

[a]Estimated change in lethality between WT and KO mice. A difference in survival percentages of (1) 0% change was estimated to be onefold increase in infectious dose (or changes that were not statistically significant); (2) 1%–49% was estimated to be greater than onefold increase in the infectious dose; (3) 50%–99% was estimated to be greater than twofold; and (4) >100% was estimated to be greater than fivefold. Where multiple doses were examined, the high dose was divided by the low dose and then multiplied by the aforementioned estimator. As an example, in Ceballos-Olvera et al. (2011), WT mice had 100% lethality at 200 CFU but many survived at 100 CFU and *Casp1*$^{-/-}$*Casp11*$^{-/-}$ mice succumbed to 25 CFU; 200/25 = 8, and so the change in lethal dose is listed as greater than eightfold since *Casp1*$^{-/-}$*Casp11*$^{-/-}$ mice may succumb to even lower doses. Note that many infectious models have only examined one dose, so the change in lethal dose may turn out to be much larger than listed in this table once additional doses are tested. A final caveat is the all these manuscripts use mice on the C57BL/6 background, which carries MX1 and Nramp1 mutations that cause susceptibility to viral and bacterial infection, respectively, it is difficult to detect increases in the lethal dose when the lethal dose is already very low in WT C57BL/6 mice.

scribed in more depth in Maltez and Miao 2016 and Box 1 of Jorgensen et al. 2017.)

Some pathogens alter their LPS structure to evade detection by caspase-11 (Hagar et al. 2013; Kayagaki et al. 2013; Paciello et al. 2013; Yang et al. 2019). Additionally, some bacteria avoid recognition by caspase-11 by remaining within the vacuolar space (Aachoui et al. 2013). There are several examples of caspase-11 responding to pathogens that are typically considered to be vacuolar. In these cases, there must be either vacuolar leakage or rupture to introduce LPS into the cytosol. In such cases, caspase-11 can reduce vacuolar pathogen burdens (Lacey et al. 2018). Nevertheless, it is likely that caspase-11 evolved to combat cytosol-invasive pathogens.

LPS is an incredibly abundant ligand. Thus, LPS sensing risks detection of LPS that enters the cytosol aberrantly, as in endotoxic shock models (Hagar et al. 2013; Kayagaki et al. 2013). This risk is partially ameliorated by tight regulation of caspase-11; caspase-11 cannot be activated in the absence of interferon (IFN)-γ or type I IFN signaling (Broz et al. 2012; Rathinam et al. 2012; Aachoui et al. 2015).

The Evolutionary Race between *Yersinia* and the Pyrin Inflammasome

One of the most notable examples of the never-ending race between host and pathogen are pathogenic *Yersinia* spp. The causative agent of plague (*Yersinia pestis*) and the enteric pathogens (*Yersinia pseudotuberculosis* and *Yersinia enterocolitica*) all encode a T3SS enabling them to infect humans and other mammals. These T3SS inject effectors called *Yersinia* outer proteins (Yops) into the cytosol of host cells (Bliska et al. 2013). Given that *Yersinia* expresses a T3SS, it should be detected by the NLRC4 inflammasome. Additionally, the NLRP3 inflammasome may detect the YopB and YopD translocon proteins. However, YopK reportedly restricts both these detection events although through an unclear mechanism (Brodsky et al. 2010; Zwack et al. 2015). A more explicit example of *Yersinia* species running the Red Queen's race has recently been shown in *Yersinia*'s ability to evade the pyrin inflammasome.

During bacterial infection, neutrophils are the first immune cells recruited to the site of infection. Neutrophils phagocytose bacteria by activating Rho GTPases, such as RhoA, Rac1, and CDC42, which polymerize actin driving phagocytosis (Mao and Finnemann 2015). The primary virulence strategy of *Yersinia* spp. is to inhibit phagocytosis and replicate extracellularly (Ke et al. 2013). Once neutrophils arrive, they are the predominant cell type targeted by the *Yersinia* T3SS (Pechous et al. 2013). *Yersinia* prevents actin polymerization in part by using YopE and YopT. YopE facilitates GTPase hydrolysis, keeping RhoA in the inactive GDP-bound state, whereas YopT is a cysteine protease that cleaves the carboxyl terminus of Rho GTPases, releasing them from the membrane (Black and Bliska 2000; Shao et al. 2003). Thus, both YopE and YopT disable Rho GTPase activity to prevent phagocytosis (Grosdent et al. 2002). At this point, the pathogen is winning the evolutionary race (Fig. 2).

In an effort to combat antiphagocytic effectors, the host evolved a functional guard called pyrin (encoded by *Mefv*). Pyrin essentially monitors for normal biochemical function of the Rho GTPases; when the Rho GTPases are perturbed, pyrin activates caspase-1 (Xu et al. 2014). This guard function is accomplished not by direct interaction between pyrin and Rho GTPases, but via monitoring Rho effector protein kinases N1 and N2 (PKN1 and PKN2; also called PRK1/PRK2). Although the exact biochemical mechanism remains unclear, current literature suggests that RhoA activates PKN1/PKN2, activating their normal effector functions (Thumkeo et al. 2013) and also permitting PKN1/PKN2 to phosphorylate pyrin (Gao et al. 2016; Park et al. 2016). Thus, when RhoA is present and is capable of inducing phagocytosis, PKN1/PKN2 phosphorylate pyrin and repress inflammasome assembly. However, if RhoA is degraded or enzymatically modified by YopT and/or YopE, PKN1/PKN2 no longer phosphorylate pyrin. By an unclear mechanism, this lack of phosphorylation results in pyrin activation (Chung et al. 2016). Exactly how inflammasome activation at this point helps the host remains to be elucidated. Induction of pyroptosis should delete phagocytes that have

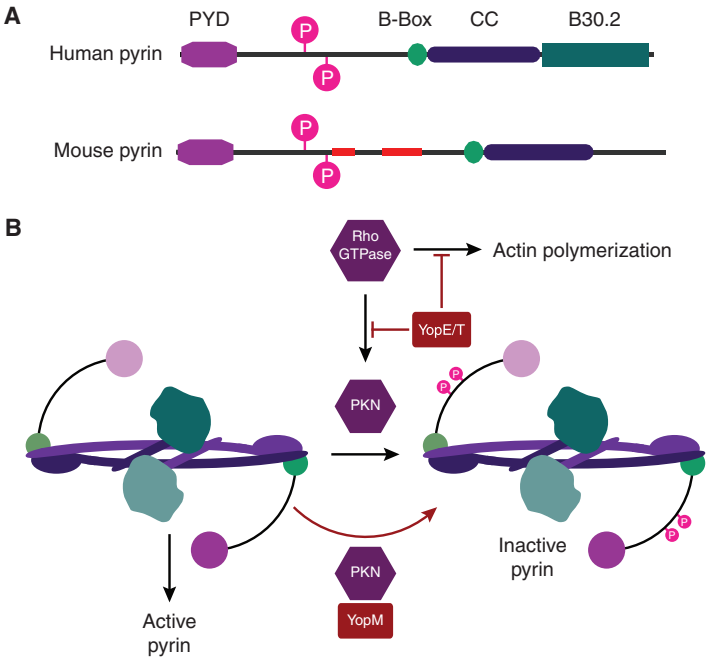

Figure 2. The pyrin inflammasome guards Rho GTPases. Pyrin was the first gene described to encode the pyrin domain (PYD) that is also found in many other inflammasomes. PYD signals through the PYD-CARD adaptor protein ASC, and thereby to caspase-1. In addition to the PYD, pyrin is a member of the tripartite motif (TRIM) family, and thus contains a B-Box, a coiled coil (CC), and a B30.2 domain (Kawai and Akira 2011; Weinert et al. 2015). (*A*) Domain structure of human and mouse pyrin. Slim red boxes indicate regions that are present in the human but absent in the mouse, or vice versa. (*B*) Rho GTPases are activated in response to signals for immune cell motility and/or phagocytosis. As part of their effector functions, Rho GTPases activate actin polymerization as well as effector protein kinases (including PKN1 and PKN2). Pyrin (shown in dimeric form) guards these Rho GTPase signaling pathways by mechanisms that are only partially understood. Successful Rho GTPase signaling will activate PKN1/PKN2, which phosphorylate and thereby inactivate pyrin. Thus, pyrin acts as a checkpoint to verify Rho GTPase function. If phosphorylation fails, pyrin becomes an active inflammasome. *Yersinia* encodes YopE and YopT that prevent phagocytosis by attacking Rho GTPases, but at the cost of preventing pyrin phosphorylation. Another *Yersinia* effector, YopM, reattaches PKN1/PKN2 to pyrin, driving pyrin phosphorylation. Thus, *Yersinia* successfully blocks phagocytosis with YopE/YopT while simultaneously defusing the pyrin guard with YopM.

been debilitated by the Yops. Additionally, the capase-1-driven release of IL-1β should recruit new neutrophils to the site of infection. In total, the pyrin inflammasome prevents the pathogen from creating a favorable environment for extracellular bacterial replication. Pyrin is also important for detection of a variety of pathogens that produce toxins or T3SS effectors that perturb Rho GTPases, indicating that pyrin is a general guard for actin cytoskeletal function (Xu et al. 2014; Aubert et al. 2016).

The evolutionary race was not finished; *Yersinia* spp. evolved an additional virulence factor in an effort to overthrow the host. *Yersinia* developed the effector YopM, which recruits PKN1/PKN2 to pyrin, driving pyrin phosphorylation even in the absence of RhoA activity (Mcdonald et al. 2003; Chung et al. 2016). Thus, WT mice are susceptible to WT *Yersinia* infection, but resistant to *yopM* mutants. *Yersinia yopM* mutants, on the other hand, are virulent in mice deficient in pyrin or caspase-1 (LaRock and Cookson 2012; Chung et al. 2016) (also see Table 1).

The basic function of YopM, to inhibit pyrin, is conserved among *Yersinia* spp.

Nevertheless, the Red Queen's race appears to be ongoing as YopM is polymorphic between *Yersinia* isolates and species (Chung et al. 2016). Similarly, there are several key differences between mouse and human pyrin, suggesting some YopM variants may work effectively against the pyrin of one host, but fail against others (Fig. 2). This extreme evolutionary pressure may push the host into a precarious position. Autosomal recessive mutations in pyrin result in the most common autoinflammatory disease worldwide, familial Mediterranean fever (FMF) (Özen 2018). These mutant pyrin proteins are not phosphorylated to the same degree by PKN1/PKN2 (Park et al. 2016). Thus, pyrin is more easily activated, resulting in high IL-1β levels and reoccurring inflammation and fever, but only in the homozygous state (Park et al. 2016). FMF is highly prevalent in parts of the Mediterranean region, thus populations that are native to the area may have evolved this gain of function if it confers a selective advantage in the heterozygous state to a pathogen. Given the impact of plague throughout history, it is tempting to speculate that *Y. pestis* provided the selective pressure to induce expansion of pyrin mutations.

NLRP1—One NLR to Guard Them All

Although it was the first inflammasome identified (Martinon et al. 2002), how the NLRP1 inflammasome detects pathogens has only recently been elucidated. NLRP1 in humans and mice contains a unique domain structure among NLRs (Fig. 3). Like NLRC4, NLRP1 has a CARD that directly activates caspase-1, yet it is located on the carboxyl terminus rather than the amino terminus. NLRP1 is also unique in that it includes a carboxy-terminal function-to-find domain (FIIND) directly upstream of its CARD. The FIIND undergoes constitutive autoproteolysis, but remains noncovalently associated with the rest of NLRP1. This noncovalent association is required for activation of NLRP1 (D'Osualdo et al. 2011; Finger et al. 2012; Frew et al. 2012). Furthermore, the minimal active component of NLRP1 is actually the cleaved FIIND-CARD fragment alone; the NOD and LRR are surprisingly dispensable. Activation of this cleaved-FIIND-CARD inflammasome occurs only when the other domains of NLRP1 are degraded (Xu et al. 2018; Chui et al. 2019; Sandstrom et al. 2019). This amino-terminal degradation is thought to be induced, at least in part, by pathogen-mediated mechanisms (Frew et al. 2012).

NLRP1 was first recognized as a sensor of anthrax lethal toxin (LT), a metalloprotease produced by *Bacillus anthracis* (Boyden and Dietrich 2006). LT cleaves MAP kinase kinases (MAPKKs) to prevent innate immune signaling (Turk 2007). The host response to this in certain mouse strains seems to be to integrate a decoy domain into NLRP1b, such that now LT cleaves NLRP1b in addition to its primary target. This cleavage event occurs at the amino terminus and activates NLRP1 (Levinsohn et al. 2012). Proteolysis of amino-terminal residues induces a process known as the N-end rule (Lucas and Ciulli 2017), wherein amino acid residues at the new amino terminus are modified by cellular E3 ubiquitin ligases and targeted for degradation by the proteasome. Thus, when LT cleaves NLRP1b, the amino-terminal portion of NLRP1 is ubiquitinated and degraded by the proteasome. The cleaved-FIIND-CARD disassociates from the rest of NLRP1 during this process because of its noncovalent association (Squires et al. 2007; Wickliffe et al. 2008; Xu et al. 2018; Chui et al. 2019; Sandstrom et al. 2019), and is then free to oligomerize and form potent inflammasomes (Xu et al. 2018; Chui et al. 2019; Sandstrom et al. 2019).

FIIND or similar domains are found in other innate immune genes, including *CARD8* (present in humans but absent in mice) and *PIDD*, suggesting that activation by proteolysis may be useful for regulating other pathways (Tinel et al. 2007; D'Osualdo et al. 2011). Given this mechanism of NLRP1 activation, there needs to be a way to safely degrade old NLRP1, CARD8, and PIDD during cell homeostasis without activating caspase-1. In this regard, inhibitors of serine dipeptidases, Dpp, induce activation of NLRP1 and/or CARD8 without proteolytic cleavage, but the proteasome is still required (Okondo et al. 2017; 2018; Johnson et al. 2018; Zhong

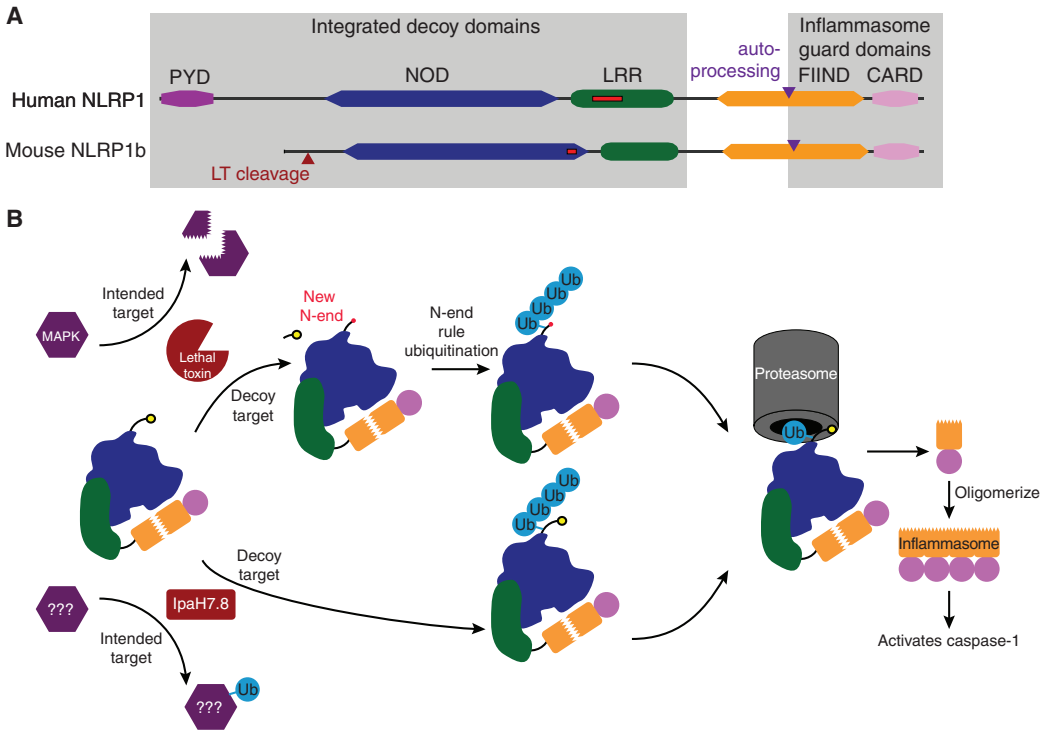

Figure 3. The NLRP1 inflammasome has integrated decoy domains to detect virulence factor attack. (*A*) Diagram of the human NLRP1 protein, and one of the NLRP1 proteins in mice (NLRP1b). Domains are indicated by colored boxes. Slim red boxes indicate regions that are present in the human, but absent in the mouse protein, or vice versa. Red triangle indicates the lethal toxin cleavage site in mouse NLRP1b. Purple triangle indicates the autoprocessing site within the FIIND domain. (*B*) Schematic of murine NLRP1b pathways. In one mode of activation (*top*), anthrax lethal toxin intends to cleave MAP kinase kinases; however, a decoy sequence in NLRP1b is also cleaved by lethal toxin. This exposes a new amino terminus (New N-end) in NLRP1b, which is detected by the N-end rule ubiquitinases that attach a ubiquitin to a nearby lysine residue. In a second mode (*bottom*) a bacterial effector such as IpaH7.8 intends to attack and ubiquitinate a different cellular protein, but also inadvertently ubiquitinates the decoy domains of NLRP1b. Ubiquitinated NLRP1b is then degraded by the proteasome, but when the precleaved FIIND domain approaches the proteasome, the carboxy-terminal FIIND fragment and attached CARD domain dissociate and are therefore not degraded. This dissociation results from the FIIND-CARD domains not being covalently attached to the rest of the protein. The liberated FIIND-CARD then oligomerizes to form an inflammasome, clustering the CARD domains that activate caspase-1. The FIIND-CARD inflammasome is different from typical inflammasomes in which the NOD domain drives oligomerization and clustering of an amino-terminal CARD domain. (From Lacey and Miao 2019; adapted, with permission, from the authors.)

et al. 2018). Thus, Dpp may participate in controlled NLRP1 and/or CARD8 degradation during normal homeostasis, thereby reducing the risk of NLRP1 autoactivation.

Nonproteolytic pathogen-mediated mechanisms may also target NLRP1 for degradation in the proteasome. *Shigella flexneri* has a T3SS that secretes the E3 ubiquitin ligase, IpaH7.8 (Rohde et al. 2007; Singer et al. 2008; Zhu et al. 2008), which is detected by the NLRP1b inflammasome (Sandstrom et al. 2019). NLRP1 is ubiquitinated by IpaH7.8, which targets it for proteasomal degradation, and consequently releases the FIIND-CARD fragment that activates caspase-1. Ubiquitination and degradation of inhibitory proteins is also used in normal signaling pathways; for example, the NEMO/IKKα/IKKβ complex ubiquitinates IκB to drive its degradation,

releasing nuclear factor (NF)-κB to translocate to the nucleus. In contrast, we speculate that NLRP1 acts as a decoy to detect ubiquitin attack on other NLRs. Thus, the NOD and LRR domains of NLRP1 would be defined as integrated decoy domains (Fig. 1). This idea would be supported if future research discovers that IpaH7.8 actually evolved to target a different NLR for degradation. The host may use these accessory integrated decoy domains to trick pathogens into targeting NLRP1 for degradation, creating a tripwire that activates the inflammasome.

The number of *NLRP1* genes and the domain structure of NLRP1 varies not only between species, but also within a species (Boyden and Dietrich 2006; D'Osualdo et al. 2011; Lilue et al. 2018). Unlike mouse macrophages, human macrophages exposed to IpaH7.8 do not activate caspase-1 (Muehlbauer et al. 2007). Thus, *Shigella* is winning the Red Queen's race against humans and losing against mice (Sharma et al. 2017). The versatility of NLRP1 is illustrated by the integration of an amino-terminal PYD in human NLRP1 (Moayeri et al. 2012), which perhaps evolved as an extra integrated decoy domain to lure virulence factors that attack PYDs. Pathogens that activate human NLRP1 have not yet been discovered, perhaps because NLRP1 eradicates them before disease development. If NLRs are the subject of attack by virulence factors, then NLRP1 could be considered the one NLR to guard them all.

Guarding Transcriptional Signaling with Apoptosis or Necroptosis

TNF is an important proinflammatory cytokine that is often targeted by bacterial virulence factors. Thus, it makes sense that TNF signaling is carefully guarded. TNF receptor signaling can have one of three outcomes: gene transcription, apoptosis, or necroptosis. The primary goal of TNF signaling is likely an NF-κB transcriptional response. When this transcriptional response is inhibited, guard functions detect the defective signaling pathway and, in response, trigger either apoptosis, pyroptosis, or necroptosis. These guard functions are likely the result of the ongoing evolutionary battle between host and pathogen, dating back to the dawn of primitive multicellular organisms (Quistad et al. 2014).

In the Red Queen's race, a hypothetical pathogen would attempt to inhibit the TNF transcriptional response with a virulence factor to dampen the host immune response. One key signaling point within the TNF signaling pathway is RIPK1, because it gets modified with polyubiquitin chains that recruit the TAK1 and IKK complexes needed to activate NF-κB (Dondelinger et al. 2016a). To counter this attack, the host has evolved guards for TNF transcriptional signaling mediated by the RIPK1 axis. Once the TNF guards detect that a virulence factor has intercepted the TNF to NF-κB pathway, the "interpretation" is that the cell has been irrevocably compromised. Therefore, the conservative response, erring on the side of assuming the worst, is to kill the cell.

The first pathway that evolved to guard RIPK1 was a branch to apoptosis (Lamkanfi et al. 2002; Dondelinger et al. 2016b). The DD of RIPK1 normally recruits it to the DD of TNF receptor 1 or the DD in the adaptor TRADD (Dondelinger et al. 2016a). When RIPK1 modification is perturbed, its DD becomes exposed and is detected by the guard protein Fas-associated death domain (FADD) protein (the caspase-8 adaptor composed of a DD and a DED). FADD binds to RIPK1 via DD–DD interactions, and then recruits caspase-8 to initiate apoptosis. Thus, FADD and caspase-8 are guards for RIPK1 (Fig. 1). This is mimicked in vitro through the addition of IAP antagonists, inhibitors of TAK1, or inhibitors of IKK, which inhibit the TNF transcriptional response and induce apoptosis (Dondelinger et al. 2016a). Although enacting apoptosis and killing a cell is a dramatic response, it allows the host to remove cells that have been irrevocably compromised. Thus, the host prevents the pathogen from hijacking cells and the pathogen is denied the ability to create its preferred environment.

Not only does caspase-8 guard RIPK1, but RIPK1 can conversely guard caspase-8. Many pathogens have evolved virulence factors to inhibit TNF transcriptional signaling, while simultaneously inhibiting caspase-8-mediated apoptosis (Kaiser et al. 2013). In response to

this double attack, the host appears to have evolved another guard pathway attached to RIPK1, monitoring for abnormalities in caspase-8. Now, instead of RIPK1 triggering apoptosis, it instead induces a completely different form of programmed cell death termed necroptosis (Dondelinger et al. 2016a). Evolution selected for the addition of a RHIM domain and a kinase domain to RIPK1. The RIPK1 kinase domain autophosphorylates RIPK1 to promote RHIM–RHIM interactions that recruit RIPK3. This induces RIPK3 oligomerization and autophosphorylation. Then RIPK3 phosphorylates the pseudokinase MLKL, leading to cell lysis. In this pathway, the RHIM and kinase domains of RIPK1 can be considered integrated guard domains (Fig. 1) that signal to the guard protein RIPK3.

In summary, for a pathogen to prevent TNF (or the similarly guarded TLR3 and TLR4)-induced activation of NF-κB while also maintaining cell viability, the pathogen must run through multiple steps of evolution. The pathogen must simultaneously achieve its primary goal of blocking NF-κB, secondarily it must also block apoptosis, and finally necroptosis. This network of pathways and guards probably makes it incredibly difficult for pathogens to readily add NF-κB-inhibiting virulence factors to their repertoire.

EPEC TRIPLE ATTACKS TRANSCRIPTION, APOPTOSIS, AND NECROPTOSIS

Enteropathogenic *E. coli* (EPEC) is a human-specific pathogen that causes diarrhea and lives in close association with host cells (Fig. 4). It uses the locus of enterocyte effacement (LEE) T3SS to reprogram intestinal epithelial cells (IECs), permitting extracellular adherence of EPEC in the intestinal lumen. The translocated effectors cause the IEC microvilli to efface, and then induce the formation of a dense actin network, creating a pedestal on which EPEC closely adheres. The bacterium likely evolved this strategy to gain first access to oxygen and nutrients that diffuse across the IEC, gaining a replication advantage over luminal commensals (Lopez et al. 2016). EPEC must reprogram the IEC, while also preventing the IEC from noticing it is compromised. Despite the fact that the EPEC rod protein is detected by the NAIP/NLRC4 inflammasome, NLRC4 is inefficient at detecting the live bacteria (Miao et al. 2010b). Detection by NLRC4 in IECs would otherwise trigger immediate extrusion (Rauch et al. 2017), which would be devastating to the virulence strategy of EPEC. We therefore speculate that EPEC has an undiscovered strategy to evade NLRC4.

There is mounting evidence that EPEC inhibits the transcriptional responses of the IEC to which it is attached, effectively shutting down the TNF signaling pathway (Pearson and Hartland 2014). EPEC attacks TAB2 and TAB3, which are adaptors for TAK1 downstream from RIPK1. EPEC accomplishes this by injecting the T3SS effector NleE, which methylates cysteines in the zinc finger domains of TAB2 and TAB3, thus inhibiting NF-κB responses (Zhang et al. 2011). EPEC also has two metalloproteases, NleC and NleD, which degrade key proteins in the transcriptional response. NleC degrades NF-κB by cleaving its p65 (RelA) subunit (Yen et al. 2010; Baruch et al. 2011; Mühlen et al. 2011; Pearson et al. 2011). Meanwhile, NleD degrades JNK and p38, which are activators of the AP1 transcription factor (Baruch et al. 2011). Another effector, NleL, also attacks JNK, but by ubiquitinating it, hence blocking JNK phosphorylation and activation (Sheng et al. 2017). Finally, NleH1 and NleH2 inhibit NF-κB-driven gene expression in part by NleH1, preventing IKKβ phosphorylation of RPS3, a specifier subunit of certain NF-κB complexes (Gao et al. 2009; Royan et al. 2010; Wan et al. 2011). The fact that EPEC translocates a plethora of T3SS effectors that redundantly attack NF-κB and AP1 indicate that preventing transcriptional responses is very important to its virulence strategy. However, attacks on these transcriptional signaling pathways theoretically should be detected by their guards.

EPEC must inhibit the caspase-8 apoptosis guard pathway to maintain its adherent niche on the IEC. The T3SS effector NleB attacks DD-containing proteins by adding an *N*-acetylglucosamine (called GlcNAcylation), with a preference for attacking the caspase-8 adaptor FADD

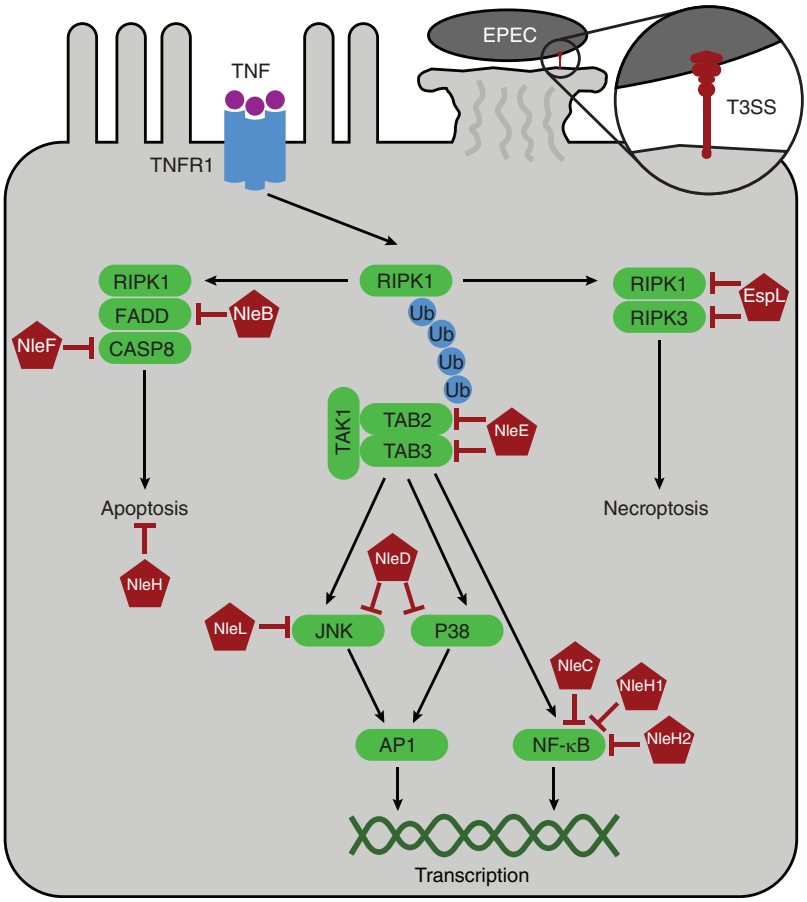

Figure 4. Enteropathogenic *Escherichia coli* (EPEC) virulence factors target pathways for transcription, apoptosis, and necroptosis. Tumor necrosis factor (TNF) receptor signaling can trigger one of three responses. The cell will first try to initiate transcriptional signaling pathways. RIPK1 acts as a scaffold for polyubiquitin chains, on which assemble TAB2 and TAB3 in a complex with TAK1. Downstream from TAK1, JNK and p38 signal to the transcription factor AP1, and other complexes signal to nuclear factor (NF)-κB. Transcription can be blocked by EPEC effectors: NleE inhibits TAB2 and TAB3, NleD obstructs JNK and p38, and NleC and NleH1/2 block NF-κB. When transcription pathways are hindered, guards of RIPK1 initiate apoptosis. RIPK1 death domain (DD) becomes exposed allowing homotypic interactions with the DD of the adaptor FADD, which in turn recruits caspase-8 to initiate apoptosis. EPEC has additional virulence strategies to inhibit the apoptotic guard pathway: NleB and NleF attack FADD and caspase-8, respectively. Finally, the host cell has a third guard pathway in which RIPK1 uses its integrated guard RHIM and kinase domains to activate the guard, RIPK3. RIPK3 signaling then induces necroptosis of the host cell. Similar to transcription and apoptosis, EPEC has a virulence factor, EspL, to inhibit signaling to necroptosis.

(Li et al. 2013; Pearson et al. 2013; Scott et al. 2017). Thus, NleB prevents guard signaling from RIPK1 to caspase-8 and prevents apoptosis. Another effector, NleF, also attacks caspase-8 (as well as some other caspases) by direct binding to inhibit its catalytic activity (Blasche et al. 2013). In addition to attack on caspase-8 apoptotic guard function, EPEC also attacks other aspects of apoptosis—NleH binds Bax inhibitor-1 (BI-1) to prevent cell-intrinsic initiation of apoptosis (Hemrajani et al. 2010).

Lastly, EPEC also attacks the necroptotic guard pathway. EspL is a cysteine protease that cleaves the RHIM domains in RIPK1, RIPK3,

and other RHIM-containing proteins, thereby preventing oligomerization and activation of RIPK3 (Pearson et al. 2017). This should prevent the IEC necroptosis guard pathway from activating in response to simultaneous attacks upon transcriptional signaling and apoptosis. The fact that EPEC delivers multiple effectors to prevent apoptosis and necroptosis illustrates that maintaining the viability of the IEC is important to the virulence strategy of EPEC, allowing it to replicate in its adherent niche.

The compendium of EPEC effectors that attack NF-κB signaling and simultaneously attack the two guard pathways to apoptosis and necroptosis are an excellent illustration of the Red Queen's race between pathogen and host. We expect that other bacterial pathogens that attack NF-κB signaling will similarly need to simultaneously block apoptosis and necroptosis. This should be particularly important for pathogens such as EPEC that replicate in intimate contact with the host cell it has reprogramed. Similarly, intracellular pathogens like *Salmonella* replicate inside a single host cell, and thus must also keep that host cell alive. *S.* Typhimurium attacks NF-κB signaling, which should trigger the apoptotic and necroptotic guard pathways. However, *S.* Typhimurium encodes SseK proteins that are similar to the EPEC apoptosis inhibitor NleB. Although homologs of the EPEC EspL necroptosis inhibitor are not present in the commonly used strains of *S.* Typhimurium, we predict that *S.* Typhimurium inhibits necroptosis by using undiscovered effectors. *Yersinia* species also attack transcriptional responses. For example, YopJ attacks TAK1, but at the cost of also triggering the apoptotic guard functions (Paquette et al. 2012). In contrast to EPEC and *S.* Typhimurium, the host cell does not need to remain viable for *Yersinia* to replicate. Thus, it remains to be determined whether *Yersinia* actually benefits from the apoptotic guard pathway triggered by its inhibition of the transcriptional response. Given that all pathogens are running the Red Queen's race against the host, it seems likely that many other pathogens will have virulence strategies that have equal complexity to those illustrated by the EPEC T3SS effectors.

CONCLUDING REMARKS

Evolution is limited by organism replication rates, thus it is easy to assume multicellular organisms are at a disadvantage as many have life span that are significantly longer than bacteria, which allows them less time to mutate and evolve between generations. The solution to being faced with a pathogen that step-for-step keeps up with the continuing evolution of the innate immune system was to generate an arm of the immune system that can evolve at a rate faster than bacterial evolution. The adaptive immune system accomplishes this feat—B cells and T cells evolve new antibody and T-cell receptors within one week. Ultimately, almost all infections in which pathogens are running the Red Queen's race against innate immunity are cleared by the adaptive immune system.

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