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Effector-Triggered Immunity

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Abstract

The innate immune system detects pathogens via germline-encoded receptors that bind to conserved pathogen ligands called pathogen-associated molecular patterns (PAMPs). Here we consider an additional strategy of pathogen sensing called effector-triggered immunity (ETI). ETI involves detection of pathogen-encoded virulence factors, also called effectors. Pathogens produce effectors to manipulate hosts to create a replicative niche and/or block host immunity. Unlike PAMPs, effectors are often diverse and rapidly evolving and can thus be unsuitable targets for direct detection by germline-encoded receptors. Effectors are instead often sensed indirectly via detection of their virulence activities. ETI is a viable strategy for pathogen sensing and is used across diverse phyla, including plants, but the molecular mechanisms of ETI are complex compared to simple receptor/ligand-based PAMP detection. Here we survey the mechanisms and functions of ETI, with a particular focus on emerging insights from animal studies. We suggest that many examples of ETI may remain to be discovered, hiding in plain sight throughout immunology.

INTRODUCTION

Although immunity is now appreciated to play roles in diverse pathologies—including cancer, metabolic disease, and neurodegeneration—pathogens have been the main force driving the evolution of immunity. The innate immune system is responsible for the initial detection of invading microbes and, as such, is situated on the front lines of the escalating arms race between hosts and their pathogens (1). Considerable efforts over the last two decades have led to a detailed understanding of the innate mechanisms used by hosts to detect pathogens. The overwhelming consensus is that pathogens are detected primarily by germline-encoded pattern recognition receptors (PRRs) that bind directly to conserved microbial molecules, such as lipopolysaccharide or double-stranded RNA (dsRNA), collectively referred to as pathogen-associated molecular patterns (PAMPs) (2) (**Figure 1*a***). Studies of PAMP-triggered immunity (PTI) have been greatly facilitated by the ability to trigger responses by using purified PAMPs. However, pathogens are not merely "bags of PAMPs" (3, p. 12), and many studies of the immune response to living, virulent pathogens indicate that PTI is not the only mechanism of pathogen recognition.

Here we consider a form of immune response widely referred to as effector-triggered immunity (ETI), a term that originated in the plant immune system literature (4, 5) but is increasingly discussed in the context of metazoans (6, 7). We define ETI as an immune reaction induced in response to a virulence-associated activity of a pathogen (**Figure 1*b***; see also the sidebar titled Key Terms and Definitions). The concept of ETI begins with the appreciation that any pathogen—in order to *be* a pathogen—must produce factors with virulence-associated activities that promote

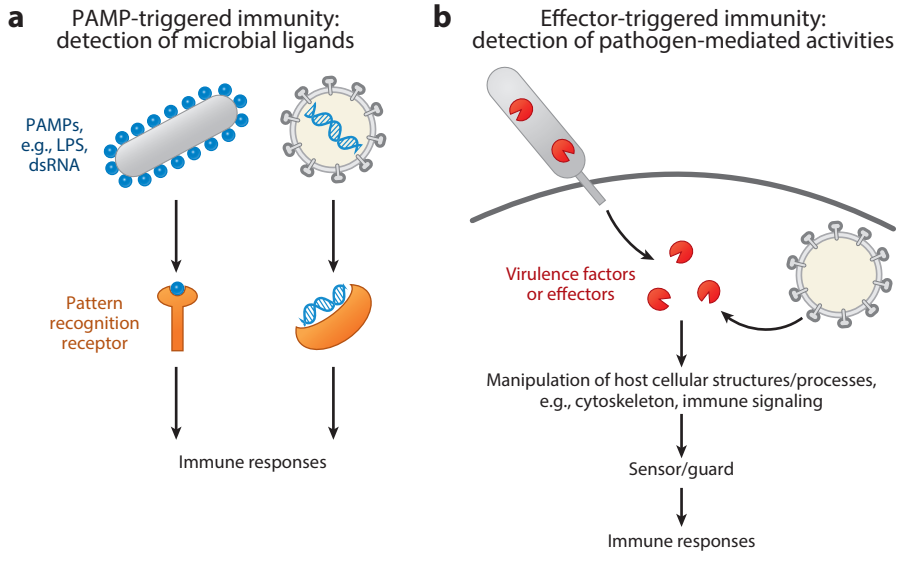


Figure 1

Overview of PAMP-triggered versus effector-triggered immunity. The innate immune system uses multiple strategies of pathogen sensing. (*a*) PAMP-triggered immunity involves detection of conserved pathogen molecules (PAMPs) by germline-encoded receptors. (*b*) Effector-triggered immunity involves sensing of pathogen-mediated activities. During infection, pathogens secrete or express proteins (referred to as virulence factors or effectors) in host cells that manipulate host cellular processes to promote pathogen replication or transmission. Often virulence factors from diverse pathogens target conserved host processes, structures, or organelles, such as the cytoskeleton or immune signaling pathways. These disruptions can be sensed in various ways by the host, leading to activation of immune responses. Abbreviations: dsRNA, double-stranded RNA; LPS, lipopolysaccharide; PAMP, pathogen-associated molecular pattern.

KEY TERMS AND DEFINITIONS

Pathogen: any microbe that can cause disease in a host by virtue of its disruption, manipulation, or evasion of host immune or barrier defenses. Some microbes, e.g., laboratory *E. coli*, can elicit host responses that can result in disease (e.g., lipopolysaccharide-induced toxic shock after intravenous injection), but we do not consider such microbes as pathogens because they do not encode virulence activities that promote their replication or transmission. Note that whether a microbe is a pathogen is by this definition relative to a host and is not an intrinsic property of the microbe; i.e., a microbe may be a pathogen in one host but not another

Virulence-associated activity: any pathogen-mediated activity that promotes pathogen replication or transmission, for example by disrupting, manipulating, or permitting evasion of a host immune or barrier defense

Effector: traditionally refers to a microbial molecule, usually an enzyme, that is delivered into host cells via a specialized secretion system (e.g., a type III or type IV secretion system) in order to manipulate the host cell. Here we adopt a broader definition of effector as any pathogen-encoded molecule that promotes pathogen replication or transmission by virtue of its activity in a host

Effector-triggered immunity (ETI): an immune response induced in response to a virulence-associated activity of a pathogen (i.e., in response to an effector, broadly defined)

Pathogen-associated molecular pattern (PAMP): a microbial molecule that is structurally conserved among many pathogens and that elicits a host immune response when bound by a germline-encoded host receptor. PAMPs are expressed by nonpathogenic microbes in addition to pathogens. Examples include lipopolysaccharide and flagellin. Most PAMPs are microbe specific and are not produced by hosts, though this is not always the case (e.g., DNA and RNA can be PAMPs)

PAMP-triggered immunity (PTI): an immune response induced by a PAMP

Danger (or damage)-associated molecular pattern (DAMP): a host-derived molecule that elicits host immune responses after release from a damaged or dying host cell, as can occur during sterile injury as well as pathogen infection. Examples include IL-1α, ATP, and DNA. Confusingly, PAMPs or effectors are sometimes referred to as danger signals, but here we avoid conflating these distinct concepts

Danger (or damage)-triggered immunity (DTI): an immune response elicited by a DAMP

Guard: a host protein that directly or indirectly monitors the functional or physical integrity of another (guarded) host protein (or protein domain) and initiates an immune response when this protein (domain) is modified or disrupted, for example, by the virulence activity of a pathogen. In some cases, the guarded protein has intrinsic anti-pathogen activity and is the intended target of disruption, in which case it is referred to as a guardee. In other cases, the guarded protein has no anti-pathogen function but structurally mimics a guardee. In this case, the guarded protein is not the intended target of disruption and is referred to as a decoy

Guardee: A host protein that has an intrinsic anti-pathogen function and that is therefore attacked by a pathogen effector, leading directly or indirectly to activation of the guard

Decoy: a host protein that is inadvertently attacked or disrupted by a pathogen, leading to activation of a guard. A decoy does not have intrinsic anti-pathogen activity but instead structurally mimics a host protein that has intrinsic anti-pathogen activity in order to elicit pathogen attack. Decoys thus function as sensors of virulence-associated activities

Integrated decoy: a decoy that is not a free-standing protein but is instead a protein domain inserted into another protein. Typically the decoy is integrated within the guard and functions as a sensing domain that detects pathogen effectors

Guard immunity: an immune response elicited when a host protein (guardee or integrated decoy) is modified or disrupted by an effector, leading to activation of a guard

Pattern of pathogenesis: a pathogen activity that promotes pathogen replication and that is conserved across multiple pathogens. Examples include cytosolic invasion or disruption of the host cytoskeleton

pathogen replication or transmission. Although the term effector traditionally refers specifically to bacterial proteins secreted into host cells via dedicated secretion systems, and thus excludes classical toxins or viral immune evasins, for ease of discussion we use effector here as an umbrella term for any pathogen factor that disrupts, manipulates, or permits evasion of a host immune or barrier defense. Unlike PTI, which can be elicited in response to harmless and commensal microbes, ETI is a specific response to pathogens, as only pathogens encode effectors. As such, ETI permits hosts to distinguish pathogenic and nonpathogenic microbes and thereby scale the magnitude and type of immune responses to be commensurate with the threat (3).

We distinguish ETI from the often conflated concept of danger- or damage-triggered immunity (DTI) (8; see also the sidebar titled Key Terms and Definitions). The main distinction is that ETI is a response to a pathogen activity or effector whereas the danger-associated molecular patterns (DAMPs) that elicit DTI are endogenous host molecules—e.g., ATP, uric acid crystals (9, 10), DNA, or IL-1α—that are released in response to tissue or cellular stress or damage (8). As such, DTI can help account for sterile-injury-induced inflammatory responses. At the same time, cell death induced by pathogens is usually the result of a host-dependent ETI response (a cell pyroptotic, apoptotic, or necroptotic "suicide") rather than directly executed by the pathogen (a "murder"). Confusion often arises because ETI-induced cell death may then result in the release of DAMPs, which may then amplify responses, even though the primary response is due to ETI. Additional confusion arises because some innate sensors, most notably NLRP3 (nucleotide-binding domain, leucine-rich repeat, and pyrin domain–containing protein 3), respond to both pathogen effectors (e.g., pore-forming toxins) and DAMPs (e.g., extracellular ATP). There is certainly a gray area between ETI and DTI in cases when pathogen replication results in cellular stress, which may result in apoptosis and release of DAMPs. In general, though, if the cellular stress can be ascribed to the activity of pathogen effectors, then we would consider the ensuing response to be primarily ETI.

Importantly, pathogen effectors tend to be diverse and rapidly evolving, making it difficult to detect them directly as ligands (PAMPs) by germline-encoded host receptors. Instead, what appear to be conserved across effectors are the host processes they target. Pathogen effectors tend to target host signaling hubs or structures that serve as barriers to pathogen replication, including the actin cytoskeleton, the secretory pathway, autophagy, inflammatory signaling pathways (e.g., NF-κB), and protein synthesis (11–13). By interfering with these common host pathways, pathogens display "patterns of pathogenesis" (3). Thus, ETI pathways focus on detecting these patterns of pathogenesis as a means to sense diverse pathogens with a limited repertoire of germline-encoded sensors. This strategy is analogous to the strategy of detecting broadly conserved PAMPs by PRRs. Indeed, one of the main virtues of PTI is that it can explain how relatively few germline-encoded receptors are able to recognize a diverse universe of pathogens, including new pathogens that were never previously encountered during evolution. Indeed, no pathogen has been described that can escape entirely from PTI. This is because the PAMPs that elicit PTI are generally highly conserved, thereby representing a microbial signature or pattern (14). We propose that ETI may possess a similar virtue.

It is important to emphasize that PTI and ETI are not mutually exclusive mechanisms of pathogen detection, and indeed, many pathogens are sensed via both mechanisms. In addition, in both plants and animals, there is considerable cross talk between PTI and ETI pathways (15–17). For example, some ETI pathways require a priming signal that originates in a PTI response. Conversely, ETI often results in upregulation or activation of canonical PTI signaling pathways. The collaboration between PTI and ETI makes sense, since fundamentally the two strategies provide distinct information to the immune system: PTI indicates the presence of a microbe, and ETI indicates that the microbe is a pathogen.

COMPLEXITIES OF EFFECTOR-TRIGGERED IMMUNITY

Investigations of ETI face several major experimental and conceptual complexities. Unlike PTI, which invariably works via a simple receptor-ligand mechanism (**Figure 1*a***), the molecular mechanisms of ETI are often extremely complex and vary substantially (**Figure 2**). In some cases, a pathogen effector modifies a host target, and this modification is detected (**Figure 2*a***). In other cases, the pathogen-induced modification results in destabilization, loss, or inhibition of a host target. In this scenario, the host target has evolved to constitutively inhibit immune responses, and thus the pathogen-induced loss or absence of this target (missing self) unleashes an immune response (**Figure 2*b***). The effector-modified or -destabilized host protein is sometimes metaphorically termed a guardee, and its modification or loss is then described as being sensed by a guard (see the sidebar titled Key Terms and Definitions). In another variant of ETI, pathogen activities can also incur host cell stress, or disrupted homeostasis (18), and this is what is detected (**Figure 2*c***). Each of these core mechanisms is elaborated in nearly infinite and sometimes baroque variations, as discussed below. The simplest version of ETI might be a direct mechanism, in which a pathogen effector is recognized as a ligand by a host receptor (**Figure 2*d***). As discussed above this situation rarely arises, due to the lack of structural conservation among effectors, though there are some examples in plants (19–22). However, even when direct effector recognition does occur, we might question whether such recognition truly falls within the definition of ETI versus PTI, especially if it is the conserved PAMP-like structure of the effector, rather than its activity, that is detected.

An additional challenge for experimental investigations of ETI is its evolutionary complexity, which arises because of ongoing and escalating arms races between hosts and pathogens, resulting in layers of pathogen attack and host counterresponse. For example, a pathogen may evolve to inhibit a host PTI pathway. In response, the host may evolve an ETI response to detect disruption of the PTI pathway. The pathogen may then evolve to inhibit the ETI response, which then imposes selective pressure on the host to evolve a secondary ETI pathway to detect disruption to the first. Alternatively, if the ETI response is effective, the pathogen (or its initial virulence strategy) may go extinct. This latter possibility poses a particular challenge to studies of ETI since it may make it difficult to find a modern example of a pathogen that triggers a given extant ETI pathway.

Lastly, when considering ETI as a host pathway that senses infection and promotes immunity, care must be taken not to confuse ETI—which is a response that evolved to protect the host—with instances in which a pathogen has evolved to stimulate inflammation for its benefit. Pathogens often compete with commensal microbes within hosts, and a strategy to do so is to induce inflammation (23). Pathogens are typically better equipped to deal with inflammation compared to commensals and thus benefit from activating the immune system. Pathogen transmission may also be promoted by triggering host responses such as diarrhea or coughing. To this end, pathogens sometimes encode proinflammatory cytokines, chemokines, or effectors to promote their replication or transmission (24, 25). While induction of inflammation by these factors may resemble ETI at first glance, the response may not promote immunity but may instead exacerbate pathology and potentially pathogen replication/spread.

ORIGINS AND BREADTH OF EFFECTOR-TRIGGERED IMMUNITY

The most in-depth description of ETI has arisen from studies of plant immunity. Pioneering work by plant breeders identified resistance (*R*) loci that confer resistance to specific pathogens, typically by initiation of a localized cell death (hypersensitive) response at the site of infection. We now recognize that most *R* genes encode nucleotide-binding leucine-rich repeat–containing (NLR) proteins (26). As is discussed below, many vertebrate ETI sensors are also NLRs, though this similarity is apparently the result of convergent evolution rather than a common ancestral

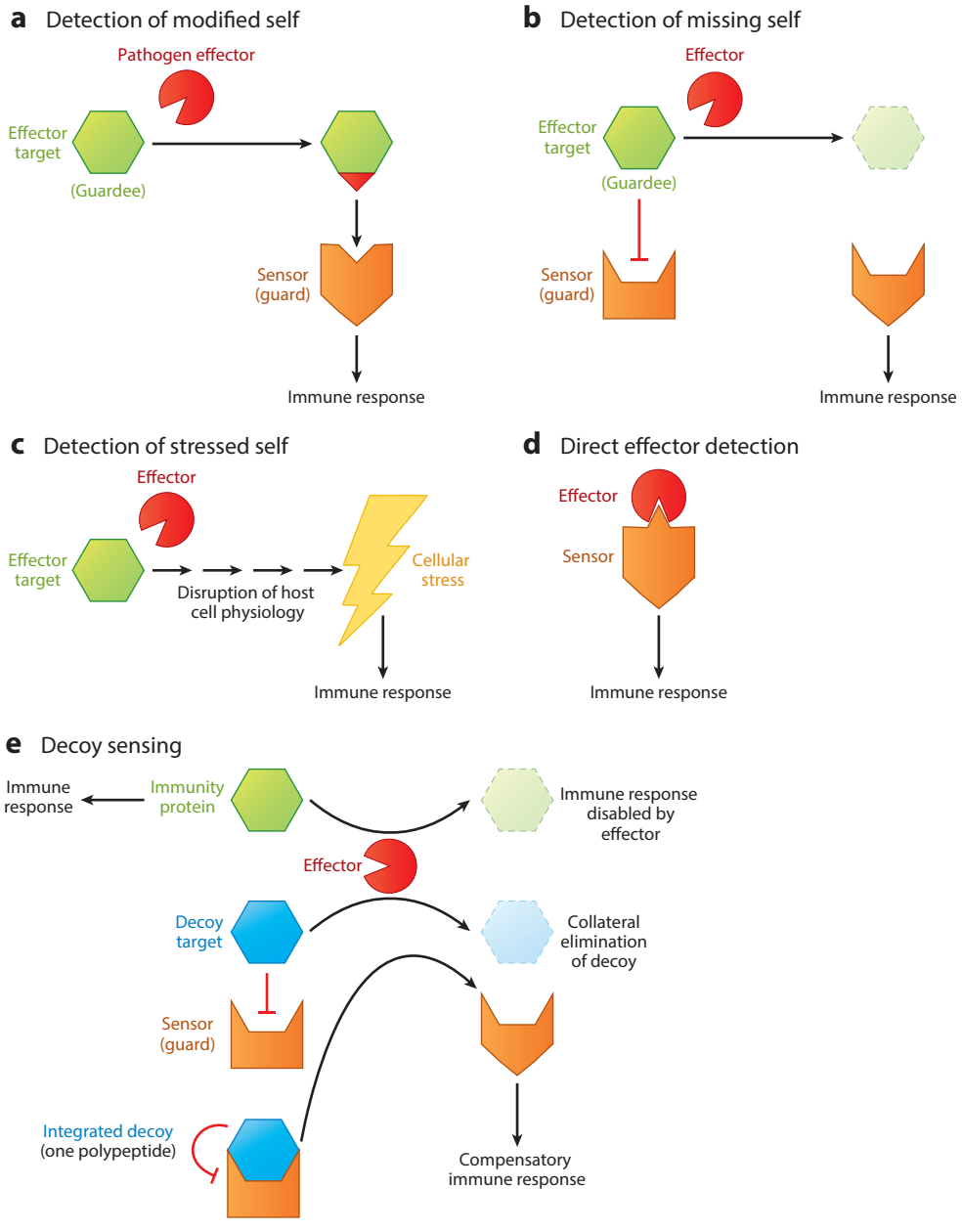


Figure 2

Diverse mechanisms of effector-triggered immunity. Pathogen activities are sensed through various mechanisms. (*a*) A pathogen effector modifies a target protein, or guardee. This modification is detected by a sensor, or guard, leading to induction of an immune response. (*b*) Pathogen activity causes destabilization or loss of a target protein that inhibits immune responses. Pathogen-induced loss of this target unleashes an immune response. (*c*) A pathogen effector induces cell stress, which leads to immune activation. (*d*) A pathogen effector is directly recognized by a host sensor. (*e*) A sensor is inhibited by a decoy, which is a mimic of an immunity protein involved in host defense. A pathogen effector that evolved to inhibit the immunity protein also inadvertently disrupts the decoy, unleashing an immune response. Decoy domain(s) can be integrated within the sensor.

origin (26–28). Interestingly, in plants, the resistance response was found to be elicited only by pathogens carrying so-called "avirulence" proteins, which are now appreciated to be pathogen effectors (that function primarily to promote pathogen virulence) (4, 5, 29). The avirulent phenotype is a consequence of a secondary ETI response elicited by the effector. Originally, each R protein was believed to detect a specific avirulence protein in a gene-for-gene relationship (30), perhaps via direct binding. It is now clear that a single NLR can detect multiple effectors, and in addition, most effectors are not detected as ligands. Instead, NLRs often function as guards (see the sidebar titled Key Terms and Definitions) that monitor the integrity of guardee virulence targets (or their decoys; see below) (29). Disruption or modification of a guardee by a pathogen effector results in guard activation. The guardees are typically proteins involved in host defense, explaining why they would be targeted by pathogen effectors. Most plant species typically express many different NLRs that sense effectors by an impressive array of distinct molecular mechanisms (26, 31, 32). The first example discovered was the RPS2 sensor in *Arabidopsis*, an NLR that senses the elimination of RIN4 by various pathogen effectors (33, 34). An additional example in *Arabidopsis* is an NLR called RPS5 that guards a kinase called PBS1 (35). PBS1 is a member of a family of kinases involved in PAMP-triggered immune signaling. A protease effector called AvrPphB from *Pseudomonas syringae* cleaves PBS1, resulting in a conformational change in PBS1 that is sensed by RPS5, leading to its activation.

In some cases, the guarded targets of plant pathogen effectors are proteins that do not appear to have a primary anti-pathogen function in host defense. It was thus unclear why they would be attacked by pathogen effectors. It now appears that many guarded proteins may actually be decoys (**Figure 2*e***; see also the sidebar titled Key Terms and Definitions), that is to say, mimics of the intended targets of pathogen effectors (36). Unlike true anti-pathogen proteins, which are under evolutionary pressure to evade targeting by effectors, decoys are free to evolve to maximize their susceptibility to effector attack, thereby improving the sensitivity of the guard to the presence of an effector (or even multiple effectors). On the other hand, the decoy strategy is limited by the possibility that pathogens could evolve effectors that avoid decoys and selectively attack their intended targets. The decoy model can be further elaborated by integration of decoys as domains within the sensors (**Figure 2*e***). The integrated decoy model (37) was originally shown for the *Arabidopsis* R protein RRS1, which contains an integrated WRKY domain (38, 39). The WRKY domain is most commonly found in transcription factors that orchestrate immune defense responses. As such, several pathogen effectors have evolved to modify (e.g., acetylate) and inactivate WRKY domain proteins. Although the WRKY domain in RRS1 does not itself appear to orchestrate transcriptional responses to infection, it is nevertheless (inadvertently) attacked by pathogen effectors. Effector-mediated modification of the RRS1 WRKY domain was shown to lead to RRS1 activation and defense responses. Genomic surveys have shown that diverse integrated decoy domains are widespread in plants (40, 41). The NLRP1 inflammasome may be an example of a mammalian immune sensor with an integrated decoy domain (discussed below in the section titled Effector-Triggered Responses by Inflammasomes).

Work in invertebrate animal species has also provided a foundation for understanding ETI. For example, it was shown that the fly *Drosophila melanogaster* mounts an antimicrobial immune response upon exposure to an *Escherichia coli* toxin called cytotoxic necrotizing factor 1 (CNF1) (42). CNF1 is sensed indirectly, as a result of the ability of CNF1 to enzymatically activate the host Rho GTPase Rac2. Active Rac2 engages the IMD signaling pathway, leading to transcription of antimicrobial peptide genes. Interestingly, the nematode *Caenorhabditis elegans* encodes few if any PTI pathways and instead relies primarily on various stress-responsive pathways for host defense, including pathways that respond to perturbations by specific bacterial toxins (43–47). For example, *Pseudomonas aeruginosa* exotoxin A blocks host protein synthesis in the worm intestine and thereby

elicits a compensatory host defense transcriptional response (44, 45). An analogous response to pathogen-induced inhibition of protein synthesis was previously also observed in mammals (48) (discussed below in the section titled Effector-Triggered Immunity Induced by Protein Synthesis Blockade).

Taken together, studies in diverse species support the idea that ETI is a trans-kingdom strategy for pathogen detection that complements PAMP-triggered immunity in most if not all domains of life. Indeed, recent evidence suggests guard-type mechanisms are also employed by bacteria to sense the activity of phage effectors that attack RecBCD, a core antiphage system (49). In light of the importance of ETI for non-mammalian immunity, we consider it surprising how few mammalian ETI pathways have been described. This might be partially explained by the importance of PTI and adaptive immunity for mammalian immunity, as well as by the challenges of studying ETI. We speculate, however, that another reason for the relative sparsity of known examples of mammalian ETI is that this important concept is not yet fully appreciated in the field of mammalian innate immunity. Thus, having acknowledged the breadth of the ETI strategy, we review below known examples of ETI responses in mammals. We propose that once the ETI concept is incorporated into our thinking, additional examples of ETI may be found throughout immunology.

EFFECTOR-TRIGGERED IMMUNITY BY NATURAL KILLER CELLS

Prior to the modern emergence of the PAMP model for pathogen sensing (2), evidence in numerous experimental systems suggested that mammalian responses to pathogens might also be initiated in response to pathogen-mediated activities. One early line of work concerned the mechanisms by which natural killer (NK) cells are activated to kill target cells. Kärre and colleagues (50) formulated a missing-self model in which NK cells are constitutively inhibited by target cell expression of MHC-I proteins. Loss of self-MHC-I by the target cell thereby unleashes killing of the target by NK cells. Although tumors and transplants were the main experimental systems for studies of NK activation, it is clear that pathogens also inhibit MHC-I expression to escape recognition by cytotoxic $CD8^+$ T cells (51). NK cells can thus be viewed as guards of MHC-I-mediated antigen presentation, a model that fits conceptually within the missing-self mode of ETI (**Figure 2*b***). Subsequent work on NK cells has identified families of MHC-specific inhibitory receptors (52, 53) that play important roles in control of viruses that attempt to evade $CD8^+$ T cells by interfering with MHC-I expression (54–56). NK cells also respond to stressed self (**Figure 2*c***), particularly via the NKG2D activating receptor (57). Viral replication often results in activation of cell stress pathways that result in upregulation of NKG2D ligands and, consequently, NK cell activation (58). The molecular connections between specific viral effectors and NKG2D ligand upregulation are generally poorly understood. However, one clear example is provided by mouse cytomegalovirus, which encodes an effector called m18 that interferes with histone deacetylation in host cells. Histone deacetylation in turn leads to upregulation of an NKG2D ligand, eliciting NK cell attack (59).

CYTOSOLIC INVASION AS A COMMON VIRULENCE ACTIVITY OF PATHOGENS

A major characteristic that distinguishes pathogens from nonpathogens is the ability to invade or otherwise access the host cytosol (3). Pathogens access the host cytosol through various mechanisms, including direct invasion, secretion of toxins, and injection of virulence factors via secretion systems. Invasion of the host cell cytosol is a critical virulence activity for most if not all pathogens. For example, intracellular bacteria and viruses require invasion of host cells to

replicate. Additionally, many bacterial pathogens that rely on secretion systems to inject virulence factors into host cells are rendered avirulent by genetic deletion of their secretion systems.

Though often critical for virulence, cytosolic access can also inadvertently result in the introduction of PAMPs into host cells, which can then be detected by various cytosolic immune sensors. For example, the type III secretion system (T3SS) used by many gram-negative bacteria inadvertently delivers flagellin, the structural component of the bacterial flagellum, into host cells (60). Flagellin, as well as the needle and inner rod structural components of the T3SS, is directly sensed by host proteins called NAIPs in the cytosol (61). Upon ligand binding, NAIPs recruit NLRC4 to assemble an active inflammasome, a multiprotein signaling complex that initiates downstream immune responses. This mechanism of pathogen-sensing is a canonical example of PTI, as a cognate PAMP is detected by a germline-encoded receptor. Importantly, however, the NAIP–NLRC4 inflammasome is activated only when flagellin and the T3SS components have gained access to the host cell cytosol. As such, while NAIP–NLRC4 directly senses PAMPs, NAIP–NLRC4 may also be considered to indirectly sense a pathogen-associated effector activity, specifically the cytosol-invasive activity of pathogen secretion systems. The detection of flagellin in the cytosol as opposed to the extracellular environment appears to be an important distinction for the host, as it results in a very different immune response: Sensing of extracellular flagellin by TLR5 (Toll-like receptor 5) initiates a transient transcriptional response, whereas detection of cytosolic flagellin by NAIP–NLRC4 triggers irreversible pyroptotic cell death. By distinguishing extracellular from cytosolic flagellin, hosts distinguish commensal versus pathogen sources of that flagellin and thereby initiate commensurate immune responses.

Other cytosolic sensors that are classically viewed as PRRs are also critical for detecting cytosolic invasion. For example, AIM2 and cGAS bind double-stranded DNA; RIG-I-like receptors, OAS, and PKR bind dsRNA; and caspases-4, -5, and -11 bind lipopolysaccharide. These and other cytosolic PRRs signal to the host that microbial molecules have gained access to the cytosol and thus can be viewed as contributing to ETI. However, since these receptors directly bind PAMPs, we do not discuss them in depth in this review.

NOD1 AND NOD2 AS SENSORS OF EFFECTOR-TRIGGERED IMMUNITY

NOD1 and NOD2 are NLR proteins that detect distinct fragments of the bacterial cell wall component peptidoglycan. Specifically, NOD1 recognizes γ-D-glutamyl-*meso*-diaminopimelic acid (iE-DAP), and NOD2 senses phosphorylated muramyl dipeptides (62–66). Upon activation, NOD1 and NOD2 assemble with receptor-interacting serine/threonine protein kinase 2 (RIPK2) to activate NF-κB and MAPK signaling pathways, leading to transcription of proinflammatory genes.

In addition to directly sensing peptidoglycan, several studies have reported that NOD1/2 also detect the activities of virulence factors that activate small Rho GTPases (67–69). Some intracellular bacterial pathogens, including *Shigella flexneri* and *Salmonella enterica* serovar Typhimurium, require rearrangement of the host actin cytoskeleton to invade epithelial cells (70). Bacteria modulate cytoskeletal dynamics by secreting effectors into host cells, many of which activate small Rho GTPases (71). For example, the *Salmonella* effector SopE is a guanine nucleotide exchange factor that activates the Rho GTPases Cdc42 and Rac1 (72). Ectopic expression of SopE activates NOD1, leading to RIPK2-dependent induction of NF-κB signaling (69). Similarly, the *S. flexneri* virulence factors IpgB2 and OspB activate NF-κB signaling in a NOD1-dependent manner that also requires the RhoA guanine nucleotide exchange factor GEF-H1 (68). Additional reports support the idea that activation of NOD1/2 signaling is closely linked to actin cytoskeletal dynamics (73, 74). Together these findings suggest that NOD1/2 guard the activity of Rho GTPases and

induce NF-κB signaling upon perturbation of the actin cytoskeleton by virulence factors. However, other reports demonstrate that Rho GTPase-activating effectors induce NF-κB signaling independently of RIPK2, suggesting that NOD1/2 are not required for sensing of these effectors (75, 76). Using genetic approaches, Sun et al. (76) demonstrated that NF-κB induction by the *Salmonella* effector SopE requires the Rho GTPase Cdc42, PAK1, TRAF6, and TAK1 but does not require RIPK2. Thus, while the underlying mechanism remains to be fully elucidated, detection of Rho GTPase activation appears to be a mechanism of pathogen sensing that leads to NF-κB-mediated proinflammatory cytokine production.

NOD1/2 have also been implicated in sensing endoplasmic reticulum (ER) stress (77, 78). Treatment of cells with the ER stress inducer thapsigargin induces NF-κB signaling by activating NOD1/2 (77). Additionally, an ER stress–inducing virulence factor encoded by *Brucella abortus*, VceC, activates NOD1/2- and RIPK2-dependent NF-κB signaling, which can be blocked by an ER stress inhibitor (77). These findings indicate that NOD1/2 may also guard ER homoeostasis. However, it has also been suggested that thapsigargin may activate NOD1/2 signaling not by inducing ER stress but rather by stimulating Ca^{2+}-dependent endocytosis of trace amounts of peptidoglycan present in serum (79). Therefore, cellular perturbations that induce endocytosis may cause internalization of peptidoglycan fragments that activate NOD1/2. In general, the molecular mechanisms of NOD1/2 activation remain poorly understood, and further work is necessary to reconcile the possible functions of NOD1/2 in detection of peptidoglycan-derived PAMPs and pathogen effectors.

EFFECTOR-TRIGGERED RESPONSES BY INFLAMMASOMES

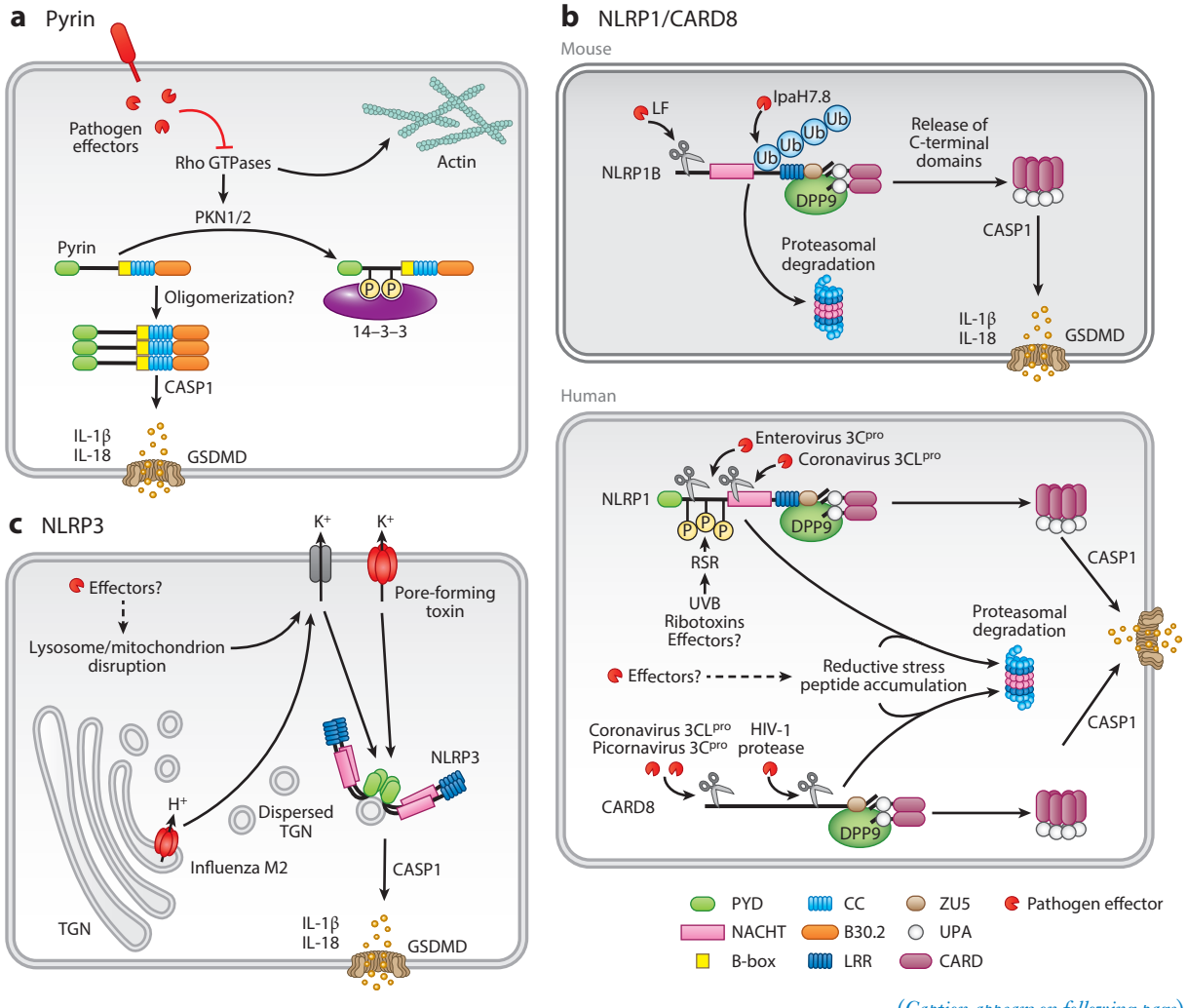
Inflammasomes are cytosolic innate immune complexes that detect various pathogen-derived ligands and activities (80, 81). Upon activation, canonical inflammasome sensors assemble into multiprotein complexes that recruit and activate the caspase-1 protease. Activated caspase-1 cleaves and activates IL-1β and IL-18 cytokines and the pore-forming protein gasdermin D (GSDMD), leading to cytokine release and an inflammatory form of cell death termed pyroptosis. Inflammasome activation provides host defense against a number of pathogens by mediating proinflammatory cytokine release and by eliminating the pathogen's replicative niche.

Pyrin

Pyrin is a non-NLR protein that forms inflammasomes and provides perhaps the best mammalian analog of the guard-type immunity in plants. In contrast to NOD1/2 detection of Rho activation discussed above, pyrin indirectly detects inactivation of the small GTPase RhoA (82) (**Figure 3*a***). Pathogens block Rho GTPases to manipulate host cytoskeletal dynamics and hinder phagocytosis. Rho-inactivating effectors include *Clostridium difficile* TcdB, *Clostridium botulinum* C3, *Vibrio parahaemolyticus* VopS, *Histophilus somni* IbpA, and *Yersinia* spp. YopE and YopT (82–84). These effectors inactivate RhoA through diverse mechanisms including glucosylation, adenylation, ADP-ribosylation, and proteolysis. Pyrin thus does not appear to directly recognize pathogen effectors or their modifications of RhoA but rather senses RhoA inactivation by monitoring the activity of downstream protein kinase N1 (PKN1) and PKN2 (85, 86). In homeostatic conditions, RhoA activates PKN1/2, which directly phosphorylate pyrin. Phosphorylated pyrin is kept in an inactive state by an interaction with 14–3–3 chaperone proteins. However, upon RhoA inactivation, PKN1/2 no longer phosphorylate pyrin, which results in release of pyrin from inhibitory 14–3–3 proteins and formation of the active inflammasome. Therefore, the pyrin inflammasome functions as a guard of RhoA activity.

A notable example of a host-pathogen evolutionary arms race has been described for the pyrin inflammasome and *Yersinia*. *Yersinia* species are extracellular bacterial pathogens that deliver

virulence factors into host cells by a T3SS. Two T3SS effectors, YopE and YopT, inactivate Rho GTPases to block phagocytosis. To prevent consequent activation of the pyrin inflammasome, *Yersinia* secretes an additional effector, YopM, which directly recruits and activates PKN1/2 to drive pyrin phosphorylation even in the absence of RhoA activity (87). A YopM-deficient strain of *Yersinia* is severely attenuated in mice, and this attenuation is fully reversed in pyrin knockout mice (87). These findings indicate that pyrin sensing of YopE/T benefits the host during *Yersinia* infection and suggest that hosts may be under evolutionary pressure to evade YopM. In fact, population genetic analyses support positive evolutionary selection of hyperactive pyrin variants that circumvent YopM (88). These pyrin variants are responsible for the autoinflammatory disease familial Mediterranean fever (FMF). However, despite the fitness costs of FMF, the carrier frequency of several FMF pyrin mutations in populations of Mediterranean origin is high. It is likely that these mutations were positively selected because they conferred an advantage against *Yersinia pestis*, the causative agent of bubonic plague. This example demonstrates the importance of ETI as a driving force in host-pathogen evolutionary conflicts. It also illustrates the challenges



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Effector-triggered responses by inflammasomes. Inflammasomes sense pathogen-mediated activities. Upon activation, inflammasomes assemble into multiprotein complexes that recruit and activate caspase-1. Activated caspase-1 cleaves and activates IL-1β and IL-18 cytokines and GSDMD, leading to cytokine release and pyroptosis. (*a*) The pyrin inflammasome indirectly detects inactivation of the small GTPase RhoA. Pathogen effectors inhibit Rho GTPases to manipulate the actin cytoskeleton and block phagocytosis. Pyrin senses RhoA inactivation by monitoring the downstream kinases PKN1 and PKN2. In resting cells, RhoA activates PKN1/2, which phosphorylate pyrin. Phosphorylated pyrin interacts with 14–3–3 chaperone proteins, which block inflammasome formation. Upon RhoA inactivation, PKN1/2 no longer phosphorylate pyrin, which results in release of pyrin from 14–3–3 and formation of the active inflammasome. (*b*) NLRP1 and CARD8 sense diverse pathogen activities. NLRP1 and CARD8 are activated when pathogen effectors induce the degradation of their N-terminal domains, resulting in release of the bioactive C-terminal fragments that seed inflammasome assembly. Mouse NLRP1B senses proteolysis by *Bacillus anthracis* LF and ubiquitylation by *Shigella flexneri* IpaH7.8. Human NLRP1 is cleaved and activated by enterovirus 3C proteases and coronavirus 3CL proteases. CARD8 is cleaved and activated by the HIV-1 protease, picornavirus 3C proteases, and coronavirus 3CL proteases. Other signals (i.e., DPP8/9 inhibition, reductive stress, peptide accumulation, ribotoxic stress) promote NLRP1 and/or CARD8 activation, but virulence factors that activate inflammasomes by inducing these signals have yet to be identified. (*c*) NLRP3 is a sensor of membrane integrity and cellular viability. Virulence factors that form pores in the plasma membrane cause potassium efflux and thus induce NLRP3 activation. The influenza M2 proton channel, which neutralizes the pH of the TGN, also activates the NLRP3 inflammasome by a mechanism that involves potassium efflux. Abbreviations: CARD8, caspase activation and recruitment domain–containing protein 8; CASP1, caspase-1; CC, coiled-coil; DPP8/9, dipeptidyl peptidase 8/9; GSDMD, gasdermin D; LF, lethal factor; LRR, leucine-rich repeat; NLRP1, nucleotide-binding domain, LRR, and pyrin domain–containing protein 1; PKN1/2, protein kinase N1/2; PYD, pyrin domain; RSR, ribotoxic stress response; TGN, trans-Golgi network; UPA, UNC5-PIDD-ankyrin; ZU5, zona occludens 1, unc5-like domain.

of studying ETI, since discovery of YopE/T-induced pyrin activation required identification and deletion of the pyrin inhibitor YopM.

NLRP1

NLRP1 is an inflammasome-forming NLR that responds to pathogen-mediated activities. Recent studies have proposed a functional degradation mechanism of NLRP1 activation (89, 90) (**Figure 3*b***). Key to this mechanism is a C-terminal function-to-find domain (FIIND) that is followed by a caspase activation and recruitment domain (CARD). The FIIND undergoes spontaneous self-cleavage between its ZU5 and UPA subdomains, resulting in N- and C-polypeptides that remain noncovalently associated in resting cells. NLRP1-activating stimuli induce destabilization and proteasome-mediated degradation of the N-terminal NLRP1 domains, resulting in release of the bioactive C-terminal UPA-CARD fragment, which seeds inflammasome assembly.

Diverse pathogen-encoded enzymatic activities are sensed by the NLRP1 inflammasome. Lethal factor (LF), a protease secreted by *Bacillus anthracis*, directly cleaves murine NLRP1B (91–93). Cleavage by LF generates a neo–N terminus that is recognized by the N-end rule proteasomal degradation pathway (90). Functional degradation of the N-terminal domains of NLRP1B liberates the C-terminal fragment, leading to inflammasome activation (89, 90). Human NLRP1 is also activated by direct cleavage by pathogen-encoded proteases. Several enterovirus 3C proteases and coronavirus 3CL proteases cleave human NLRP1, resulting in inflammasome activation via the functional degradation pathway (94–96). Furthermore, NLRP1 can detect nonprotease virulence factors as well. An E3 ubiquitin ligase encoded by *S. flexneri*, IpaH7.8, directly ubiquitylates mouse NLRP1B, resulting in proteasome-mediated functional degradation and inflammasome activation (89). The intracellular parasite *Toxoplasma gondii* also activates NLRP1 by an unknown mechanism (97, 98). Overall, NLRP1 appears to be a sensor of diverse enzymatic activities that converge on the proteasomal degradation of its N-terminal domains.

Virulence factors that are sensed by NLRP1 have various primary functions that promote pathogen replication. For example, enterovirus 3C proteases are essential for processing the viral polyprotein into mature proteins. 3C proteases cleave the polyprotein at multiple sites in a sequence-specific manner and are therefore under evolutionary constraint. Likewise, the

pathogen-beneficial function of LF is to cleave and inactivate MAPK kinases, which otherwise promote immune activation. IpaH7.8 degrades gasdermin proteins (99, 100). Therefore, NLRP1 detects pathogen enzymes by harboring integrated decoy target motifs (**Figure 2*e***) that mimic the intended enzyme substrates. NLRP1 appears to be evolving under positive selection to acquire such motifs (95, 101). Interestingly, human NLRP1 contains an N-terminal pyrin domain (PYD), which in other NLRP proteins is an essential domain to recruit the ASC adaptor and initiate downstream signaling. However, as described above, NLRP1 instead uses its C-terminal CARD to activate signaling. While there is no pathogen effector known that recognizes the NLRP1-PYD, a tempting hypothesis is that this domain is an integrated decoy that evolved to sense a pathogen (that may now be extinct) that targets PYDs to block inflammasome activation (102).

In addition to directly sensing pathogen effectors, NLRP1 may also function to guard certain host cellular factors and processes. The NLRP1 inflammasome is activated by inhibitors of the serine dipeptidyl peptidases DPP8 and DPP9 (103). DPP8/9 cleave peptides containing a proline in the P2 position, generating XP peptides (where X is any amino acid) as a by-product. DPP9 restrains NLRP1 activation by forming a ternary complex with a full-length NLRP1 molecule and a C-terminal NLRP1 fragment (104). This complex is thought to prevent the NLRP1 C-terminal domains from triggering inappropriate inflammasome activation during homeostatic protein turnover. Inhibitors of DPP8/9, such as Val-boroPro (VbP), activate NLRP1 by displacing the C-terminal NLRP1 fragment from the inhibitory ternary complex and by accelerating N-terminal degradation (104). These findings suggest that NLRP1 may have evolved to guard DPP8/9. To date, virulence factors that block DPP8/9 have not been identified, but future studies may shed light on this intriguing possibility. Additionally, small-molecule inducers of reductive stress or peptide accumulation have been shown to promote NLRP1 activation and pyroptosis (105, 106). These findings suggest that NLRP1 may also guard the cellular redox state and protein homeostasis (105–107). However, whether pathogens trigger NLRP1 activation via induction of reductive stress and peptide accumulation remains to be shown.

Human NLRP1 has also been proposed to guard translation integrity (108, 109). Diverse stimuli that trigger ribosome collisions, including ultraviolet B irradiation and microbial ribotoxins, lead to activation of the ribotoxic stress response (RSR) pathway. The RSR pathway initiates ZAKα-mediated activation of p38 kinases, which phosphorylate NLRP1 and induce inflammasome activation via N-terminal degradation. Many pathogens disrupt host translation through various mechanisms (discussed below in the section titled Effector-Triggered Immunity Induced by Protein Synthesis Blockade). Thus, it is an interesting possibility that virulence factors reported to activate the RSR pathway may also trigger activation of NLRP1 (110, 111). Other pathways that converge on p38 activation may also activate NLRP1. Alphaviruses have been found to activate NLRP1 in a p38-dependent manner, but upstream signaling remains to be elucidated (108).

CARD8

Like NLRP1, CARD8 is another inflammasome-forming sensor that contains a FIIND-CARD. It similarly undergoes FIIND auto-processing and is activated through proteasome-mediated release of its bioactive C-terminal fragment (112, 113) (**Figure 3*b***). CARD8 also senses viral protease activity. The HIV-1 protease, coronavirus 3CL proteases, and picornavirus 3C proteases directly cleave CARD8, leading to inflammasome activation and pyroptosis (113–116). Like NLRP1, CARD8 is also activated by DPP8/9 inhibitors and therefore may function to guard DPP8/9 (117). CARD8 is also activated by reductive stress and peptide accumulation (105, 106). The threshold for CARD8 activation by peptide accumulation appears to be lower than that for NLRP1. Accumulation of XP peptides, the enzymatic product of DPP8/9, weakly inhibits DPP8/9, which is sufficient to activate CARD8 but does not activate NLRP1 (118). Elevated

XP peptide levels can be induced by small-molecule inhibitors of M24B aminopeptidases, enzymes that catabolize XP peptides. M24B aminopeptidase inhibitors selectively activate CARD8, suggesting that CARD8 may guard M24B aminopeptidases. Further investigation is needed to understand whether pathogens target M24B aminopeptidases or otherwise induce XP peptide buildup during infection.

NLRP3

NLRP3 is an inflammasome-forming NLR that is a promiscuous sensor of disrupted membrane integrity and impaired cellular viability. NLRP3 activation requires at least two signals. The first is delivered by NF-κB activation to prime NLRP3 transcriptionally and/or post-translationally. Priming allows NLRP3 to sense a second activating stimulus that generally involves the efflux of potassium across the plasma membrane (119). Another hallmark of NLRP3 activation is the dispersal of the trans-Golgi network and accumulation of the membrane lipid phosphatidylinositol 4-phosphate (PI4P) at the dispersed Golgi or endosomes, which allows recruitment and assembly of the NLRP3 inflammasome (120, 121). In addition, the NEK7 kinase physically associates with NLRP3 (122, 123) and is often required for NLRP3 activation (124–126). The exact molecular activation mechanism and the relationship between potassium efflux, trans-Golgi network dispersal, PI4P accumulation, NEK7 licensing, and other proposed activation mechanisms, such as reactive oxygen species production, are not clear. Nevertheless, NLRP3 functions as a general sensor of cell integrity (**Figure 3*c***). It is thus involved in directly sensing effectors that manipulate ion homeostasis and/or cause trans-Golgi network dispersal and potassium efflux. Direct inducers of potassium efflux that activate NLRP3 include microbial toxins that disrupt ion gradients across the plasma membrane, such as *Staphylococcus aureus* alpha-toxin, *Aeromonas hydrophila* aerolysin, and *Streptomyces hygroscopicus* nigericin (119, 127–130). Manipulation of intracellular membranes by virulence factors also induces NLRP3 activation. For example, the influenza virus M2 protein is a proton-selective ion channel that neutralizes the pH of the trans-Golgi network during infection. How this activity leads to activation of the NLRP3 inflammasome is not clear, but it appears to also involve potassium efflux (131, 132). Together these studies indicate that the promiscuity of NLRP3 as a general sensor of membrane and cellular homeostasis places it at the center of ETI induced by many pathogens.

Gasdermins

The gasdermins are a family of pore-forming proteins that are the terminal effectors of pyroptotic cell death (133, 134). In humans, the gasdermin family comprises six genes: *GSDMA–GSDME* and *DFNB59*. Gasdermins are activated by proteolytic removal of an autoinhibitory C-terminal region (135). Upon activation, gasdermins drive pyroptosis by forming pores in the cell membrane. Although gasdermins are typically activated downstream of inflammasome sensors, two recent publications report that a protease secreted by *Streptococcus pyogenes*, SpeB, directly cleaves GSDMA to trigger pyroptosis in keratinocytes (136, 137). GSDMA-deficient mice exhibited increased systemic spread and decreased survival when challenged with *S. pyogenes*, suggesting that GSDMA is critical in host defense against *S. pyogenes* (136). The finding that GSDMA directly senses the proteolytic activity of SpeB to induce pyroptosis is reminiscent of NLRP1 and CARD8 sensing of pathogen-encoded proteases. Interestingly, many components of the pyroptotic pathway, including caspases, gasdermins, and IL-1 family cytokines, are activated by proteolysis. In addition to gasdermins, other pyroptosis-related proteins are directly cleaved and activated by pathogen-encoded proteases (138–140). Most notably, *S. pyogenes* SpeB cleaves and matures IL-1β and IL-18 (139, 140). One interpretation of these observations is that components

of the pyroptotic pathway have evolved the capability to directly serve as tripwires of pathogen protease activity to mediate ETI. Another interpretation is that pathogens have evolved to activate parts of the inflammasome cascade because they benefit from an inflammatory environment (e.g., while competing with commensal microbes).

EFFECTOR-TRIGGERED IMMUNITY INDUCED BY PROTEIN SYNTHESIS BLOCKADE

Blockade of host protein synthesis is a common pattern of pathogenesis of many pathogens. For example, many viruses disrupt host protein synthesis to redirect the protein synthesis machinery toward the production of viral proteins (141). Unlike viruses, which must co-opt host ribosomes, bacteria encode their own ribosomes and are not reliant on host protein synthesis. Nevertheless, host protein synthesis is blocked by diverse bacterial toxins, including diphtheria toxin from *Corynebacterium diphtheriae*, and exotoxin A from *Pseudomonas aeruginosa*, both of which ADP-ribosylate and inhibit translation mediated by eukaryotic elongation factor 2 (eEF-2). Certain *Shigella* and *E. coli* strains encode Shiga toxins, which modify the 28S ribosomal RNA to disrupt protein synthesis (142). *Legionella pneumophila* secretes multiple effectors into host cells to inhibit host protein synthesis (143). It is unclear why these diverse bacterial pathogens, which occupy different host niches, independently evolved toxins to inhibit host protein synthesis, but plausible explanations are that these toxins inhibit the host response, alleviate ER stress (144, 145), or free amino acids for bacterial consumption (146).

The diversity of pathogens that target host protein synthesis provides a rationale for immune sensing of protein synthesis inhibition. The chemical protein synthesis inhibitor cycloheximide has long been known to induce interferon responses (147), but more recent work has shown that protein synthesis inhibition also elicits immune responses during infection. One set of early findings showed Shiga toxins trigger the MAPK-dependent transcriptional superinduction and secretion of IL-8 and chemokines (148, 149). Expression of inflammatory cytokines such as GM-CSF and IL-1α was also observed to be induced by *L. pneumophila* in a manner dependent on its secreted effectors that block protein synthesis (48). As mentioned above, subsequent work in *C. elegans* demonstrated that *Pseudomonas* exotoxin A also elicits a host response (44, 45).

The above observations are counterintuitive since inhibition of protein synthesis is expected to block rather than induce the *de novo* production of anti-pathogen proteins. In vertebrates, induction of inflammatory cytokines appears to be largely due to a dramatic (3-log) transcriptional superinduction of the cytokine mRNAs, which overcomes a partial inhibition of protein synthesis and results in a net production of specific inflammatory proteins, many of which are cytokines that can act on bystander (translation-competent) cells (150–152). The transcriptional superinduction appears to arise as a result of sustained NF-κB activation (48) and MAPK activation (149, 153). Sustained NF-κB activation arises in cells that experience protein synthesis blockade due to the failure to resynthesize the unstable inhibitor protein, IκB, which therefore essentially functions as the sensor in this pathway.

The slow resynthesis of short-lived inhibitory proteins seems to be a common strategy to initiate responses to pathogen-mediated translational shutdown. For example, low levels of the short-lived proteins MCL-1/BCL-xL induce gasdermin E–mediated pyroptosis upon translational inhibition by viruses (154). In *C. elegans*, a distinct mechanism is employed in which protein synthesis inhibition leads to selective translation of the ZIP-2 transcription factor in a manner dependent on an upstream open reading frame in the ZIP-2 mRNA (44, 45). Selective translation of key immune response genes may also occur in macrophages experiencing protein synthesis inhibition (155–157).

Together, these observations suggest host cells have evolved multiple strategies to sense disruption of translation and redirect their limited protein synthesis capacity to initiate an immune response. Even pathogens that do not encode effectors to directly disrupt protein synthesis may alter host protein synthesis indirectly as a consequence of virulence-associated activities, e.g., via consumption of amino acids or induction of ER or integrated stress responses (158, 159). For example, a drop in amino acid concentrations impairs mTOR activity and results in autophagy induction, an antimicrobial response. Low amino acid levels also activate GCN2, a kinase that activates an integrated stress response that also likely inhibits pathogen replication. Thus, multiple host pathways guard the integrity of protein synthesis, and pathogen interference with protein synthesis appears to be an important activator of ETI.

EFFECTOR-TRIGGERED IMMUNITY INDUCED BY BLOCKADE OF TNF SIGNALING

Many pathogens block the TNF and TLR signaling pathways to suppress induction of antimicrobial factors and proinflammatory cytokines. To counteract this pathogen activity, mammalian hosts have evolved a multilayered strategy to guard the signaling pathways downstream of TNF receptor 1 (TNFR1) and the TRIF-dependent TLRs, TLR3 and TLR4 (160) (**Figure 4**). The primary outcome of activation of these receptors is induction of NF-κB- and MAPK-dependent signaling, leading to transcription of host defense genes. However, when these signaling pathways are blocked, a backup pathway culminating in cell death is induced. A key mediator of this cell fate decision is RIPK1. Activation of TNFR1 and TLR3/4 leads to recruitment of the adaptor proteins TRADD and TRIF, respectively, which both interact with RIPK1. Polyubiquitylation of RIPK1 generates a scaffold for activation of the NF-κB and MAPK pathways (161). However, when TNF/TLR signaling is inhibited, RIPK1 kinase activity is induced and mediates initiation of caspase-8-dependent apoptosis (162). Therefore, RIPK1 guards the TNF/TLR signaling pathways and induces cell death when these pathways are disrupted. Mechanistically, this process involves negative regulation of RIPK1 and cell death signaling by several components of the TNF/TLR signaling pathways [e.g., direct phosphorylation of RIPK1 by IκB kinases (IKKs) (163) and transcriptional activation of cFLIP (164)]. Interestingly, in the context of TNF/TLR signaling blockade, caspase-8 has been shown to directly cleave IL-1β and GSDMD and to drive potassium efflux leading to NLRP3 inflammasome activation and IL-1 cytokine processing (165–167). Caspase-8 also cleaves a suppressor of cytokine signaling called NEDD4-binding protein 1 (N4BP1) (168), leading to enhanced inflammatory gene expression. These findings indicate that, despite the classical view of apoptosis as an immunologically silent form of cell death, an apoptotic caspase can drive inflammation when TNF/TLR signaling is blocked.

Perhaps to avoid the above inflammatory responses or to preserve their replicative niche, many pathogens have evolved to inhibit caspase-8. However, when caspase-8 is inhibited, the host employs a second backup pathway that induces an alternative form of cell death termed necroptosis. Necroptosis is mediated by the proteins RIPK3 and MLKL (mixed-lineage kinase domain–like pseudokinase) and is characterized by rupture of the plasma membrane. Active caspase-8 normally suppresses necroptosis via cleavage of RIPK1; thus, inhibition of caspase-8 unleashes necroptosis (169–172). This multilayered host defense system is likely challenging for pathogens to overcome, as it requires pathogens to simultaneously block TNF/TLR signaling, apoptosis, and necroptosis (173).

Indeed, virulence factors that target each layer of this host defense strategy have been identified (**Figure 4**). For example, the *Yersinia* effector YopJ is an acetyltransferase that blocks TNF/TLR signaling by inhibiting TAK1, IKK, and MAPK proteins (174–176). Disruption of TNF/TLR

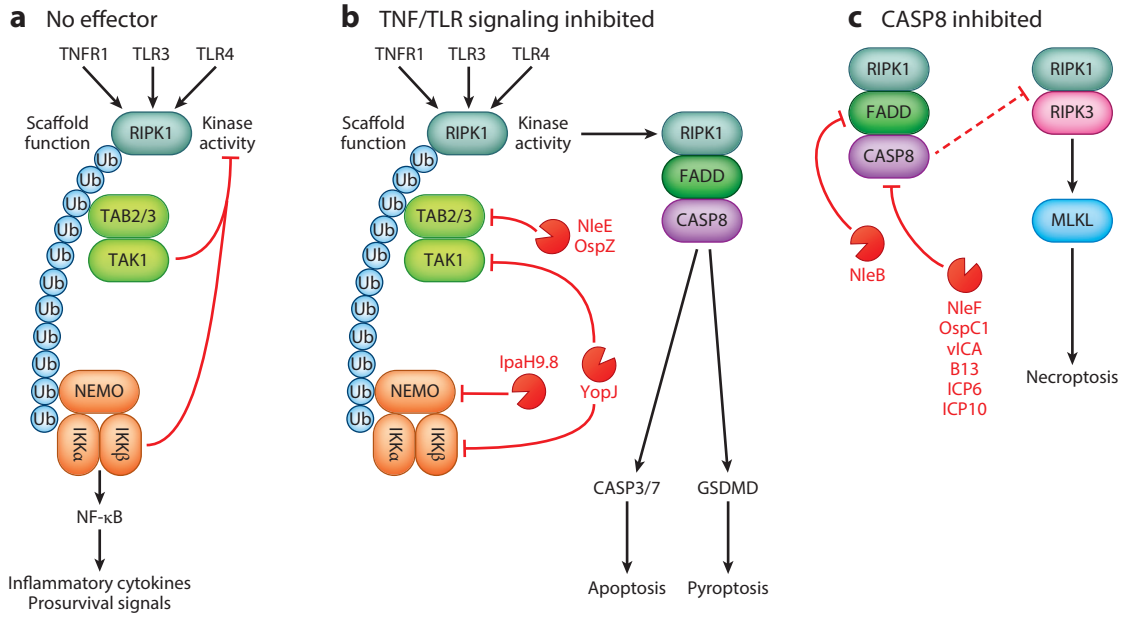


Figure 4

Guarding of TNF and TLR signaling by effector-triggered immunity. A multilayered strategy guards the signaling pathways downstream of TNFR1, TLR3, and TLR4. (*a*) The primary outcome of activation of these receptors is induction of NF-κB signaling, resulting in transcription of inflammatory cytokines and prosurvival signals. Polyubiquitylated RIPK1 serves as a scaffold for the activation of NF-κB signaling. Kinases activated downstream of RIPK1 block induction of cell death by suppressing RIPK1 kinase activity. (*b*) Multiple virulence factors inhibit TNF/TLR signaling to block induction of host defense genes. When TNF/TLR signaling is blocked, RIPK1 kinase activity is induced and promotes caspase-8-dependent cell death. In this context, caspase-8 also directly cleaves GSDMD, driving inflammation. (*c*) To block caspase-8-mediated inflammation and cell death, pathogens encode virulence factors that inhibit caspase-8. When caspase-8 is inhibited, RIPK3/MLKL-mediated necroptosis is unleashed. Abbreviations: CASP8, caspase-8; FADD, FAS-associated death domain protein; GSDMD, gasdermin D; IKK, IκB kinase; MLKL, mixed-lineage kinase domain–like pseudokinase; NEMO, NF-κB essential modulator; RIPK1, receptor-interacting serine/threonine protein kinase 1; TAB2/3, TGF-β-activated kinase 1–binding protein 2/3; TAK1, TGF-β-activated kinase 1; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNFR1, TNF receptor 1.

signaling by YopJ is sensed by the host, leading to caspase-8-dependent cell death and inflammation (165, 177). YopJ-induced cell death is critical for host survival during *Yersinia* infection, demonstrating the importance of this host defense mechanism (177). Other bacterial pathogens that block TNF/TLR signaling encode additional virulence factors to inhibit apoptosis and necroptosis. For example, enteropathogenic *E. coli* (EPEC), which encodes multiple effectors that redundantly target TNF/TLR signaling, also encodes the effectors NleB and NleF, which inhibit apoptosis, as well as EspL, which inhibits necroptosis (173, 178–180). *S. flexneri* likely employs a similar strategy to maintain cell viability while blocking TNF/TLR signaling, as the effectors OspC1 and OspD3 were reported to inhibit apoptosis and necroptosis, respectively (181).

Many viruses also inhibit apoptosis. For example, the orthopoxvirus vaccinia virus (VACV) encodes the virulence factor B13, which inhibits caspase-8. However, inhibition of caspase-8 by B13 sensitizes cells to necroptosis (182). Mice deficient for necroptosis (e.g., *Ripk3*$^{-/-}$) fail to control VACV replication, suggesting that necroptosis is an important antiviral immune response (183). Interestingly, other poxviruses encode orthologs of B13 that inhibit caspase-8 but, unlike that of VACV, do not sensitize cells to necroptosis. This difference is likely due to a virulence factor, vIRD, that blocks necroptosis by promoting degradation of RIPK3 (184). vIRD orthologs

are encoded by certain orthopoxviruses, such as cowpox virus, monkeypox virus, and ectromelia virus, but are missing from laboratory-adapted VACV, which causes relatively benign disease, and the distantly related leporipoxvirus myxoma virus, which infects RIPK3-deficient hosts (184). A vIRD-deficient cowpox virus strain exhibits reduced replication in mice compared to wild-type virus, which is dependent on RIPK3 and MLKL, further highlighting the role of necroptosis in antiviral defense. Herpesviruses also encode inhibitors of apoptosis and necroptosis. For example, mouse cytomegalovirus encodes vICA and vIRA, which target caspase-8 and RIPK1/3, respectively (185–187). Herpes simplex virus 1 (HSV-1) and HSV-2 produce virulence factors—ICP6 and ICP10, respectively—that block apoptosis and necroptosis simultaneously (188). Together, these examples demonstrate that innate immune signaling pathways themselves are guarded and that diverse pathogens have evolved strategies to counteract both PTI and ETI. These multilayered responses provide an elegant example of host-pathogen coevolution.

EFFECTOR-TRIGGERED IMMUNITY–MEDIATED INDUCTION OF TYPE I INTERFERONS

Type I interferons are a class of cytokines comprising IFN-β and numerous IFN-α isoforms that are critical for induction of antiviral immune responses. Type I interferons are induced primarily by PAMP-triggered signaling pathways, mainly involving cytosolic or endosomal sensors of nucleic acids, the hallmark PAMPs associated with viral infection (189, 190). The canonical interferon-inducing pathways all converge on key signaling components, in particular TBK1 kinase and its substrate IRF3 transcription factor. In some cell types, a MyD88- and IRF7-dependent PTI pathway can also induce type I interferons. However, cells deficient in both IRF3 and IRF7 are highly deficient in interferon induction in response to nucleic acid PAMPs. Given the central role of type I interferons in antiviral immunity, it is not surprising that viral pathogens have evolved numerous inhibitors of interferon-inducing, nucleic acid–sensing pathways (191). This in turn places pressure on hosts to evolve alternative pathways for interferon induction that do not rely on the canonical signaling pathways or components.

MORC3

The recently described MORC3 (microrchidia family CW-type zinc finger 3) pathway represents an alternative effector-triggered pathway for type I interferon induction (192) (**Figure 5**). This pathway was shown to be induced by an important effector of HSV-1, an E3 ubiquitin ligase called ICP0. HSV-1 encodes ICP0 to ubiquitylate and degrade numerous host proteins that otherwise restrict lytic viral replication (193). ICP0 is recruited to several of its targets by virtue of an ability to bind SUMO, a ubiquitin-like modification that decorates many proteins associated with mysterious structures called nuclear bodies. Though the functions of nuclear bodies remain largely obscure, it is clear they play a role in sequestering and silencing the genomes of many DNA viruses, including HSV-1. MORC3 is itself a sumoylated nuclear body protein that restricts HSV-1 replication (194). As such, it makes sense that MORC3 is targeted for ubiquitylation and degradation by ICP0 (194, 195). However, viral attack of MORC3 bodies was recently shown to be guarded by a secondary effector-triggered function of MORC3, in which MORC3 acts an essential repressor of *IFNB1* expression (192). Genetic loss of MORC3, or its degradation by ICP0, is sufficient to result in potent *IFNB1* induction. Importantly, *IFNB1* induction that occurs after MORC3 loss is independent of TBK1 and IRF3/7; thus, the MORC3 pathway is potentially operable even in a cell in which PTI pathways for interferon induction are disabled. The MORC3 pathway was also shown to be activated by an adenovirus effector, E4ORF3 (192). The transcription factors required for *IFNB1* induction in *MORC3*-deficient cells remain to be identified, but

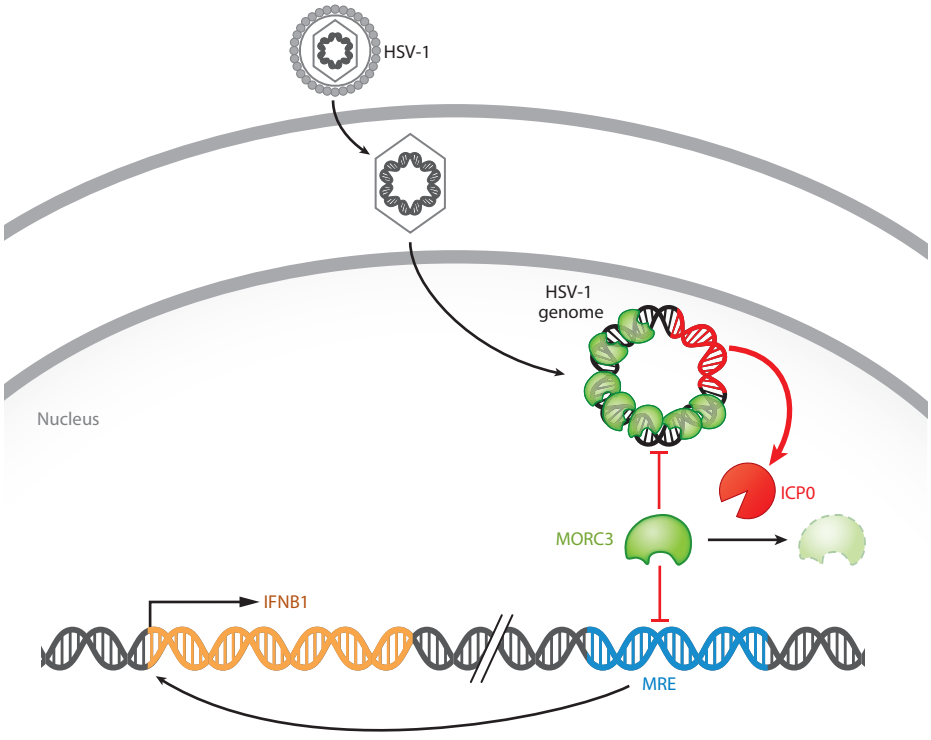


Figure 5

MORC3 pathway. MORC3 is a nuclear body protein that restricts HSV-1 replication and is thus targeted for ubiquitylation and degradation by the HSV-1 virulence factor ICP0. Viral attack of MORC3 is guarded by a secondary function of MORC3, in which MORC3 represses *IFNB1* expression. Degradation of MORC3 by ICP0 results in potent *IFNB1* induction. A genetic element adjacent to the *IFNB1* gene, called the MORC3-regulated element, is required for ICP0-induced IFNB1 production. Abbreviations: HSV-1, herpes simplex virus 1; IFNB1, IFN-β1; MORC3, microrchidia family CW-type zinc finger 3; MRE, MORC3-regulated element.

at least one essential player appears to be a genetic element adjacent to the *IFNB1* gene, called the MORC3-regulated element (MRE) (192). The MRE is essential for ICP0-induced IFNB1 production but is not required for interferon induction via PTI. Interestingly, genetic interference with sumoylation, which presumably also results in MORC3 destabilization, also results in a very similar IRF3/7-independent type I interferon response (196, 197). The MORC3 pathway appears to be primarily operational in monocytes, and its in vivo function during HSV-1 or adenovirus infection remains to be elucidated.

Other Potential Effector-Triggered Immunity Pathways for Interferon Induction

We speculate that many examples of ETI are not recognized as such and remain to be discovered. Non-inflammasome responses, such as type I interferon responses, may be a rich source of examples of ETI. Indeed, several negative regulators of type I interferons have been described in addition to MORC3. Many of these negative regulators were identified because loss-of-function mutations result in Aicardi-Goutières Disease, a serious autoinflammatory disease characterized

by excessive type I interferon production (198). Though usually viewed as mere negative regulators of type I interferons, many of the Aicardi-Goutières genes (e.g., *ADAR1*, *TREX1*, *SAMHD1*, *RNASEH2*, *ISG15*) notably encode products with known or speculated antiviral enzymatic activities, such as degradation or mutation of viral genomes or other antiviral functions (199–201). In some cases, there are viral factors that degrade or antagonize these factors, thereby providing an evolutionary rationale for why loss of the factor would elicit an interferon response (192).

Indeed, the formal difference between a simple negative regulator and an ETI sensor is the existence of a pathogen effector that targets the regulator, though additional superimposed viral evasion strategies may make it difficult to demonstrate that the ETI response has bona fide antiviral effects. Indeed, other negative regulators of type I interferons have been described. For example, TRIM24/28/33 (202–204) and SP140 (205) are negative regulators of interferons. It remains to be shown whether these proteins are targeted by viruses, though they are in protein families with known antiviral functions (206, 207), raising the possibility that these proteins function as guardees or decoys.

CONCLUSION

Once one is open to the concept of ETI, potential examples abound throughout immunology, hiding in plain sight. A shift in perspective is possible once one realizes that potentially any negative regulator of an immune response pathway could function as a sensor (target) of a pathogen effector. Thus, identification of virulence factors that attack or disrupt negative regulators, thereby eliciting a host response, is a fundamental approach to discover ETI pathways.

Awareness of ETI as a mechanism of immune activation can lead to reinterpretation of existing data. For example, systematic genetic studies have identified numerous host proteins that function as dependency factors, i.e., host proteins that are necessary for viral replication (208). Dependency factors are the opposite of restriction factors and are defined as host genes whose deletion results in decreased viral replication. Dependency factors are usually interpreted as host factors that the virus exploits for its benefit, e.g., entry receptors. However, some apparent dependency factors may actually be examples of negative regulators that function in ETI pathways. For example, MORC3 was originally proposed to be a host factor required for influenza replication (209). This result can now be reinterpreted with the knowledge that loss of MORC3 results in interferon induction (192), which likely explains the observed repression of influenza replication seen in *MORC3*-deficient cells. Similarly, genetic data led to the provocative proposal that the intracellular ubiquitin-like protein ISG15 is a secreted factor required for IFN-γ-mediated responses (210). This result can be reinterpreted: Loss of ISG15 results in type I interferon induction, which in turn represses IFN-γ signaling (211). The reinterpretation makes abundant sense when one considers that the primary function of ISG15 is likely antiviral (199), thus rationalizing why it would be a conserved target of viruses and why its loss would lead to a type I interferon response.

We fully expect—and are even excited to acknowledge—that our survey of mammalian ETI pathways will likely prove woefully incomplete. We anticipate, for example, that type 2 immune responses will prove a rich source of ETI pathways, as suggested by the existence of protease allergens (212) and the overall lack of PAMPs in type 2 immunity. Other unanticipated ETI pathways also likely exist—we hope to be enthralled by their diversity and elegance.

DISCLOSURE STATEMENT

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