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Activation and pathogenic manipulation of the sensors of the innate immune system

Charlotte Odendall^a, Jonathan C. Kagan^{b,*}

^aDepartment of Infectious Diseases, King's College London, London SE1 9RT, UK

^bHarvard Medical School and Division of Gastroenterology, Boston Children's Hospital, Boston, MA 02115, USA

Abstract

The innate immune system detects the presence of microbes through different families of pattern-recognition receptors (PRRs). PRRs detect pathogens of all origins and trigger signaling events that activate innate and adaptive immunity. These events need to be tightly regulated in order to ensure optimal activation when required, and minimal signaling in the absence of microbial encounters. This regulation is achieved, at least in part, through the precise subcellular positioning of receptors and downstream signaling proteins. Consequently, mislocalization of these proteins inhibits innate immune pathways, and pathogens have evolved to alter host protein localization as a strategy to evade immune detection. This review describes the importance of subcellular localization of various PRR families and their adaptors, and highlights pathogenic immune evasion strategies that operate by altering immune protein localization.

Keywords

Innate immunity; Toll-like receptors; Myddosome; Inflammasome; Infection; Immune evasion

1. Introduction

Species from all kingdoms are subject to infection by a range of pathogens and in response have developed some form of immunity. These 'immune systems' enable, for example, insects to combat viruses using RNA interference (RNAi), plants to fight bacterial infections through effector-triggered immunity and the hypersensitivity response, mammals to sense and eliminate infections via innate and adaptive immunity, and even bacteria to battle bacteriophages via CRISPR/Cas and restriction enzymes (Fig. 1).

An absolute prerequisite in immunity against pathogens is the recognition of microbes via receptors that survey extra and intracellular spaces. In 1989, Charles Janeway Jr predicted the now validated existence of germ-line encoded pattern-recognition receptors (PRRs) in all multicellular organisms, and in doing so set the foundation for the field of innate immunity

*Corresponding author. jonathan.kagan@childrens.harvard.edu (J.C. Kagan).

Conflict of interest

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[1]. PRRs detect and respond to conserved patterns expressed by microbes, termed pathogen-associated molecular patterns (PAMPs). These molecules are produced uniquely by pathogens, and as such are recognized as infectious non-self by the innate immune system. PRRs are divided into families, based on sequence homology, localization and downstream signaling. The first identified PRR family was the toll-like receptor (TLR) family, named after their homology with the *Drosophila* Toll receptor [2–5]. Later, Nucleotide binding, leucine rich repeat containing proteins (NLRs), C-type lectin receptors (CLRs), RIG-I-like receptors (RLRs), AIM-2-like receptors (ALRs) and several DNA sensors were identified. Together, these PRRs recognize ligands of all origins, ranging from bacterial proteins and lipids to fungal sugars, as well as nucleic acids containing structures or sequences that are unique to viruses or bacteria. Collectively, these sensors survey all intracellular and extracellular environments for microbes and their products. TLRs and CLRs are localized at the plasma membrane and monitor the extracellular space for microbes. The lumen of endosomes is monitored by additional TLRs, while the DNA sensor IFI16 is in the nucleus. Finally, the cytosol contains an array of PRRs: RLRs, ALRs, NLRs, cGAS and the autophagic machinery.

Appropriate localization of PRRs is required for their ability to detect PAMPs, as well as signal to downstream partners. An instrumental step downstream of ligand binding is the interaction between PRRs and their adaptors, which induces the assembly of large complexes recently termed supramolecular organizing centres (SMOCs) [6]. SMOCs are assembled on specific organelles and other intracellular sites, thus ensuring that signaling occurs from discrete subcellular positions. For example, ligation of certain TLRs leads to the formation of a SMOC called the myddosome, which is composed of the adaptors TIRAP and MyD88 and members of the IRAK family of serine/threonine kinases (discussed further below). NLRs and several other cytosolic PRRs induce the formation of a different class of SMOCs, called inflammasomes, and the RLRs induce the formation of a SMOC consisting of the prion-like protein MAVS. Because myddosomes (and other SMOCs) are not present in resting cells, but are assembled upon PRR activation, these structures can be considered organelles that are assembled on-demand, or as needed by the cell. As their name indicates, these organelles are organizing centers for the innate immune system, serving as the principal subcellular site of innate immune signal transduction. In the case of activated TLRs and RLRs, the myddosome or MAVS complex operates as a platform for the recruitment and/or activation of various signaling proteins and enzymes that are shared between multiple pathways. These include MAP kinases, E3 ubiquitin ligases, and I κ B kinases (IKKs), which ultimately activate inflammatory transcription factors such as NF- κ B, AP-1 and several interferon (IFN) regulatory factors (IRFs). Consequently, a potent transcriptional response is induced upon myddosome or MAVS complex assembly, leading to the expression of cytokines, chemokines and IFNs of the type I and III families. A similar sequence of events applies to the operation of the PRRs that promote inflammasome formation, with the notable exception being that inflammasomes do not promote inflammatory gene expression. Rather, inflammasomes induce the cleavage and release of IL-1 family cytokines from the cytosol into the extracellular space. The mechanisms of inflammasome-mediated cytokine release are poorly understood, but are considered to be either a consequence of a lytic cell death program called pyroptosis, or some other atypical mechanism of secretion. Many cytokines

are pro-inflammatory, acting to recruit immune mediators to the site of infection, as well as promote adaptive immunity. Therefore, innate immunity is often described as the first line of defense against pathogens, and is required for the eradication of many microbial infections.

A common theme shared by PRR families and their adaptors is that appropriate subcellular localization is required not only for the ability of PRRs to detect PAMPs, but also for the PRRs to induce SMOC assembly and downstream inflammatory signal transduction. Mislocalization of innate immune proteins is therefore a simple way used by pathogens to disrupt immune recognition and signaling. Herein, we review the importance of subcellular localization in innate immunity. We discuss where the known PRRs and their adaptors are located, how subcellular localization is important for signaling, which adaptors assemble SMOCs downstream of each PRR and where they are located. In addition, we discuss mechanisms through which viral and bacterial pathogens inhibit innate immune sensing. We detail the many enzymatic functions of virulence proteins that include proteolytic cleavage of the signal sequences required for proper localization of host proteins, inhibition of chaperones, degradation of lipids that serve as anchors for membrane proteins or hijacking of the host trafficking machinery.

2. TLR signaling occurs at the plasma membrane and on endosomes

Depending on the nature of the ligand they recognize, TLRs localize at the cell surface or endosomes. Cell-surface localized TLRs include TLR 1, 2, 4, 5 and 6. These receptors recognize a range of bacterial cell surface components. TLR4 is the best characterized TLR, and also localizes to endosomes. Together with CD14 and MD-2, TLR4 recognizes the lipid A portion of lipopolysaccharide (LPS) present on gram-negative bacteria. TLR5 recognizes bacterial flagellin, a subunit of the flagella apparatus. TLR2 forms dimers with TLR1 or 6. Even though it is best known for its ability to detect bacterial lipoproteins, TLR2 recognizes a large range of ligands of different origins, including fungi, helminths, and viruses [7].

TLR3, 7, 8, 9, 13 localize to endosomal compartments and recognize nucleic acids: TLR3 detects dsRNA, TLR7 and 8 detect ssRNA and TLR9 has specificity towards DNA sequences containing unmethylated CpG motifs. TLR10 does not have a known ligand but appears to have anti-inflammatory functions [8]. TLRs 11–13 are only present in mice, with TLR11 being a pseudogene in humans while the TLR12 and 13 genes are absent. TLR11 and 12 detect profilin from *Toxoplasma gondii*, a protein that is critical for parasitic movement [9]. Finally, TLR13 detects specific sequences present in bacterial ribosomal RNA [10].

The first step in TLR signaling is the detection of the active (dimerized) receptor by a sorting adaptor. TLR sorting adaptors include TIRAP and TRAM, and are localized at the site of signaling prior to PAMP encounter. These proteins therefore determine the subcellular sites of SMOC assembly. Upon detection of active TLRs, sorting adaptors mediate the recruitment of another class of adaptors termed “signaling adaptors”, which represent the core proteins within SMOCs and promote signal transduction [11]. The best-characterized sorting adaptor is TIRAP, whose function is to induce the formation of a myddosome upon detection of active TLRs. MyD88, the most abundant protein in the

myddosome, interacts directly with TLR-bound TIRAP to promote assembly of this SMOC, and subsequent signal transduction. Similarly, the sorting adaptor TRAM detects active TLRs and subsequently, promotes the recruitment of the signaling adaptor TRIF to promote signal transduction. In the TLR4 signaling pathway, which is best understood, the functions of TIRAP and TRAM are distinct spatially, as TLR4 signaling from the cell surface proceeds via TIRAP-dependent myddosome assembly, and TLR4 signaling from endosomes proceeds via TRAM/TRIF.

TLRs 2, 4, 5 and 7–9 also signal via MyD88 and are therefore expected to assemble myddosomes to promote signaling. In contrast, TLR3 signals via TRIF exclusively, and should not assemble a myddosome. Importantly, activation of TLRs yields different transcriptional responses depending on TLR and adaptor localization: while all TLRs induce pro-inflammatory cytokines, only endosomal TLRs (including TLR4 in endosomes) induce type I IFNs [11–21].

Since TLR adaptors are shared among the TLR family, disabling one of these proteins is sufficient to inhibit many or most TLR pathways. It seems that pathogens have identified this weakness in our immune system, and several have elected to target TLR adaptors rather than receptors themselves. Indeed, degradation of TIRAP and MyD88 by the *Brucella* protein TcpB blocks signaling by TLR2, 4 and 5 [22,23]. Similarly, ICP0 protein from herpes simplex virus type 1 (HSV-1) inhibits TLR2 responses by inducing TIRAP and MyD88 degradation [24]. Remarkably vaccinia virus disables TIRAP, TRAM, MyD88, TRIF and TLR4, via one single protein: A46R. A46R contains a TIR domain and achieves this function by competing for TIR binding [25].

As mentioned above, preventing the correct localization of innate immune proteins can be achieved by several mechanisms. One mechanism is the degradation of membrane lipids required for adaptor localization. TIRAP localization to the plasma membrane requires binding to the lipid phosphatidylinositol (PI) 4, 5 bisphosphate (PI(4,5)P2) [13]. The *Salmonella* effector SopB is a phosphatidylinositol phosphatase that dephosphorylates PI(4,5)P2 [26]. This was shown to contribute to *Salmonella* invasion of non-phagocytic cells [27], but also prevents TIRAP recruitment to the plasma membrane. As a result, SopB has the potential to inhibit TLR4 responses [13].

TLR3 is important for the defense against many viruses and requires TRIF signaling from endosomes. Many viral proteins target TRIF. Hepatitis C virus (HCV) NS3/4a induces TRIF proteolysis [28], and Enterovirus 68 3C cleaves and inactivates TRIF [29]. Viral factors also affect TRIF function by interfering with its endosomal recruitment. This includes Coxsackievirus 3C, a protease that relocates TRIF to the cytosol [30].

As previously mentioned, LPS sensing by TLR4 in a complex with CD14 and MD-2 occurs at the cell surface. Following a first wave of signaling via the myddosome, TLR4 is internalized into endosomes, where it induces a separate signaling pathway with a distinct transcriptional output: endosomal TLR4 induces type I IFNs. This process is dependent on CD14 [31]. Indeed, CD14-deficient cells do not induce TLR4 signaling from endosomes and are unable to produce type I IFNs. It would be an interesting strategy to block endosomal

TLR4 signaling by blocking its endocytosis mediated by CD14. A pathogen able to actively block CD14 has yet to be identified. However, LPS from *Francisella tularensis* has biochemical properties that enable it to evade detection by CD14. As a consequence, *Francisella tularensis* does not trigger TLR4 endocytosis or signaling [32].

Proper biosynthetic trafficking of TLRs is required for their function. Several chaperones and other proteins have been implicated in this process. These include UNC93B, which drives transport of TLRs from the endoplasmic reticulum (ER) to endolysosomes, and remains associated with TLRs even after Golgi processing [33,34]. GP96 is a chaperone that, along with PRAT4A, is required for the proper folding of TLRs in the ER, and delivery of both cell surface and endosomal TLRs [35]. Other proteins are required for trafficking of TLRs to their site of function, as has been reviewed elsewhere [35]. Improper function of these proteins strongly compromises the functions of many TLRs. For example, UNC93B-deficient cells are unresponsive to ligands for endosomal TLRs [36] and UNC93B-deficient mice and humans are susceptible to viral infections [37]. Therefore, interfering with the function of these chaperones is predicted to be an efficient strategy to disrupt many or all TLR pathways. UNC93B was shown recently to be cleaved by enteroviral proteases but this didn't affect its trafficking function or TLR signaling [38]. To date, there hasn't been any report of inhibition of TLR pathways by bacterial or viral factors through inhibition of UNC93B function. Monitoring UNC93B function, localization or integrity during infection with a range of viral or bacterial pathogens could be instrumental in elucidating the mechanisms through which some of these pathogens block TLR signaling.

3. RLR signaling proceeds on mitochondria and peroxisomes following detection of viral RNA

RLRs detect cytosolic foreign RNA species. Unlike the TLRs, RLRs are expressed by most cell types. The family is composed of Retinoic acid-inducible gene-I (RIG-I), melanoma-differentiation 5 (MDA5) and Laboratory of Genetics and Physiology 2 (LGP2) [39–41]. The functions of LGP2 are still unclear [42–44], but RIG-I and MDA5 are well-characterized and detect distinct viral RNA species based on their biochemical properties and RNA fragment length [45–47]. Like most PRRs, RLRs are the target of viral evasion mechanisms. These strategies include preventing PAMP detection by RLRs, such as the N protein of rabiesvirus that shields viral RNA from RIG-I [48], or Dengue viruse-mediated prevention of RIG-I activation [49]. Even though RLRs are cytosolic, a common evasion mechanism is their mislocalization. RLR signaling requires binding to an adaptor protein termed mitochondria-antiviral signaling protein (MAVS, also known as IPS-1, Cardiff or VISA) [50–53]. MAVS localizes to mitochondria [52], peroxisomes [54] and mitochondria-associated ER membranes [55]. Following activation, RIG-I traffics to mitochondria, a process that is dependent on the chaperone 14–3–3 ϵ [56]. This is necessary for MAVS interactions and the activation of the downstream signal transduction cascade that leads to IFN production. A recent study has shown that Dengue virus NS3 binds 14–3–3 ϵ through a sequence that mimics a RIG-I-binding motif. This interaction prevents RIG-I binding to its chaperone, and therefore trafficking to mitochondria. NS3 is therefore an example of an

immune evasion mechanism through sequestration of a chaperone, and prevention of receptor access to its site of signaling [57].

Cells lacking MAVS do not induce type I or III IFNs, or pro-inflammatory cytokines downstream of RNA virus infection [52,58,59]. In addition, MAVS KO mice are susceptible to viral infections [60]. As described above, MAVS localizes on membranes. The importance of membrane localization can be demonstrated by experimental relocation of MAVS to the cytosol (via truncation of the transmembrane domain). These conditions render the protein inactive upon overexpression [52] or in response to viral infection [54]. Several viral pathogens are able to cleave MAVS off these membranes as part of their virulence strategies. The best-described viral protein to perform this function is Although first described as viral sensors, RLRs are important for the detection of some intracellular bacteria, including *Listeria monocytogenes* the HCV protein NS3/4a [50,61]. NS3/4a is a serine protease, a heterodimer composed of the catalytic subunit NS3 and its activating cofactor NS4. This protease is required for the processing of the HCV poly-protein precursor into 10 functional proteins, but also for innate immune silencing. NS3/4a cleaves 5e7 amino acids at the MAVS C-terminus, causing its relocation to the cytosol, thereby blocking RLR signaling [50,61]. Impressively, NS3/4a also cleaves and inhibits TRIF, providing an additional means of inhibiting antiviral signaling [28] (discussed above). HCV NS3/4a therefore constitutes an elegant example of immune evasion through mislocalization of adaptors.

Although first described as viral sensors, RLRs are important for the detection of some intracellular bacteria, including *Listeria monocytogenes* [59,62] and *Legionella pneumophila* [63,64]. Despite this fact, only one bacterial factor, the *Legionella* type IV effector SdhA, is capable of blocking this pathway. However, it was later revealed that *sdhA* mutant bacteria are cytosolic and thus more readily detected by RLRs, while wild-type *Legionella* remain in a vacuole [65]. Therefore, it is likely that SdhA does not block RLR signaling specifically and the search for bacterial factors that specifically inhibit RLRs or MAVS remains open.

Organelles serve as platforms for PRR signaling, and intact organelle networks are necessary for pathway activation. For example, chemical depolarization of mitochondria with the pharmacological inhibitor CCCP blocks MAVS-dependent type I IFN expression in response to viral infection [66]. Therefore, some pathogens have opted to disrupt organelle networks directly, rather than inducing the mislocalization of target proteins through proteolytic cleavage or inhibition of chaperones. For example, *Listeria monocytogenes* [67] and many viruses [68] induce the collapse of the mitochondrial network. This is predicted to inhibit type I IFN signaling. Similarly Dengue virus was recently shown to alter mitochondria morphology, promoting infection [69].

MAVS also localizes to peroxisomes, from which it induces type III IFNs [54,59,70,71]. Type III IFN expression is linked directly to the cellular abundance of peroxisomes, as increasing peroxisomal numbers strengthens type III IFN responses [59]. Interestingly, a number of flaviviruses impair peroxisome biogenesis in infected cells, thereby reducing peroxisomal numbers. This manipulation of peroxisome biogenesis resulted in an inhibition of type III IFN induction [72]. As many pathogens subvert or hijack host membranes and

affect organelle function as part of their intracellular lifecycle, it would be interesting to study the extent to which these actions affect innate immune responses.

4. DNA sensing requires STING trafficking through the ER and the Golgi

Cytosolic DNA sensors have been described with relative importance depending on the nature and origin of the DNA and the observed cell type (reviewed in Ref. [73]). Among these DNA sensors, the most important are cGAMP synthase (cGAS) [74,75], and IFI16 [76], the latter of which also localizes to the nucleus [77]. A common adaptor for these sensors was identified as stimulator of IFN genes (STING) [78–80], an ER-localized protein. STING binds IFI16 directly [76], and detects cyclic di-GMP-AMP (cGAMP), a cyclic dinucleotide synthesized by cGAS upon DNA stimulation. Therefore, STING is important for the detection of DNA viruses. Importantly, STING is also required for IFN expression following infection with a number of intracellular bacterial pathogens. Indeed, STING directly detects the bacterial second messengers cyclic di-GMP and cyclic di-AMP [81]. Upon ligand binding, STING interacts with TBK1 and its transcription factor substrate IRF3. This trimeric complex leads to IRF3 phosphorylation, which promotes its transcriptional activity [82]. As STING and its upstream activators are instrumental in the defense against a large number of DNA viruses, many pathogens attempt to evade this pathway via different mechanisms (reviewed in Ref. [83]). However, few of them interfere with localization or trafficking, with the exception of human cytomegalovirus (HCMV) that causes IFI16 relocalization to multivesicular bodies where it cannot detect HCMV infection [84].

An example of STING inhibition through manipulation of its localization comes from the intracellular bacterial pathogen *Shigella flexneri* and its virulence proteins IpaJ and VirA. Following its activation, STING translocates to perinuclear structures [85]. These trafficking events are required for downstream TBK1 activation, but exactly where activation takes place was unclear. IpaJ is a cysteine protease that cleaves proteins myristoylated at their N-terminus [86]. Among these proteins is Arf1, a small GTPase that regulates cargo transport in the secretory pathway. VirA acts as a GTPase activating protein and inhibits Rab1 function, another small GTPase important for protein secretion [87]. The actions of IpaJ and VirA cause Golgi fragmentation, and disrupt host cargo trafficking and many cellular functions. Studying IpaJ and VirA-mediated disruption of Golgi trafficking shone light on STING trafficking [88]. IpaJ blocks translocation of STING to the ERGIC while VirA blocks trafficking from the ERGIC to the Golgi. Interestingly, VirA-mediated inhibition of STING trafficking does not affect IFN expression while IpaJ does [88]. These data suggest that activation of TBK1 and IRF3 occurs at the ERGIC. This work is a telling example that studying pathogenic effectors or toxins often enables the clarification of a host trafficking and signaling pathways.

5. NLRs can assemble into SMOCs in the cytosol called inflammasomes

NLRs are intracellular PRRs that detect a number of ligands. This family can be further subdivided in two, based on their functions. Nod1 and Nod2 are similar to other PRRs, in that they activate the transcription of cytokines upon microbial detection. They recognize

bacterial peptidoglycan fragments derived from gram-negative or gram-positive bacteria, respectively. Although originally described as cytosolic, recent work has shown that Nod1 and 2 signal from endosomal membranes [89,90]. Whether pathogens are able to prevent Nod recruitment to endosomes remains an open question.

Other NLRs: NLRP1, NLRP3 and NLRC4 and NAIPs are involved in the formation of SMOCs called inflammasomes. Although not NLRs, the proteins Aim2 and Pyrin are also inflammasome receptors. Rather than inducing transcription, inflammasomes promote the post-translational activation of the pro-inflammatory cytokines IL-1 and IL-18. 'Canonical' inflammasomes are formed through either one of the five aforementioned receptor complexes [91]. A 'non-canonical' inflammasome was more recently described. It relies on caspase 11 in mice, or caspases 4/5 in humans, and recognizes cytosolic LPS. Non-canonical inflammasomes can indirectly induce IL-1 processing, as they act upstream of the canonical inflammasome NLRP3 [92–94]. NLRC4 is unusual in that it does not bind PAMPs directly, but rather signals downstream of NAIPs, which bind directly to bacterial products [95,96]. In addition to being assembled in response to PAMPs like LPS or flagellin, inflammasomes are also assembled in response to self-encoded indicators of stress termed damage-associated molecular patterns (DAMPs). Dysregulation of host proteins (e.g. Rho GTPases) by bacterial virulence factors can also lead to inflammasome activation. For example bacterial pore forming toxins activate the NLRP3 inflammasome, and type III secretion systems rod proteins NAIP2-NLRC4 [97]. At the core of most inflammasomes is the adaptor ASC. In this regard, ASC and MyD88 can be considered analogous proteins, which function is to seed the formation of a SMOC, which serves as the principle subcellular site of PRR signal transduction. In the case of inflammasomes, the best-characterized effector response emanating from this SMOC is caspase-1 activation, which induces the processing of IL-1 and IL-18 into their mature forms. Caspase-1 also processes the cytosolic protein Gasdermin D, which activates its pore forming activity, leading to pyroptosis and IL-1 release [98,99]. Given their range of ligands and importance in the defense against many infections, it is not surprising that intracellular pathogens have developed mechanisms to evade or inhibit inflammasomes, at the sensor or adaptor level. These mechanisms include modulation of expression of inflammasome components, inhibition of assembly through direct binding with inflammasome components or ligand masking. For example *Yersinia* YopK associates with type III secretion system translocon components, preventing detection by the NLRP3 and NLRC4 inflammasomes [100]. *Yersinia* also blocks activation of the Pyrin inflammasome by co-opting host kinases via the effector YopM [101]. The myxoma virus protein M13L and the Shope fibroma virus protein S013L interact with ASC through their pyrin domain and block its ability to interact with downstream partners [102,103]. However perhaps because these proteins are cytosolic, interfering with trafficking or localization of inflammasome components does not seem to be a favoured method for inflammasome interference. An exception is perhaps the measles virus V protein that suppresses the NLRP3 inflammasome. Interestingly, V interacts with NLRP3 and promotes its recruitment to perinuclear structures where presumably it is unable to interact with ASC and downstream partners although this was not formally demonstrated [104]. Whether other viral or bacterial factors are able to perform comparable functions remains to be studied.

6. Conclusion

Innate sensing must fulfil three criteria to be efficient and safe. First, the presence of microbes of all species and lifestyles, including extracellular, cytosolic and intra-vacuolar pathogens must be detected. Secondly, innate sensing must be activated rapidly and efficiently. Third, recognition of self and accidental misfiring of innate sensing pathways must be avoided. These three aspects are regulated through localization of PRRs and their adaptors. Collectively, PRRs survey all subcellular compartments: TLRs detect extracellular and vacuolar pathogens, while multiple receptors are present in the cytosol. Rapidly upon sensing, PRRs and adaptors bind and initiate the formation of SMOCs, large complexes that serve as signaling platforms. At steady state, PRRs, adaptors and other SMOC components are located in distinct subcellular compartments, thus preventing misfiring of innate pathways. Although extremely effective, this regulation through localization and compartmentalization constitutes a weakness that is exploited by many pathogens. Simply affecting the correct localization or preventing the recruitment of innate immune proteins is indeed very efficient at blocking innate immune pathways. As assays to monitor early events associated with PRR signaling emerge, a more direct assessment of the spectrum of virulence strategies that target these steps in the innate immune response will certainly follow. Thus, we expect the analysis of receptor-proximal virulence strategies to be a rich area of investigation in the coming years.

References

- [1]. Janeway JCA. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symposia Quantitative Biol 1989;54: 1–13.
- [2]. Gay NJ, Keith FJ. Drosophila toll and IL-1 receptor. Nature 1991;351: 355–6. [PubMed: 1851964]
- [3]. Anderson KV, Jürgens G, Nüsslein-Volhard C. Establishment of dorsalventral polarity in the Drosophila embryo: genetic studies on the role of the Toll gene product. Cell 1985;42:779–89. [PubMed: 3931918]
- [4]. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette *spätzle*/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 1996;86:973–83. [PubMed: 8808632]
- [5]. Medzhitov R, Preston-Hurlburt P, Janeway CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 1997;388:394–7. [PubMed: 9237759]
- [6]. Kagan JC, Magupalli VG, Wu H. SMOCs: supramolecular organizing centres that control innate immunity. Nat Rev Immunol 2014;14:821–6. [PubMed: 25359439]
- [7]. Oliveira-Nascimento L, Massari P, Wetzler LM. The role of TLR2 in infection and immunity. Front Immunol 2012;3:79. [PubMed: 22566960]
- [8]. Oosting M, Cheng S-C, Bolscher JM, Vestering-Stenger R, Plantinga TS, Verschueren IC, et al. Human TLR10 is an anti-inflammatory pattern-recognition receptor. Proc Natl Acad Sci U. S. A 2014;111:E4478–84. [PubMed: 25288745]
- [9]. Koblansky AA, Jankovic D, Oh H, Hieny S, Sungnak W, Mathur R, et al. Recognition of profilin by Toll-like receptor 12 is critical for host resistance to *Toxoplasma gondii*. Immunity 2013;38:119–30. [PubMed: 23246311]
- [10]. Oldenburg M, Krüger A, Ferstl R, Kaufmann A, Nees G, Sigmund A, et al. TLR13 recognizes bacterial 23S rRNA devoid of erythromycin resistance-forming modification. Science 2012;337:1111–5. [PubMed: 22821982]
- [11]. Kagan JC. Defining the subcellular sites of innate immune signal transduction. Trends Immunol 2012;33:442–8. [PubMed: 22817912]

- [12]. Horng T, Barton GM, Flavell RA, Medzhitov R. The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. *Nature* 2002;420:329–33. [PubMed: 12447442]
- [13]. Kagan JC, Medzhitov R. Phosphoinositide-mediated adaptor recruitment controls Toll-like receptor signaling. *Cell* 2006;125:943–55. [PubMed: 16751103]
- [14]. Kagan JC, Su T, Horng T, Chow A, Akira S, Medzhitov R. TRAM couples endocytosis of Toll-like receptor 4 to the induction of inter-feron-beta. *Nat Immunol* 2008;9:361–8. [PubMed: 18297073]
- [15]. Bonham KS, Orzalli MH, Hayashi K, Wolf AI, Glanemann C, Weninger W, et al. A promiscuous lipid-binding protein diversifies the subcellular sites of toll-like receptor signal transduction. *Cell* 2014;156: 705–16. [PubMed: 24529375]
- [16]. Rowe DC, McGettrick AF, Latz E, Monks BG, Gay NJ, Yamamoto M, et al. The myristoylation of TRIF-related adaptor molecule is essential for Toll-like receptor 4 signal transduction. *Proc Natl Acad Sci U. S. A* 2006;103:6299–304. [PubMed: 16603631]
- [17]. Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T. TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. *Nat Immunol* 2003;4:161–7. [PubMed: 12539043]
- [18]. Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science* 2003;5633:640–3.
- [19]. Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshino K, Kaisho T, et al. TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat Immunol* 2003;4: 1144–50. [PubMed: 14556004]
- [20]. Fitzgerald KA, Palsson-McDermott EM, Bowie AG, Jefferies CA, Mansell AS, Brady G, et al. Mal (MyD88-adaptor-like) is required for Toll-like receptor-4 signal transduction. *Nature* 2001;413:78–83. [PubMed: 11544529]
- [21]. Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, et al. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* 1998;2:253–8. [PubMed: 9734363]
- [22]. Cirl C, Wieser A, Yadav M, Duerr S, Schubert S, Fischer H, et al. Subversion of Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain [ndash] containing proteins. *Nat Med* 2008;14:399–406. [PubMed: 18327267]
- [23]. Sengupta D, Koblansky A, Gaines J, Brown T, West AP, Zhang D, et al. Subversion of innate immune responses by *Brucella* through the targeted degradation of the TLR signaling adapter, MAL. *J Immunol* 2010; 184:956–64. [PubMed: 20018612]
- [24]. van Lint AL, Murawski MR, Goodbody RE, Severa M, Fitzgerald KA, Finberg RW, et al. Herpes simplex virus immediate-early ICP0 protein inhibits Toll-like receptor 2-dependent inflammatory responses and NF-kappaB signaling. *J Virol* 2010;84:10802–11. [PubMed: 20686034]
- [25]. Stack J, Haga IR, Schröder M, Bartlett NW, Maloney G, Reading PC, et al. Vaccinia virus protein A46R targets multiple Toll-like-interleukin-1 receptor adaptors and contributes to virulence. *J Exp Med* 2005;201: 1007–18. [PubMed: 15767367]
- [26]. Terebiznik MR, Vieira OV, Marcus SL, Slade A, Yip CM, Trimble WS, et al. Elimination of host cell PtdIns(4,5)P2 by bacterial SigD promotes membrane fission during invasion by *Salmonella*. *Nat Cell Biol* 2002;4: 766–73. [PubMed: 12360287]
- [27]. Zhou D, Chen LM, Hernandez L, Shears SB, Galan JE. A *Salmonella* inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host cell actin cytoskeleton rearrangements and bacterial internalization. *Mol Microbiol* 2001;39:248–60. [PubMed: 11136447]
- [28]. Li K, Foy E, Ferreón JC, Nakamura M, Ferreón ACM, Ikeda M, et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. *Proc Natl Acad Sci U. S. A* 2005;102:2992–7. [PubMed: 15710891]
- [29]. Xiang Z, Li L, Lei X, Zhou H, Zhou Z, He B, et al. Enterovirus 68 3C protease cleaves TRIF to attenuate antiviral responses mediated by Toll-like receptor 3. *J Virol* 2014;88:6650–9. [PubMed: 24672048]

- [30]. Mukherjee A, Morosky SA, Delorme-Axford E, Dybdahl-Sissoko N, Oberste MS, Wang T, et al. The coxsackievirus B 3C protease cleaves MAVS and TRIF to attenuate host type I interferon and apoptotic signaling. *PLoS Pathog* 2011;7:e1001311. [PubMed: 21436888]
- [31]. Zanoni I, Ostuni R, Marek LR, Barresi S, Barbalat R, Barton GM, et al. CD14 controls the LPS-induced endocytosis of Toll-like receptor 4. *Cell* 2011;147:868–80. [PubMed: 22078883]
- [32]. Tan Y, Zanoni I, Cullen TW, Goodman AL, Kagan JC. Mechanisms of toll-like receptor 4 endocytosis reveal a common immune-evasion strategy used by pathogenic and commensal bacteria. *Immunity* 2015; 43:909–22. [PubMed: 26546281]
- [33]. Kim Y-M, Brinkmann MM, Paquet M-E, Ploegh HL. UNC93B1 delivers nucleotide-sensing toll-like receptors to endolysosomes. *Nature* 2008;452:234–8. [PubMed: 18305481]
- [34]. Lee BL, Moon JE, Shu JH, Yuan L, Newman ZR, Schekman R, et al. UNC93B1 mediates differential trafficking of endosomal TLRs. *Elife* 2013;2:e00291. [PubMed: 23426999]
- [35]. Lee CC, Avalos AM, Ploegh HL. Accessory molecules for Toll-like receptors and their function. *Nat Rev Immunol* 2012;12:168–79. [PubMed: 22301850]
- [36]. Tabeta K, Hoebe K, Janssen EM, Du X, Georgel P, Crozat K, et al. The Unc93b1 mutation 3d disrupts exogenous antigen presentation and signaling via Toll-like receptors 3, 7 and 9. *Nat Immunol* 2006;7: 156–64. [PubMed: 16415873]
- [37]. Casrouge A, Zhang S-Y, Eidenschenk C, Jouanguy E, Puel A, Yang K, et al. Herpes simplex virus encephalitis in human UNC-93B deficiency. *Science* 2006;314:308–12. [PubMed: 16973841]
- [38]. Harris KG, Coyne CB. Unc93b induces apoptotic cell death and is cleaved by host and enteroviral proteases. *PLoS One* 2015;10: e0141383. [PubMed: 26509685]
- [39]. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* 2004;5:730–7. [PubMed: 15208624]
- [40]. Kang D-C, Gopalkrishnan RV, Wu Q, Jankowsky E, Pyle AM, Fisher PB. mda-5: an interferon-inducible putative RNA helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties. *Proc Natl Acad Sci U. S. A* 2002;99: 637–42. [PubMed: 11805321]
- [41]. Andrejeva J, Childs KS, Young DF, Carlos TS, Stock N, Goodbourn S, et al. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. *Proc Natl Acad Sci U. S. A* 2004;101:17264–9. [PubMed: 15563593]
- [42]. Satoh T, Kato H, Kumagai Y, Yoneyama M, Sato S, Matsushita K, et al. LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. *Proc Natl Acad Sci U. S. A* 2010;107:1512–7. [PubMed: 20080593]
- [43]. Malur M, Gale M, Krug RM. LGP2 downregulates interferon production during infection with seasonal human influenza A viruses that activate interferon regulatory factor 3. *J Virol* 2012;86:10733–8. [PubMed: 22837208]
- [44]. Rothenfusser S, Goutagny N, DiPerna G, Gong M, Monks BG, Schoenemeyer A, et al. The RNA helicase Lgp2 inhibits TLR-independent sensing of viral replication by retinoic acid-inducible Gene-I. *J Immunol* 2005;175:5260–8. [PubMed: 16210631]
- [45]. Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, et al. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature* 2006;441:101–5. [PubMed: 16625202]
- [46]. Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, Kato H, et al. 5'-Triphosphate RNA is the ligand for RIG-I. *Science* 2006;314: 994–7. [PubMed: 17038590]
- [47]. Kato H, Takeuchi O, Mikamo-Sato E, Hirai R, Kawai T, Matsushita K, et al. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. *J Exp Med* 2008;205:1601–10. [PubMed: 18591409]
- [48]. Masatani T, Ito N, Shimizu K, Ito Y, Nakagawa K, Sawaki Y, et al. Rabies virus nucleoprotein functions to evade activation of the RIG-I-mediated antiviral response. *J Virol* 2010;84:4002–12. [PubMed: 20130065]

- [49]. Manokaran G, Finol E, Wang C, Gunaratne J, Bahl J, Ong EZ, et al. Dengue subgenomic RNA binds TRIM25 to inhibit interferon expression for epidemiological fitness. *Science* 2015;350:217–21. [PubMed: 26138103]
- [50]. Meylan E, Curran J, Hofmann K, Moradpour D. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature* 2005;437:1167–72. [PubMed: 16177806]
- [51]. Xu L-G, Wang Y-Y, Han K-J, Li L-Y, Zhai Z, Shu H-B. VISA is an adapter protein required for virus-triggered IFN-beta signaling. *Mol Cell* 2005;19:727–40. [PubMed: 16153868]
- [52]. Seth RB, Sun L, Ea C-K, Chen ZJ. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. *Cell* 2005;122:669–82. [PubMed: 16125763]
- [53]. Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, et al. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat Immunol* 2005;6:981–8. [PubMed: 16127453]
- [54]. Dixit E, Boulant S, Zhang Y, Lee ASY, Odendall C, Shum B, et al. Peroxisomes are signaling platforms for antiviral innate immunity. *Cell* 2010;141:668–81. [PubMed: 20451243]
- [55]. Horner SM, Liu HM, Park HS, Briley J, Gale M. Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus. *Proc Natl Acad Sci U. S. A* 2011;108:14590–5. [PubMed: 21844353]
- [56]. Liu HM, Loo Y-M, Horner SM, Zornetzer GA, Katze MG, Gale M. The mitochondrial targeting chaperone 14-3-3e regulates a RIG-I translocon that mediates membrane association and innate antiviral immunity. *Cell Host Microbe* 2012;11:528–37. [PubMed: 22607805]
- [57]. Chan YK, Gack MU. A phosphomimetic-based mechanism of dengue virus to antagonize innate immunity. *Nat Immunol* 2016;17:523–30. [PubMed: 26998762]
- [58]. Kumar H, Kawai T, Kato H, Sato S, Takahashi K, Coban C, et al. Essential role of IPS-1 in innate immune responses against RNA viruses. *J Exp Med* 2006;203:1795–803. [PubMed: 16785313]
- [59]. Odendall C, Dixit E, Stavru F, Bierne H, Franz KM, Durbin AF, et al. Diverse intracellular pathogens activate type III interferon expression from peroxisomes. *Nat Immunol* 2014;15:717–26. [PubMed: 24952503]
- [60]. Sun Q, Sun L, Liu H-H, Chen X, Seth RB, Forman J, et al. The specific and essential role of MAVS in antiviral innate immune responses. *Immunity* 2006;24:633–42. [PubMed: 16713980]
- [61]. Li X, Sun L, Seth R. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. *Proc Natl Acad Sci U. S. A* 2005;99:17717–22.
- [62]. Abdullah Z, Schlee M, Roth S, Mraheil MA, Barchet W, Böttcher J, et al. RIG-I detects infection with live *Listeria* by sensing secreted bacterial nucleic acids. *Embo J* 2012;31:4153–64. [PubMed: 23064150]
- [63]. Chiu Y-H, MacMillan JB, Chen ZJ. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell* 2009;138:576–91. [PubMed: 19631370]
- [64]. Monroe KM, McWhirter SM, Vance RE. Identification of host cytosolic sensors and bacterial factors regulating the type I interferon response to *Legionella pneumophila*. *PLoS Pathog* 2009;5:e1000665. [PubMed: 19936053]
- [65]. Creasey EA, Isberg RR. The protein SdhA maintains the integrity of the *Legionella*-containing vacuole. *Proc Natl Acad Sci U. S. A* 2012;109: 3481–6. [PubMed: 22308473]
- [66]. Koshihara T, Yasukawa K, Yanagi Y, Kawabata S-I. Mitochondrial membrane potential is required for MAVS-mediated antiviral signaling. *Sci Signal* 2011;4:ra7. [PubMed: 21285412]
- [67]. Stavru F, Cossart P. *Listeria* infection modulates mitochondrial dynamics. *Commun Integr Biol* 2011;4:364–6. [PubMed: 21980582]
- [68]. Anand SK, Tikoo SK. Viruses as modulators of mitochondrial functions. *Adv Virol* 2013;2013:1–17.
- [69]. Chatel-Chaix L, Cortese M, Romero-Brey I, Bender S, Neufeldt CJ, Fischl W, et al. Dengue virus perturbs mitochondrial morphodynamics to dampen innate immune responses. *Cell Host Microbe* 2016;20: 342–56. [PubMed: 27545046]

- [70]. Odendall C, Kagan JC. The unique regulation and functions of type III interferons in antiviral immunity. *Curr Opin Virol* 2015;12:47–52. 10.1016/j.coviro.2015.02.003. [PubMed: 25771505]
- [71]. Odendall C, Kagan JC. Peroxisomes and the antiviral responses of Mammalian cells. *Subcell Biochem* 2013;69:67–75. [PubMed: 23821143]
- [72]. You J, Hou S, Malik-Soni N, Xu Z, Kumar A, Rachubinski RA, et al. Flavivirus infection impairs peroxisome biogenesis and early anti-viral signaling. *J Virol*. 2015 12;89(24):12349–61. [PubMed: 26423946]
- [73]. Dempsey A, Bowie AG. Innate immune recognition of DNA: a recent history. *Virology* 2015;479–480:146–52.
- [74]. Wu J, Sun L, Chen X, Du F, Shi H, Chen C, et al. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science* 2012;339:826–30. [PubMed: 23258412]
- [75]. Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science* 2012;339:786–91. [PubMed: 23258413]
- [76]. Unterholzner L, Keating SE, Baran M, Horan KA, Jensen SB, Sharma S, et al. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol* 2010;11:997–1004. [PubMed: 20890285]
- [77]. Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* 2009;458: 514–8. [PubMed: 19158675]
- [78]. Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature* 2008;455: 674–8. [PubMed: 18724357]
- [79]. Ishikawa H, Barber GN. The STING pathway and regulation of innate immune signaling in response to DNA pathogens. *Cell Mol Life Sci* 2010;68:1157–65. [PubMed: 21161320]
- [80]. Zhong B, Yang Y, Li S, Wang Y-Y, Li Y, Diao F, et al. The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immunity* 2008;29:538–50. [PubMed: 18818105]
- [81]. Burdette DL, Monroe KM, Sotelo-Troha K, Iwig JS, Eckert B, Hyodo M, et al. STING is a direct innate immune sensor of cyclic di-GMP. *Nature* 2011;478:1–5.
- [82]. Abe T, Barber GN. Cytosolic-DNA-mediated, STING-dependent proinflammatory gene induction necessitates canonical NF- κ B activation through TBK1. *J Virol* 2014;88:5328–41. [PubMed: 24600004]
- [83]. Chan YK, Gack MU. Viral evasion of intracellular DNA and RNA sensing. *Nat Rev Microbiol* 2016;14:360–73. [PubMed: 27174148]
- [84]. Dell'Oste V, Gatti D, Gugliesi F, De Andrea M, Bawadekar M, Cigno Lo I, et al. Innate nuclear sensor IFI16 translocates into the cytoplasm during the early stage of in vitro human cytomegalovirus infection and is entrapped in the egressing virions during the late stage. *J Virol* 2014; 88:6970–82. [PubMed: 24696486]
- [85]. Barber GN. STING-dependent cytosolic DNA sensing pathways. *Trends Immunol* 2014;35:88–93. [PubMed: 24309426]
- [86]. Burnaevskiy N, Fox TG, Plymire DA, Ertelt JM, Weigele BA, Selyunin AS, et al. Proteolytic elimination of N-myristoyl modifications by the Shigella virulence factor IpaJ. *Nature* 2013;496: 106–9. [PubMed: 23535599]
- [87]. Dong N, Zhu Y, Lu Q, Hu L, Zheng Y, Shao F. Structurally distinct bacterial TBC-like GAPs link Arf GTPase to Rab1 inactivation to counteract host defenses. *Cell* 2012;150:1029–41. [PubMed: 22939626]
- [88]. Dobbs N, Burnaevskiy N, Chen D, Gonugunta VK, Alto NM, Yan N. STING activation by translocation from the ER is associated with infection and autoinflammatory disease. *Cell Host Microbe* 2015;18: 157–68. [PubMed: 26235147]
- [89]. Nakamura N, Lill JR, Phung Q, Jiang Z, Bakalarski C, de Mazi ere A, et al. Endosomes are specialized platforms for bacterial sensing and NOD2 signalling. *Nature* 2014;509:240–4. [PubMed: 24695226]
- [90]. Irving AT, Mimuro H, Kufer TA, Lo C, Wheeler R, Turner LJ, et al. The immune receptor NOD1 and kinase RIP2 interact with bacterial peptidoglycan on early endosomes to promote autophagy and inflammatory signaling. *Cell Host Microbe* 2014;15:623–35. [PubMed: 24746552]

- [91]. Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol* 2016;16:407–20. [PubMed: 27291964]
- [92]. Kayagaki N, Wong MT, Stowe IB, Ramani SR, Gonzalez LC, Akashi-Takamura S, et al. Noncanonical inflammasome activation by intra-cellular LPS independent of TLR4. *Science* 2013;341:1246–9. [PubMed: 23887873]
- [93]. Kayagaki N, Warming S, Lamkanfi M, Vande Walle L, Louie S, Dong J, et al. Non-canonical inflammasome activation targets caspase-11. *Nature* 2011;479:117–21. [PubMed: 22002608]
- [94]. Shi J, Zhao Y, Wang Y, Gao W, Ding J, Li P, et al. Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature* 2014; 514:187–92. [PubMed: 25119034]
- [95]. Zhao Y, Yang J, Shi J, Gong Y-N, Lu Q, Xu H, et al. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature* 2011;477:596–600. [PubMed: 21918512]
- [96]. Kofoed EM, Vance RE. Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. *Nature* 2011;477: 592–5. [PubMed: 21874021]
- [97]. Latz E, Xiao TS, Stutz A. Activation and regulation of the inflammasomes. *Nat Rev Immunol* 2013;13:397–411. [PubMed: 23702978]
- [98]. Kayagaki N, Stowe IB, Lee BL, O'Rourke K, Anderson K, Warming S, et al. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 2015;526:666–71. [PubMed: 26375259]
- [99]. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 2015;526:660–5. [PubMed: 26375003]
- [100]. Brodsky IE, Palm NW, Sadanand S, Ryndak MB, Sutterwala FS, Flavell RA, et al. A Yersinia effector protein promotes virulence by preventing inflammasome recognition of the type III secretion system. *Cell Host Microbe* 2010;7:376–87. [PubMed: 20478539]
- [101]. Chung LK, Park YH, Zheng Y, Brodsky IE, Hearing P, Kastner DL, et al. The Yersinia virulence factor YopM hijacks host kinases to inhibit type III effector-triggered activation of the pyrin inflammasome. *Cell Host Microbe* 2016;20:296–306. [PubMed: 27569559]
- [102]. Johnston JB, Barrett JW, Nazarian SH, Goodwin M, Ricciuto D, Ricuttio D, et al. A poxvirus-encoded pyrin domain protein interacts with ASC-1 to inhibit host inflammatory and apoptotic responses to infection. *Immunity* 2005;23:587–98. [PubMed: 16356857]
- [103]. Dorfleutner A, Talbott SJ, Bryan NB, Funya KN, Rellick SL, Reed JC, et al. A Shope Fibroma virus PYRIN-only protein modulates the host immune response. *Virus Genes* 2007;35:685–94. [PubMed: 17676277]
- [104]. Komune N, Ichinohe T, Ito M, Yanagi Y. Measles virus V protein inhibits NLRP3 inflammasome-mediated interleukin-1 β secretion. *J Virol* 2011;85:13019–26. [PubMed: 21994456]

Fig. 1.

Localization of receptors and adaptors, and their targeting by virulence factors. Pattern recognition receptors are localized at various sub-cellular localizations: Toll-like receptors (TLRs) are at the cell surface and endosomes, RIG-I-like receptors (RLRs), cGAS, and inflammasomes are in the cytosol, IFI16 is in the nucleus. Signaling occurs at membranes: TLR recruit the adaptors TIRAP, MyD88, TRAM and TRIF. RLR signaling occurs via the adaptor MAVS that is localized on mitochondria and peroxisomes. DNA sensors (here shown are cGAS and IFI16) occurs via STING that is localized in the ER at steady state and traffics through the Golgi via the ER-Golgi intermediate compartment (ERGIC). Additional proteins are involved in the trafficking of receptors and adaptors. These include for CD14 that mediates TLR4 internalization, UNC93B that mediates TLR trafficking and 14-3-3ε that contributes to RIG-I migration to mitochondria. Examples of virulence factors are indicated in blue. These alter the subcellular localization of host proteins, either at steady state or following stimulation. Included for example are the type III-secreted *Shigella* proteins IpaJ and VirA that affect different steps of STING trafficking through the ERGIC. RLR signaling is inhibited by Hepatitis C virus (HCV) NS3/4a that cleaves MAVS or NS3 from Dengue Virus (DenV) that prevents 14-3-3ε mediated RIG-I trafficking to mitochondria. Also included is *Salmonella* SopB that cleaves lipids necessary for TIRAP localization, or *Francisella* LPS that is not detected by CD14, a step required for TLR4

endocytosis and function. Finally, Measles virus V protein affects the localization of IFI16 and some inflammasome components, and Coxsackievirus virus 3C protein cleaves TRIF.

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