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Tripping the wire: sensing of viral protease activity by CARD8 and NLRP1 inflammasomes

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Abstract

Host innate immune sensors are vital for the initial detection of pathogen infection. Such sensors thus need to constantly adapt in escalating evolutionary arms races with pathogens. Recently, two inflammasome-forming proteins, CARD8 and NLRP1, have emerged as innate immune sensors for the enzymatic activity of virus-encoded proteases. When cleaved within a rapidly evolving ‘tripwire’ region, CARD8 and NLRP1 assemble into inflammasomes that initiate pyroptotic cell death and pro-inflammatory cytokine release as a form of effector-triggered immunity (ETI). Short motifs in the CARD8 and NLRP1 tripwires mimic the protease-specific cleavage sites of picornaviruses, coronaviruses, and HIV-1, providing virus-specific sensing that can rapidly change between closely related hosts and within the human population. Recent work highlights the evolutionary arms races between viral proteases and NLRP1 and CARD8, including insights into the mechanisms of inflammasome activation, host diversity of viral sensing, and means that viruses have evolved to avoid tripping the wire.

Introduction

Viruses and their hosts are locked in molecular arms races that lead to rapid evolution at host-virus interfaces [1–3]. Such evolutionary conflicts drive innovations in host antiviral mechanisms and select for adaptations that evade viral counterstrategies. Importantly, the diversity that results from these host-virus arms races shapes host-specific immune responses, the ability of viruses to ‘jump’ species, and the susceptibility of humans to emerging zoonotic viruses [4,5]. Even within humans, variability in innate immune-associated genes impacts viral susceptibility and pathogenesis, indicating that human polymorphism is an important but understudied determinant of severity of infectious diseases [6].

A central function of the innate immune system is the ability to sense and respond to viral infection. Extensive research has focused on the roles of innate immune sensing through the recognition of highly conserved pathogen-associated molecular patterns (PAMPs) such as double-stranded RNA (dsRNA) [7]. However, there is increasing appreciation of the role of mammalian effector-triggered immunity (ETI), which can sense the activity of pathogen-encoded effectors, such as toxins and proteases [8–11]. Although less broadly conserved

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than PAMPs (e.g. all RNA viruses generate dsRNA), pathogen-encoded activities sensed by ETIs are often important for pathogen fitness and are therefore evolutionary constrained in a manner that allows ETI sensors to be effective at detecting a wide diversity of pathogens [8].

Recently, two inflammasome proteins, NLRP1 and CARD8, have emerged as ETI sensors of the enzymatic activities of diverse viral proteases. Proteases are encoded by many human viruses, including +ssRNA viruses in *Coronaviridae* (e.g. SARS-CoV-2), *Picornaviridae* (e.g. enteroviruses, rhinovirus), and *Flaviviridae* (e.g. dengue virus, hepatitis C virus) families, as well as members of *Retroviridae* (e.g. HIV-1). These proteases are critical to viral propagation, as they both liberate functional proteins from virally-encoded polyproteins and cleave host factors to further facilitate viral replication [12–14]. Importantly, viral protease specificity is constrained to cleave the viral polyprotein at multiple sequence-specific locations, limiting protease evolvability [12]. Taking advantage of this evolutionary constraint on viral protease substrate specificity, NLRP1 and CARD8 have evolved N-terminal disordered ‘tripwire’ sequences that mimic the preferred cleavage sequences of several virus-encoded proteases. Cleavage of NLRP1 and CARD8 at these sites results in immune activation and cell death in a virus- and host-specific manner [15–21] (Table 1). Here we discuss the mechanism and outcomes of tripwire ETI sensing of protease activity by NLRP1 and CARD8, the consequences of host and viral evolution on inflammasome sensing of protease activity, and additional possibilities for ETI sensing of viral proteases.

NLRP1 and CARD8 are innate immune ETI sensors activated by viral protease cleavage

NLRP1 and CARD8 are homologous proteins that each assemble into an inflammasome, a cytoplasmic immune complex whose activation triggers pyroptotic cell death and release of pro-inflammatory cytokines, including IL-1β and IL-18 [22–26] (Figure 1A). The first indication that NLRP1/CARD8 homologs may contribute to ETI sensing of pathogen infection was observed in mice. Mouse NLRP1B is cleaved in a disordered N-terminal region, which we refer to as the ‘tripwire’, by the secreted Lethal Factor (LF) protease from *Bacillus anthracis*, resulting in protective immune response against bacterial challenge [27,28]. The mechanism by which LF protease cleavage leads to inflammasome activation has been termed ‘functional degradation’ [29,30] (Figure 1B). Important for this mechanism of ETI sensing is the unusual domain architecture of NLRP1 and CARD8, in which the C-terminal end comprises a function-to-find domain (FIIND) followed by a caspase activation and recruitment domain (CARD) [24,26]. A constitutive self-cleavage event occurs in the FIIND, separating the protein into an N-terminal ‘sensing’ region and a C-terminal CARD-containing region that remain non-covalently associated [31–33]. Following cleavage by pathogen-encoded proteases like LF, the new N-terminus serves as an N-degron for ubiquitylation and subsequent proteasomal degradation by the ‘N-end rule’ pathway, thus liberating the CARD-containing C-terminus to self-oligomerize and recruit and activate Caspase-1 (CASP1) [29,30]. CASP1 initiates pyroptotic cell death through cleavage of gasdermin D (GSDMD) and inflammatory cytokine signaling through pro-inflammatory cytokine (IL-1β and IL-18) processing [24,26,29,30]. Thus, functional degradation of the N-terminus following tripwire cleavage is mechanistically tied to the release of a CARD-containing C-terminus and activation of pyroptosis and inflammation (Figure 1B). Notably, NLRP1/CARD8 homologs differ in their N-terminal domains (Figure 1A), which may be important for homolog-specific functions outside of ETI sensing, and human NLRP1, but not mouse NLRP1 or human CARD8, requires ASC for the recruitment and activation of CASP1[18,34–38]. Irrespective of these differences, all homologs have a conserved C-terminal FIIND-CARD architecture that enables ETI sensing by functional degradation.

This system of activation by functional degradation suggested that NLRP1 and CARD8 may have the capacity to sense a variety of pathogen-encoded proteases beyond LF. Indeed, NLRP1 and CARD8 can sense cleavage by proteases encoded by diverse viruses from at least three distinct families: *Picornaviridae*, *Coronaviridae*, and *Retroviridae* [15–21] (Table 1). For instance, the disordered human NLRP1 tripwire region, analogous to the LF-sensing tripwire region in mouse NLRP1B, contains a mimic of the preferred cleavage sequence of the 3C protease ($3C^{pro}$) encoded by enteroviruses such as human rhinovirus (HRV) and coxsackievirus B3 (CVB3) within *Picornaviridae* [15,16] (Figure 2A). Viral protease-mediated cleavage within this human NLRP1 tripwire activates the inflammasome and results in pro-inflammatory cytokine release and pyroptotic cell death during HRV infection of primary human airway epithelial cells [15] or CVB3 infection of human keratinocytes [16]. NLRP1 also contains a cleavage site within the NACHT domain for the 3CL protease ($3CL^{pro}$) of coronaviruses, resulting in cell death upon SARS-CoV-2 infection, which induces a non-canonical pathway involving CASP3, CASP8, and GSDME [17]. Similarly, CARD8 can sense a diverse array of viral proteases through protease site mimics in a disordered N-terminal tripwire region (Figure 2B). For instance, HIV-1 protease cleavage of CARD8 results in pyroptotic cell death and pro-inflammatory cytokine release in HIV-1 infected immune cells, which can both eliminate latently infected cells [18,39] and prevent HIV-1 infection [20]. CARD8 also contains a site mimic for both picornavirus $3C^{pro}$ and coronavirus $3CL^{pro}$, resulting in inflammasome-mediated death of SARS-CoV-2 infected human monocytes [19] and CVB3-infected cardiomyocytes [21]. These results establish the importance of human NLRP1 and CARD8 at ETI sensors of diverse viral infections. However, as described below, the molecular arms race between inflammasome sensors and viral proteases is ongoing, and both host and virus evolution have the capacity to turn the tables in the arms race.

Rapid NLRP1 and CARD8 tripwire evolution results in host-specific sensing of viral infection.

The NLRP1/CARD8 mechanism for recognition of viral proteases relies on the presence of viral polyprotein cleavage site mimics in the disordered tripwire region, suggesting such sequences may evolve rapidly and recurrently. Indeed, both NLRP1 and CARD8 are evolving under strong positive (diversifying) selection in their respective disordered tripwire regions [19,34]. This suggests that evolution of the tripwire region is driven by host-virus arms races in which host evolution of short linear motifs (SLiMs), specifically viral protease cleavage site mimics, allows hosts to exploit the evolutionarily constrained viral protease to gain a selective advantage [12]. For instance, phylogenetic analyses of NLRP1 revealed that the $3C^{pro}$ cleavage site mimic in NLRP1 evolved in the primate lineage, but is lacking in other mammals [16] (Figure 2C). Importantly, genetic and functional differences in the tripwire region are observed among closely related primate species, and even a single-nucleotide polymorphism (SNP) within the human population can prevent cleavage and inflammasome activation by enterovirus 3C^{pros} [16] (Figure 2C). Human NLRP1 also contains sites elsewhere in the tripwire that can be cleaved by 3C^{pros} from non-enterovirus picornaviruses, suggestive of independent acquisition of novel virus sensing functions. Similarly, NLRP1 homologs in other species, such as mice, have convergently evolved site mimics for 3C^{pros} [16]. Together, these data suggest that novel SLiMs can be easily evolved in protein sequences [40] and that ETI tripwires are a robust and highly evolvable system that underlies host- and virus-specific protease recognition. While the divergent pathogen specificity of NLRP1 sensing between different host species may appear paradoxical at first, rapid sequence evolution and changes in pathogen specificity are hallmarks of innate immune sensors that are engaged in arms races with pathogens [1,2,5,8], providing further evidence for the importance of NLRP1 in ETI sensing.

The tripwire region of CARD8 is also rapidly evolving under positive selection and is cleaved and activated by viral proteases in a virus- and host-specific manner [19]. Proteases from endemic and pandemic human coronaviruses, including SARS-CoV-2, cleave and activate human CARD8, but the site mimic is lacking in other publicly available primate CARD8 sequences, and many species, including rodents, lack CARD8 altogether [19] (Figure 2D). Intriguingly, bats are one group of species that lack CARD8-mediated 3CL^{pro} sensing. Like rodents, microbats lack a CARD8 ortholog, and CARD8 from the megabat, *Rousettus aegypticus*, is not activated by 3CL^{pros}, suggesting one mechanism of viral tolerance in this important reservoir of coronaviruses [19]. Additionally, a SNP found at >20% frequency in some human populations attenuates cleavage and activation of CARD8 by coronavirus 3CL^{pro} [19]. Strikingly, this same SNP potentiates the ability of CARD8 to sense 3C^{pros} from some picornaviruses, including enteroviruses such as HRV. Thus, a single amino acid substitution within the tripwire region in humans toggles CARD8 sensing of protease activity from one family of respiratory viruses, coronaviruses, to another, enteroviruses [19]. Finally, human CARD8 contains a site unique among known primate sequences that confers the ability to sense proteases from HIV-1 and a related simian immunodeficiency virus from chimpanzees (SIVcpz) (Figure 2D) [20]. Although the selective pressure that drove this change in human CARD8 is unknown, these data suggest that the functional consequences of CARD8 evolution may be a potential mechanism for differential pathogenesis of lentiviruses among primates. Together, the evolutionary dynamics of NLRP1 and CARD8 across species and within humans reveals that ETI sensing is influenced by host genetic diversity, which can in turn possibly impact host susceptibility to viral infection and viral pathogenesis.

The virus strikes back: protease evolution leads to evasion or antagonism of inflammasome sensing

While deeply constrained by the need to retain cleavage of multiple independent polyprotein sites, viral proteases do evolve in a manner that affects their sequence specificity [12] and these changes impact NLRP1 and CARD8-mediated sensing. For instance, divergence of picornavirus 3C^{pros} results in differential ability to be sensed by human NLRP1 and mouse NLRP1B [16] (Figure 3A). This has the functional consequence of human NLRP1 inflammasome sensing and activation in response to infection by one picornavirus, CVB3, but not by another picornavirus, EMCV [16]. Intriguingly, protease specificity evolution can not only evade sensing by NLRP1 but can also actively antagonize NLRP1 inflammasome function. As one example, several non-enterovirus 3C^{pros} can attenuate NLRP1 function when it is activated by a heterologous protease. Although the mechanism for this antagonism is unknown, these proteases do cleave NLRP1 near or within the FIIND-CARD C-terminus [16]. In addition, both 3C^{pro} and 3CL^{pro} can cleave and inactivate GSDMD, thus antagonizing inflammasome-mediated pyroptosis [17,41]. These data suggest that protease evolution can not only result in lack of tripwire cleavage, but also in cleavage of novel sites that may antagonize inflammasome function (Figure 3A). Similar antagonistic effects of viral proteases are seen with megabat CARD8 by coronavirus 3CL^{pros}. In this case, the site of 3CL^{pro} cleavage within the C-terminal region of megabat CARD8 is specific to the megabat lineage [19], providing another example of host-specific inflammasome antagonism by viral proteases. The need to evade or antagonize ETI sensors may therefore be one driving force for diversification of viral proteases that are otherwise expected to evolve very slowly [12].

Just the beginning: outstanding questions and additional innate immune sensors of viral proteases

Much remains to be explored at the intersection of the ongoing molecular arms race between viral proteases and host ETI sensors. For instance, expression of NLRP1 and CARD8 varies across tissues and cell types [23,42,43], and other host factors such as DPP8/9 impact responsiveness of NLRP1 and CARD8 [44–46]. This indicates that the inflammasome response may be an important but underexplored determinant of tissue tropism of viral replication and pathogenesis. Likewise, the consequences of viral protease-mediated activation of NLRP1 and CARD8 on systemic viral replication and immunopathology in humans remains to be elucidated. Development of animal models for NLRP1/CARD8 inflammasome sensing of viral infection, possibly taking advantage of the one known example of mouse NLRP1B sensing of viral proteases [19], will be critical for assessing the *in vivo* contribution of ETI inflammasome activation on viral control and pathogenesis. Additionally, human polymorphism of NLRP1 contributes to autoinflammatory diseases [46–50], but how this human genetic variation impacts protease or pathogen sensing is not known. Finally, although ETI sensing appears to be the unifying function of both CARD8 and NLRP1, other sensing functions have been described for these inflammasome proteins, including activation of NLRP1 by dsRNA and ribotoxic stress during infection [51–53]. Whether there is a common mechanism relating these functions and protease sensing, or whether they are independent, remains to be determined.

As the role of ETI in animal innate immunity is increasingly appreciated, it is tempting to speculate that host evolution of protease cleavage site mimics might be a broadly employed strategy in host-pathogen arms races (Figure 3B). For instance, within pyroptotic cell death pathways, both IL-1β and GSDMA can be directly cleaved and activated by bacterial proteases to provide antibacterial immunity [54–56]. Similarly, GSDMD can be directly cleaved near its natural site of CASP1-mediated maturation by Zika virus protease to initiate pyroptotic cell death [57]. Whether other inflammasome sensors from the NLR superfamily also contain a tripwire region sensitive to virally-encoded protease cleavage is unknown. However, other NLR inflammasomes sensors do not share the unique domain architecture seen in NLRP1 and CARD8, where the CARD is at the C-terminus and separated from the rest of the protein by the self-cleaving FIIND (Figure 1A). In fact, many NLR inflammasome sensors contain a CARD at the N-terminus or not at all [58]. Thus, it is unlikely that cleavage of these sensors would result in activation by functional degradation, although cleavage of these sensors could result in activation via an unknown mechanism. More broadly, a variety of innate immune proteins have been described as ‘guards’ that could represent ETI protease sensors, similar to what has been observed with ETI sensing in plants [8,9,59]. For instance, CVB3 $3C^{pro}$ cleaves an inhibitor of NF-κB signaling, IκBα, resulting in increased apoptosis and decreased viral replication [60]. These examples suggest that NLRP1 and CARD8 are likely just the first of many innate immune sensors that mimic viral protease cleavage sites to promote an immune response. We envision that further studies will not only uncover novel mechanisms of effector-triggered immunity in humans but also strategies employed by viruses to escape them. Understanding how such ETI-protease interactions evolve across species will shed further light on virus-specific and host-specific determinants of host range, tissue tropism, pathogenesis, and chance of virus spillover into humans.

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References

* - of special interest

** - of outstanding interest

1. Daugherty MD, Malik HS: Rules of engagement: molecular insights from host-virus arms races. Annu Rev Genet 2012, 46:677–700. [PubMed: 23145935]
2. Sironi M, Cagliani R, Forni D, Clerici M: Evolutionary insights into host-pathogen interactions from mammalian sequence data. Nat Rev Genet 2015, 16:224–236. [PubMed: 25783448]
3. Enard D, Cai L, Gwennap C, Petrov DA: Viruses are a dominant driver of protein adaptation in mammals. Elife 2016, 5.
4. Warren CJ, Sawyer SL: How host genetics dictates successful viral zoonosis. PLoS Biol 2019, 17:e3000217. [PubMed: 31002666]
5. Rothenburg S, Brennan G: Species-Specific Host-Virus Interactions: Implications for Viral Host Range and Virulence. Trends Microbiol 2020, 28:46–56. [PubMed: 31597598]
6. Casanova JL, Abel L: From rare disorders of immunity to common determinants of infection: Following the mechanistic thread. Cell 2022, 185:3086–3103. [PubMed: 35985287]
7. Medzhitov R, Janeway CA Jr.: Innate immunity: the virtues of a nonclonal system of recognition. Cell 1997, 91:295–298. [PubMed: 9363937]
8. Remick BC, Gaidt MM, Vance RE: Effector-Triggered Immunity. Annu Rev Immunol 2022.
9. Jones JD, Vance RE, Dangl JL: Intracellular innate immune surveillance devices in plants and animals. Science 2016, 354.
10. Lopes Fischer N, Naseer N, Shin S, Brodsky IE: Effector-triggered immunity and pathogen sensing in metazoans. Nat Microbiol 2020, 5:14–26. [PubMed: 31857733]

11. Orzalli MH, Parameswaran P: Effector-triggered immunity in mammalian antiviral defense. Trends Immunol 2022, 43:1006–1017. [PubMed: 36369102]

12. Tsu BV, Fay EJ, Nguyen KT, Corley MR, Hosuru B, Dominguez VA, Daugherty MD: Running With Scissors: Evolutionary Conflicts Between Viral Proteases and the Host Immune System. Front Immunol 2021, 12:769543. [PubMed: 34790204]

13. Chin CV, Saeed M: Surgical Strikes on Host Defenses: Role of the Viral Protease Activity in Innate Immune Antagonism. Pathogens 2022, 11. [PubMed: 36678359]

14. Lei J, Hilgenfeld R: RNA-virus proteases counteracting host innate immunity. FEBS Lett 2017, 591:3190–3210. [PubMed: 28850669]

15. Robinson KS, Teo DET, Tan KS, Toh GA, Ong HH, Lim CK, Lay K, Au BV, Lew TS, Chu JJH, et al. : Enteroviral 3C protease activates the human NLRP1 inflammasome in airway epithelia. Science 2020, 370. [PubMed: 32703862] ** - This paper was the first demonstration that human NLRP1 could respond to viral infection by detecting viral protease activity. Robinson et al. show that during human rhinovirus infection in primary human airway epithelial cells, the virally-encoded 3C protease cleaves NLRP1 at a specific sequence to activate the NLRP1 inflammasome via the ‘functional degradation’ mechanism.

16. Tsu BV, Beierschmitt C, Ryan AP, Agarwal R, Mitchell PS, Daugherty MD: Diverse viral proteases activate the NLRP1 inflammasome. Elife 2021, 10.* - This paper describes the host and viral determinants of NLRP1 cleavage and activation by a wide range of picornavirus 3C proteases. This paper also reveals that some 3C proteases antagonize, rather than activate, the NLRP1 inflammasome.

17. Planes R, Pinilla M, Santoni K, Hessel A, Passemar C, Lay K, Paillette P, Valadao AC, Robinson KS, Bastard P, et al. : Human NLRP1 is a sensor of pathogenic coronavirus 3CL proteases in lung epithelial cells. Mol Cell 2022, 82:2385–2400 e2389. [PubMed: 35594856] * - This paper describes the cleavage and activation of NLRP1 by coronavirus 3CL protease during virus infection. This work also identifies that 3CL protease cleaves GSDMD, resulting in non-canonical inflammasome activation requiring CASP3, CASP8, and GSDME.

18. Wang Q, Gao H, Clark KM, Mugisha CS, Davis K, Tang JP, Harlan GH, DeSelm CJ, Presti RM, Kutluay SB, et al. : CARD8 is an inflammasome sensor for HIV-1 protease activity. Science 2021, 371.** - This paper is the first to identify CARD8 as an inflammasome sensor of protease activity. Using small molecule treatment to stimulate cytoplasmic HIV-1 protease activity, Wang et al. demonstrate that CARD8 is cleavage results in inflammasome activation and clearance of latently infected immune cells.

19. Tsu BV, Agarwal R, Gokhale NS, Kulsuptrakul J, Ryan AP, Fay EJ, Castro LK, Beierschmitt CM, Yap C, Turcotte EA, Delgado-Rodriguez SE, Vance RE, et al. : Host specific sensing of coronaviruses and picornaviruses by the CARD8 inflammasome. PLoS Biol 2023, In press.** - This paper demonstrates that CARD8 can be cleaved and activated in a host-specific manner by coronavirus 3CL proteases and picornavirus 3C proteases. In addition to characterizing differences between CARD8s from different species, Tsu et al. demonstrate that within humans, a high frequency polymorphism results in CARD8 toggling between sensing coronavirus proteases to sensing enterovirus proteases.

20. Kulsuptrakul J, Turcotte EA, Emerman M, Mitchell PA: A human-specific motif facilitates CARD8 inflammasome activation after HIV-1 infection. bioRxiv 2022.* - This paper describes the host and viral specificity of CARD8 cleavage and activation by proteases from HIV-1 and a related SIV from chimpanzees, SIVcpz. This paper also identifies that CARD8 inflammasome activation prevents HIV-1 infection of human cells.

21. Nadkarni R, Chu WC, Lee CQE, Mohamud Y, Yap L, Toh GA, Beh S, Lim R, Fan YM, Zhang YL, et al. : Viral proteases activate the CARD8 inflammasome in the human cardiovascular system. J Exp Med 2022, 219.* - This paper describes the cleavage and activation of CARD8 by enterovirus 3C protease during coxsackievirus B3 infection. This work also identifies that CARD8 sensing of viral infection can occur in endothelial cells and cardiomyocytes.

22. Broz P, Dixit VM: Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol 2016, 16:407–420. [PubMed: 27291964]

23. Johnson DC, Taabazuing CY, Okondo MC, Chui AJ, Rao SD, Brown FC, Reed C, Peguero E, de Stanchina E, Kentsis A, et al. : DPP8/DPP9 inhibitor-induced pyroptosis for treatment of acute myeloid leukemia. Nat Med 2018, 24:1151–1156. [PubMed: 29967349]
24. Taabazuing CY, Griswold AR, Bachovchin DA: The NLRP1 and CARD8 inflammasomes. Immunol Rev 2020.
25. Martinon F, Burns K, Tschopp J: The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 2002, 10:417–426. [PubMed: 12191486]
26. Mitchell PS, Sandstrom A, Vance RE: The NLRP1 inflammasome: new mechanistic insights and unresolved mysteries. Curr Opin Immunol 2019, 60:37–45. [PubMed: 31121538]
27. Moayeri M, Crown D, Newman ZL, Okugawa S, Eckhaus M, Cataisson C, Liu S, Sastalla I, Leppla SH: Inflammasome sensor Nlrp1b-dependent resistance to anthrax is mediated by caspase-1, IL-1 signaling and neutrophil recruitment. PLoS Pathog 2010, 6:e1001222. [PubMed: 21170303]
28. Terra JK, Cote CK, France B, Jenkins AL, Bozue JA, Welkos SL, LeVine SM, Bradley KA: Cutting edge: resistance to Bacillus anthracis infection mediated by a lethal toxin sensitive allele of Nalp1b/Nlrp1b. J Immunol 2010, 184:17–20. [PubMed: 19949100]
29. Chui AJ, Okondo MC, Rao SD, Gai K, Griswold AR, Johnson DC, Ball DP, Taabazuing CY, Orth EL, Vittimberga BA, et al. : N-terminal degradation activates the NLRP1B inflammasome. Science 2019, 364:82–85. [PubMed: 30872531]
30. Sandstrom A, Mitchell PS, Goers L, Mu EW, Lesser CF, Vance RE: Functional degradation: A mechanism of NLRP1 inflammasome activation by diverse pathogen enzymes. Science 2019, 364.
31. D'Osualdo A, Weichenberger CX, Wagner RN, Godzik A, Wooley J, Reed JC: CARD8 and NLRP1 undergo autoproteolytic processing through a ZU5-like domain. PLoS One 2011, 6:e27396. [PubMed: 22087307]
32. Finger JN, Lich JD, Dare LC, Cook MN, Brown KK, Duraiswami C, Bertin J, Gough PJ: Autolytic proteolysis within the function to find domain (FIIND) is required for NLRP1 inflammasome activity. J Biol Chem 2012, 287:25030–25037. [PubMed: 22665479]
33. Frew BC, Joag VR, Mogridge J: Proteolytic processing of Nlrp1b is required for inflammasome activity. PLoS Pathog 2012, 8:e1002659. [PubMed: 22536155]
34. Chavarria-Smith J, Mitchell PS, Ho AM, Daugherty MD, Vance RE: Functional and Evolutionary Analyses Identify Proteolysis as a General Mechanism for NLRP1 Inflammasome Activation. PLoS Pathog 2016, 12:e1006052. [PubMed: 27926929]
35. Linder A, Bauernfried S, Cheng Y, Albanese M, Jung C, Keppler OT, Hornung V: CARD8 inflammasome activation triggers pyroptosis in human T cells. EMBO J 2020, 39:e105071. [PubMed: 32840892]
36. Ball DP, Taabazuing CY, Griswold AR, Orth EL, Rao SD, Kotliar IB, Vostal LE, Johnson DC, Bachovchin DA: Caspase-1 interdomain linker cleavage is required for pyroptosis. Life Sci Alliance 2020, 3.
37. Guey B, Bodnar M, Manie SN, Tardivel A, Petrilli V: Caspase-1 autoproteolysis is differentially required for NLRP1b and NLRP3 inflammasome function. Proc Natl Acad Sci U S A 2014, 111:17254–17259. [PubMed: 25404286]
38. Van Opdenbosch N, Gurung P, Vande Walle L, Fossoul A, Kanneganti TD, Lamkanfi M: Activation of the NLRP1b inflammasome independently of ASC-mediated caspase-1 autoproteolysis and speck formation. Nat Commun 2014, 5:3209. [PubMed: 24492532]
39. Clark KM, Kim JG, Wang Q, Gao H, Presti RM, Shan L: Chemical inhibition of DPP9 sensitizes the CARD8 inflammasome in HIV-1-infected cells. Nat Chem Biol 2022.
40. Chemes LB, de Prat-Gay G, Sanchez IE: Convergent evolution and mimicry of protein linear motifs in host-pathogen interactions. Curr Opin Struct Biol 2015, 32:91–101. [PubMed: 25863584]
41. Lei X, Zhang Z, Xiao X, Qi J, He B, Wang J: Enterovirus 71 Inhibits Pyroptosis through Cleavage of Gasdermin D. J Virol 2017, 91.

42. Johnson DC, Okondo MC, Orth EL, Rao SD, Huang HC, Ball DP, Bachovchin DA: DPP8/9 inhibitors activate the CARD8 inflammasome in resting lymphocytes. Cell Death Dis 2020, 11:628. [PubMed: 32796818]
43. Sand J, Haertel E, Biedermann T, Contassot E, Reichmann E, French LE, Werner S, Beer HD: Expression of inflammasome proteins and inflammasome activation occurs in human, but not in murine keratinocytes. Cell Death Dis 2018, 9:24. [PubMed: 29348630]
44. Hollingsworth LR, Sharif H, Griswold AR, Fontana P, Mintseris J, Dagbay KB, Paulo JA, Gygi SP, Bachovchin DA, Wu H: DPP9 sequesters the C terminus of NLRP1 to repress inflammasome activation. Nature 2021, 592:778–783. [PubMed: 33731932]
45. Sharif H, Hollingsworth LR, Griswold AR, Hsiao JC, Wang Q, Bachovchin DA, Wu H: Dipeptidyl peptidase 9 sets a threshold for CARD8 inflammasome formation by sequestering its active C-terminal fragment. Immunity 2021, 54:1392–1404 e1310. [PubMed: 34019797]
46. Zhong FL, Robinson K, Teo DET, Tan KY, Lim C, Harapas CR, Yu CH, Xie WH, Sobota RM, Au VB, et al. : Human DPP9 represses NLRP1 inflammasome and protects against autoinflammatory diseases via both peptidase activity and FIIND domain binding. J Biol Chem 2018, 293:18864–18878. [PubMed: 30291141]
47. Grandemange S, Sanchez E, Louis-Plence P, Tran Mau-Them F, Bessis D, Coubes C, Frouin E, Seyger M, Girard M, Puechberty J, et al. : A new autoinflammatory and autoimmune syndrome associated with NLRP1 mutations: NAIAD (NLRP1-associated autoinflammation with arthritis and dyskeratosis). Ann Rheum Dis 2017, 76:1191–1198. [PubMed: 27965258]
48. Herlin T, Jorgensen SE, Host C, Mitchell PS, Christensen MH, Laustsen M, Larsen DA, Schmidt FI, Christiansen M, Mogensen TH: Autoinflammatory disease with corneal and mucosal dyskeratosis caused by a novel NLRP1 variant. Rheumatology (Oxford) 2020, 59:2334–2339. [PubMed: 31873740]
49. Zhong FL, Mamai O, Sborgi L, Boussofara L, Hopkins R, Robinson K, Szeverenyi I, Takeichi T, Balaji R, Lau A, et al. : Germline NLRP1 Mutations Cause Skin Inflammatory and Cancer Susceptibility Syndromes via Inflammasome Activation. Cell 2016, 167:187–202 e117. [PubMed: 27662089]
50. Drutman SB, Haerynck F, Zhong FL, Hum D, Hernandez NJ, Belkaya S, Rapaport F, de Jong SJ, Creytens D, Tavernier SJ, et al. : Homozygous NLRP1 gain-of-function mutation in siblings with a syndromic form of recurrent respiratory papillomatosis. Proc Natl Acad Sci U S A 2019, 116:19055–19063. [PubMed: 31484767]
51. Jenster LM, Lange KE, Normann S, vom Hemdt A, Wuerth JD, Schiffelers LDJ, Tesfamariam YM, Gohr FN, Klein L, Kaltheuner IH, et al. : P38 kinases mediate NLRP1 inflammasome activation after ribotoxic stress response and virus infection. J Exp Med 2023, 220.
52. Robinson KS, Toh GA, Rozario P, Chua R, Bauernfried S, Sun Z, Firdaus MJ, Bayat S, Nadkarni R, Poh ZS, et al. : ZAKalpha-driven ribotoxic stress response activates the human NLRP1 inflammasome. Science 2022, 377:328–335. [PubMed: 35857590]
53. Bauernfried S, Scherr MJ, Pichlmair A, Duderstadt KE, Hornung V: Human NLRP1 is a sensor for double-stranded RNA. Science 2021, 371.
54. LaRock CN, Todd J, LaRock DL, Olson J, O’Donoghue AJ, Robertson AA, Cooper MA, Hoffman HM, Nizet V: IL-1beta is an innate immune sensor of microbial proteolysis. Sci Immunol 2016, 1.
55. Deng W, Bai Y, Deng F, Pan Y, Mei S, Zheng Z, Min R, Wu Z, Li W, Miao R, et al. : Streptococcal pyrogenic exotoxin B cleaves GSDMA and triggers pyroptosis. Nature 2022, 602:496–502. [PubMed: 35110732]
56. LaRock DL, Johnson AF, Wilde S, Sands JS, Monteiro MP, LaRock CN: Group A Streptococcus induces GSDMA-dependent pyroptosis in keratinocytes. Nature 2022, 605:527–531. [PubMed: 35545676]
57. Yamaoka Y, Matsunaga S, Jeremiah SS, Nishi M, Miyakawa K, Morita T, Khatun H, Shimizu H, Okabe N, Kimura H, et al. : Zika virus protease induces caspase-independent pyroptotic cell death by directly cleaving gasdermin D. Biochem Biophys Res Commun 2021, 534:666–671. [PubMed: 33208231]
58. Tschopp J, Martinon F, Burns K: NALPs: a novel protein family involved in inflammation. Nat Rev Mol Cell Biol 2003, 4:95–104. [PubMed: 12563287]

59. Cui H, Tsuda K, Parker JE: Effector-triggered immunity: from pathogen perception to robust defense. Annu Rev Plant Biol 2015, 66:487–511. [PubMed: 25494461]

60. Zaragoza C, Saura M, Padalko EY, Lopez-Rivera E, Lizarbe TR, Lamas S, Lowenstein CJ: Viral protease cleavage of inhibitor of kappaBalpha triggers host cell apoptosis. Proc Natl Acad Sci U S A 2006, 103:19051–19056. [PubMed: 17138672]

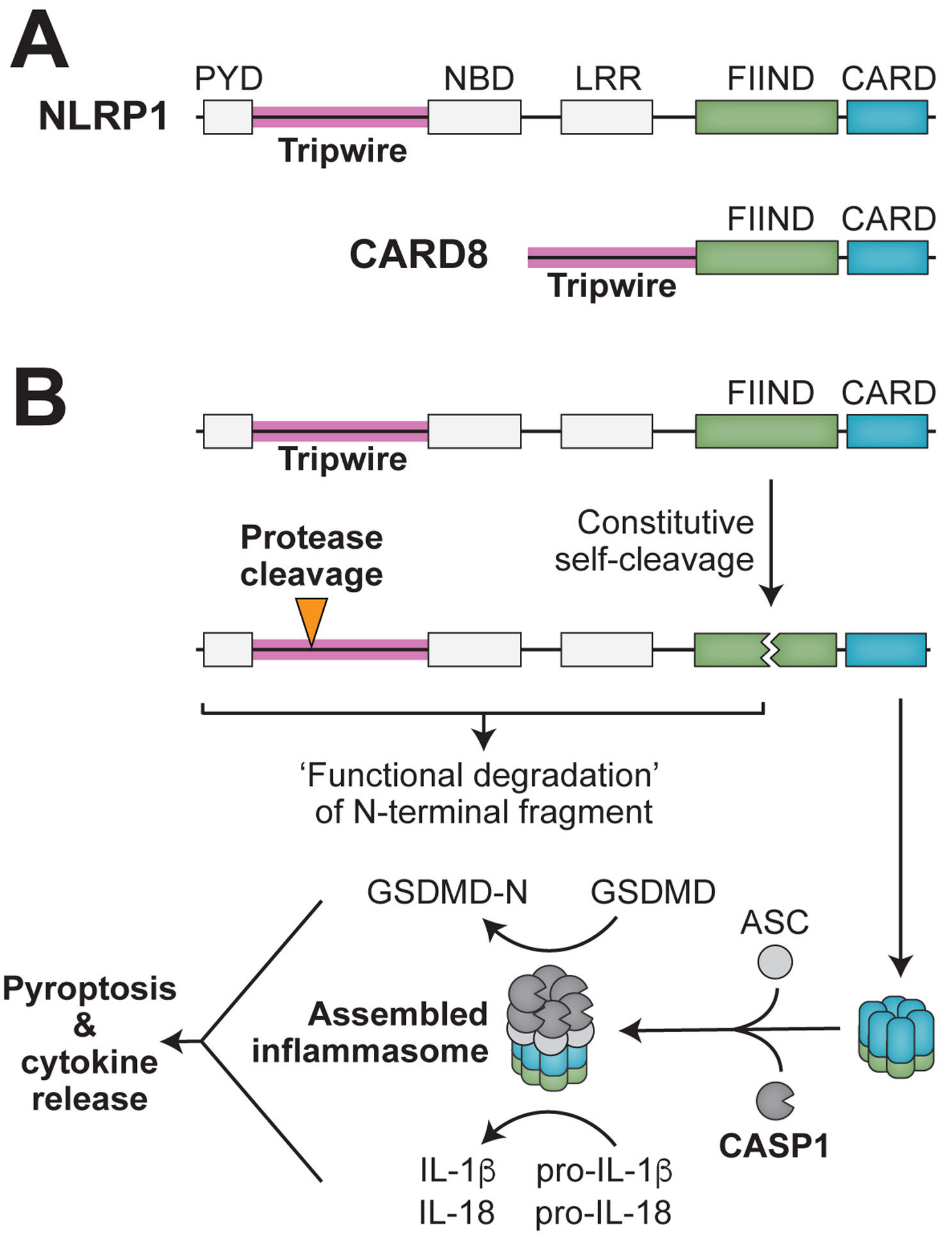

Figure 1. NLRP1 and CARD8 inflammasomes are effector-triggered immune sensors that are activated by 'functional degradation'.

(A) Schematic of the domain structure of human NLRP1 and CARD8. Both NLRP1 and CARD8 contain a rapidly evolving disordered region in their N-terminus that we refer to as the 'tripwire' (pink shading) for its ability to sense cleavage by diverse pathogen-encoded proteases. (B) 'Functional degradation' model for NLRP1 inflammasome activation. The FIIND, shared by both NLRP1 and CARD8, undergoes constitutive self-cleavage, resulting in a CARD-containing C-terminus being non-covalently bound to the N-terminus of the respective proteins. Cleavage by a pathogen-encoded protease in a rapidly evolving and disordered 'tripwire' region exposes an N-degron, which subsequently results in proteasomal degradation of the N-terminal domains. The bioactive CARD-containing C-terminus is liberated allowing for recruitment and activation of CASP1, requiring ASC in the case of human NLRP1 but not CARD8. Inflammasome activation results in maturation and release of pro-inflammatory cytokines, IL-1β and IL-18, and initiation of pyroptotic cell death via GSDMD.

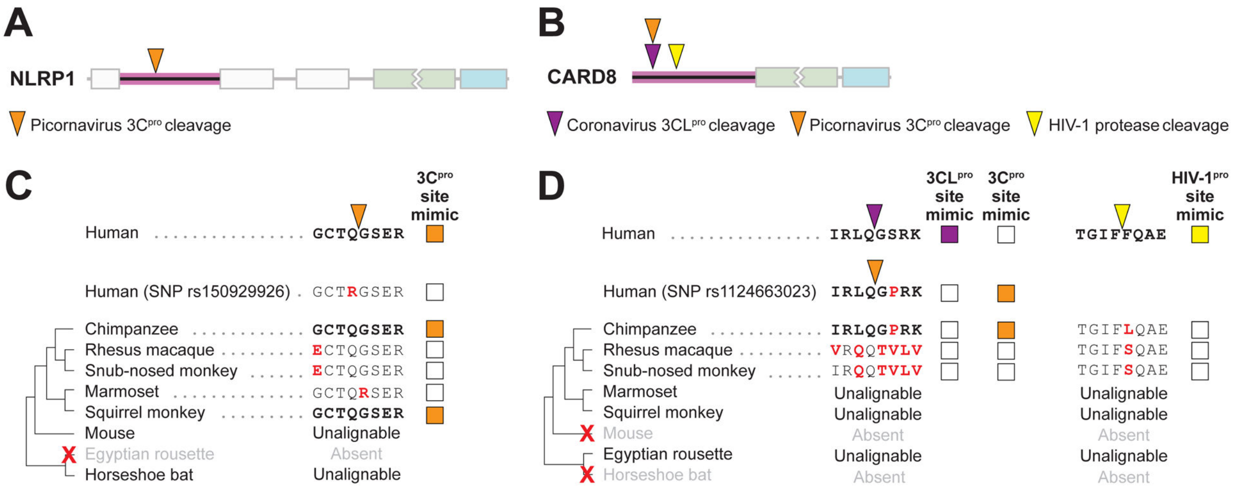

Figure 2. Host- and virus-specific activation of NLRP1 and CARD8 inflammasomes by virus-encoded proteases.

(A) Site of picornavirus 3C protease ($3C^{pro}$) cleavage (orange triangle) within the rapidly evolving tripwire region (pink shading) of human NLRP1 [16,19]. (B) Sites of coronavirus 3CL protease ($3CL^{pro}$) (purple triangle) [19], picornavirus 3Cpro (orange triangle) [19,21], and HIV-1 protease (HIV-1^{pro}) (yellow triangle) [18] cleavage within the tripwire region (pink shading) of human CARD8. (C) Sequence within human NLRP1 that mimics the preferred enteroviral $3C^{pro}$ cleavage site is shown at top. Below are NLRP1 sequences from other human individuals or non-human mammalian species that align to this region. NLRP1 homologs from mouse and microbat (represented by the horseshoe bat) are unalignable to this region of human NLRP1. Megabats (represented by the Egyptian rousette), have lost NLRP1 altogether, as indicated by a red X on the phylogenetic tree to the left. Amino acid changes relative to the human NLRP1 reference sequence are highlighted in red. NLRP1 sequences that are cleaved and activated by $3C^{pro}$ are in bold and are marked with a filled orange box. Data from [16]. (D) Sequence within human CARD8 that mimics the preferred cleavage sites of the indicated proteases are shown. Megabat CARD8 lack any sequence that is alignable to this region of primate CARD8, and mice and microbats lack a CARD8 ortholog altogether. Amino acid changes relative to the human CARD8 reference sequence are highlighted in red. CARD8 sequences that are cleaved and activated by viral proteases are in bold and are marked with filled boxes of the appropriate color. Data on $3CL^{pro}$ and $3C^{pro}$ from [19], data on HIV-1^{pro} from [20].

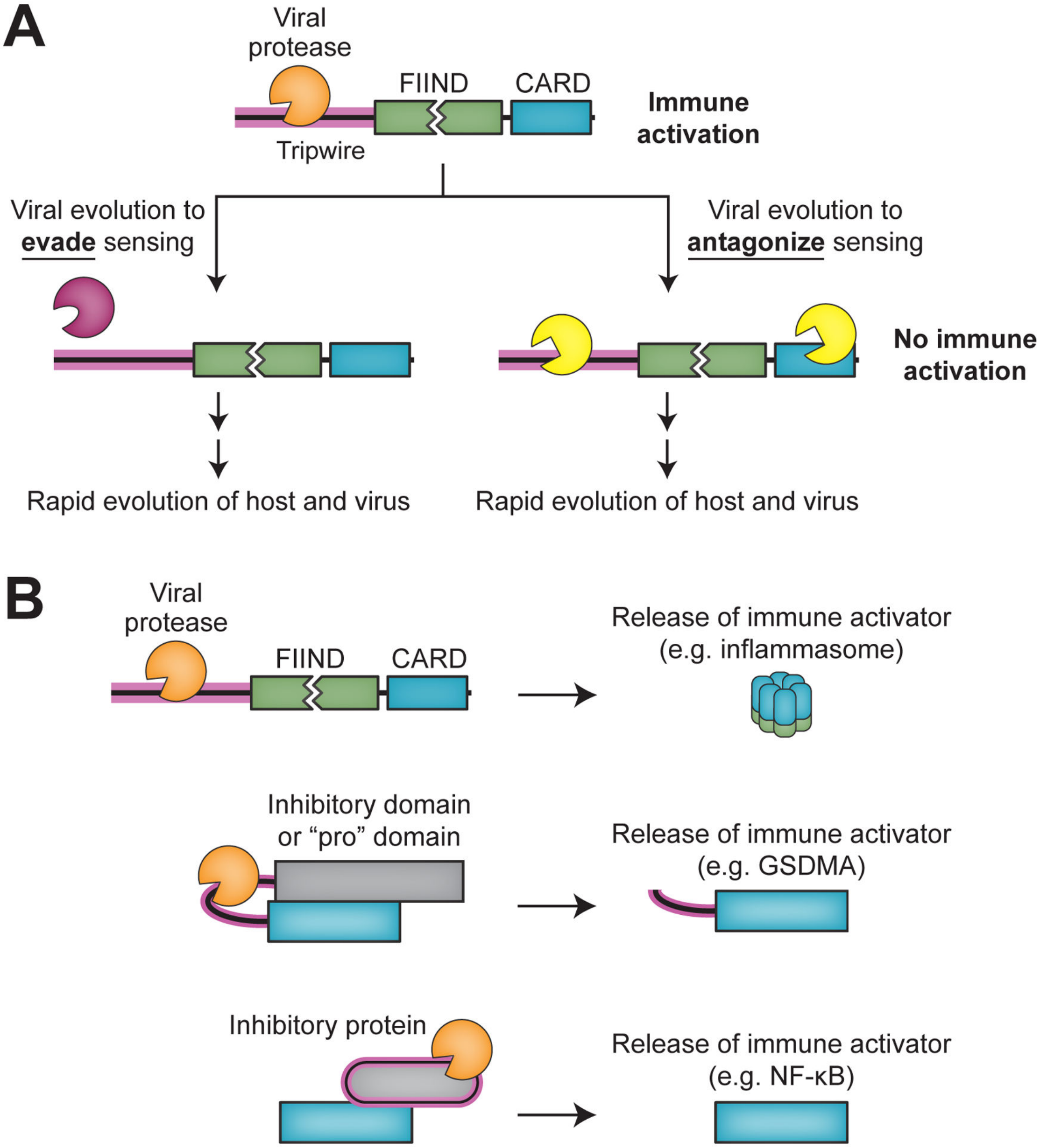

Figure 3. Potential mechanisms of innate immune sensing of viral proteases and routes of viral escape.

(A) Two possible routes for how viral protease evolution can evade inflammasome sensing and immune activation. On the left, viral evolution may result in a protease that no longer cleaves the tripwire region of the inflammasome sensor. On the right, viral evolution may result in a protease that cleaves the tripwire region of the inflammasome sensor but also cleaves in another position that antagonizes inflammasome activation. (B) Proposed models for diverse mechanisms for ETI sensing of viral protease activity. At the top is the inflammasome-mediated mechanism characterized by CARD8 and NLRP1. Other potential mechanisms of innate immune activation as a result of viral protease sensing include viral protease-mediated cleavage to release inhibitory or ‘pro’ domains that attenuate immune activation (middle) or cleavage of inhibitory proteins that attenuate immune activation (bottom). In all cases, rapidly evolving sequences (pink shading) may allow host adaptation to specific viral proteases.

Table 1.

Viral proteases sensed by NLRP1 and CARD8 inflammasomes.

Host protein	Virus	Protease	Reference	Notes
NLRP1	HRV	3C	Robinson et al, 2020 [15]	Activation in primary airway epithelial cells.
	CVB3, others	3C	Tsu et al, 2021 [16]	Host- and virus-specific activation described.
	SARS-CoV-2	3CL	Planes et al, 2022 [17]	Cleaves outside tripwire. Activation via caspase-3 and GSDME.
CARD8	HIV-1	PR	Wang et al, 2021 [18]	Inflammasome-dependent death of latently infected immune cells.
	HIV-1, SIVcpz	PR	Kulsuptrakul et al, 2022 [20]	Host- and virus-specific activation described.
	SARS-CoV-2 HRV, others	3CL 3C	Tsu et al, 2023 [19]	Host- and virus-specific activation described. Activation sensitive to human SNP.
	CVB3	3C	Nadkarni et al, 2022 [21]	Activation in endothelial cells and cardiomyocytes.

Viral abbreviations: human rhinovirus (HRV), coxsackievirus B3 (CVB3), chimpanzee SIV (SIVcpz).