



Epithelial inflammasomes in the defense against *Salmonella* gut infection[☆]

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The gut epithelium prevents bacterial access to the host's tissues and coordinates a number of mucosal defenses. Here, we review the function of epithelial inflammasomes in the infected host and focus on their role in defense against *Salmonella* Typhimurium. This pathogen employs flagella to swim towards the epithelium and a type III secretion system (TTSS) to dock and invade intestinal epithelial cells. Flagella and TTSS components are recognized by the canonical NAIP/NLRC4 inflammasome, while LPS activates the non-canonical Caspase-4/11 inflammasome. The relative contributions of these inflammasomes, the activated cell death pathways and the elicited mucosal defenses are subject to environmental control and appear to change along the infection trajectory. It will be an important future task to explain how this may enable defense against the challenges imposed by diverse bacterial enteropathogens.

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Introduction

Salmonella Typhimurium (*S.Tm*) is a common foodborne pathogen. It is closely related to other bacterial enteropathogens infecting humans and animals, for example enteropathogenic *Escherichia coli*, *Citrobacter rodentium* or *Shigella flexneri*. All these pathogens employ type III secretion systems (TTSS) to manipulate gut epithelial cells, express

lipopolysaccharide (LPS) on their surface, and appear to interact with host cellular inflammasomes during the infection (Table 1). In spite of these similarities, some aspects of the pathogens' attack on the gut epithelium, that is, the requirement for flagella, the actin structures at the epithelial surface, and/or the capacity for actin-based propulsion into neighbouring epithelial cells may differ between these enteropathogens. This may contribute to differences in the pathogen's host range, or aspects of the pathophysiology of the infectious disease. Nevertheless, general principles are emerging, including the basic function of epithelial inflammasome defense. Here, we will focus on epithelial inflammasome defense against *S.Tm*, while other enteropathogens are covered elsewhere in this issue.

Over the last decades, *S.Tm* has been extensively studied in cell culture and animal infection models (reviewed in Refs. [1,2]), which has substantially advanced our general understanding of enterobacterial infection mechanisms. This has revealed important inflammasome functions in the complex setting of a gut infection. In our review, we will discuss the experimental evidence from orogastric mouse infections and selected data from human and murine tissue culture models.

Murine models for studying *Salmonella* gut infection

In order to interpret animal data, it is important to consider the experimental details. In mice, colonization resistance, that is, the ability of the complex gut microbiota to suppress *S.Tm* growth in the gut lumen, limits enteric disease to a few percent of infected hosts [3,4]. Therefore, *in vivo* studies as a rule employ antibiotic pre-treated mice and gnotobiotic mice associated with defined microbiotas of reduced complexity, which permit highly reproducible gut colonization and enteric disease kinetics [5–9]. Shifts in food composition may provide another option for enhancing the infection in mice with a complex microbiota [4] (reviewed in Ref. [10]). The associated changes in microbiota composition, metabolite or vitamin concentrations may modulate the pathogen's virulence or mucosal immune response kinetics and could explain subtle differences between data from different studies [11,12] (reviewed in Ref. [13]). Moreover, oral infection models are vulnerable to confounding effects from pathobionts present in the gut luminal microbiota. Another review in this issue discusses this phenomenon in depth. When studying mice with mucosal immune system defects, the use of littermate controls is the best way to avoid such confounding microbiota effects [14,15].

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Table 1

Enteropathogenic bacteria infecting the gut epithelium are targeted by epithelial inflammasome responses

Pathogen	Host	Virulence factors for IEC attachment/ invasion		Inflammasome ligands		Actin manipulation		Main replication niche in host tissue		References (reviews, key primary work)	
		Motility in gut lumen	Adhesion	TTSS	TTSS	LPS	Flagellin	Effectors enabling actin based attachment/ invasion	Actin based intracellular motility		Extracellular
Enteropathogenic <i>Escherichia coli</i>	Human, cattle	No ^a	Fimbriae, HCP, ECP, Intimin, Tir	Yes	Yes	Yes	No	Tir, TccP, Map, EspM, EspT (rare)	No	A/E lesions	[88–90]
<i>Citrobacter rodentium</i>	Mouse	No	Fimbriae, AdcA, EspA, Intimin, Tir	Yes	Yes	Yes	No	Tir, Map, EspM, EspT	No	A/E lesions	[88,90–92]
<i>Shigella flexneri</i>	Human	No	OspE1/2, IcsA	Yes	Yes	Yes	No	IpaA, IpaC, VirA, IpgB1, IpgB2, IpgD	IcsA	Mainly cytosolic	[93]
<i>Salmonella enterica</i>	Human, cattle, mouse, other	Flagella	Fimbriae, BapA, Misl, SiiE, TTSS translocon	Yes	Yes	Yes	Yes	SipA, SopB, SopE, SopE2	No	Vacuolar and cytosolic	[19,25 [*]]
Typhimurium											

^a Flagellated pathogenic *E. coli* strains do exist.

(reviewed in Refs. [16–18]). By carefully controlling the mouse infection and by exploring the immune responses and their effects at different time points post infection (p. i.), first important concepts have emerged. Given that orogastric *Salmonella* infection models mimic key disease symptoms observed in human gastroenteritis, including epithelial erosion, crypt abscesses, and inflammatory changes within the epithelium and the underlying lamina propria [5,8,9], the concepts may also apply to the human infection.

Mouse models have shed light onto the initial stages of gut colonization by *S.Tm*, which have been reviewed elsewhere [2,19,20]. Importantly, *S.Tm* expresses flagella to navigate gaps in the mucus layer [21*,22,23]. When arriving at the apical surface of the gut epithelium, the pathogen remains flagellated and expresses a pre-formed TTSS to dock, inject bacterial effector proteins and invade intestinal epithelial cells (IECs) [21*,22–24,25*]. Thus, it arrives at the IECs ‘pre-loaded’ with PAMPs (discussed, below) and elicits inflammation. The latter limits pathogen tissue loads and also alters the gut luminal nutrient pool, which may enhance pathogen growth within the gut and promote transmission [26–32].

Here, we focus on the innate immune responses elicited by IEC inflammasomes upon *S.Tm* gut infection. We review the well-characterized inflammasome responses that dominate during the first day of the infection, and discuss recent findings suggesting how inflammasome responses may change at later time points. We summarize validated concepts and present hypotheses about the epithelial cell death pathways triggered during *S.Tm* infection.

Inflammasomes

Inflammasomes are signal processing machines executing important sensor and signal transduction functions of the innate immune system, that is, by surveying the host cell’s cytosol for pathogen- or danger associated molecular patterns (PAMPs, DAMPs respectively). They are extensively reviewed elsewhere in this issue. Briefly, inflammasomes are divided into canonical and non-canonical inflammasomes [33–35]. Canonical inflammasomes include the NLRP family with NLRP1, NLRP3, the NLRC family with its single member NAIP/NLRC4, and the non-NLR family with pyrin and AIM2 inflammasomes. All these canonical inflammasomes share a common signalling cascade: Upon sensing PAMPs or DAMPs, a Caspase-1 activation platform is assembled, leading to the recruitment and processing of pro-Caspase-1 into its active form. Activated Caspase-1 cleaves downstream targets such as pro-inflammatory cytokines pro-IL-1 β and pro-IL-18 and Gasdermin D (GsdmD). GsdmD forms pores in the cell membrane leading to pyroptosis - a specific type of cell death featuring cell membrane lysis and pro-inflammatory cytokine secretion

into the extracellular space. The Caspase-11 inflammasome in mice and its human orthologue, the Caspase-4/5 inflammasome, do not follow this common signalling pathway and are therefore dubbed non-canonical inflammasomes. Caspase-4/5/11 can directly sense cytosolic lipopolysaccharide (LPS), which is a common outer membrane component of gram-negative bacteria, including *S. Tm* cells when invading the host's IECs. Subsequently, it can cleave GsdmD, which induces membrane damage similar to canonical inflammasomes. While most of this knowledge is based on studies in macrophages, IECs have also been shown to employ inflammasome signalling [36–39]. However, in IECs only the canonical NAIP/NLRC4 inflammasome and the non-canonical Caspase-4/11 inflammasome are thought to significantly affect the *S. Tm* infection. We discuss these inflammasomes, and their interconnection, in detail below.

NAIP/NLRC4 and Caspase-11 inflammasomes appear to work sequentially during *S. Tm* murine gut infection

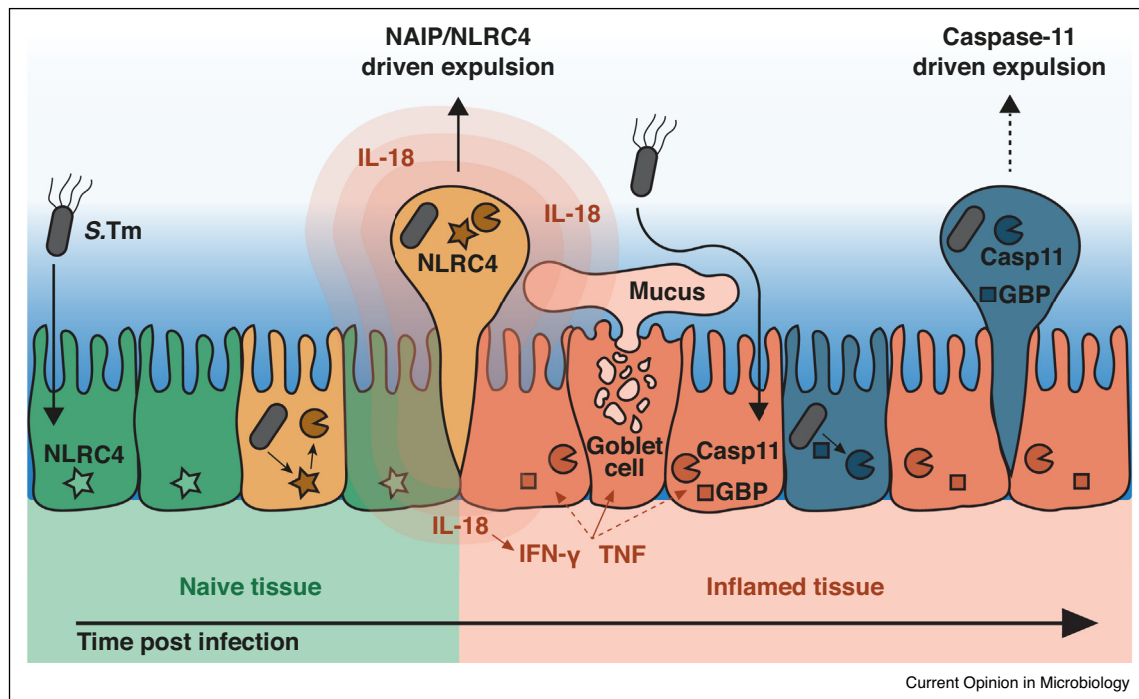
In 2006, it was shown that Caspase-1 deficient mice are more susceptible to orogastric *S. Tm* infection than WT mice [40,41]. This included shortened time to death and increased pathogen loads in the mesenteric lymph nodes and spleens of the Caspase-1 deficient mice (which were later found to also lack Caspase-11; [42,43]). This pathogen control deficiency of mice lacking Caspase-1 has been confirmed by independent follow-up studies [44,45,46,47]. Similar observations were made in NLRC4 inflammasome deficient mice [48,49], which suggested a NLRC4/Caspase-1 dependent restriction of systemic *S. Tm* spread. At this time, it remained unknown at which stage of the infection, in which cell type, and how NLRC4/Caspase-1 signalling can restrict *S. Tm*. The following years unveiled that the NAIP/NLRC4 inflammasome (partially including Caspase-1) in IECs is responsible for *S. Tm* restriction [37,39]. Littermate controlled experiments with bone marrow chimeras and IEC-specific knockout mice revealed that epithelial NAIP/NLRC4 promotes the expulsion of infected IECs during the first day of infection (Figure 1). The lack of this host response resulted in up to 100 times elevated *S. Tm* cecal tissue loads at 18 hour pi. [39]. These findings were later confirmed by an independent study [50], and protection against systemic *S. Tm* spread was also assigned to the gut epithelium [45]. Barcoded *S. Tm* strains, mathematical modelling and epithelium-specific NAIP1-6-ablation established that NAIP/NLRC4, which is highly expressed in IECs [35,51,52], prevents pathogen access to the mucosal tissue and thereby reduces subsequent pathogen dissemination to the mLN [45]. In contrast, during the first day of infection, there was no discernible contribution of NAIP/NLRC4 in immune cells, in spite of the role of phagocytes in systemic *S. Tm* dissemination [53]. This can be explained by the fact that *S. Tm* has to express

PAMPs such as flagellin and the TTSS to invade IECs, but downregulates these PAMPs within the host tissues to evade recognition by the NAIP/NLRC4 inflammasome (reviewed in Ref. [54]).

The non-canonical Caspase-4/11 inflammasome can elicit a similar response as NAIP/NLRC4 in *S. Tm* infected epithelial cell lines, and this may have implications *in vivo* [36]. Similar to NAIP/NLRC4, intracellular *S. Tm* (as well as LPS and extracellular *E. coli* infection) induce epithelial Caspase-4/11 signalling in infected IECs and WT mice showed lower mucosal pathogen loads compared to Caspase-11 deficient animals at day 7 p.i. While littermate controls were lacking, a recent follow up study expanded these findings [55]. After exposure to IFN γ , which is expressed in copious amounts in the infected gut [56–58], IECs upregulate pro-Caspase-11 and shift towards Caspase-11 dependent expulsion of *S. Tm* infected cells [55]. Accordingly, Caspase-11 can limit mucosal pathogen loads in *S. Tm* infected mice by days 3–7 p.i. [36,55]. Notably, independent work showed that other pro-inflammatory cytokines such as TNF can also induce pro-Caspase-11 expression in intestinal epithelial organoids (enteroids) [52] and that IFN signalling can influence Caspase-4/11 activation through GBPs [59,60,61]. Taken together, it seems plausible that gut inflammation provides multiple signals to optimize defense. In the murine gut, this may shift the response driving infected IEC expulsion from NAIP/NLRC4 dependence at \sim day 1 p.i. towards Caspase-11 dependence at \sim days 3–7 of the infection (Figure 1).

NAIP/NLRC4-deficient mice show a delayed onset of inflammation during the first 12–18 hour p.i. with reduced levels of pro-inflammatory IL-18, which is known to induce IFN γ production [30,39,50]. Thus, it is reasonable to speculate that NAIP/NLRC4 drives initial IEC expulsion and generates an inflammatory environment fuelling Caspase-11 dependent IEC expulsion as observed later in the infection (Figure 1). This would be in line with the observed negligible Caspase-11 dependent restriction of *S. Tm* within the first day of infection [39,45], but elevated gut tissue loads in Caspase-11 deficient mice at later time points (>1 day p.i.) [36,55]. Thereby, Caspase-11 dependent IEC expulsion might partially rely on NAIP/NLRC4, that is, through NAIP/NLRC4-inflammasome elicited IL-18, IFN γ , and/or TNF signalling. In mice, Caspase-11 dependent IEC expulsion may hence be regarded as a complementary defense system. However, this remains to be formally tested. One should also quantify the relative contributions of the canonical and non-canonical triggers of infected IEC expulsion during later phases of the infection. Time-resolved littermate-controlled infection experiments in single and double knockout mice should provide interesting answers. Importantly, NLRC4 as well as Caspase-11 contribute to restricting systemic *S. Tm*

Figure 1



Epithelial NAIP/NLRC4 and Caspase-11 inflammasomes may sequentially contribute to *S. Tm* restriction in mice.

In mice, *S. Tm* invasion into IECs promotes NAIP/NLRC4 driven expulsion and soluble mediator release, which may generate an inflammatory environment fueling IEC expulsion by Caspase-11 and involving GBPs. Thus, Caspase-11 dependent expulsion may partially rely on NAIP/NLRC4, that is, through IL-18, IFN γ , and/or TNF signalling. In particular, IFN γ and TNF increase the expression of Caspase-11 and GBPs, which may facilitate activation of the Caspase-11 inflammasome. IFN γ is also known to promote mucus secretion by goblet cells.

burden at later time points (based on systemic infection studies; [62–65]). This warrants a careful assessment of epithelial and systemic protection alike, while studying NLRC4 and Caspase-11 defenses at >1 day p.i.

IEC inflammasomes – epithelial cell state and species-specific differences

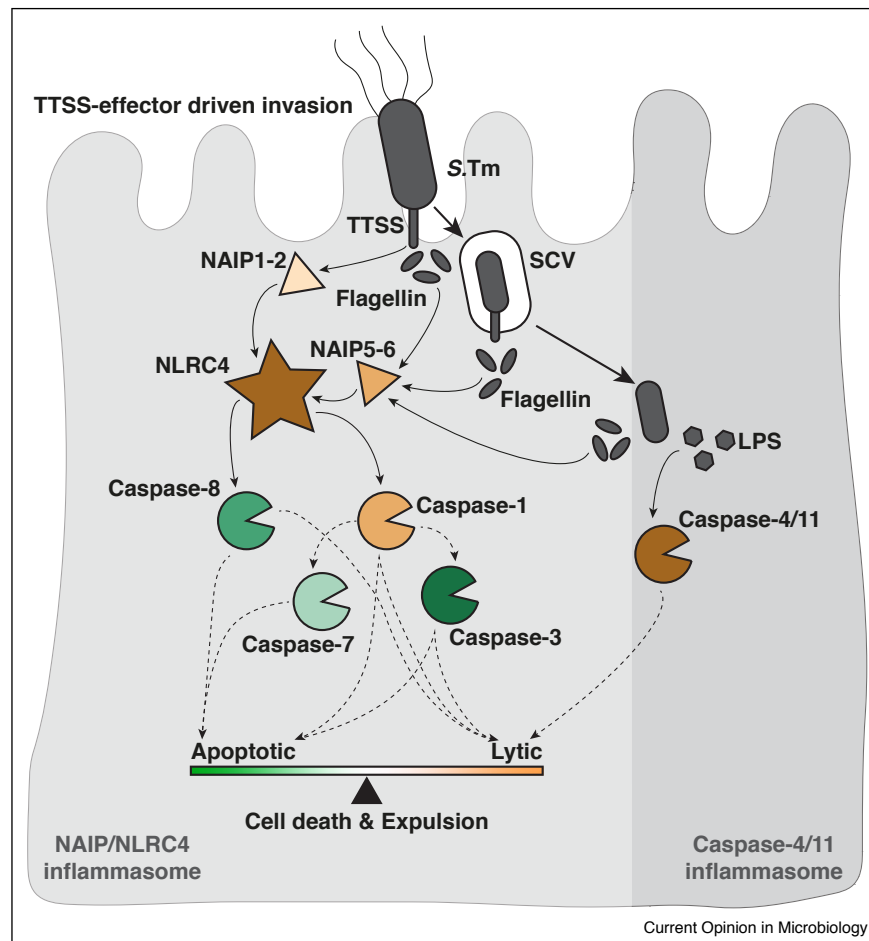
The relative importance of different inflammasomes in naïve IECs might vary dependent on the growth and differentiation state of the epithelium. Inflammasome expression varies substantially between immortalized/transformed cell lines and primary epithelial cells [52*,66**]. Therefore, inflammasome signalling at early and late infection could be further influenced by the IEC differentiation status. It is plausible that increased IEC proliferation observed during *S. Tm* infection might lead to poorly differentiated cells and thereby affect the relative expression and contribution of NAIP/NLRC4 or Caspase-11 inflammasomes. A recent study moreover observed considerable interspecies variations [66**]. In particular, non-canonical inflammasome signalling seems more important in human than in murine IECs, as demonstrated in enteroid culture infections. In contrast, Caspase-1/5 seemed to be dispensable. Based on these findings it is important to acknowledge potential cell-state

and species-specific differences in IEC inflammasome signaling when interpreting experimental data.

Inflammasome signalling within IECs upon *S. Tm* infection

While non-canonical inflammasome signaling employs Caspase-4/11 as both the sensor and executor, NAIP/NLRC4 signaling is organized in a more complex cascade. The NAIP/NLRC4 inflammasome integrates signals elicited by several different PAMPs. This hinges on the respective receptors. In murine immune cells, this includes NAIP1-2 recognizing the TTSS and NAIP5-6 recognizing flagellin [67–72]. Similarly, flagellin delivery into the IEC cytosol is a potent trigger of the NAIP/NLRC4 inflammasome [50]. NAIP1-6 receptors are highly expressed in IECs [35,39,45**,51,52*], and permit the epithelial NAIP/NLRC4 inflammasome to also integrate multiple PAMP signals (Figure 2). Early studies suggested that *S. Tm* effectors such as SipB or SopE may also induce Caspase-1 dependent defenses [47,73]. However, it remains unclear if this is indeed the case *in vivo*. Alternatively, SipB and SopE-driven enhancement of host cell invasion [9,25*,74] may determine the dose of TTSS or flagellar proteins arriving in the IEC's cytosol, thereby indirectly fueling IEC inflammasome signaling. Further work will have to conclusively address this question.

Figure 2



Epithelial inflammasome signalling and potential crosstalk upon *S. Tm* infection leading to apoptotic and/or lytic IEC expulsion. *S. Tm* invading into IECs can be sensed by the NAIP/NLRC4 and the Caspase-4/11 inflammasomes. NAIP1-2 recognize the TTSS and flagellin is sensed by NAIP5-6. Caspase-11 is activated by cytosolic LPS. While Caspase-11 serves as both the sensor and executor, NAIP receptors activate the NAIP/NLRC4 inflammasome, leading to an interconnected downstream Caspase signaling. This may result in apoptotic and/or lytic cell death and expulsion. SCV - *Salmonella* containing vacuole.

In murine epithelia, NAIP/NLRC4 induced IEC expulsion is only partially dependent on Caspase-1, suggesting Caspase-1 dependent and independent downstream signalling [39]. This finding was confirmed by an independent report [50]. Moreover, by using a toxin fusion protein that delivers flagellin into the host cellular cytosol, it was shown that epithelial NAIP/NLRC4 signalling can activate either Caspase-1/GsdmD or ASC/Caspase-8 resulting in pyroptosis or apoptosis, respectively [50,75]. However, this has left unanswered if both pathways are fully engaged during *S. Tm* infection and if pyroptosis, apoptosis or a mixed cell death response dominates. Notably, recent studies using macrophages as the main assay system suggest that cell death signalling can be highly interconnected. This has given rise to a new concept called 'PANoptosis' (discussed in another chapter of this issue). Caspase-1 can activate apoptosis associated targets

such as Caspase-3 and Caspase-7 [76–80] and Caspase-3 and Caspase-8 can under some conditions trigger pyroptosis [81–84]. It is therefore reasonable to speculate that a similar crosstalk as in *S. Tm* infected macrophages [85] might occur downstream of epithelial NAIP/NLRC4, resulting in a mixed cell death and expulsion response (Figure 2). Along these lines, a recent publication observed increased *S. Tm* susceptibility in epithelial Caspase-8-deficient mice at day 3 p.i. [86]. The PANoptosome response concept of epithelial defense should be probed in time-resolved and littermate-controlled *S. Tm* using *in vivo* infection series.

Complex control of the inflammatory output of *S. Tm*-mediated IEC inflammasome activation

Epithelial inflammasome signalling leads to eicosanoid and IL-18 secretion, promoting diarrhea, and eliciting

inflammatory pathology in the intestinal mucosa [30,36,39,50,66**]. In naïve streptomycin pretreated mice, IL-18 was shown to be dispensable for IEC expulsion [39], but important to elicit a number of defenses including NK cell recruitment, IFN γ production by NK-cells, T-cells and IEL, as well as perforin-dependent enteropathy [30]. IFN γ in turn can activate phagocytes and triggers mucus secretion by goblet cells [58]. Considering that the colonic mucus layer can reduce mucosal *S.Tm* invasion by as much as 10-fold [21*], this hints towards a complex array of defenses that are elicited by IEC inflammasomes. Moreover, these defenses appear to be regulated in response to chemical cues derived from the food or the microbiota. Vitamin feeding experiments and infections in mice with retinoic acid-signaling deficient IECs suggest that vitamin A not only controls epithelial maturation, but also modulates IL-18 and IFN γ responses to an acute *S.Tm* infection [11*]. In these mice, IL-18 supplementation might for instance shift the epithelial response to *S.Tm* towards caspase-3 dependent cell death. Thus, careful control of the experimental conditions is warranted when studying the epithelial inflammasome functions *in vivo*.

Conclusions and perspectives

Research over the last years has identified epithelial inflammasomes as key coordinators of the defense against infection. Since IECs are at the very frontline of host-pathogen interactions, it makes intuitively sense that they take active part in the early immune response against *S.Tm*. We have just begun to understand certain aspects of IEC inflammasomes during *S.Tm* infection. Further research will be needed to gain a comprehensive understanding of the IEC inflammasomes at different stages of infection and the diversity of the triggered responses. Single cell techniques described elsewhere in this issue will help to decipher the diversity of the responses on how this contributes to defense. Much of this knowledge will also apply to other closely related enteropathogenic bacteria like *C. rodentium*, enteropathogenic *E. coli* and *S. flexneri*, which are known to trigger, and are subject to control by, epithelial inflammasomes (Table 1). The recent advances in *ex vivo* culture of primary epithelial enteroids and colonoids will help to dissect the underlying molecular mechanisms and the immediate downstream effects of IEC inflammasome signalling. Interesting discoveries in this field of research can be anticipated in the near future. This will contribute to our general understanding of enteropathogen-elicited host responses and may help to prevent acute gut infections as well as chronic mucosal inflammation, which can occur in the aftermath of such disease [87].

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. LaRock DL, Chaudhary A, Miller SI: ***Salmonellae* interactions with host processes.** *Nat Rev Microbiol* 2015, **13**:191-205 <http://dx.doi.org/10.1038/nrmicro3420>.
 2. Wotzka SY, Nguyen BD, Hardt WD: ***Salmonella* Typhimurium diarrhea reveals basic principles of enteropathogen infection and disease-promoted DNA exchange.** *Cell Host Microbe* 2017, **21**:443-454 <http://dx.doi.org/10.1016/j.chom.2017.03.009>.
 3. Velazquez EM *et al.*: **Endogenous enterobacteriaceae underlie variation in susceptibility to *Salmonella* infection.** *Nat Microbiol* 2019, **4**:1057-1064 <http://dx.doi.org/10.1038/s41564-019-0407-8>.
 4. Wotzka SY *et al.*: ***Escherichia coli* limits *Salmonella* Typhimurium infections after diet shifts and fat-mediated microbiota perturbation in mice.** *Nat Microbiol* 2019, **4**:2164-2174 <http://dx.doi.org/10.1038/s41564-019-0568-5>.
 5. Barthel M *et al.*: **Pretreatment of mice with streptomycin provides a *Salmonella enterica* serovar Typhimurium colitis model that allows analysis of both pathogen and host.** *Infect Immun* 2003, **71**:2839-2858 <http://dx.doi.org/10.1128/iai.71.5.2839-2858.2003>.
 6. Brugiroux S *et al.*: **Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium.** *Nat Microbiol* 2016, **2**:16215 <http://dx.doi.org/10.1038/nmicrobiol.2016.215>.
 7. Nguyen BD *et al.*: **Import of aspartate and malate by DcuABC drives H₂/fumarate respiration to promote initial *Salmonella* gut-lumen colonization in mice.** *Cell Host Microbe* 2020, **27**:922-936 <http://dx.doi.org/10.1016/j.chom.2020.04.013> e926.
 8. Stecher B *et al.*: **Like will to like: abundances of closely related species can predict susceptibility to intestinal colonization by pathogenic and commensal bacteria.** *PLoS Pathog* 2010, **6**: e1000711 <http://dx.doi.org/10.1371/journal.ppat.1000711>.
 9. Zhang K *et al.*: **Age-dependent enterocyte invasion and microcolony formation by *Salmonella*.** *PLoS Pathog* 2014, **10**: e1004385 <http://dx.doi.org/10.1371/journal.ppat.1004385>.
 10. Kreuzer M, Hardt WD: **How food affects colonization resistance against enteropathogenic bacteria.** *Annu Rev Microbiol* 2020, **74**:787-813 <http://dx.doi.org/10.1146/annurev-micro-020420-013457>.
 11. Iyer N *et al.*: **Epithelium intrinsic vitamin A signaling coordinates pathogen clearance in the gut via IL-18.** *PLoS Pathog* 2020, **16**: e1008360 <http://dx.doi.org/10.1371/journal.ppat.1008360>.
 - This study investigates the effect of IEC-intrinsic vitamin A signalling on the defense against *S.Tm* infection. They show that mice deficient in retinoic acid receptor (RAR) signalling feature higher *S.Tm* burden.
 12. Miki T, Goto R, Fujimoto M, Okada N, Hardt WD: **The bactericidal lectin RegIII β prolongs gut colonization and enteropathy in the streptomycin mouse model for *Salmonella* diarrhea.** *Cell Host Microbe* 2017, **21**:195-207 <http://dx.doi.org/10.1016/j.chom.2016.12.008>.
 13. Luan HH, Medzhitov R: **Food fight: role of itaconate and other metabolites in antimicrobial defense.** *Cell Metab* 2016, **24**:379-387 <http://dx.doi.org/10.1016/j.cmet.2016.08.013>.

14. Mamantopoulos M *et al.*: **Nlrp6- and ASC-dependent inflammasomes do not shape the commensal gut microbiota composition.** *Immunity* 2017, **47**:339-348 <http://dx.doi.org/10.1016/j.immuni.2017.07.011> e334.
15. Robertson SJ *et al.*: **Comparison of co-housing and littermate methods for microbiota standardization in mouse models.** *Cell Rep* 2019, **27**:1910-1919 <http://dx.doi.org/10.1016/j.celrep.2019.04.023> e1912.
- This study reports the impact on intestinal microbiota of co-housed animals versus F2-generation littermates. The authors conclude that F2 littermate animals from a unidirectional P1 cross should be used as a standard method to minimize the influence of the microbiota in genotype-phenotype studies.
16. Kaiser P, Diard M, Stecher B, Hardt WD: **The streptomycin mouse model for *Salmonella* diarrhea: functional analysis of the microbiota, the pathogen's virulence factors, and the host's mucosal immune response.** *Immunol Rev* 2012, **245**:56-83 <http://dx.doi.org/10.1111/j.1600-065X.2011.01070.x>.
17. Mamantopoulos M, Ronchi F, McCoy KD, Wullaert A: **Inflammasomes make the case for littermate-controlled experimental design in studying host-microbiota interactions.** *Gut Microbes* 2018, **9**:374-381 <http://dx.doi.org/10.1080/19490976.2017.1421888>.
18. Wullaert A, Lamkanfi M, McCoy KD: **Defining the impact of host genotypes on microbiota composition requires meticulous control of experimental variables.** *Immunity* 2018, **48**:605-607 <http://dx.doi.org/10.1016/j.immuni.2018.04.001>.
19. Hausmann A, Hardt WD: **The interplay between *Salmonella enterica* Serovar Typhimurium and the intestinal mucosa during oral infection.** *Microbiol Spectr* 2019, **7** <http://dx.doi.org/10.1128/microbiolspec.BAI-0004-2019>.
20. Litvak Y, Byndloss MX, Tsois RM, Baumler AJ: **Dysbiotic Proteobacteria expansion: a microbial signature of epithelial dysfunction.** *Curr Opin Microbiol* 2017, **39**:1-6 <http://dx.doi.org/10.1016/j.mib.2017.07.003>.
21. Furter M, Sellin ME, Hansson GC, Hardt WD: **Mucus architecture and near-surface swimming affect distinct *Salmonella* Typhimurium infection patterns along the murine intestinal tract.** *Cell Rep* 2019, **27**:2665-2678 <http://dx.doi.org/10.1016/j.celrep.2019.04.106> e2663.
- This study investigates how the mucus affects S.Tm infection. The authors show with microscopy-based approaches that the mucus layer shields the colonic epithelium from S.Tm invasion. Hence, the mucus layer can efficiently reduce the levels of inflammasome stimuli.
22. Stecher B *et al.*: **Motility allows *S. Typhimurium* to benefit from the mucosal defence.** *Cell Microbiol* 2008, **10**:1166-1180 <http://dx.doi.org/10.1111/j.1462-5822.2008.01118.x>.
23. Stecher B *et al.*: **Flagella and chemotaxis are required for efficient induction of *Salmonella enterica* serovar Typhimurium colitis in streptomycin-pretreated mice.** *Infect Immun* 2004, **72**:4138-4150 <http://dx.doi.org/10.1128/IAI.72.7.4138-4150.2004>.
24. Ackermann M *et al.*: **Self-destructive cooperation mediated by phenotypic noise.** *Nature* 2008, **454**:987-990 <http://dx.doi.org/10.1038/nature07067>.
25. Fattinger SA *et al.*: ***Salmonella* Typhimurium discreet-invasion of the murine gut absorptive epithelium.** *PLoS Pathog* 2020, **16**: e1008503 <http://dx.doi.org/10.1371/journal.ppat.1008503>.
- This study identifies SipA as the main TTSS-1 effector enabling discreet-invasion of S.Tm into IECs *in vivo*. These findings highlight that TTSS-1 effectors are essential for efficient IEC invasion and thereby influence inflammasome activation.
26. Byndloss MX *et al.*: **Microbiota-activated PPAR-gamma signaling inhibits dysbiotic Enterobacteriaceae expansion.** *Science* 2017, **357**:570-575 <http://dx.doi.org/10.1126/science.aam9949>.
27. Koscsó B *et al.*: **Gut-resident CX3CR1(hi) macrophages induce tertiary lymphoid structures and IgA response *in situ*.** *Sci Immunol* 2020, **5** <http://dx.doi.org/10.1126/sciimmunol.aax0062>.
28. Lawley TD *et al.*: **Host transmission of *Salmonella enterica* serovar Typhimurium is controlled by virulence factors and indigenous intestinal microbiota.** *Infect Immun* 2008, **76**:403-416 <http://dx.doi.org/10.1128/IAI.01189-07>.
29. Maier L *et al.*: **Granulocytes impose a tight bottleneck upon the gut luminal pathogen population during *Salmonella typhimurium* colitis.** *PLoS Pathog* 2014, **10**:e1004557 <http://dx.doi.org/10.1371/journal.ppat.1004557>.
30. Muller AA *et al.*: **An NK cell perforin response elicited via IL-18 controls mucosal inflammation kinetics during *Salmonella* gut infection.** *PLoS Pathog* 2016, **12**:e1005723 <http://dx.doi.org/10.1371/journal.ppat.1005723>.
31. Stecher B *et al.*: ***Salmonella enterica* serovar Typhimurium exploits inflammation to compete with the intestinal microbiota.** *PLoS Biol* 2007, **5**:2177-2189 <http://dx.doi.org/10.1371/journal.pbio.0050244>.
32. Winter SE *et al.*: **Gut inflammation provides a respiratory electron acceptor for *Salmonella*.** *Nature* 2010, **467**:426-429 <http://dx.doi.org/10.1038/nature09415>.
33. Bauer R, Rauch I: **The NAIP/NLRC4 inflammasome in infection and pathology.** *Mol Aspects Med* 2020:100863 <http://dx.doi.org/10.1016/j.mam.2020.100863>.
34. Broz P: **Recognition of intracellular bacteria by inflammasomes.** *Microbiol Spectr* 2019, **7** <http://dx.doi.org/10.1128/microbiolspec.BAI-0003-2019>.
35. Winsor N, Krustev C, Bruce J, Philpott DJ, Girardin SE: **Canonical and noncanonical inflammasomes in intestinal epithelial cells.** *Cell Microbiol* 2019, **21**:e13079 <http://dx.doi.org/10.1111/cmi.13079>.
36. Knodler LA *et al.*: **Noncanonical inflammasome activation of caspase-4/caspase-11 mediates epithelial defenses against enteric bacterial pathogens.** *Cell Host Microbe* 2014, **16**:249-256 <http://dx.doi.org/10.1016/j.chom.2014.07.002>.
37. Knodler LA *et al.*: **Dissemination of invasive *Salmonella* via bacterial-induced extrusion of mucosal epithelia.** *Proc Natl Acad Sci U S A* 2010, **107**:17733-17738 <http://dx.doi.org/10.1073/pnas.1006098107>.
38. Nordlander S, Pott J, Maloy KJ: **NLRC4 expression in intestinal epithelial cells mediates protection against an enteric pathogen.** *Mucosal Immunol* 2014, **7**:775-785 <http://dx.doi.org/10.1038/mi.2013.95>.
39. Sellin ME *et al.*: **Epithelium-intrinsic NAIP/NLRC4 inflammasome drives infected enterocyte expulsion to restrict *Salmonella* replication in the intestinal mucosa.** *Cell Host Microbe* 2014, **16**:237-248 <http://dx.doi.org/10.1016/j.chom.2014.07.001>.
40. Lara-Tejero M *et al.*: **Role of the caspase-1 inflammasome in *Salmonella typhimurium* pathogenesis.** *J Exp Med* 2006, **203**:1407-1412 <http://dx.doi.org/10.1084/jem.20060206>.
41. Raupach B, Peuschel SK, Monack DM, Zychlinsky A: **Caspase-1-mediated activation of interleukin-1beta (IL-1beta) and IL-18 contributes to innate immune defenses against *Salmonella enterica* serovar Typhimurium infection.** *Infect Immun* 2006, **74**:4922-4926 <http://dx.doi.org/10.1128/IAI.00417-06>.
42. Kayagaki N *et al.*: **Non-canonical inflammasome activation targets caspase-11.** *Nature* 2011, **479**:117-121 <http://dx.doi.org/10.1038/nature10558>.
43. Kenneth NS *et al.*: **An inactivating caspase 11 passenger mutation originating from the 129 murine strain in mice targeted for c-IAP1.** *Biochem J* 2012, **443**:355-359 <http://dx.doi.org/10.1042/BJ20120249>.
44. Broz P *et al.*: **Redundant roles for inflammasome receptors NLRC3 and NLRC4 in host defense against *Salmonella*.** *J Exp Med* 2010, **207**:1745-1755 <http://dx.doi.org/10.1084/jem.20100257>.
45. Hausmann A *et al.*: **Intestinal epithelial NAIP/NLRC4 restricts systemic dissemination of the adapted pathogen *Salmonella* Typhimurium due to site-specific bacterial PAMP expression.** *Mucosal Immunol* 2020, **13**:530-544 <http://dx.doi.org/10.1038/s41385-019-0247-0>.

This study shows that NAIP/NLRC4 solely in IECs restricts systemic *S. Tm* migration during the first day of infection. Furthermore, the study shows that other inflammasomes such as NLRP3 and Caspase-11 do not contribute to *S. Tm* restriction at <1 day p.i.

46. Lai MA *et al.*: **Innate immune detection of flagellin positively and negatively regulates salmonella infection.** *PLoS One* 2013, **8**:e72047 <http://dx.doi.org/10.1371/journal.pone.0072047>.
 47. Muller AJ *et al.*: **The *S. typhimurium* effector SopE induces caspase-1 activation in stromal cells to initiate gut inflammation.** *Cell Host Microbe* 2009, **6**:125-136 <http://dx.doi.org/10.1016/j.chom.2009.07.007>.
 48. Carvalho FA *et al.*: **Cytosolic flagellin receptor NLRC4 protects mice against mucosal and systemic challenges.** *Mucosal Immunol* 2012, **5**:288-298 <http://dx.doi.org/10.1038/mi.2012.8>.
 49. Franchi L *et al.*: **NLRC4-driven production of IL-1 β discriminates between pathogenic and commensal bacteria and promotes host intestinal defense.** *Nat Immunol* 2012, **13**:449-456 <http://dx.doi.org/10.1038/ni.2263>.
 50. Rauch I *et al.*: **NAIP-NLRC4 inflammasomes coordinate intestinal epithelial cell expulsion with eicosanoid and IL-18 release via activation of caspase-1 and -8.** *Immunity* 2017, **46**:649-659 <http://dx.doi.org/10.1016/j.immuni.2017.03.016>.
 51. Allam R *et al.*: **Epithelial NAIPs protect against colonic tumorigenesis.** *J Exp Med* 2015, **212**:369-383 <http://dx.doi.org/10.1084/jem.20140474>.
 52. Hausmann A *et al.*: **Germ-free and microbiota-associated mice yield small intestinal epithelial organoids with equivalent and robust transcriptome/proteome expression phenotypes.** *Cell Microbiol* 2020, **22**:e13191 <http://dx.doi.org/10.1111/cmi.13191>.
- The authors compare proteomes and transcriptomes of enteroids established from germ-free and microbiota-associated mice. They conclude that the long-term global impact of donor microbiota on organoid expression patterns is negligible. In addition, they observe high baseline expression of Naip1-6 and Nlrc4 and note that Caspase-11 expression in IECs is stimulated by TNF.
53. Hapfelmeier S *et al.*: **Microbe sampling by mucosal dendritic cells is a discrete, MyD88-independent step in DeltainvG *S. Typhimurium* colitis.** *J Exp Med* 2008, **205**:437-450 <http://dx.doi.org/10.1084/jem.20070633>.
 54. Brewer SM, Brubaker SW, Monack DM: **Host inflammasome defense mechanisms and bacterial pathogen evasion strategies.** *Curr Opin Immunol* 2019, **60**:63-70 <http://dx.doi.org/10.1016/j.coi.2019.05.001>.
 55. Crowley SM *et al.*: **Intestinal restriction of *Salmonella* Typhimurium requires caspase-1 and caspase-11 epithelial intrinsic inflammasomes.** *PLoS Pathog* 2020, **16**:e1008498 <http://dx.doi.org/10.1371/journal.ppat.1008498>.
- The authors show that Caspase-11 can restrict *S. Tm* infection by an IEC intrinsic mechanism. While Caspase-1 is expressed already at baseline, IFN γ can stimulate Caspase-11 expression, which promotes Caspase-11 driven *S. Tm* restriction in primed IECs.
56. Godinez I *et al.*: **T cells help to amplify inflammatory responses induced by *Salmonella enterica* serotype Typhimurium in the intestinal mucosa.** *Infect Immun* 2008, **76**:2008-2017 <http://dx.doi.org/10.1128/IAI.01691-07>.
 57. Rhee SJ, Walker WA, Cherayil BJ: **Developmentally regulated intestinal expression of IFN-gamma and its target genes and the age-specific response to enteric *Salmonella* infection.** *J Immunol* 2005, **175**:1127-1136 <http://dx.doi.org/10.4049/jimmunol.175.2.1127>.
 58. Songhet P *et al.*: **Stromal IFN-gammaR-signaling modulates goblet cell function during *Salmonella* Typhimurium infection.** *PLoS One* 2011, **6**:e22459 <http://dx.doi.org/10.1371/journal.pone.0022459>.
 59. Meunier E *et al.*: **Caspase-11 activation requires lysis of pathogen-containing vacuoles by IFN-induced GTPases.** *Nature* 2014, **509**:366-370 <http://dx.doi.org/10.1038/nature13157>.
 60. Santos JC *et al.*: **Human GBP1 binds LPS to initiate assembly of a caspase-4 activating platform on cytosolic bacteria.** *Nat Commun* 2020, **11**:3276 <http://dx.doi.org/10.1038/s41467-020-16889-z>.

Santos *et al.* [60] shows that GBP coating of cytosolic Gram-negative bacteria promotes non-canonical Caspase-4 inflammasome activation. This highlights the importance of IFN γ induced GBP expression for the response against cytosolic *S. Tm*.

61. Wandel MP *et al.*: **Guanylate-binding proteins convert cytosolic bacteria into caspase-4 signaling platforms.** *Nat Immunol* 2020, **21**:880-891 <http://dx.doi.org/10.1038/s41590-020-0697-2>.
- Wandel *et al.* [61] shows that GBP coating of cytosolic Gram-negative bacteria promotes non-canonical Caspase-4 inflammasome activation. This highlights the importance of IFN γ induced GBP expression for the response against cytosolic *S. Tm*.
62. Chen KW *et al.*: **Noncanonical inflammasome signaling elicits gasdermin D-dependent neutrophil extracellular traps.** *Sci Immunol* 2018, **3** <http://dx.doi.org/10.1126/sciimmunol.aar6676>.
 63. Karki R *et al.*: **IRF8 regulates transcription of NAIPs for NLRC4 inflammasome activation.** *Cell* 2018, **173**:920-933 <http://dx.doi.org/10.1016/j.cell.2018.02.055> e913.
 64. Shutinoski B, Patel R, Tomlinson JJ, Schlossmacher MG, Sad S: **Ripk3 licenced protection against microbial infection in the absence of caspase-1-11 inflammasomes.** *Microbes Infect* 2020, **22**:40-45 <http://dx.doi.org/10.1016/j.micinf.2019.08.002>.
 65. Thurston TL *et al.*: **Growth inhibition of cytosolic *Salmonella* by caspase-1 and caspase-11 precedes host cell death.** *Nat Commun* 2016, **7**:13292 <http://dx.doi.org/10.1038/ncomms13292>.
 66. Holly MK, Han X, Zhao EJ, Crowley SM, Allaire JM, Knodler LA *et al.*: ***Salmonella enterica* infection of murine and human enteroid-derived monolayers elicits differential activation of epithelial-intrinsic inflammasomes.** *Infect Immun* 2020, **88**:e00017-e00020 <http://dx.doi.org/10.1128/IAI.00017-20>.
- This study compares the relative importance of inflammatory Caspases in human and murine enteroids during *S. Tm* infection. The authors conclude that the relative contribution of IEC Caspases may differ between species, with murine IECs relying predominantly on Caspase-1, and human IECs predominantly on Caspase-4.
67. Kofoed EM, Vance RE: **Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity.** *Nature* 2011, **477**:592-595 <http://dx.doi.org/10.1038/nature10394>.
 68. Rauch I *et al.*: **NAIP proteins are required for cytosolic detection of specific bacterial ligands in vivo.** *J Exp Med* 2016, **213**:657-665 <http://dx.doi.org/10.1084/jem.20151809>.
 69. Rayamajhi M, Zak DE, Chavarria-Smith J, Vance RE, Miao EA: **Cutting edge: mouse NAIP1 detects the type III secretion system needle protein.** *J Immunol* 2013, **191**:3986-3989 <http://dx.doi.org/10.4049/jimmunol.1301549>.
 70. Yang J, Zhao Y, Shi J, Shao F: **Human NAIP and mouse NAIP1 recognize bacterial type III secretion needle protein for inflammasome activation.** *Proc Natl Acad Sci U S A* 2013, **110**:14408-14413 <http://dx.doi.org/10.1073/pnas.1306376110>.
 71. Zhao Y *et al.*: **Genetic functions of the NAIP family of inflammasome receptors for bacterial ligands in mice.** *J Exp Med* 2016, **213**:647-656 <http://dx.doi.org/10.1084/jem.20160006>.
 72. Zhao Y *et al.*: **The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus.** *Nature* 2011, **477**:596-600 <http://dx.doi.org/10.1038/nature10510>.
 73. Hersh D *et al.*: **The *Salmonella* invasin SipB induces macrophage apoptosis by binding to caspase-1.** *Proc Natl Acad Sci U S A* 1999, **96**:2396-2401 <http://dx.doi.org/10.1073/pnas.96.5.2396>.
 74. Zhang K *et al.*: **Minimal SPI1-T3SS effector requirement for *Salmonella* enterocyte invasion and intracellular proliferation in vivo.** *PLoS Pathog* 2018, **14**:e1006925 <http://dx.doi.org/10.1371/journal.ppat.1006925>.
 75. Van Opdenbosch N *et al.*: **Caspase-1 engagement and TLR-induced c-FLIP expression suppress ASC/Caspase-8-dependent apoptosis by inflammasome sensors NLRP1b and NLRC4.** *Cell Rep* 2017, **21**:3427-3444 <http://dx.doi.org/10.1016/j.celrep.2017.11.088>.
 76. Goncalves AV *et al.*: **Gasdermin-D and Caspase-7 are the key Caspase-1/8 substrates downstream of the NAIP5/NLRC4**

- inflammasome required for restriction of *Legionella pneumophila*.** *PLoS Pathog* 2019, **15**:e1007886 <http://dx.doi.org/10.1371/journal.ppat.1007886>.
77. Lamkanfi M *et al.*: **Targeted peptidecentric proteomics reveals Caspase-7 as a substrate of the caspase-1 inflammasomes.** *Mol Cell Proteomics* 2008, **7**:2350-2363 <http://dx.doi.org/10.1074/mcp.M800132-MCP200>.
 78. Mahib MR *et al.*: **Caspase-7 mediates caspase-1-induced apoptosis independently of Bid.** *Microbiol Immunol* 2020, **64**:143-152 <http://dx.doi.org/10.1111/1348-0421.12756>.
 79. Malireddi RK, Ippagunta S, Lamkanfi M, Kanneganti TD: **Cutting edge: proteolytic inactivation of poly(ADP-ribose) polymerase 1 by the Nlrp3 and Nlr4 inflammasomes.** *J Immunol* 2010, **185**:3127-3130 <http://dx.doi.org/10.4049/jimmunol.1001512>.
 80. Tsuchiya K *et al.*: **Caspase-1 initiates apoptosis in the absence of gasdermin D.** *Nat Commun* 2019, **10**:2091 <http://dx.doi.org/10.1038/s41467-019-09753-2>.
 81. Gurung P *et al.*: **FADD and caspase-8 mediate priming and activation of the canonical and noncanonical Nlrp3 inflammasomes.** *J Immunol* 2014, **192**:1835-1846 <http://dx.doi.org/10.4049/jimmunol.1302839>.
 82. Orning P *et al.*: **Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death.** *Science* 2018, **362**:1064-1069 <http://dx.doi.org/10.1126/science.aau2818>.
 83. Sarhan J *et al.*: **Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during Yersinia infection.** *Proc Natl Acad Sci U S A* 2018, **115**:E10888-E10897 <http://dx.doi.org/10.1073/pnas.1809548115>.
 84. Wang Y *et al.*: **Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin.** *Nature* 2017, **547**:99-103 <http://dx.doi.org/10.1038/nature22393>.
 85. Christgen S *et al.*: **Identification of the PANoptosome: a molecular platform triggering pyroptosis, apoptosis, and necroptosis (PANoptosis).** *Front Cell Infect Microbiol* 2020, **10**:237 <http://dx.doi.org/10.3389/fcimb.2020.00237>.
 86. Hefele M *et al.*: **Intestinal epithelial Caspase-8 signaling is essential to prevent necroptosis during *Salmonella* Typhimurium induced enteritis.** *Mucosal Immunol* 2018, **11**:1191-1202 <http://dx.doi.org/10.1038/s41385-018-0011-x>.
 87. Axelrad JE *et al.*: **Gastrointestinal infection increases odds of inflammatory bowel disease in a nationwide case-control study.** *Clin Gastroenterol Hepatol* 2019, **17**:1311-1322 <http://dx.doi.org/10.1016/j.cgh.2018.09.034> e1317.
 88. Arbeloa A *et al.*: **Distribution of espM and espT among enteropathogenic and enterohaemorrhagic *Escherichia coli*.** *J Med Microbiol* 2009, **58**:988-995 <http://dx.doi.org/10.1099/jmm.0.010231-0>.
 89. Croxen MA, Finlay BB: **Molecular mechanisms of *Escherichia coli* pathogenicity.** *Nat Rev Microbiol* 2010, **8**:26-38 <http://dx.doi.org/10.1038/nrmicro2265>.
 90. Orchard RC, Alto NM: **Mimicking GEFs: a common theme for bacterial pathogens.** *Cell Microbiol* 2012, **14**:10-18 <http://dx.doi.org/10.1111/j.1462-5822.2011.01703.x>.
 91. Collins JW *et al.*: ***Citrobacter rodentium*: infection, inflammation and the microbiota.** *Nat Rev Microbiol* 2014, **12**:612-623 <http://dx.doi.org/10.1038/nrmicro3315>.
 92. Petty NK *et al.*: **The *Citrobacter rodentium* genome sequence reveals convergent evolution with human pathogenic *Escherichia coli*.** *J Bacteriol* 2010, **192**:525-538 <http://dx.doi.org/10.1128/JB.01144-09>.
 93. Schnupf P, Sansonetti PJ: **Shigella pathogenesis: new insights through advanced methodologies.** *Microbiol Spectr* 2019, **7** <http://dx.doi.org/10.1128/microbiolspec.BAI-0023-2019>.