HHS Public Access

Author manuscript

Macrophage (Houst). Author manuscript; available in PMC 2015 December 18.

Published in final edited form as:

Macrophage (Houst). 2015 April 26; 2: e779–. doi:10.14800/Macrophage.779.

Macrophage cell death upon intracellular bacterial infection

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Abstract

Macrophage-pathogen interaction is a complex process and the outcome of this tag-of-war for both sides is to live or die. Without attempting to be comprehensive, this review will discuss the complexity and significance of the interaction outcomes between macrophages and some facultative intracellular bacterial pathogens as exemplified by *Francisella, Salmonella, Shigella* and *Yersinia*. Upon bacterial infection, macrophages can die by a variety of ways, such as apoptosis, autophagic cell death, necrosis, necroptosis, oncosis, pyronecrosis, pyroptosis *etc*, which is the focus of this review.

Keywords

macrophage polarization; bacterial pathogen; apoptosis; autophagic cell death; necrosis; necroptosis; oncosis; pyronecrosis; pyroptosis

Introduction

Before initiating an infection, bacterial pathogens come into contact with the human skin, respiratory or gastro-intestinal system and interact with different host cells, such as epithelial cell, PMN cell, and macrophage. As sentinels of infection, macrophages are one of the first cell types to encounter pathogens, and the frontline of defense when combating bacterial infection. During the host cell-pathogen interaction, macrophages can die in many ways such as apoptosis, necrosis, pyroptosis and autophagy, and sometimes they are intertwined

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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involving with different and complex underlying mechanisms[1]. This review will start in the introduction section with a brief summary of the four different facultative intracellular bacterial pathogens about their virulence factors and lifestyle most relevant to pathogen-macrophage interactions and follow with discussion of various macrophage cell death by a very concise introduction of the different cell death definition under each individual section.

Of these four facultative intracellular bacteria except *Francisella*, all the other 3 Genus of enteropathogens harbor one or even two Type three secretion systems (T3SS) which is important for their pathogenesis. These secreted T3SS molecules, *i.e.*, SipB (*Salmonella*), IpaB (*Shigella*), YopJ (*Y. pseudotuberculosis*) and YopP (*Y. enterocolitica*) are required for *Salmonella, Shigella* and *Yersinia* spp. to kill infected macrophages *in vitro*[2–5].

With some controversy, *F. tularensis* is described to consist of four subspecies: *tularensis, holarctica, mediasiatica* and *novicida*[6] and its virulence is partly reflected by its ability to replicate within host cells and its cytopathogenicity. Besides carrying one or two copies of the pathogenicity island (*F. novicida* has only one copy) where its major virulence arsenal and the T6SS locate, *F. tularensis* does not produce potent toxin nor harbor any T3SS[7]. Among the T6SS proteins, IglA and IglB are believed to form the putative outer tube (the “needle” part) and IglC the inner tube of the T6SS whereas IglI and VgrG are secreted[7].

Two distinct species have been described for the Genus of *Salmonella*: *S. bongori* and *S. enterica*, and the latter contains two T3SS which secrete effector proteins into the host cytosol. Virulence factors encoded by *Salmonella* Pathogenicity Island-1 (SPI-1) are crucial for invasion while those 30-ish molecules secreted by SPI-2 play a key role in the intracellular bacterial survival[8, 9].

S. dysenteriae, S. flexneri, S. boydii and *S. sonnei* are the four species in the genus of *Shigella* and the 214 kb virulence plasmid of *S. flexneri* contains T3SS including the *mxi* and *spa* genes encoding IpaB acting as a prominent component of the Mxi-Spa translocon[10, 11].

Human pathogenic *Yersinia* species include *Y. pestis, Y. pseudotuberculosis*, and *Y. enterocolitica*, and they all contain a virulence plasmid encoding T3SS and *Yersinia* outer proteins (Yops)[12].

After internalization, these four genus of facultative intracellular pathogens are initially enclosed in spacious[13] or compact[14] phagosomes (the *Francisella*-, *Salmonella-, Yersinia*-containing vacuole). *Salmonella* are capable of persisting in the relatively mild phagosome uncoupled from the normal endocytic route and live inside the macrophage by subverting the formation of phagolysosome thus inhibiting digestion by lysosomal action, which provides an environment for the pathogen to hide from the immune system and replicate[14]. Within a certain period of time varying between pathogens, phagosomes are destroyed through known or unknown bacterial factors, allowing microbe access to the cytosol where the cytoplasmic bacteria replicate[14]. *Y. pestis* is armed with a certain arsenals when encountering with macrophages and can resist the engulfment of the macrophage and choose to remain extracellular[12].

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For years scientists have proposed several versions of recommendations to standardize the nomenclature of cell death for the purpose of better communication among scientists and ultimate acceleration of the scientific discovery pace[1]. With the progress in this field and judging from genetic and biochemical changes, the border between different cell deaths becomes blurry. As per nomenclature proposed by the Nomenclature Committee on Cell Death[1], we use these definitions therein in each category if available. For the purpose of creating less confusion, this review will quote the different sometimes controversial names of macrophage cell death as used in the original paper.

Apoptosis

Intrinsic apoptosis is a mitochondrion-centered control mechanism mediated by mitochondrial outer membrane permeabilization (MOMP), and extrinsic apoptosis can be suppressed by chemical or viral inhibitors and is a caspase-dependent cell death via at least one of three major lethal signaling cascades with the involvement of death receptors, caspases (-3, -8, -9), bid, and MOMP at different order and time[1]. Challenging the traditional belief that apoptotic cell death is generally viewed as non-inflammatory or immunosuppressive, a recent study indicates that apoptosis may not necessarily be immunologically silent[15].

Shigella

Shigella is the first demonstration that a bacterial pathogen and its clinical cytotoxic isolates of both invasive *S. flexneri* and *S. Sonnei* can trigger macrophage apoptosis whereas its non-invasive, plasmid-cured isogenic strain and *ipa*B (invasion protein antigen) mutant cannot[2, 16–18]. To verify that the macrophage death is really apoptosis and not oncosis as questioned[19], Hilbi *et al.*[20] repeated the *Shigella* infection on human monocyte-derived macrophages (HMDM), analyzed the infected macrophages with similar assays as in reference[19], and stood firm that HMDM indeed still die of apoptosis. There were two major differences in their research approaches though. Hilbi *et al.*[20] checked the TUNEL/DNA fragmentation at one hour post infection (PI) of the macrophages and had seen the DNA ladder pattern of apoptosis whereas the other group did that at 30 min and failed to see the ladder. Probably the concentration of the caspase inhibitor used in the work of Fernandez-Prada *et al.*[19] was also too low to see any inhibition of caspase activity since protection of casp-1 inhibitor is inhibitor-dose and infection-dose dependent.

Contrary to the above mentioned case where virulence is needed for apoptosis induction, *Shigella* is found to induce apoptosis in interferon-γ differentiated U937 cells in a virulence-independent fashion, *i.e.*, it does not require bacterial internalization as antibiotic-killed wildtype (WT) *Shigella* or its live avirulent mutant, or even *Escherichia coli* strain JM109 can do it[21, 22]. It appears that interferon-γ treatment sensitizes U937 cells and lowers its threshold for some bacterial product(s) to trigger apoptosis.

Since *ipa*B mutant is no longer able to translocate many effector proteins by T3SS, the following experiments using the purified IpaB protein make it more convincing than the mutant strain only to demonstrate that IpaB is the molecule that induces apoptosis. IpaB was found to colocalize with casp-1 in the macrophage cytoplasm after microinjecting

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macrophages with purified IpaB protein[23, 24], on the bacterial surface and vesicular membranes[25] and bound directly to casp-1 but not to casp-2 or casp-3[26]. Cholesterol is needed for casp-1 activation, and thus *Shigella*-induced macrophage apoptosis because casp-1 is host cell membrane associated when colocalizing with IpaB[25].

Francisella

Most of the *Francisella* molecules critical for apoptosis induction are encoded by the pathogenicity island, either secreted by T6SS or being the core components[7, 27]. *F. holarctica* live vaccine strain (LVS) and its *tol*C mutant induces J774 apoptosis by different kinetics but similar mechanism via an intrinsic apoptotic pathway with mitochondrial damage, PS expression, casp-3 activation, and DNA fragmentation[28–33]. The *tol*C mutant is hypercytotoxic with a kinetic as early as 7 h PI and causes apoptosis with faster cleavage of casp-3, casp-9, and PARP but independent of casp-1 and casp-8 in murine macrophages[29, 31–34] whereas most hypercytotoxic *Francisella* mutants induce pyroptosis[35]. Although *F. novicida* WT strain U112 triggers casp-3 and casp-1 activation by 6 h PI, apoptosis is delayed in infected HMDM[36]. Modulation of Fas and SHIP expression, or regulation of PI3K/Akt and MAPK pathways also contribute to apoptosis induction by *Francisella* infection[37, 38].

Francisella mutants unable to proliferate intracellularly are unable to induce apoptosis with two exceptions[32, 39]. *igl*I mutant can replicate as well as its parental strain LVS but unable to kill macrophages whereas the *pdp*C mutant cannot replicate but still can induce macrophage apoptosis with a delayed pace[32] by unknown mechanism. Other mutants that are partially compromised in their ability escaping out of the phagosome can induce macrophage apoptosis but with a slower kinetics.

As the first example that inflammasome is involved in apoptosis induction[40], casp-1$^{-/-}$ bone marrow-derived macrophages (BMDM) die via casp-3-dependent apoptosis upon sensing cytosolic *F. novicida* DNA, a process where active casp-8 as the initiator caspase, interacts with ASC using the AIM2/ASC inflammasome complex as a novel activation platform. With *F. novicida*, WT macrophages start to die at 6h PI, casp-8 and casp-9 processing in casp-1$^{-/-}$ BMDM macrophages starts at 8h PI and LDH release at 10h PI, which is in contrast with the death of ASC$^{-/-}$ macrophages observed only at 24h PI[40].

In vivo, casp-3 activation and apoptotic cell death have been observed in C57BL/6 mice challenged with type A *F. tularensis* by the intranasal route[31] and in female C3H/HeN mice infected with the hypercytotoxic *tol*C mutant[33]. At 4 days PI, extensive cell death is within tissues of type A *F. tularensis*-infected WT and casp-1$^{-/-}$ but not casp-3$^{-/-}$ mice, and dying cells express activated casp-3 but very little activated casp-1, confirming that apoptosis *in vivo* was not mediated by activated casp-1[31]. In infected female C3H/HeN mice, 80% splenocytes are casp-3-positive at day 3 PI with the *tol*C mutant as compared to the 8% for the WT LVS[33].

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Salmonella

Three groups of researchers in 1996 demonstrated that *Salmonella* induces apoptosis respectively in J774, RAW264.7, and murine BMDM macrophages[3, 4, 41], which requires bacterial internalization but is strictly dependent upon the expression of the invasion-associated SPI-II T3SS. Mutations in invJ, spaO, sipB, sipC and sipD, but not sipA and sptP abolishes apoptosis[3] and mutants unable to cause host cell membrane ruffling also fail to induce apoptosis[4]. The rapid, extensive macrophage apoptosis caused by the Lon protease mutant of *S. typhimurium* involves both casp-1 and -3[42]. Purified SipB interacts with casp-1 and results in its activation after microinjection into macrophages, which leads to apoptosis[43].

Subsequent studies report apoptosis induction by *Salmonella* with different death kinetics[44–50]. *Salmonella* of a particular growth phase (transition from the exponential to the stationary) is reported to induce 90% of the macrophages apoptosis within 30 min PI[44]. Another relatively rapid apoptosis occurs within 2 hours PI with *S. typhimurium* grown logarithmically (SPI-1 expression), which depends on *sip*B and casp-1 but without activation of casp-3 and -8[3]. A delayed type of apoptosis occurs between 5 to 12 h PI in casp-1$^{-/-}$ macrophages with stationary-phase cultures (SPI-II expression) which is *sip*B independent[45, 48, 49] or even without intracellular bacterial growth[50], similar to the *Francisella* case[32].

Salmonella-induced apoptosis can be a casp-1–independent pathway that involves the release of cytochrome *c* from mitochondria and sequentially targets casp-2, -3, -6, and -8[48]. At the top of the caspase activation cascade in *Salmonella*-infected casp-1$^{-/-}$ macrophages is casp-2, already activated 10 min PI, with casp-3 in between and followed by casp-6 and -8 in the late phase processed 3 h PI. Under anaerobic conditions *S. typhi* can induce macrophage apoptosis mediated by casp-3, reactive nitrogen intermediates and monokines[51].

Raf-1 suppresses casp-1 activation, and thus macrophages with conditional inactivation of Raf-1 become more sensitive towards *Salmonella* infection[52]. Casp-1 activity and inflammasome receptors NLRP3, NLRC4 are required to inhibit *Salmonella* to cause delayed apoptosis in primary BMBM as monitored with the activity-based probe AWP28[53]. Without inflammasome-mediated casp-1 activation or in the absence of inflammasome receptors NLRP3, NLRC4, BMDM infected with *Salmonella* can undergo apoptosis[53]. The increased resistance of *casp-1*$^{-/-}$ mice to *Salmonella* infection appears specific since they remain susceptible to colonization by *Y. pseudotuberculosis*[54].

Co-activation of Akt by tyrosine kinase and PI-3K in receptor-mediated phagocytosis protects cells from apoptosis whereas direct activation of Cdc42 and Rac1 is needed for invasive *Salmonella* to kill U937 macrophages[55]. A geranylgeranyltransferase-1 inhibitor that prevents prenylation of Cdc42 and Rac1, GGTI-298, remarkably inhibits apoptosis induction of *Salmonella*[55]. Neither p65 nor IKKβ protects against *Salmonella*-induced apoptosis.

Yersinia

All the three *Yersinia* species pathogenic to humans are able to induce apoptosis in naïve macrophages. Early in 1997 three independent laboratories reported that two species of *Yersinia* causes apoptosis in human and murine macrophages respectively, all showing that Yop effector(s) secreted via the T3SS by bacteria adherent to but outside the macrophages is necessary for inducing apoptosis[5, 56, 57], two reports specifically point out that YopJ in *Y. pseudotuberculosis* and YopP in *Y. enterocolitica* are required for apoptosis[56, 57] and one mentions that *yop*E mutant is more efficient than WT in apoptosis induction for reasons unknown[56]. It is hard to make the third species of *Yersinia* (*Y. pestis*) cause apoptosis even with high MOIs and initial close contact of the bacteria with macrophages by centrifugation[58]. A decade later since the finding of YopJ(P) induction of macrophage apoptosis, Lilo *et al.* found that infection with *Y. pestis* Kim strains can easily induce BMDM apoptosis[59]. Definitely there are other genetic differences between the Kim strains and other *Y. pestis* strains, it is the two amino acid (F177L and K206E) differences in the sequence of YopJ isoform in the *Y. pestis* Kim strains from that of *Y. pestis* CO92[59] that make it easier for Kim strains to induce macrophage apoptosis. Unlike the cell death caused by strain CO92 *pgm, Y. pestis* Kim strains induce apoptosis when casp-1 activity in macrophages is either inhibited (by inhibitor YVAD) or absent (in *casp-1*$^{-/-}$ macrophages).

Yersinia infection cleaves Bid to its truncated form tBid and tBid translocation to mitochondria induces cytochrome c release, which further leads to cascade activation of procasp-9, -3 and -7[60]. Bid cleavage clearly occurs before cytochrome *c* release so its cleavage is not affected by the inhibition of casp-3 and -7[60], suggesting a separate death pathway. *Y. pestis* can induce YopJ-dependent apoptosis of RAW264.7 macrophages via the intrinsic or extrinsic pathways and YopK is required for the extrinsic pathway independent of casp-9 and acting upstream of casp-8 to regulate YopJ-mediated apoptosis[61], which can be blocked by the inhibition of casp-8. Although macrophages primed by IFN-γ are less sensitive to apoptosis, *Y. pestis* can overcome the protection of LcrV antibody and the counterattack from IFN-γ stimulation and still manage to induce apoptosis in murine macrophages[62]. Although MAPK pathway promotes macrophage survival independently of the NF-κB pathway in response to *Yersinia* infection, macrophage cells can use both pathways to up-regulate apoptosis inhibitor gene expression, and IKKβ and the subunit p65 of NF-κB to protect cells from apoptosis, so disrupting these pathways by YopJ is important for rapid apoptosis induction[63].

Early following infection *in vivo*, YopK can modulate macrophage apoptosis[61] but ultimately WT *Yersinia* can cause Mac-1^{+} cell apoptosis in mesenteric lymph nodes and spleens[64].

Autophagic cell death

Autophagy is an activated response to cellular stress by dying cells, and can be inhibited by specific chemicals and/or suppressed by genetic means by knocking down the expression of some distinct essential autophagic proteins, which will accelerate, rather than prevent, cell death[1].

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Macrophages infected with *S. typhimurium* can die by autophagy via disrupting mitochondria making them swollen and devoid of christae through the membrane fusion activity of sipB[65]. Upon infection, casp-1$^{-/-}$ BMDM macrophages form numerous unusual multimembrane-bound autophagosomes which contain both mitochondrial and endoplasmic reticulum markers.

Although intended to function as a host defense mechanism to eliminate invading pathogens, certain virulent pathogens can manipulate the endosomal membrane system and induce autophagy to enhance their intracellular replication but autophagy does not actually execute host cell demise. Of note, those autophagy that cannot be blocked by inhibitor(s) should not be classified as autophagic cell death.

Necrosis

Recent discoveries find that necrosis may also be programmed, thus a regulated process, and by definition is oversimplified and may include other forms of cell death as discussed elsewhere in this review.

Salmonella-infected macrophages can be killed by an unusual necrosis depending on casp-1 that glycine can completely block the cytotoxicity[66].

The intracellular ATP levels of HMDM but not monocytes infected by virulent *Shigella* drop by >50% within 30 min PI, and within 2 h, 59% ± 6% of HMDM cell membrane is permeable[67]. The *ipa* mutant strain N1411 is unable to cause this rapid necrosis and cytochalasin D pretreatment of macrophages can prevent *Shigella*-induced necrosis[21, 67]. Within one hour of infection, *S. flexneri* induces necrotic cell death in J774 and all-*trans*-retinoic acid differentiated U937 macrophages with pores estimated of about 2.87 nm in diameter inserted into the host cell membrane[21, 67], where DNA fragmentation in the nuclei of dead macrophages by TUNEL staining is diffuse and without casp-1 and casp-3/-7 activation as measured fluorometrically. In contrast, the infected macrophages in another study die of necrosis with cleavage and activation of casp-1, -3, and -9, which is independent of TLR4 or IpaB activity and potentially mediated by bacterial lipid A translocation into macrophage cytosol[68]. What causes this difference is currently unknown. Necrosis in casp-1$^{-/-}$ macrophages occurs later as compared with the WT macrophages[68].

Necroptosis

Necroptosis is sometimes induced by TNF-mediated TNFR1 ligation through activation of the RIP family kinases and dependent on activation of RIP1, RIP3, or mixed lineage kinase domain-like[69], which is inhibited by the RIP1-targeting necrostatin[1]. Since casp-8 is the central canonical inhibitor of kinases of the RIP family and necroptosis, necroptosis can be suppressed by casp-8/FADD-mediated apoptosis[69], or conversely when casp-8 activity is blocked it leads to necroptosis[70]. Up to date, there is only one example in the literature describing that facultative intracellular bacterium causes infected macrophages necroptosis[71].

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BMDM of the WT or of the TNFR1&2$^{-/-}$, *Ifnar1*$^{-/-}$ (IFNAR-deficient), *Rip3*$^{-/-}$ mice with C57BL/6J background are infected with *S. typhimurium* and cell death is defined as necroptosis based on the following findings that treatment with necrostatin, or YVAD-CHO, or RIP3-specific small interfering RNA, results in substantially less macrophage death. The PARP cleavage pattern into approximately 72 kDa/50 kDa fragments is specific for necrotic death, which is quite different in size from the 89 kDa/24 kDa fragments of apoptotic cells. TUNEL staining in infected WT macrophages is diffuse, which is in sharp contrast to the typical condensed chromatin staining in apoptosis. Casp-8 is downregulated in infected macrophages. Moreover, macrophages from *Rip3*$^{-/-}$ mice undergo significantly less *S. typhimurium*–induced death. Anti-TNF treatment of WT macrophages has no effect in preventing necroptosis and TNFR1&2$^{-/-}$ macrophages are not resistant to death from infection of *S. typhimurium*, which is different from the initial study where necroptosis is induced by TNFR1 ligation[70, 71].

Compared to the massive necroptosis and induction of type I interferon (IFN-α and IFN-β) in the WT macrophages, *Ifnar1*$^{-/-}$ macrophages can resist necroptosis induction by *S. typhimurium* infection even primed with LPS. Consistent with its intact cytokine signaling, these *Ifnar1*$^{-/-}$ macrophages have almost the same level of NF-κB subunit p65, phosphorylated IκB, phosphatidylinositol-3-OH kinase, p10 fragment of casp-1 and the transcription factor STAT1, STAT3 and secretion of type I interferon, IL-6 and IL-12. The only observed difference is that *Ifnar1*$^{-/-}$ macrophages do release more IL-1β than the WT macrophages but its resistance to *S. typhimurium* infection seems to be independent of IL-1β release. Although neutralization of IL-1β has no substantial influence on the burden of *S. typhimurium* in *Ifnar1*$^{-/-}$ mice, treatment with anti-IFN-β but not anti-IFN-α, or L-NMMA prevents necroptosis of WT macrophages *in vitro*.

In the absence of type I interferon signaling, survival of intravenous and intraperitoneal *S. typhimurium*-infected *Ifnar1*$^{-/-}$ mice is enhanced and the bacterial burden in spleen and liver is much lower, whereas those TNFR1 and TNFR2, or iNOS2 or IFN-r deficient mice have a slightly higher susceptibility to *S. typhimurium* infection and IL-6 seems insignificant for infection. Furthermore, 5 days PI of *S. typhimurium*, spleens of *Rip3*$^{-/-}$ mice have remarkably more CD11b^{+}F4/80^{+} macrophages and contain less TUNEL^{+} or propidium iodide positive staining macrophages than those of the WT. In summary, *S. typhimurium* infection leads to casp-8 downregulation, type I interferon (IFN-α and IFN-β) induction and necroptosis mediated by RIP1- and RIP3, and ultimately lost control of pathogen in macrophages.

Oncosis

Oncosis is eukaryotic cell death featured by cellular swelling[72, 73] with unclear mechanism, although ATP depletion or increase of intracellular calcium level has been suggested to be the cues that eventually leads to cellular swelling and further malfunction of ion channels[74].

HMDM undergo oncosis one hour PI with *S. flexneri* uninhibitable with caspase inhibitor ZVAD-fmk[19, 75], and human monoblastic U937 cells undifferentiated or differentiated

with all-*trans*-retinoic acid die with features of oncosis 2 h after *S. flexneri* infection[22]. Intracellular *Salmonella* can induce oncosis in three kinds of macrophages (RAW264.7, J774A.1, and BALB/c peritoneal macrophage cells)[76]. RAW264.7 macrophages infected with opsonized log phase *Salmonella* show morphology of oncosis after 6 h and exhibit casp-1 and -3 activities but are TUNEL negative[76]. PARP activity or DNA fragmentation is not required in the lysis of *S. typhimurium*-infected macrophages, which distinguishes oncosis from pyroptosis[77]. How macrophages will react to stationary phase *Salmonella* infection under this experimental setting remains to be tested.

Pyronecrosis

A novel type of necrotic macrophage death termed pyronecrosis mediated by the CIAS1/Cryopyrin/NLRP3 and ASC is reported in *S. flexneri* infection[78].

Peritoneal macrophages and BMDM from WT or *CIAS1*$^{-/-}$ mice, and ASC-deficient THP-1 macrophages infected with *S. flexneri* undergo pyronecrosis within 6 h with no cleavage of PARP. WT *Shigella* but not the plasmid-cured BS103 strain causes *CIAS1*-dependent pyronecrosis in primary macrophages. In contrast, *S. flexneri*-induced pyronecrosis is substantially reduced in *CIAS1*$^{-/-}$ BMDM and ASC-deficient THP-1 cells. Whereas casp-1$^{-/-}$ BMDM die a similar rate relative to its WT counterpart upon *S. flexneri* infection, casp-1 remains essential for IL-1β activation. In addition, *Shigella*-induced pyronecrosis triggers the release of HMGB1, a chromatin-associated protein and a proinflammatory mediator from necrotic cells. Although the casp-1-specific inhibitor YVAD abrogates IL-1β and IL-18 secretion substantially, it fails to block pyronecrosis. Glycine, previously effective for reducing *Shigella*-induced apoptosis and IL-1β release[79], is not effective at all for pyronecrosis. Among all the inhibitors tested, only the cathepsin B inhibitor Ca-074-Me substantially blocks cell death. Taken together, these results suggest that *Shigella*-induced pyronecrosis proceeds through cathepsin B, is mediated by cryopyrin and ASC, independent of either casp-1, IL-1β, IL-18 or the inflammasome. Macrophage cell death induced by *Francisella, S. typhi* and *S. typhimurium* are cryopyrin-independent, in sharp contrast to the cryopyrin-dependence of *Shigella*, demonstrating that the role of cryopyrin is pathogen specific and cannot be generalized to any intracellular bacteria.

Pyroptosis

Depending on casp-1 and whether suppressible by genetic means or with specific exogenous caspase inhibitors, pyroptosis is related to the generation of pyrogenic mediators and regulated by inflammasome which contains cytosolic pattern recognition receptors (PRRs), casp-1, and often the adapter protein ASC[1]. There are at least 8 different inflammasomes identified so far[80], and 5 of them are involved in bacterium-induced pyroptosis[81]. NLRP1, AIM2, IPAF, NLRP3 are activated by anthrax lethal toxin, cytosol DNA, flagellin or T3SS component, and a broad range of stimuli, respectively. Aggregation of ASC with NLR/PYHIN and casp-1 leads to the formation of one large ASC focus, which mediates very efficient processing of pro-inflammatory cytokines and release of cytokines[82].

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Salmonella

First used in 2000 to define the cell death in macrophages infected by *Salmonella*[72], the proinflammatory pyroptosis is induced by *Salmonella* which is detected by NLRP3 and NLRC4 inflammasomes, resulting in casp-1 activation which is required to bypass apoptosis[83, 84]. Although both *S. typhi* and *S. typhimurium* can induce pyroptosis in naive macrophages[85], prior activation by LPS or IFN-γ lowers the threshold to pyroptosis for RAW 264.7 macrophages[4]. *Salmonella*-induced pyroptosis in IFN-γ sensitized RAW264.7 cells that express guanylate binding protein 5 requires T3SS SPI-1 and the activation of casp-1[86]. *S. typhimurium* infection of the non-mammal sea bream macrophages also induces a casp-1-dependent pyroptotic cell death, and processing and secretion of IL-1β that is casp-1-independent[87], indicating that pyroptosis is reserved among vertebrate animals. Ca^{2+} and potassium fluxes are not necessarily required for *Salmonella* to activate casp-1 and induce inflammasome formation[88], different to occasions where they are needed for casp-1 activation. *Salmonella* induces expression and activation of casp-11 through a Toll-like receptor 4 (TLR4)-dependent and TIR-domain containing adaptor-inducing IFN-β (TRIF)-mediated IFNβ signaling pathway. Consistent with this, *Ifnar1*$^{-/-}$ or *Irf3*$^{-/-}$, or *Stat-1*$^{-/-}$ macrophages infected with mutant *Salmonella* does not process casp-11 or activate the non-canonical cell death pathway. Furthermore, c*asp1*$^{-/-}$ mice are significantly more susceptible to *Salmonella* infection than the double knockout mice (*casp-1*$^{-/-}$/*casp-11*$^{-/-}$)[89].

Francisella

Although seems dispensable for normal NF-kB and ERK signaling, ASC is essential for casp-1 activation in WT *F. novicida* and LVS-induced pyroptosis whereas NLRP3, NLRC4, IPAF are not, and Nod2 is not needed for detection of intracellular *F. tularensis*[90]. Cytosol *Francisella* or their DNA induces type I IFN in an IRF3-dependent manner[91] and Aim2 inflammasome activation and pyroptosis[90–92], and in *Irf3*$^{-/-}$ and *Ifnar*$^{-/-}$ BMDM, Aim2 inflammasome activation and IL-1β secretion are abrogated[93]. Further study reveals that a group of *F. novicida* hypercytotoxic mutants lyse more intracellularly, thus cause more AIM2-dependent pyroptosis and enhance other innate immune signaling pathways[94]. During *F. novicida* infection *in vivo*, extensive type I IFN–dependent pyroptosis occurs, resulting in macrophage depletion and control of bacterial replication[91].

Shigella

Shigella needs an intact T3SS to induce the IPAF-dependent, flagellin and ASC-independent macrophage pyroptosis[95]. A recent study reports that other proteases can contribute to proIL-1β cleavage in addition of casp-1[95]. Purified IpaB can form ion channels by spontaneous oligomerization and inserting into the host plasma membrane, which perturbs homeostasis of monovalent anorganic cations in the endolysosomal compartments. The chain reaction of membrane disintegration, endolysosomal leakage, IPAF/ASC inflammasome formation and casp-1 activation ultimately causes macrophage pyroptosis[96].

Yersinia

Pyroptosis of activated macrophages infected with *Y. pseudotuberculosis* requires the T3SS, a process without involvement of either YopJ or any of the other known translocated

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effector molecules from the bacterial side[97], or RIPK1, FADD, or casp-8 from the host cell side[98]. Casp-1 activation by a yet unknown ligand translocated by the T3SS of *Y. pestis* leads to casp-1-dependent pyroptosis in activated macrophages[97]. Currently two Yops are found to be able to inhibit casp-1 activation, in particular YopK can inhibit inflammasome activation in naïve, LPS-primed or LPS-activated macrophages[99]. YopM blocks pyroptosis in LPS-activated macrophages *in vitro* by inhibiting casp-1 activity via binding directly to and sequestering casp-1 and aborting inflammasome formation, and its role for *Yersinia* pathogenesis *in vivo* is demonstrated in disease development[99].

IPAF is critical in pathogen-induced pyroptosis but the role of the host adaptor protein ASC is controversial. For *Salmonella*, one study claims ASC is required for[100] whereas another suggests it is dispensable in casp-1 activation[95] and the resolution awaits. In the absence of ASC, casp-1 activation may be dispensable to *Shigella*-induced pyroptosis, which is a defining point much different from other cases of pyroptosis. While *Shigella* induces pyroptosis depending on cryopyrin, *S. typhimurium* or *F. tularensis* do in a cryopyrin-independent fashion[90, 101]. AIM2 is critical to *Francisella* but dispensable to *S. typhimurium* induction of pyroptosis[93].

Summary

Macrophage response during host–pathogen interactions is a complex process and bacterial pathogens can manipulate macrophage death pathways to influence the fate and outcome of infection/disease and the integrity of the host defense barrier/immunity. Some seemingly differences regarding the nature of macrophage death may simply attribute to the different experimental settings, such as the differences of host species, host cell types and pretreatments, bacterial strain and its preparation (for instance, culture), infection protocol *in vitro/in vivo* (route, dose/ multiplicity of infection, and duration), and the methodology used to analyze cells, and so forth. It appears that caspases can tip the balance and make the delicate decision which mode of macrophage cell death to go. In the absence (inhibition or depletion) of active apoptotic caspases, macrophages can die by an inflammatory cell death such as necrosis, oncosis, pyroptosis with the participation of IFNs and interleukins. Activation of the apoptotic caspase cascade can block the cGAS/STING pathway, inactivate the IFN response, and generate the classical "eat me" signal[102–104] to make the macrophage death immunologically silent.

Activated macrophages can be classified as M1 and M2[105] and some pathogens have evolved strategies to actively polarize cells toward the M2 phenotype as a virulence mechanism. *S. typhimurium* associates with M2 macrophages at later stages of infection[106] and requires them as a unique niche for long-term intracellular survival and persistence[107]. *Francisella* can redirect macrophage differentiation from M1 to M2 and survive at the expense of the host[108]. Whether M1 or M2 macrophages would die differently upon infection *in vivo* and *in vitro* remains to be investigated. Better understanding the molecular details of the macrophage death response during host–pathogen interactions will definitely provide new avenues for better control of bacterial infection and inflammatory disease progression.

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Acknowledgement

This work was supported by the National Natural Scientific Fund (81371748) and National Institutes of Health (R01GM100474-04).

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