The *Salmonella* Effector Protein SopB Protects Epithelial Cells from Apoptosis by Sustained Activation of Akt*

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Invasion of epithelial cells by *Salmonella enterica* is mediated by bacterial “effector” proteins that are delivered into the host cell by a type III secretion system. Although primarily known for their roles in actin rearrangements and membrane ruffling, translocated effectors also affect host cell processes that are not directly associated with invasion. Here, we show that SopB/SigD, an effector with phosphoinositide phosphatase activity, has anti-apoptotic activity in *Salmonella*-infected epithelial cells. *Salmonella* induced the sustained activation of Akt/protein kinase B, a pro-survival kinase, in a SopB-dependent manner. Failure to activate Akt resulted in increased levels of apoptosis after infection with a sopB deletion mutant (ΔsopB). Furthermore, cells infected with wild type bacteria, but not the ΔsopB strain, were protected from camptothecin-induced cleavage of caspase-3 and subsequent apoptosis. The anti-apoptotic activity of SopB was dependent on its phosphatase activity, because a catalytically inactive mutant was unable to protect cells from the effects of camptothecin. Finally, small interfering RNA was used to demonstrate the essential role of Akt in SopB-mediated protection against apoptosis. These results provide new insights into the mechanisms of apoptosis and highlight how bacterial effectors can intercept signaling pathways to manipulate host responses.

The bacterial species *Salmonella enterica* consists of over 2,000 closely related serovars that cause a variety of diseases in humans as well as other animals. *S. enterica* serovar Typhimurium, one of the most common food-borne pathogens, causes self-limiting gastroenteritis in humans and a similar diarrheal disease in calves and pigs. In contrast, infection of mice with *Salmonella* serovar Typhimurium, which is one of the most common food-borne pathogens, causes self-limiting gastroenteritis in humans and a similar diarrheal disease in calves and pigs.
identified SPI2 and spv genes (21). Because both SPI2 (33) and spv (34) genes are induced rapidly following internalization into host cells, but caspase-3 is not activated, we considered that a SPI1 effector could be actively delaying or inhibiting the onset of apoptosis in infected epithelial cells.

Thus, based on the observations that (i) SopB activates Akt, (ii) Akt is an important regulator of apoptosis in epithelial cells, and (iii) apoptosis is delayed in Salmonella-infected epithelial cells, we have investigated the role of the SPI1 effector SopB in survival of infected epithelial cells. Using several complementary approaches we show that SopB has an anti-apoptotic activity in infected epithelial cells, which is dependent on the phosphorylation activity of SopB and the presence of Akt.

**MATERIALS AND METHODS**

**Chemicals and Supplies**—Disposable tissue cultureware was from Corning Life Sciences (Acton, MA). Biochemicals were obtained from Sigma-Aldrich unless otherwise stated. Antibodies against Akt and secondary antibodies conjugated to horseradish peroxidase were obtained from Cell Signaling Technology, Inc. (Beverly, MA). Typhimurium, Cytodeath, a fluorescein-conjugated monoclonal antibody that recognizes a caspase-cleaved, formalin-resistant epitope of human cytokeratin 18, was obtained from Roche Applied Science. Secondary antibodies conjugated to Alexa 594 were from Molecular Probes (Eugene, OR). Recombinant human epidermal growth factor was from Calbiochem.

**Cells and Bacterial Strains**—HeLa cells (human adenocarcinoma cervix epithelial, ATCC CCL-2) and IEC-6 cells (rat small intestine epithelial, ATCC CRL-1592) were used within 10 passages of receipt from the American Type Culture Collection (Manassas, VA). HeLa cells were cultured in Eagle’s minimum essential medium (Mediatech, Inc., Herndon, CA) supplemented with 10% (v/v) fetal bovine serum (FBS, Invitrogen). IEC-6 cells were cultured in Dulbecco’s modified Eagle’s medium (Mediatech, Inc.) supplemented with 5% (v/v) FBS and 0.1 unit/ml bovine insulin. *S. enterica* serovar Typhimurium wild type strain SL1344 and the ΔsopB mutant were as described previously (12, 35). pDE is a pACYC184-based plasmid (New England Biolabs) that encodes SopB and its chaperone SigE under the control of the sopB promoter (36). The plasmid pDE C460S encodes a catalytically inactive SopB point mutant. To construct this mutant, pDE was partially digested with KpnI and blunt-ended by treatment with T4 DNA polymerase (New England Biolabs) and religated. A clone was chosen that retained the KpnI site within the sopB open reading frame, but lost the KpnI site downstream of sigE, and was designated pACDEΔKpnI. A 376-bp KpnI-Stul fragment containing the C460S mutation was then excised from pMWDE C460S (12) and ligated into the corresponding sites of pACDEΔKpnI to create pDE C460S. This mutant was originally described previously (36) based on the original published sequence of sopB (GenBank™ accession number AAC46234) but has now been amended to pDE C460S based on the sopB sequence from the *S. enterica* serovar Typhimurium LT2 genome (accession number NP_600604).

**Preparation of SPI1-induced Bacteria**—For bacterial infections, SPI1-induced bacteria were prepared by diluting 0.3 ml of overnight LB culture in 10 ml of fresh LB and incubating at 37 °C with shaking (200 rpm). At late log phase (3.5 h), the bacteria were pelleted at 7,000 × g for 2 min and resuspended in an equal volume of Hanks’ balanced salt solution (Invitrogen) or phosphate-buffered saline. This suspension of hyperinvasive bacteria was then used to inoculate cells using invasion times of 10–45 min as indicated.

**Apoptosis Assays**—For microscopic analysis of apoptosis, cells were seeded on glass coverslips in 24-well plates 24 h prior to infection and serum-starved (0.5% FBS) for 3 h prior to infection. 5 (HeLa) or 10 μl (IEC-6) of SPI1-induced bacteria were then added directly to each well to give an multiplicity of infection of ~100. After 15 (HeLa) or 45 min (IEC-6) of infection, the monolayers were washed three times in phosphate-buffered saline and then incubated in fresh culture medium containing 0.5% FBS. After 20 min, fresh culture medium (0.5% FBS) containing 50 μM gentamycin was added for 1 h followed by 5 μg/ml gentamycin maintained throughout the remainder of the experiment. For HeLa cells, apoptosis was measured at 4 h postinfection (p.i.) using M30 Cytodeath staining according to the manufacturer’s instructions. Levels of apoptosis in infected IEC-6 cells were measured at 4 h p.i. by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) using an In Situ Cell Death Detection kit (Roche Applied Science) according to the manufacturer’s instructions. For both cell types, nuclei were counterstained with 4',6-diamidino-2-phenylindole. Using a Zeiss Axioscope fluorescence microscope, cells were observed and scored for the number of Cytodeath-positive or TUNEL-positive cells/total cell nuclei (n ≥ 100/ experiment). Results are the mean ± S.D. from at least three separate experiments.

**RESULTS**

SopB Induces Sustained Akt Phosphorylation in Epithelial Cells—Previously we have shown that SopB induced Akt phosphorylation in *S. enterica* serovar Typhimurium-infected epithelial cells for at least 2 h p.i. (12). Here we have further analyzed the kinetics of Akt phosphorylation using an antibody that specifically recognizes Akt when it is phosphorylated on Ser-473. Treatment of cells with stimuli such as epidermal growth factor caused a transient increase in phospho-Akt that peaks at 2–5 min (Fig. 1) and is no longer detectable after ~10–20 min (12). In contrast, infection with wild type *S. enterica* serovar Typhimurium induced prolonged Akt phosphorylation that peaked at ~20 min and was detectable for at least 4 h postinfection. This phosphorylation was dependent on SopB, because an isogenic sopB deletion strain (ΔsopB) induced no significant phospho-Akt at any time point studied (Fig. 1). Taken together with our previous data (12) this shows that SopB is required for the sustained phosphorylation and activation of Akt in infected epithelial cells.
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Epithelial Cells Infected with the ∆sopB Mutant Have Increased Levels of Apoptosis at 4 h p.i.—Because Akt is a well characterized prosurvival kinase that mediates survival of intestinal epithelial cells, we next analyzed the levels of apoptosis in epithelial cells infected with S. enterica serovar Typhimurium. In HeLa cells the cleavage of cytokeratin 18, one of several keratins cleaved by caspases (37), was used as an early indicator of apoptosis. The cleavage of cytokeratin 18 generates a stable fragment that accumulates in the cytosol and can be detected using an epitope-specific monoclonal antibody (M30) (37, 38). Cells grown on glass coverslips were infected with Salmonella, fixed at 4 h p.i., and processed for immunofluorescence using the M30 antibody. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole to facilitate the identification of all cells. Infection with the ∆sopB mutant caused a greater than 9-fold increase in apoptotic cells, whereas wild type S. enterica serovar Typhimurium increased the number of M30-reactive cells by only 5.0-fold (Fig. 2). In contrast, when the ∆sopB strain was complemented with a pACYC184-based plasmid (pDE), which expresses the sopBsigE operon (SigE is the type III chaperone for SopB) under the control of the sopB promoter (36, 39), apoptosis was increased by only 3.1-fold. We confirmed these results in IEC-6 cells, a non-transformed crypt cell line derived from the rat small intestine, using a TUNEL assay to detect apoptotic cells. Overall, we detected slightly higher levels of apoptosis in IEC-6 cells compared with HeLa cells, with -fold increases of 10.4, 17.2, and 9.0 after infection for 4 h by wild type, ∆sopB, and ∆sopB pDE strains, respectively. Thus, in both of the cell types studied, using different assays for apoptosis, the ∆sopB strain induced significantly higher levels of apoptosis than either the wild type or the sopB complemented strain at 4 h p.i. (Fig. 2). Together these experiments suggest a role for SopB in preventing or delaying the onset of apoptosis in epithelial cells.

SopB Reduces Caspase-3 Activity in Infected Epithelial Cells—Caspases are the major executors of the apoptotic program. Therefore, to explore in more detail the mechanistic role of SopB, we measured caspase activity in infected HeLa cells. Cell-free lysates, prepared 3 h p.i., were incubated with fluorogenic caspase substrates and caspase activity was assayed by the release of the fluorescent compound AMC. Although substrates specific for caspases 3, 8, and 9 were compared, we only detected a statistically significant change in caspase-3 activity in cells infected with the ∆sopB strain compared with uninfected cells (a 1.6-fold increase). Complementation of ∆sopB with pDE abrogated the ability of this strain to induce caspase-3 activity (Fig. 3). These results confirm those of the previous experiment (Fig. 2) demonstrating that SopB mediates cellular survival. Furthermore, they suggest that the apoptosis induced by the ∆sopB mutant involves caspase-3 activation.

SopB Protects Epithelial Cells against Camptothecin-induced Apoptosis—We next considered the possibility that SopB can actively protect epithelial cells from apoptosis by preventing the activation of caspase-3. Therefore, we investigated the ability of S. enterica serovar Typhimurium to protect cells against the activity of camptothecin (CPT), a topoisomerase inhibitor that induces apoptosis in a caspase-3-dependent manner (40, 41). For these experiments bacteria were internalized into HeLa cells for 10 min, extracellular bacteria were then removed by washing, CPT was added, and the cells incubated for a further 3 h. The cytokeratin 18 cleavage assay was then used to detect apoptotic cells. Under these conditions ~13% of CPT-treated uninfected cells were M30 reactive and therefore apoptotic, compared with ~1% of untreated HeLa cells (p < 0.001) (Fig. 4A). Infection with wild type S. enterica serovar Typhimurium reduced CPT-induced apoptosis by ~50% (p = 0.003). This decrease was dependent on SopB, because the ∆sopB strain did not significantly affect the levels of CPT-induced apoptosis unless complemented with pDE. Furthermore, complementation of ∆sopB with a catalytically inactive mutant, SopB C460S, did not protect cells from drug-induced apoptosis. This indicates that the conserved cysteine residue Cys-460, which is essential for the inositol phosphatase activity of SopB and Akt activation (8, 12), is also necessary for protection against apoptosis.

SopB Inhibits Camptothecin-mediated Cleavage of Procaspase-3—Caspase-3 activity involves the cleavage of a 32-kDa precursor (p32) into 20-kDa (p20) and 12-kDa (p12) subunits, with p20 undergoing further conversion to a 17-kDa (p17) active subunit (42). Therefore, to confirm that SopB antagonizes caspase-3 activation, immunoblot analysis was used to monitor proteolytic conversion of the procaspase into the active subunits (Fig. 4B). Using an antibody that recognizes p32, p20, and p17, but not p12, only the p32 procaspase form was detected in untreated HeLa cells. Following treatment with CPT a significant proportion of p32 was cleaved as indicated by the appearance of p17. CPT treatment of cells infected with wild type S. enterica serovar Typhimurium resulted in decreased levels of p17, and the intermediate form (p20) was now also detected. In contrast, the ∆sopB mutant did not affect the CPT-induced levels of p17. Confirming the role of SopB in p32 protection, complementation of ∆sopB with pDE, but not C460S, restored the ability to block p32 processing. Immunoblot analysis of phospho-Akt in these samples revealed a direct correlation between Akt phosphorylation and protection against camptothecin-induced p32 processing (Fig. 4B, lower panel). These observations show that SopB can protect epithelial cells from CPT-induced apoptosis by preventing activation of caspase-3 and also implicate the involvement of Akt phosphorylation.

SopB-dependent Protection against Apoptosis Requires Akt— Having shown that SopB is required for a rapid and sustained increase in Akt phosphorylation and activation (Fig. 1 and Ref. 12), delays the onset of apoptosis, and protects epithelial cells from the effects of CPT, we next asked whether Akt is required for SopB-mediated protection against apoptosis. To do this, siRNA was used to specifically deplete the two Akt isoforms (Akt1 and Akt2) present in HeLa cells. Fig. 5 (Total, lanes 4–6, both panels) demonstrates that Akt was significantly depleted 48 h after siRNA transfection using pooled Akt1- and Akt2-specific siRNAs compared with Akt3-specific siRNA (lanes I–3). Analysis of phospho-Akt levels after infection with wild...
type *S. enterica* serovar Typhimurium (3 h p.i.) revealed that Ser-473 phosphorylation was drastically reduced in Akt1/Akt2-depleted cells (compare lane 5 with 2). If, as according to our hypothesis, Akt is required for SopB-mediated protection against apoptosis then wild type *S. enterica* serovar Typhimurium should not be able to protect Akt-depleted cells from the effects of CPT. Fig. 5 shows that, indeed, infection with wild type *S. enterica* serovar Typhimurium could no longer block CPT-induced cleavage of caspase-3 when Akt1 and Akt2 were depleted (right panel, compare lane 5 with 2). We also investigated whether Akt is required for SopB-dependent inhibition of apoptosis in the absence of drug treatment. In these samples the p32 procaspase predominates, and p17 and p20 are only visible after overexposure of the immunoblot (Fig. 5, bottom left panel). In cells transfected with control siRNA, the ΔsopB strain induced more caspase-3 processing then wild type *S. enterica* serovar Typhimurium (Fig. 5, compare lane 2 with 3). However, in Akt1/Akt2-depleted cells both *S. enterica* serovar Typhimurium strains induced comparable amounts of caspase-3 cleavage (Fig. 5, compare p17 levels in lane 5 with 6). These observations support our model where Akt is essential for the anti-apoptotic activity of SopB in epithelial cells.

### DISCUSSION

For intracellular pathogens such as *Salmonella* the establishment of a protected niche is a crucial step in the development of infection. Countermeasures by the host reduce the chances of pathogenic success, but these countermeasures can also drive the pathogen to develop even more sophisticated methods to manipulate cellular responses. For example, pathogens have evolved multiple and distinct mechanisms to modulate apoptotic pathways in host cells, and the diverse nature of these interactions reflects the complexity of the apoptotic program itself (43, 44). Viruses and other obligate intracellular pathogens, such as *Chlamydia* sp. (45), have presumably evolved under considerable selective pressure to modulate apoptotic pathways, because premature death of the host cell would prevent the completion of their replication cycle. However, in some situations, such as virus egress, apoptosis may be beneficial, and many viruses have also evolved pro-apoptotic mechanisms (43). Facultative intracellular pathogens such as *Salmonella* have also co-evolved with their host albeit under different selective pressures. From the bacterial perspective the most remarkable consequence of this process is TTSS, used specifically to deliver bacterial effector proteins into host cells. Once translocated into the host cell, effector proteins
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Infection with *S. enterica* serovar Typhimurium protects cells against camptothecin-induced apoptosis. A, HeLa cells were grown on glass coverslips and infected with *S. enterica* serovar Typhimurium wild type (wt), ΔsopB or ΔsopB complemented with a plasmid encoding either wild type SopB (pDE) or a catalytically inactive SopB mutant (C460S). Camptothecin (CPT) (5 μg/ml) was added to the cells immediately after the 10-min invasion. After 3 h the cells were fixed and immunostained with the monoclonal M30 antibody to detect apoptotic cells. Nuclei were counterstained with 4',6-diamidino-2-phenylindole. The number of apoptotic cells in the total population was then evaluated by fluorescence microscopy. Results are the mean ± S.D. from three separate experiments. B, immunoblot showing levels of procaspase-3 and cleaved caspase-3 in infected cells with *S. enterica* serovar Typhimurium wild type or the ΔsopB mutant. Where indicated the ΔsopB strain was complemented with a plasmid encoding wild type sopB (pDE) or a catalytically inactive sopB mutant (C460S). CPT (5 μg/ml) was added to the cells immediately after the 10-min invasion. After 3 h the cells were solubilized and equal volumes loaded onto a 12% polyacrylamide gel. Caspase-3 cleavage was detected by immunoblotsing using a polyclonal antibody that recognizes the pro-caspase (p32) as well as the 17-kDa active form (p17) and a 20-kDa intermediate form (p20). The experiment was repeated three times, and the results shown are from one representative experiment.

**FIG. 4.** Infection with *S. enterica* serovar Typhimurium protects cells against camptothecin-induced apoptosis. A, HeLa cells were transfected with siRNA sequences targeting human Akt isoforms 48 h prior to infection with *S. enterica* serovar Typhimurium. Akt3 serves as a siRNA control, because this isoform is not expressed in HeLa cells. Where indicated CPT (5 μg/ml) was added to the cells immediately after the 10-min invasion. At 3 h p.i. cells were solubilized, and proteins were separated by SDS-PAGE under reducing conditions then electrotransferred onto nitrocellulose membranes. Caspase-3 cleavage, Akt Ser-473 phosphorylation, and total Akt (Akt1 and Akt2 isoforms) were detected by immunoblotting using polyclonal antibodies. The experiment was repeated three times, and the results shown are from one representative experiment.

**FIG. 5.** Akt is required for *S. enterica* serovar Typhimurium-mediated protection of epithelial cells from apoptosis. Representative immunoblot showing cleavage of caspase-3 in HeLa cells transfected with siRNA sequences targeting human Akt isoforms 48 h prior to infection with *S. enterica* serovar Typhimurium. Akt3 serves as a siRNA control, because this isoform is not expressed in HeLa cells. Where indicated CPT (5 μg/ml) was added to the cells immediately after the 10-min invasion. At 3 h p.i. cells were solubilized, and proteins were separated by SDS-PAGE under reducing conditions then electrotransferred onto nitrocellulose membranes. Caspase-3 cleavage, Akt Ser-473 phosphorylation, and total Akt (Akt1 and Akt2 isoforms) were detected by immunoblotting using polyclonal antibodies. The experiment was repeated three times, and the results shown are from one representative experiment.

Salmonella can induce apoptosis by both SPI1- and SPI2-dependent mechanisms, the details of which remain unclear. The best characterized of these pathways, which occurs within 2 h of invasion, is mediated by the SPI1 effector SipB and involves caspase-1 activation (16, 30, 31). Delayed apoptosis can also be induced in macrophages via at least two pathways, which require caspase-1 or other caspases (14, 23) and can involve the disruption of mitochondria and autophagy (15, 19). In epithelial cells, apoptosis is not detectable until 12–28 h after invasion and is mediated by the SPI-TTSS, the *Salmonella* virulence plasmid (spv) and caspase-3 activation (21, 48). Because SPI2 and spv genes are induced rapidly after bacterial internalization into host cells (33), but there is an apparent lag in the induction of apoptosis in epithelial cells, we hypothesized that *Salmonella* can actively delay apoptosis in these early hours after infection. A likely candidate was the SPI1 effector SopB, because it has been shown to induce the Akt prosurvival signaling pathway in infected epithelial cells (12).

Here we have shown that SopB does suppress apoptosis in epithelial cells, specifically via the sustained activation of Akt and inhibition of caspase-3 cleavage. Compared with wild type bacteria, sopB deletion mutants do not activate Akt and consequently induce higher levels of apoptosis and caspase-3 activation after infection. Furthermore, epithelial cells infected with wild type *Salmonella*, but not the ΔsopB strain, are protected against the chemical induction of apoptosis. The promotion of cell survival via Akt kinase activation and the subsequent suppression of caspase-3 activity has been reported in response to diverse stimuli, including nutrient depletion (13) and growth factors (49, 50). From the results we present here, it appears that *Salmonella* is cleverly “tapping into” a signaling pathway used by mammalian cells to suppress apoptosis in response to non-pathogenic stimuli. Interestingly, the hepatitis B virus has also been shown to co-opt this signaling pathway to suppress apoptosis (51).

The intestinal epithelium has an extremely high turnover rate that is determined by the rates of cell proliferation and death. Apoptosis plays a critical role in maintenance of this balance and is regulated by numerous factors (40, 52). In normally maturing human intestinal epithelial cells caspase-3 is activated as the cells migrate and differentiate along the crypt-
villus axis toward the intestinal lumen (53). Normal intestinal epithelial cell growth depends on a supply of multiple factors, including polyamines, which are present at high concentrations in the lumen (54). Removal of polyamines from normal intestinal epithelial cells stimulates Akt activity thereby decreasing caspase-3 activity and increasing resistance to apoptosis (13). Perhaps by circumventing the normal polyamine-mediated down-regulation of Akt, SopB can delay the unavoidable, and usually rapid, apoptotic progression of intestinal epithelial cells. The most obvious advantage for the bacteria would be a “gain in time,” allowing Salmonella to establish a niche permissive for replication, because apoptotic intestinal epithelial cells are usually extremely rapidly destroyed or shed into the lumen.

During the intestinal phase of salmonellosis it is well established that SopB contributes to fluid secretion and the inflammatory response (5, 8, 22, 55, 56). Our data suggest that this SPI1 effector is also involved in the delay of apoptosis, although, the role of apoptosis in gastroenteritis is still unclear. A recent study using pig jejunal loops showed that though, the role of apoptosis in gastroenteritis is still unclear. SPI1 effector is also involved in the delay of apoptosis, although, the role of apoptosis in gastroenteritis is still unclear. Our data suggest that this SPI1 effector is also involved in the delay of apoptosis, although, the role of apoptosis in gastroenteritis is still unclear. SPI1 effector is also involved in the delay of apoptosis, although, the role of apoptosis in gastroenteritis is still unclear. Perhaps by circumventing the normal polyamine-mediated down-regulation of Akt, SopB can delay the unavoidable, and usually rapid, apoptotic progression of intestinal epithelial cells. The most obvious advantage for the bacteria would be a “gain in time,” allowing Salmonella to establish a niche permissive for replication, because apoptotic intestinal epithelial cells are usually extremely rapidly destroyed or shed into the lumen.

In conclusion, our results show that the SPI1 TTSS effector SopB acts as a prosurvival factor by preventing the execution of early onset apoptosis in epithelial cells. Therefore, in Salmonella-infected epithelial cells there exists an early SPI1-dependent anti-apoptotic effect that precedes the previously described late onset SPI2- and virulence plasmid-dependent pro-apoptotic effect. In comparison, in Salmonella-infected macrophages, rapid cell death is evident and SPI1-dependent (Table I). Comparing the anti-versus pro-apoptotic activities of SPI1 in epithelial cells and macrophages, respectively, it is apparent that Salmonella can selectively modulate host cell events depending on the type of cell infected, a further example of the remarkable ability of this pathogen to adapt to its host cell environment.

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| Cell death         | Caspase activity | Virulence factor(s) | Refs. |
|--------------------|------------------|---------------------|-------|
| Epithelial         |                  |                     |       |
| Early              |                  |                     |       |
| No cell death      | Caspase-3        | SopB                | This paper |
| Late               | Caspase-3 inhibition | SPI1/SPV, AvrA   |       |
| 18–24 h p.i.       |                  |                     | 21, 48, 58 |
| Macrophage         |                  |                     |       |
| Rapid              |                  |                     |       |
| Cell death         | Caspase-1        | SipB                | 15, 19 |
| <1 h p.i.          |                  |                     |       |
| Early Autophagy    | Not caspase-1, multiple caspases | SPI1 |       |
| 1–6 h p.i.         |                  |                     |       |
| Late Apoptosis     | Caspase-1        | SpvB                | 14, 19, 20, 22, 23, 60 |
| >12 h p.i.         |                  |                     |       |
| Dendritic          |                  |                     |       |
| Rapid              | Caspase-1        | SipB                | 24    |
| Cell death <1 h p.i. |                  |                     |       |

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