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Distinct Roles of TLR2 and the Adaptor ASC in IL-1β/IL-18 Secretion in Response to *Listeria monocytogenes*\(^1\)

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Apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (ASC) is an adaptor molecule that has recently been implicated in the activation of caspase-1. We have studied the role of ASC in the host defense against the intracellular pathogen *Listeria monocytogenes*. ASC was found to be essential for the secretion of IL-1β/IL-18, but dispensable for IL-6, TNF-α, and IFN-β production, in macrophages infected with *Listeria*. Activation of caspase-1 was abolished in ASC-deficient macrophages, whereas activation of NF-κB and p38 was unaffected. In contrast, secretion of IL-1β, IL-6, and TNF-α was reduced in TLR2-deficient macrophages infected with *Listeria*; this was associated with impaired activation of NF-κB and p38, but normal caspase-1 processing. Analysis of *Listeria* mutants revealed that cytosolic invasion was required for ASC-dependent IL-1β secretion, consistent with a critical role for cytosolic signaling in the activation of caspase-1. Secretion of IL-1β in response to lipopptide, a TLR2 agonist, was greatly reduced in ASC-null macrophages and was abolished in TLR2-deficient macrophages. These results demonstrate that TLR2 and ASC regulate the secretion of IL-1β via distinct mechanisms in response to *Listeria*. ASC, but not TLR2, is required for caspase-1 activation independent of NF-κB in *Listeria*-infected macrophages. *The Journal of Immunology*, 2006, 176: 4337–4342.

The elimination of infectious agents by the host is fundamental for the survival of multicellular organisms. In mammals, detection of microbial agents is mediated by the recognition of conserved and unique pathogen structures by specific host pattern recognition molecules, such as the TLRs (1). TLRs mediate host immune responses by inducing the secretion of proinflammatory cytokines and costimulatory surface molecules through the activation of transcriptional factors, including NF-κB and kinases of the MAPK family, including p38, ERK, and JNK (2). TLRs recognize microbial ligands at the cell surface or within intravesicular structures (1). Recent studies have identified a family of proteins called nucleotide-binding oligomerization domain

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7 Abbreviations used in this paper: NOD-LRR, nucleotide-binding oligomerization domain and leucine-rich repeat (NOD-LRR, or CATERPILLER) that are involved in cytosolic recognition of bacterial components and induction of innate immune responses (2, 3). The human NOD-LRR protein family contains >20 members, including Nod1, Nod2, cryopyrin/Nalp3, and Ipaf (2, 3). Nod1, Nod2, and cryopyrin/Nalp3 act as bacterial peptidoglycan recognition molecules through detection of distinct and conserved structures (4–7). The signaling pathways activated by NOD-LRR family members are beginning to emerge. Nod1 and Nod2 activate NF-κB and MAPK signaling, whereas Ipaf, cryopyrin, and Nalp1/Defcap have been implicated in the activation of caspase-1 and NF-κB and in apoptosis (8–10).

Apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (ASC) is an adaptor molecule originally identified in an insoluble cytosolic fraction called the speck, found in cells undergoing apoptosis (11). ASC is composed of an N-terminal pyrin domain and a C-terminal caspase recruitment domain (11). Recent studies have shown that Ipaf, cryopyrin, and Nalp1 regulate, in the presence of ASC, the activation of caspase-1 (8–10, 12). ASC has been proposed to link intracellular NOD-LRR proteins such as cryopyrin to caspase-1 through direct physical association (10, 12). Consistent with this, Nalp1 and cryopyrin can form an endogenous multiprotein complex containing ASC and caspase-1 called the inflammasome, which promotes caspase-1 activation (9, 12). Caspase-1 is synthesized as an inactive zymogen that becomes activated by cleavage after aspartic residues to generate the enzymatically active heterodimer in response to proinflammatory stimuli and bacteria (13). Activated caspase-1 is essential for the processing and release of biologically active IL-1β and IL-18, two proinflammatory cytokines with critical roles in
in innate and adaptive immunities (14). The secretion of IL-1β is also dependent on the levels of pro-IL-1β induced at the transcriptional level by proinflammatory stimuli, including several TLR ligands (14). Recent results suggest that ASC plays an important role in caspase-1 activation as well as IL-1β and IL-18 processing and secretion induced by stimulation with bacterial products and Salmonella infection ex vivo (15, 16). However, the precise signaling pathways that regulate the secretion of IL-1β/IL-18 in response to live bacteria and the relationship between TLR and ASC signaling remain poorly understood. In addition, the role of ASC against pathogenic bacteria in vivo has not been studied.

Listeria monocytogenes is a Gram-positive, facultative intracellular bacterium that causes life-threatening infection in neonates, pregnant women, and immune-compromised hosts (17). After internalization through phagocytosis, Listeria escapes the vacuole by producing listeriolysin O (LLO) and replicates efficiently within the macrophage cytosol (18). The early host defense against Listeria is regulated by the secretion of several cytokines and chemokines, including IFN-γ, TNF-α, CCL2, IL-1β, IL-6, IL-12, and IL-18 (17). Cytokine and chemokine production in Listeria-infected macrophages is induced at least in part via TLR signaling (19–21). In this study we generated mutant mice deficient in ASC to understand the contributions of this adaptor molecule to cytokine production and innate immunity to Listeria. Furthermore, we compared the responses of TLR2-null and ASC-null macrophages to gain insight into the mechanism by which these molecules regulate the secretion of IL-1β during Listeria infection in macrophages.

Materials and Methods

Mice

ASC knockout (ASC-KO) mice were generated by homologous recombination in embryonic stem (ES) cells. The strategy involved replacement of the coding exons of the ASC gene with an internal ribosome entry site-β-galactosidase-neomycin resistance cassette via a targeting vector. A positive ES clone was used to generate chimeric mice. 129/C57BL/J6 chimeric mice were crossed with C57BL/J6 females to generate ASC heterozygous mice. ASC-KO mice were generated by crossing male and female ASC heterozygous mice. The ASC-KO and wild-type mice used in these experiments were in a mixed 129/C57BL/J6 background. C57BL/J6 and TLR2-KO mice, in a C56BL/J6 background, were purchased from The Jackson Laboratory.

Isolation of peritoneal macrophages

Mice older than 8 wk were injected i.p. with 2 ml of 4% thioglycolate solution, and peritoneal macrophages were collected via peritoneal lavage 4 or 5 days later. Adherent macrophages were cultured in IMDM supplemented with 10% heat-inactivated FBS in 48-well plates at 2 × 10⁵ cells/well for ELISAs and at 2 × 10⁶ cells/well in six-well dishes for immunoblotting analysis.

Isolation of dendritic cells

Bone marrow was obtained from mice femur and tibia, and the cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS, 0.1 mM nonessential amino acids, 50 μM 2-ME, 2 mM L-glutamine, 1% fungizone, 1 mM sodium pyruvate, and 10 ng/ml recombinant GM-CSF for 5 days. Floating cells were collected and labeled with anti-mouse CD11c-conjugated magnetic MicroBeads (Miltenyi Biotec). The labeled cells were separated with the autoMACS separator (Miltenyi Biotec) using a positive selection procedure.

Bacterial culture and stimulation of macrophages

The Listeria strains used were a wild-type strain, 10403S, or strains containing in-frame deletions of the hly gene (LLO−, DP-L2161) or DP-L3078 (ActA−) strain (a gift from Dr. M. O’Riordan, University of Michigan, Ann Arbor, MD). Single colonies were inoculated into 3 ml of brain-heart infusion medium and grown overnight at 37°C without shaking. On the day of the infection, a 1:10 dilution of the overnight culture was prepared and allowed to grow at 37°C with shaking to A₅₀₀ = 0.5, which corresponds to ~10⁶ CFU/ml. Bacteria were diluted to the desired concentration in complete IMDM and used to infect peritoneal macrophages at different macrophage/bacterial ratios (see figure legends). After 30-min infection at 37°C, the macrophages were washed twice with PBS, and complete IMDM containing 33 μg/ml gentamicin was added to limit the growth of extracellular bacteria. Macrophages were stimulated with synthetic bacterial lipopeptide Pam3CSK4 (EMC Microcollections) for 18 h, and the supernatants were analyzed for IL-6 secretion. For analysis of IL-1β secretion, macrophages were incubated with Pam3CSK4 for 18 h, then with medium containing 2 mM ATP (Sigma-Aldrich) for 30 min, washed, and cultured for an additional 5 h. Culture supernatants were collected and assayed for cytokine production by ELISA.

Western analysis

Cells were lysed in buffer containing 1% Nonidet P-40 supplemented with Complete protease inhibitor Cocktail (Roche) and 2 mM DTT. Lysates were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes by electroph blotting. Anti-mouse ASC mAb was generated by immunizing rats with mouse recombinant ASC, and hybridoma culture medium was used for immunoblotting. The rabbit anti-mouse caspase 1 was a gift from Dr. Vandanaehele (Ghent University, Ghent, Belgium). The Abs for mouse IκB-α, phospho-IκB-α, p38, and phospho-p38 were obtained from Cell Signaling Technology and were used as recommended by the manufacturer. Mouse anti-tubulin Ab was purchased from Sigma-Aldrich.

ELISA and RT-PCR analyses

The ELISA kits for mouse IL-1β, IL-18, IL-6, and TNF-α (R&D Systems) were used according to the manufacturer’s recommendations. Real-time quantitative PCR analysis of IFN-β RNA transcripts was performed using SYBR Green reagents on an Applied Biosystems 7700 sequence detector after normalization to β-actin. Primer sequences are available upon request.

Results

Generation of ASC-KO mice

To assess the physiological role of ASC, we generated ASC-KO mice by homologous recombination in ES cells. The strategy involved replacement of the coding exons of the ASC gene with an internal ribosome entry site-β-galactosidase-neomycin-resistance cassette via a targeting vector (Fig. 1a). Homologous recombination was confirmed by Southern blotting analysis of BamHI digests of genomic DNA from mouse progeny. Genotyping analysis revealed the presence of progeny mice with homozygous KO (−/−), heterozygous (+/−), and wild-type (+/+ ) ASC genotypes (Fig. 1b). To verify that the ASC protein was absent in KO mice, we developed an mAb reactive to mouse ASC by immunizing rats with recombinant ASC protein. Immunoblotting revealed that endogenous ASC was present in spleen and bone marrow of wild-type mice, but not in homozygous ASC mutant mice (Fig. 1c). ASC-KO mice were born at the expected Mendelian ratio, were fertile, showed no gross abnormalities, and appeared healthy when housed in a standard specific pathogen-free environment.

ASC is essential for IL-1β/IL-18 secretion, but not TNF-α, IL-6, or IFN-β, in peritoneal macrophages infected with Listeria

To study the involvement of ASC in innate signaling triggered by intracellular pathogens, thioglycolate-elicited peritoneal macrophages from wild-type and ASC-KO mice were infected with Listeria. Macrophages were infected with Listeria for 30 min, then washed and cultured in medium containing gentamicin to inhibit the growth of extracellular bacteria. Supernatants were collected 24 h later and analyzed for the secretion of IL-1β, IL-18, IL-6, and TNF-α (Fig. 2a). ASC-KO macrophages failed to secrete IL-1β and IL-18, but they produced levels of IL-6 and TNF-α similar to those of wild-type cells (Fig. 2a). In contrast, induction of IFN-β mRNA in macrophages infected with Listeria was unimpaired in ASC-null macrophages (Fig. 2e). Consistent with previous results (22, 23), induction of IFN-β required LLO in Listeria (Fig. 2e),
suggestion that cytosolic invasion of the bacterium is critical for induction of type I IFN. Under our culture conditions, we found no significant death of wild-type or ASC-KO peritoneal macrophages after *Listeria* infection (data not shown).

**ASC is essential for activation of caspase-1 in *Listeria*-infected macrophages**

Secretion of mature IL-1β in response to microbial components is controlled by several events, including transcriptional induction of pro-IL-1β via NF-κB and activation of caspase-1 (14, 24). To assess the role of ASC in NF-κB and caspase-1 activation, macrophage extracts were prepared at different times after *Listeria* infection and immunoblotted with Abs that recognize activated forms of caspase-1 and NF-κB. By 3 h of infection, *Listeria* induced the proteolytic processing of procaspase 1, as determined by the detection of the mature 20-kDa subunit in wild-type macrophages, which was enhanced by 6 h after infection (Fig. 3a). Such proteolytic processing of procaspase 1 was abolished in ASC-KO macrophages (Fig. 3a). In contrast, phosphorylation and degradation of IκBα and p38 activation in response to *Listeria* were unaffected in ASC-KO macrophages compared with wild-type cells (Fig. 3b). There was some variation in the kinetics of induced p38 and IκBα phosphorylation among individual experiments, but there was no difference between wild-type and ASC-KO macrophages. We tested ERK and JNK activation and found that they were also unaffected in ASC-KO macrophages compared with wild-type cells (data not shown). These results indicate that ASC is critical for the activation of caspase-1, but is dispensable for that of NF-κB and MAPK signaling pathways, in response to *Listeria* infection.

**Presence of *L. monocytogenes* in cytosol is required for IL-1β secretion in macrophages**

TLR2 recognizes bacterial products at the cell surface, whereas NOD-LRR proteins appear to sense microbes in the cytosol (1–3). To determine whether cytosolic recognition of *Listeria* is important for ASC-dependent IL-1β secretion, macrophages and dendritic cells were infected with wild-type and two mutant strains of *Listeria*: LLO−, which cannot escape the phagosome, and ActA−, which can escape the phagosome, but cannot spread from cell to cell due to the lack of actin-based motility (18). The results revealed that the production of IL-1β in response to wild-type and ActA− *Listeria* was greatly impaired in macrophages and dendritic cells deficient in ASC compared with that observed in wild-type cells (Fig. 4, a and b). Notice that the production of IL-1β was 20-fold higher in macrophages than in dendritic cells (Fig. 4, a and b). Notably, wild-type and ActA−, but not LLO−, *Listeria*, induced IL-1β production in wild-type macrophages (Fig. 4a). In contrast, the LLO− mutant was capable of eliciting IL-1β secretion in dendritic cells, although at a lower level than wild-type and ActA− *Listeria* (Fig. 4b). Significantly, the production of TNF-α and IL-6 induced by infection with wild-type, ActA−, or LLO− *Listeria* was comparable in WT and ASC-KO dendritic cells (Fig. 4, c and d) and macrophages (data not shown). These results indicate that the invasion of the bacterium into the cytosol is required for *Listeria* to induce high levels of IL-1β production in macrophages.

**TLR2 and ASC regulate IL-1β secretion via distinct mechanisms**

TLR2 has been implicated in the recognition and innate immune response to *Listeria* (19). To understand the role of TLR2 in IL-1β...
secretion, macrophages from wild-type and TLR2-KO mice were exposed to *Listeria*, and the production of cytokines was assessed 24 h after infection. In contrast to what was observed in ASC-KO macrophages, the secretion of IL-6 and TNF-α was greatly reduced in TLR2-null macrophages compared with wild-type cells (Fig. 5a). Moreover, the secretion of IL-1β was significantly inhibited in TLR2-KO macrophages (Fig. 5a). The role of TLR2 in IL-1β production is presumably mediated through the transcriptional induction of pro-IL-1β via NF-κB and MAPK activation. Consistent with the latter, IκB-α degradation was delayed, and p38 activation was reduced in TLR2-KO macrophages (Fig. 5b). In contrast, the activation of caspase-1 was unimpaired in TLR2-KO macrophages infected with *Listeria*, as determined by the production of the mature 20-kDa subunit of caspase-1 (Fig. 5c).

**ASC and TLR2 are required for IL-1β secretion in response to lipopeptide**

To assess the roles of ASC and TLR2 in the production of IL-1β, we tested next whether cytokine production in response to the Pam3CSK4 lipopeptide, a TLR2 agonist, was impaired in macrophages deficient in ASC or TLR2. In these experiments, macrophages were stimulated with various concentrations of synthetic lipopeptide for 18 h, then incubated for a short time with ATP, a signal that is necessary for maximal IL-1β release after stimulation with purified TLR ligands (14). The results showed that the production of IL-1β in response to lipopeptide was reduced in ASC-KO macrophages and was abolished in TLR2-KO macrophages (Fig. 6, a and c). The decrease in IL-1β was specific, in that production of IL-6 was unimpaired in ASC-KO macrophages and, as expected, was abolished in TLR2-KO macrophages (Fig. 6, b and d). Control experiments showed that TLR2-KO macrophages responded normally to lipid A, a TLR4 ligand (data not shown).

**Discussion**

Based on the results shown in this study, we conclude that ASC is critical for the activation of caspase-1 and the production of IL-1β/IL-18 in response to *Listeria*. In monocytes and macrophages, the secretion of IL-1β is dependent on both the induction of pro-IL-1β and the activation of caspase-1 (12). We show in this study that IL-1β secretion is greatly reduced in the absence of TLR2, which is associated with delayed IκB-α degradation and reduced p38 activation, whereas processing of caspase-1 was unaltered. The opposite was found in ASC-null macrophages after infection with *Listeria*. Previous work showed that macrophages lacking MyD88 or Toll/IL-1 receptor (TIR)-domain-containing adaptor-inducing IFN-β, two adaptor molecules that are critical for TLR-mediated NF-κB activation, are deficient in the up-regulation of pro-IL-1β, but exhibit normal caspase-1 activation upon LPS stimulation (13). Collectively, these results strongly suggest that TLR signaling promotes IL-1β secretion primarily through NF-κB and p38 signaling, not by promoting caspase-1 activation. We found no evidence that ASC regulates the production of cytokines via NF-κB or MAPK activation in response to *Listeria*, which is in accord with recent studies performed with *Salmonella* (9). In contrast, the production of TNF-α, IL-6, and IL-1β was greatly reduced in TLR2-null macrophages infected with *Listeria*, indicating that TLR2 is a general regulator of inducible cytokine production in *Listeria*-infected macrophages (19, 20). The partial effect on the production of cytokines and NF-κB and p38 activation observed in TLR2-null cells is consistent with the observation that other TLRs, including TLR5, are involved in host recognition of *Listeria* (25).

ASC is an adaptor molecule that links upstream receptors involved in bacterial recognition to caspase-1. Several NOD-LRR proteins, including Nalp1, cryopyrin, and Ipaf, have been shown to interact with ASC and have been suggested to be involved in the recognition of bacterial components through their cytosolic LRRs (2, 3, 7). We found that ASC-dependent caspase-1 activation was unimpaired in TLR2-null macrophages, indicating that ASC does not act downstream of TLR2 to activate caspase-1 in response to *Listeria*. Thus, ASC signaling, which is required for caspase-1 activation, could be induced via direct recognition of *Listeria* by NOD-LRR proteins in the host cytosol independently of TLRs. Consistent with the latter is our observation that translocation of *Listeria* to the cytosol is required for IL-1β secretion in macrophages. In the macrophage cytosol, *Listeria* is known to activate...
we found that the production of IL-1 

tidoglycan, and flagellin (15, 16). Consistent with these findings, 

that TLR2-mediated signaling is not used by 

TLR2 macrophages infected with 

casprase-1 activation. We have found 

ASC-deficient macrophages in response to synthetic lipopeptide, a 


described in Fig. 3 

a

result in caspase-1 activation. The experiment 

was performed simultaneously and un-

der the same conditions as those de-

scribed in Fig. 3 a.

several signaling pathways that involve NF-κB, MAPK, and IFN 

responses (22, 23). Our results suggest that in addition to the latter 

signaling pathways, Listeria activates caspase-1 through ASC in the 

host cytosol.

Previously, two different groups generated mutant mice lacking 

ASC and showed that ASC is required for caspase-1 activation and 

IL-1β secretion in response to LPS, lipoteichoic acid, PGN-pepti-

doglycan, and flagellin (15, 16). Consistent with these findings, 

we found that the production of IL-1β was greatly reduced in 

ASC-deficient macrophages in response to synthetic lipopeptide, a 

TLR2 agonist. These results suggest that ASC could act down-

stream of TLR signaling in caspase-1 activation. We have found 

no evidence that TLR2 is used by Listeria to activate caspase-1, 

because processing of this inflammatory casps was unaffected in 

TLR2 macrophages infected with Listeria. These results suggest 

that TLR2-mediated signaling is not used by Listeria to induce 

caspase-1 activation. Alternatively, TLR2 could be involved in 

Listeria-induced caspase-1 activation, but this represents a minor 

mechanism that was not revealed by immunoblotting analysis. It is 

also possible that bacteria other than Listeria use TLR2 and/or 

other TLRs for caspase-1 activation. LPS, a TLR4 agonist, has 

been reported to activate caspase-1 in an ASC-dependent manner 

(15, 16). LPS preparations are not pure, and they typically contain 

peptidoglycan-related molecules and other products that are 

known to activate NOD-LRR proteins (6, 7). Thus, in the case of 

LPS preparations, bacterial components other than LPS could 

induce ASC-dependent activation of caspase-1 independent of TLR 

signaling (7). Furthermore, it is also formally possible that NOD-

LRRs could be directly activated by certain TLR ligands in the 

cytosol. In contrast to macrophages, the Listeria LLO− mutant 

was able to induce low levels of IL-1β production in dendritic 

cells. The molecular basis to account for this difference between 

macrophages and dendritic cells is unclear. Because the LLO− 

mutant presumably cannot escape the phagosome, it is possible 

that the response is mediated through TLR signaling, and the ob-

served differences could be explained by differential expression 

of TLRs or signaling molecules. Clearly, additional studies using a 

variety approaches and additional bacteria are needed to clarify the 

signaling pathway(s) that leads to caspase-1 activation through 

NOD-LRR proteins and ASC.

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References


