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ASC CONTROLS *LEGIONELLA PNEUMOPHILA* INFECTION IN HUMAN MONOCYTES

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Running head: ASC regulates *Legionella pneumophila* infection.

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The ability of *Legionella pneumophila* (*L. pneumophila*) to cause pneumonia is determined by its capability to evade the immune system and grow within human monocytes and their derived macrophages. Human monocytes efficiently activate caspase-1 in response to *Salmonella* but not to *L. pneumophila*. The molecular mechanism for the lack of inflammasome activation during *L. pneumophila* infection is unknown. Evaluation of the expression of several inflammasome components in human monocytes during *L. pneumophila* infection revealed that the expression of the apoptosis associated speck-like protein (ASC) and the NOD-Like Receptor (NLR) NLRC4 are significantly down regulated in human monocytes. Exogenous expression of ASC maintained the protein level constant during *L. pneumophila* infection and conveyed caspase-1 activation and restricted the growth of the pathogen. Further depletion of ASC with siRNA was accompanied with improved NF-κB activation and enhanced *L. pneumophila* growth. Therefore, our data demonstrate that *L. pneumophila* manipulates ASC levels to evade inflammasome activation and grow in human monocytes. By targeting ASC, *L. pneumophila* modulates the inflammasome, the apoptosome and NF-κB pathway simultaneously. *L. pneumophila* is an intracellular bacterium and the causative agent of Legionnaire's pneumonia; a severe form of pneumonia that affects the immune compromised and the elderly (1-4). The ability of *L. pneumophila* to cause pneumonia in humans is dependent on its capability to evade the immune system and multiply within human monocytes and derived macrophages (5-9). In murine macrophages, *L. pneumophila* activates the Nlrc4 inflammasome leading to the production of active caspase-1 (10-15). Then Naip5 cooperates with Nlrc4 to mediate caspase-7 activation downstream of caspase-1 which restricts the intracellular survival of the organism (16-19). For reasons that are still not understood, human monocytes and their derived macrophages do not activate caspase-1 in response to *L. pneumophila* and permit intracellular replication of the pathogen (17).

The apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) is an adaptor molecule that mediates inflammatory and apoptotic signals and is predominantly expressed in monocytes and mucosal epithelial cells (20). ASC is an integral component of the inflammasome, which is a large protein complex responsible for caspase-1 activation (21-24). Within the inflammasome ASC links caspase-1 and NOD-like receptors (NLR) leading to the activation of caspase-1 (16-19). Many Gram-negative bacteria, such as *Salmonella typhimurium*, *Pseudomonas aeroginosa*,

Shigella flexneri, and *Legionella pneumophila* (*L. pneumophila*), are recognized in murine macrophages by the NLR Ipaf/Nlrc4 leading to caspase-1 activation through the inflammasome (25-26).

ASC has been reported to cooperate with NLRP3 (cryopyrin) and NLRP12 (PYPAF7) in inducing NF-κB activity in an over expression system (27-28). However, other reports show that ASC uses its CARD domain for interaction to down-regulate NF-κB signaling (20,29). Therefore, ASC can be an inducer or an inhibitor of NF-κB depending on expression level, cell type and location within the cell (30).

ASC has also been suggested to induce apoptosis by recruiting caspase-8 and/or Bax (31). Thus, ASC can regulate the inflammasome and other signaling pathways depending on the stoichiometry of its expression and on whether other PYD family proteins are expressed upon activation (27,32). Moreover, emerging reports show novel roles for ASC in disease conditions such as arthritis (33).

Here we demonstrate that *L. pneumophila* down regulates human ASC in primary human monocytes. Remarkably, exogenous expression of ASC in primary human monocytes, promoted caspase-1 activation in response to *L. pneumophila* and hindered bacterial intracellular growth. Moreover, our data revealed that ASC controls *L. pneumophila* infection in human cells by modulating host cell survival through limiting NF-κB activation leading to early host cell death. Taken together, our data demonstrate that ASC is a central molecule that modulates the fate of *L. pneumophila* within human monocytes by different mechanisms. Therefore, designated down regulation of ASC by *L. pneumophila* established the necessary environment for its replication within human monocytes.

MATERIALS AND METHODS

Quantitative PCR- Total RNA was extracted from primary human monocytes, lyzed in Trizol (Invitrogen Life Technologies) and 1-2 μg of the RNA was converted to cDNA by ThermoScript RNase H^- Reverse Transcriptase (Invitrogen, Life Technologies). 20-60 ng of the converted cDNA was used for quantitative PCR with SYBR Green I PCR Master Mix in the StepOne Plus Real Time PCR System (Applied Biosystems). The target gene Ct values were normalized to the Ct values of two housekeeping genes (human GAPDH and CAP-1, accordingly to the cell origin), and expressed as relative copy number (RCN), as described earlier (34). Primers used in the study are presented in Supplementary Tables 1.

Bacterial Strains- Legionella pneumophila (*L. pneumophila*) strain Lp02, is a thymine auxotrophic derivative of Philadelphia-1 (3). *L. pneumophila* was cultured as described previously (17). All experiments were performed at a low MOI of 0.5 or 1, followed by centrifugation and rinsing of the wells after 30 minutes except when otherwise indicated (35). All experiments were performed in the absence of ferric nitrate and L-cysteine from the monocytes or macrophage culture medium, to allow *L. pneumophila* multiplication only intracellularly. Viable bacteria per 5 x 10^5 cells per well were quantified as previously described (8). The quantification of the colony-forming units (CFU) *in vitro* was performed more than four independent times as described (8).

Primary human monocytes- Human buffy coats were obtained from the local Red Cross and monocytes were isolated by CD14 positive selection as previously described (36).

Generation of THP1 cell line stably expressing YFP-ASC- To make a fusion protein, ASC was amplified from cDNA by PCR and inserted at the C-terminus of yellow fluorescent protein (YFP) on the basis of pLenti6/V5 plasmid (Invitrogen Life Technologies), described in (37). After plasmid verification by sequencing, we generated lentivirus and transduced THP-1 cells (ATCC, lot 385653) with 10-15% efficiency, as described in details earlier (37). Stably transduced cells were selected with blasticidin (Invitrogen Life Technologies) for 10 days followed by two rounds of flow sorting using FACS ARIA (Becton

Dickinson), resulting in nearly 100% yield of stably transducedTHP-1 cells.

L. pneumophila growth intracellularly- Monocytes or macrophages were infected as described before (8). At designated time points, monocytes or macrophages were lysed and plated on AYE plates for colony forming units (CFUs).

Immunoblotting- Cell lysates or supernatants were prepared and immunoblotted with an antibody that recognizes phoshpho-p65, phospho-IKKαβ and IKKα (Cell Signaling), Actin (Abcam), NLRC4 and ASC (Alexis biochemicals), human caspase-1 and pro-IL-1 β (Dr. Mark Wewers). Then, the blots were treated with appropriate secondary antibody as described (8).

Cytotoxicity assays- *In vitro* quantification of cytoplasmic (apoptosis) histone-associated DNA fragments was performed using The Cell Death Detection ELISAplus photometric enzyme immunoassay kit from Roche to the specifications of the manufacturer.

Transfection of primary human monocytes with small interfering RNA- Small interfering RNA (siControl and siASC) was purchased from Dharmacon and nucleofected into the cells with the Lonza apparatus, as described earlier (17,37). A plasmid coding ASC tagged with YFP was nucleofected into the cells with the Lonza apparatus according to the manufacturer's protocol.

Statistical Analysis- All experiments were done at least three independent times and yielded similar results. Comparisons of groups for statistical difference were done using Student's two-tailed t test. P value $\leq$ 0.05 was considered significant.

RESULTS

Primary human monocytes do not activate caspase-1 in response L. pneumophila- Since recognition of *L. pneumophila* by the murine Nlrc4 inflammasome is accompanied with caspase-1 activation and restriction of infection (8,13,17,38), we examined if *L. pneumophila* infection activates the inflammasome in human monocytes. Primary human monocytes were infected with *L. pneumophila* and the cleavage of caspase-1 was examined. Given that cleaved caspase-1 and IL-1β are released from macrophages once activated, we examined their amount in culture supernatants. Caspase-1 was not cleaved in response to *L. pneumophila* after 8 hrs of infection (Figure 1 A). Consequently, IL-1β production was minimal after similar incubation time (Figure 1 B). It is possible that *L. pneumophila* deliberately avoids its own detection by the NLRC4 inflammasome by an unknown mechanism. It is also possible that the NLRC4 inflammasome is poorly functional in primary human monocytes. To distinguish between these possibilities and since *Salmonella* is detected by NLRC4, primary human monocytes were infected with *Salmonella* and the release of active caspase-1 and IL-1β in culture supernatants was examined. Within 4 hrs of *Salmonella* infection caspase-1 was efficiently cleaved as observed by the detection of the p20 in culture supernatants (Figure 1 A). Accordingly, significant amounts of IL-1β were detected in culture supernatants of primary human monocytes infected with *Salmonella* (Figure 1 B). Furthermore, human monocytes were able to detect Gram-positive organisms such as *Listeria* monocytogenes which was accompanied with caspase-1 activation (Figure 1 A). Therefore, the human inflammasome complex in general and the NLRC4 inflammasome in particular are perfectly functional in primary human monocytes, yet it is not activated in response to *L. pneumophila*.

L. pneumophila reduces ASC and NLRC4 mRNA and their expression in primary human monocytes- To understand the role of the inflammasome during *L. pneumophila* infection of human monocytes, primary human monocytes were infected with *L. pneumophila* for 4 and 24 hrs. Caspase-1, pro-IL-1β, ASC and NLRC4 expression were examined at the mRNA and protein levels using quantitative RT-PCR and Western blot respectively (Figure 2). Our results demonstrate that the expression of caspase-1 and pro-IL-1β were significantly increased at 4 hrs then decreased after 24 hrs of *L.*

pneumophila infection (Figure 2 A and B). Western blot analysis showed that the levels of caspase-1 and IL-1β proteins correlated with mRNA levels revealed by RT-PCR (Figure 2 A and B, lower panel).

On the other hand, the NLRC4 message was significantly suppressed at 4 hrs and remained low after 24 hrs of infection by *L. pneumophila* (Figure 2 C). Western blot analysis showed that NLRC4 protein levels decreased after the decrease in mRNA (Figure 2 C). Next, we explored the expression of PYCARD/ASC mRNA in primary human monocytes since it is a general adaptor molecule in the inflammasome. We found that *L. pneumophila* infection diminished PYCARD mRNA expression within 4 hrs of infection and remained low at 24 hrs (Figure 2 D). The ASC protein level was decreased within 24 hrs after infection in the same samples (Figure 2 D). As an independent control, mRNA levels of other molecules such as annexin1 and pyrin were not altered during *L. pneumophila* infection (Supplementary Figure 1). To further confirm our observation, we examined the expression of the same inflammasome components in the human monocytic cell line THP-1. Supplementary figure 2 shows that both NLRC4 and ASC were down regulated in response to *L. pneumophila* infection in THP-1 cells which are also permissive to *L. pneumophila* growth. Therefore, our results reveal that, *L. pneumophila* infection decreases the expression of essential inflammasome components such as NLRC4 and ASC in permissive primary human monocytes and in human monocytic cell lines.

Exogenous expression of ASC in human monocytes restores caspase-1 activation in response to L. pneumophila- Despite its up regulation, caspase-1 was not activated or released in culture supernatants of *L. pneumophila*-infected human monocytes (Figure 1). Because mRNA for ASC and NLRC4, were down regulated in response to *L. pneumophila*, we postulated that their down regulation contributes to the absence of caspase-1 activation during *L. pneumophila* infection. To test this hypothesis, we transfected primary human monocytes with a plasmid coding ASC tagged with yellow fluorescent protein (YFP) (Figure 3 A). GFP plasmid was used as control. Exogenous expression of ASC was calibrated as to maintain moderate ASC levels during *L. pneumophila*. After transfection, cells were infected with *L. pneumophila* and the cleavage of caspase-1 was examined by Western blot. Active caspase-1 (p-20) was detected only in the supernatants of human monocytes expressing Y-ASC infected with *L. pneumophila* but not in supernatants derived from human monocytes transfected with the GFP- plasmid prior to *L. pneumophila* infection (Figure 3 B). This finding supports the idea that the down regulation of ASC mRNA by *L. pneumophila* may contribute to the lack of caspase-1 activation in human monocytes.

Exogenous expression of ASC restricts L. pneumophila infection and diminishes NF-κB activation- To further investigate the role of ASC during *L. pneumophila* infection, both THP-1 cells stably over expressing Y-ASC and normal THP-1 cells were infected with *L. pneumophila*, then, the ability of *L. pneumophila* to grow intracellularly was examined by scoring CFUs within 24 hrs. Notably, endogenous ASC was decreased during *L. pneumophila* infection, yet exogenous ASC levels remained unchanged (Supplementary Figure 3). THP-1 cells allowed *L. pneumophila* replication whereas those expressing Y-ASC did not (Figure 4 A). To understand the mechanism by which ASC may regulate *L. pneumophila* infection, apoptosis was evaluated in THP-1 cells after 24 hrs of infection (Figure 4 B). Exogenous expression of ASC in THP-1 cells increased cell death induced in response to *L. pneumophila* infection (Figure 4 B). *L. pneumophila* activates NF-κB to extend host cell survival till it replicates intracellularly. Given that ASC may activate or inhibit NF-κB activation depending on the circumstances, we examined the effect of ASC on the phosphorylation of IKKαβ and p65 (Figure 4 C). Exogenous expression of ASC decreased the phosphorylation of IKKαβ

and p65. Therefore, ASC restricts *L. pneumophila* growth by hindering NF-κB activation.

Down regulation of ASC in human monocytes by siRNA allows more L. pneumophila growth and more NF-κB activation- ASC expression in human monocytes is decreased during *L. pneumophila* infection and that is accompanied by permissiveness to infection. To further confirm the contribution of ASC in restriction of *L. pneumophila* infection, primary human monocytes were transfected with siRNA specific for ASC or control siRNA (Figure 5 A). Intracellular growth of *L. pneumophila* was evaluated by scoring CFU within 24 hrs of infection. Notably, depletion of ASC allowed more *L. pneumophila* growth when compared to human monocytes harboring scrambled siRNA as a control (Figure 5 B). Therefore, ASC also controls *L. pneumophila* infection in human monocytes independently of caspase-1. To discern the mechanism by which depletion of ASC may allow more *L. pneumophila* growth, NF-κB activation was examined in human monocytes harboring siRNA specific for ASC or si control after *L. pneumophila* infection for 20, 40 or 60 minutes. Our data show that human monocytes treated with siRNA specific for ASC permitted more phosphorylation of IKKαβ and p65 than cells treated with control siRNA (Figure 5 C). Thus, in the context of *L. pneumophila* infection, ASC hinders NF-κB activation. Taken together, ASC controls *L. pneumophila* infection in a caspase-1 dependent and independent manner.

DISCUSSION

Pathogens have developed different mechanisms to avoid the engagement of the inflammasome and its activation (39). Viruses utilize proteins to bind ASC and prevent inflammasome assembly or disrupt the proteolytic activity of caspase-1. *Yersinia enterocolitica* express proteins that prevent caspase-1 oligimerization. *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* generate molecules that block the activation of the NLRC4 and NLRP3 inflammasomes respectively (39). In this report, we demonstrate that *L. pneumophila* suppresses the expression of ASC, thus preventing the activation of the NLRC4 inflammasome. This finding explains the lack of caspase-1 activation release during *L. pneumophila* infection of human monocytes. This also provides mechanistic insight for the permissiveness of human monocytes to *L. pneumophila* infection.

Caspase-1 and IL-1β were up regulated in human monocytes during *L. pneumophila* infection, yet, they were not activated or released in culture supernatants of infected monocytes. Caspase-1 activation in response to *L. pneumophila* was re-instated when ASC was exogenously expressed under a different promoter impervious to *L. pneumophila*. Concurrently, the activation of caspase-1 was accompanied with restriction of infection. Thus, the down regulation of ASC during *L. pneumophila* infection dampened caspase-1 activation and its bactericidal consequences. However, we believe that the down regulation of ASC impinge on several pathways not only on caspase-1 activation.

Because *L. pneumophila* resides in macrophages for 6-8 hours before replication starts, it must insure the survival of the host cell for extended duration (10,14-15). To achieve this, *L. pneumophila* activates NF-κB in a TLR5-dependent manner at early stages and in a TLR5-independent manner at latter stages of infection for up to 30 hrs (10,15). The mechanism for the prolonged activation of NF-κB for several hours is still not clear. Here we demonstrate that ASC prevents NF-κB activation induced by *L. pneumophila*, thus its down regulation could be an elegant virulence strategy employed by *L. pneumophila* to extend host cell survival allowing more intracellular growth (10,15).

Optiz group demonstrated that exogenous expression of human NLRC4 and human NAIP restricts of *L. pneumophila* (40). Yet, their study did not offer a mechanism for restriction and did not examine caspase-1 activation. Anders and his group recently showed that NLRs tend to be down regulated in human cells and up regulated in murine cells in response to LPS (41). Thus, it is possible that, similar to NLR expression

patterns, the down regulation of ASC during *L. pneumophila* infection is an inherent response of human monocytes to infection exploited by the pathogen to avoid caspase-1 activation and to establish infection. Alternatively, it is plausible that *L. pneumophila* intentionally down regulates ASC in human monocytes to avoid the detrimental consequences of caspase-1 activation. We favor the second possibility since *Salmonella* infection of human monocytes was not accompanied with down regulation of ASC and caspase-1 was efficiently activated (data not shown and Figure 1). This prospect is supported by a study in clinically ill patients showing that the ASC gene expression levels are elevated irrespective of the infectious agent (42). This is also supported by several studies demonstrating that ASC is up regulated in human monocytes and neutrophils during inflammation (31,43). Taken together, it is likely that *L. pneumophila* uses many of virulence factors such as eukaryotic-like molecules to modulate human ASC functions to its advantage (44-45).

Therefore, by down regulating ASC, *L. pneumophila* achieves several aims; it avoids caspase-1 activation, evades bacterial clearance and extends host cell survival. This is an elegant mechanism by which *L. pneumophila* exploits an adaptor molecule to establish infection in human monocytes.

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Footnotes

1. Studies in Dr. Amer A. laboratory are supported by grants R01HL094586 and R21AI083871 from the National Institute of Health (NIH) and GRT00013604 from the American Lung Association (ALA).
2. Abbreviations used in this paper: ASC, The apoptosis-associated speck-like protein containing a caspase recruitment domain; NLR, NOD-like receptors; PYD, pyrin domain; CARD, caspase recruitment domain; IKK, IKB kinase. 1A.

FIGURE LEGENDS

Figure 1: **Human monocytes do not activate caspase-1 or IL-1β in response to *Legionella pneumophila* (*L. pneumophila*) infection.** Primary human monocytes were infected or not (NT) with *L. pneumophila*, *Liseria monocytogenes* for 8 h or *Salmonella typhimurium* for 4 h. (A) Cell culture supernatants were collected and analyzed by Western blot with anti-caspase-1 antibody. (B) Culture supernatants of primary human monocytes infected with *L. pneumophila* or *Salmonella typhimurium* were analyzed for active IL-1β by ELISA.

Figure 2: **The effect of *L. pneumophila* infection on the expression of caspase-1, IL-1β, NLRC4 and ASC in human monocytes.** Primary human monocytes were infected or not (NT) with *L. pneumophila* for 4 hrs and 24 hrs at MOI of 1. The expression of caspase-1 (A), IL-1β (B), NLRC4 (C), and PYCARD/ASC (D) , mRNA (upper panels) and protein levels (lower panels) were assessed using quantitative RT-PCR and Western blotting respectively. The results are displayed as the mean of three independent experiments ± SD. (∗ $P \leq 0.05$, ∗∗ $P \leq 0.01$). Actin was used as a loading control in all panels.

Figure 3: **Exogenous expression of ASC in human monocytes restores caspase-1 activation.** (A) Primary human monocytes were nucleofected with a plasmid coding ASC tagged with YFP (Y-ASC). A plasmid encoding the green florescent protein (GFP) was used as a control. Twenty four hours after nucleofection, cells were lysed and Y-ASC (52 kD) and normal ASC (25kD) were assessed using Western blotting. (B) Human monocytes were nucleofected with a plasmid encoding GFP or Y-ASC then they were either left untreated (NT) or infected with *L. pneumophila* for 24h at MOI of 1. Supernatant was collected and cleaved caspase-1 (p-20) was assessed via immunoblotting.

Figure 4: **Exogenous expression of ASC in THP-1 cells restricts *L. pneumophila* infection, increases apoptosis and deters NF-κB activation.** (A) THP-1 over expressing Y-ASC and normal THP-1 were infected with *L. pneumophila*, and bacterial growth was assessed at 1h and 24h after infection by counting the colony forming units (CFU). The bacterial count is displayed as log CFU/ml. The results are displayed as the mean of three independent experiments ± SD. (B) THP-1 over expressing Y-ASC and normal THP-1 were infected or not (NT) with *L. pneumophila* and apoptosis was evaluated after 24 hrs of infection. (C) THP-1 over expressing Y-ASC and normal THP-1 were infected or not (0) with *L. pneumophila* for 20, 40 or 60 min. Levels of phosphorylation of IKKαβ and p65 were detected using immunoblots with corresponding antibodies. Actin was used as loading control.

Figure 5: **Depletion of ASC in primary human monocytes with specific siRNA allows more *L. pneumophila* growth and improves NF-κB activation. .** (A) Human monocytes were transfected with ASC specific or control si RNA then the expression of ASC was examined by qRT-PCR and by Western blot. (B) Transfected cells described in (A) were infected with *L. pneumophila* and bacterial growth was evaluated by scoring CFU. The results are displayed as log CFU/ml. the mean of three independent experiments ± SD. (∗∗$P \leq 0.01$). Primary human monocytes transfected with siRNA targeting ASC or control siRNA were infected or not (0) with *L. pneumophila* for 20, 40 or 60 min. The phosphorylation of IKKαβ and p65 was examined by Western blots using corresponding antibodies. Actin was used as loading control.

Supplementary figure 1:
Primary human monocytes were infected or not (NT) with *L. pneumophila* for 4 hrs and 24 hrs. The levels for mRNA for Annexin1 and Pyrin were analyzed by qRT-PCR.

Supplementary figure 2:
The effect of *L. pneumophila* on caspase-1, IL-1β, NLRC4 and PYCARD/ASC expression in THP-1 cells. THP-1 monocyte cell line were infected or not (NT) with *L. pneumophila* for 4h and 24h. Then cells were lysed and the levels of mRNA for caspase-1 (A), IL-1β (B), NLRC4 (C) and PYCARD/ASC (D) were evaluated by qRT-PCR (upper panels). Corresponding protein expression was detected by Western blots with corresponding antibodies (lower panels).

Supplementary figure 3:
The effect of *L. pneumophila* on ASC protein level in both THP-1 and THP-1 stably over-expressing Y-ASC. THP-1 monocyte cell line and THP-1 over-expressing ASC tagged with YFP (Y-ASC) were infected or not (NT) with *L. pneumophila* for 4h and 24h. Then cells were lysed and both normal ASC (25 KD) and Y-ASC (52 KD) level was detected by Western blots. Actin was used as loading control

Supplementary Table 1: Primers used in qRT-PCR.

Figure 1

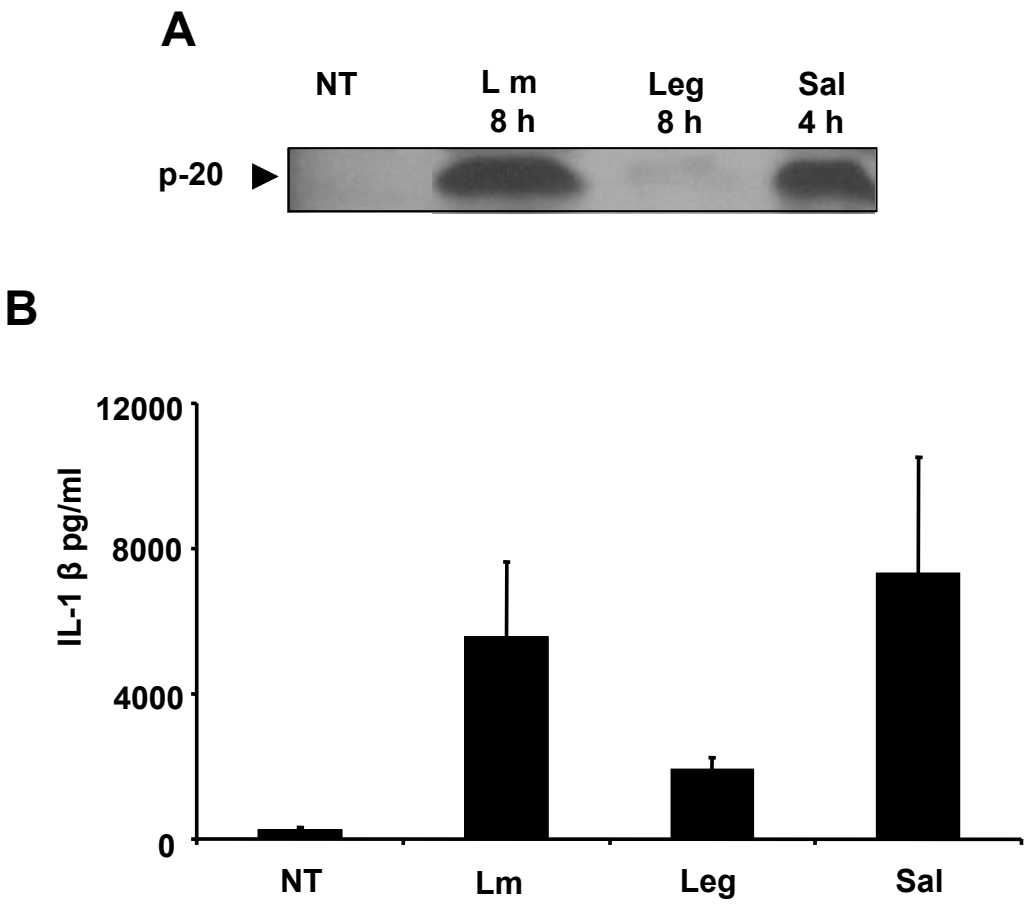

A
NT
L m
8 h
Leg
8 h
Sal
4 h
p-20
B
12000
8000
4000
0
IL-1 β pg/ml
NT
Lm
Leg
Sal

Figure 2

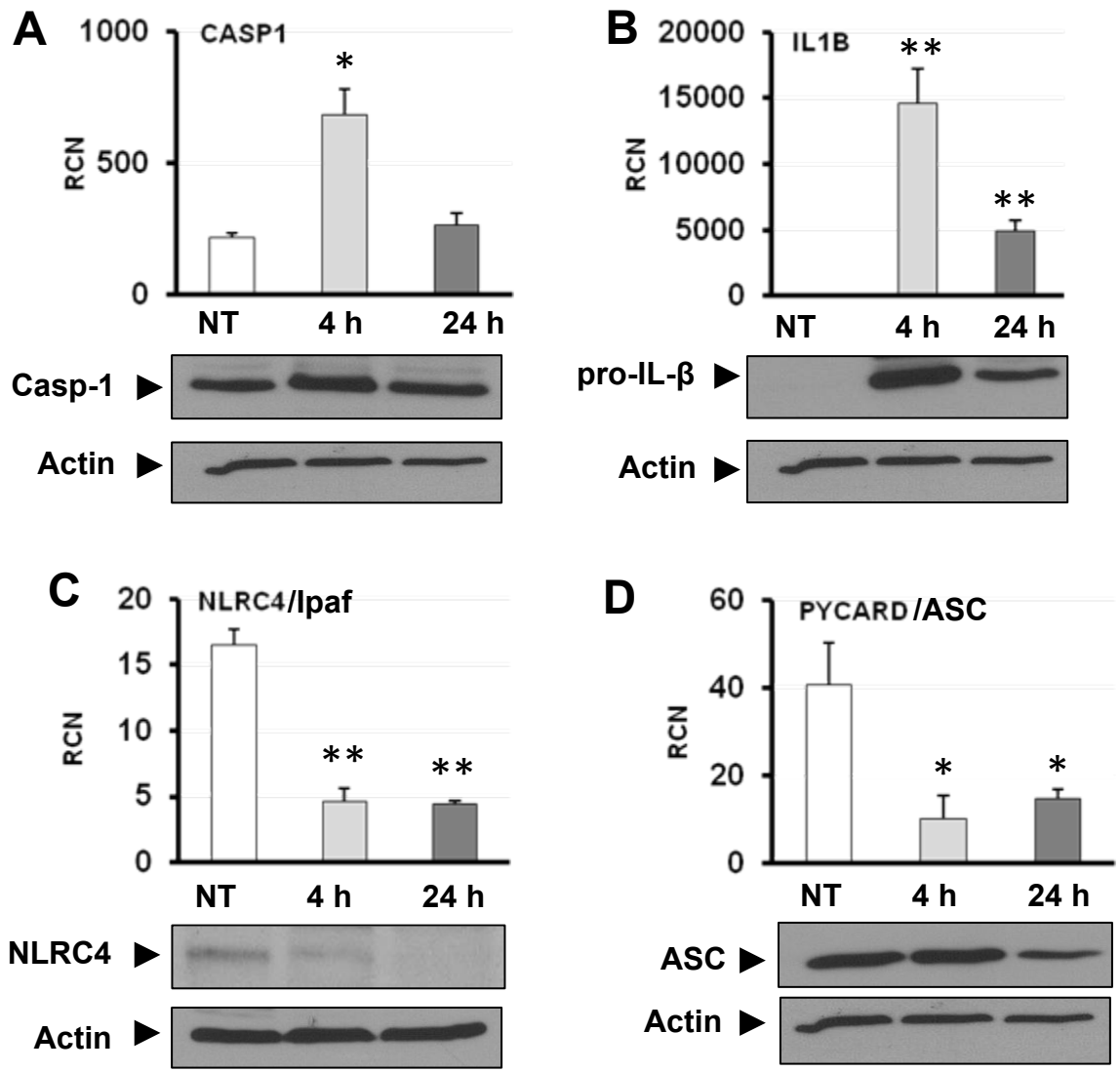

A
CASP1
RCN
1000
500
0
*
NT
4 h
24 h
Casp-1
Actin
B
IL1B
RCN
20000
15000
10000
5000
0
**
**
NT
4 h
24 h
pro-IL-β
Actin
C
NLRC4/Ipaf
RCN
20
15
10
5
0
**
**
NT
4 h
24 h
NLRC4
Actin
D
PYCARD/ASC
RCN
60
40
20
0
*
*
NT
4 h
24 h
ASC
Actin

Figure 3

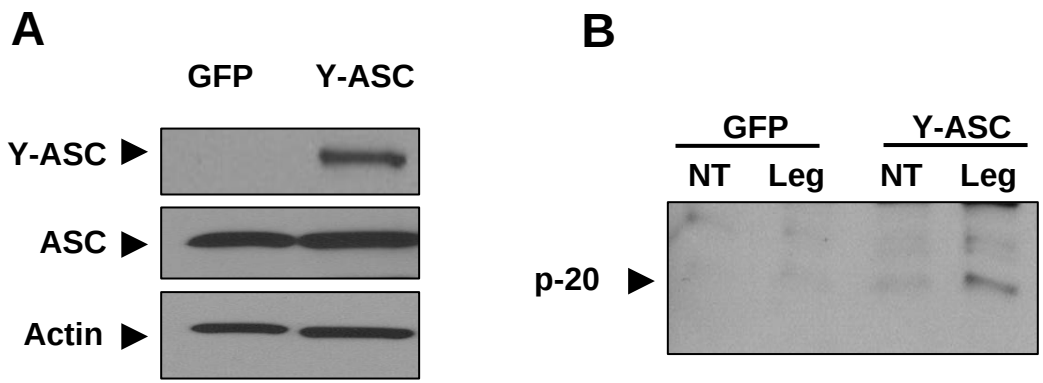


Figure 4

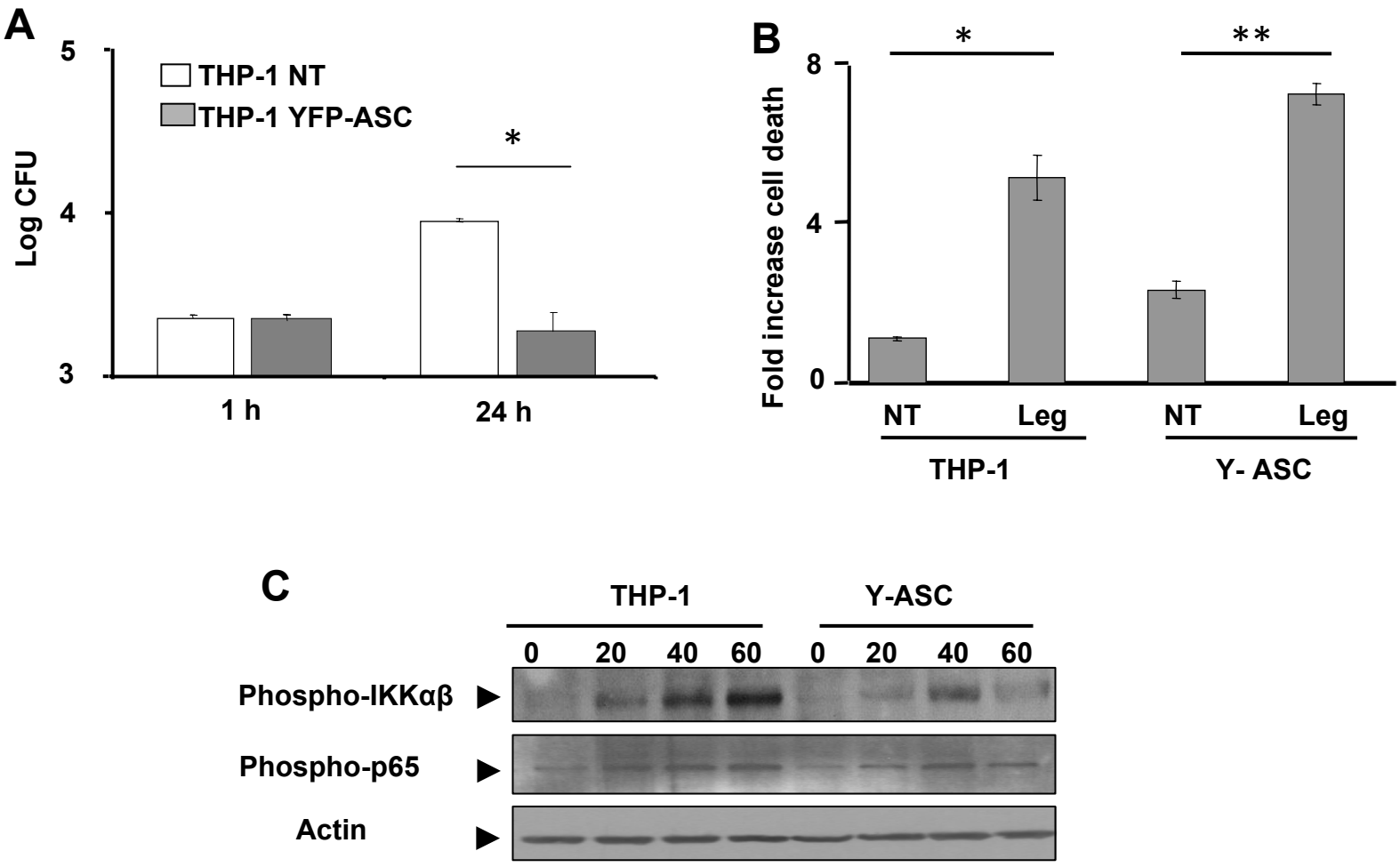
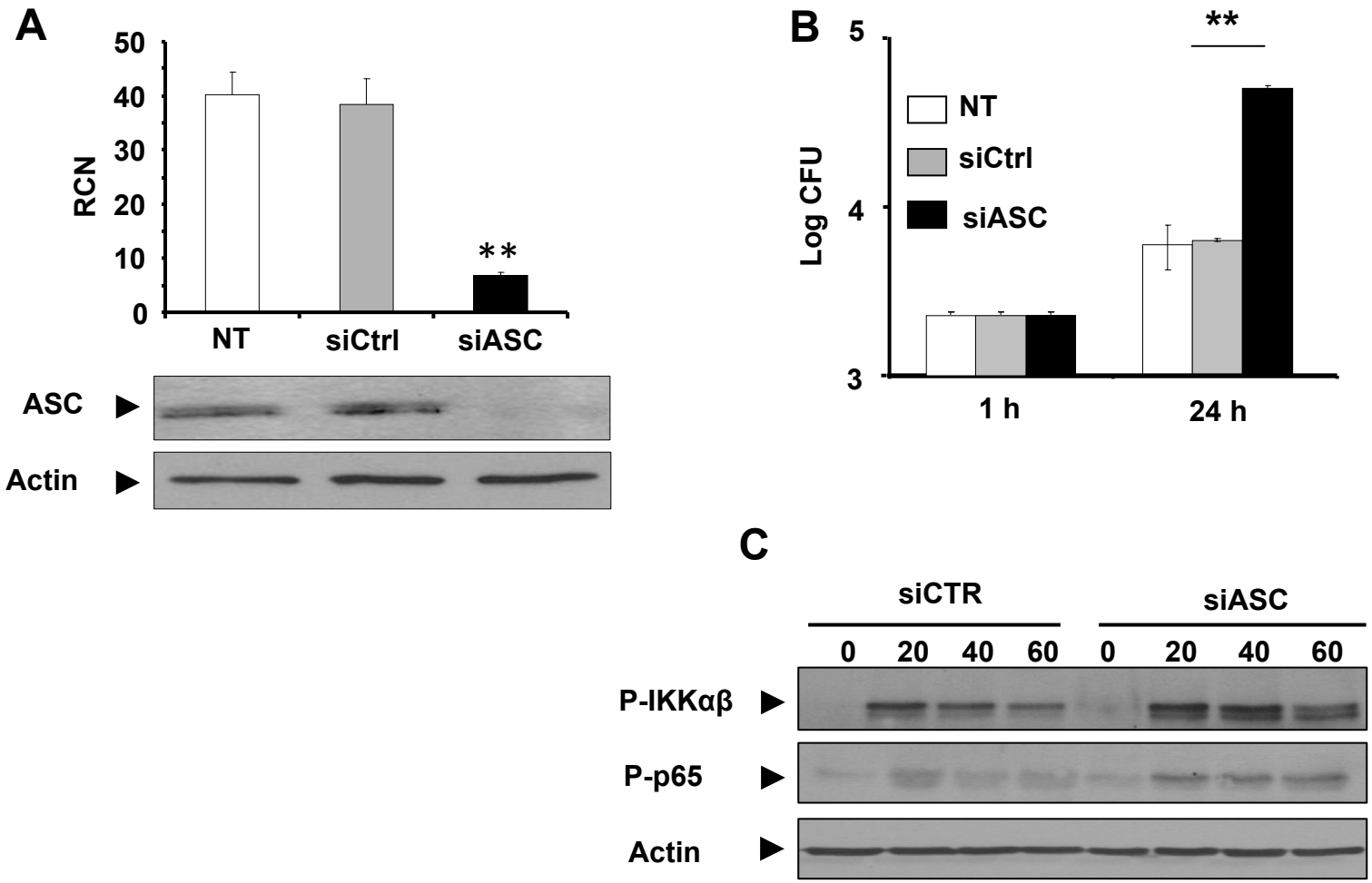

A
5
4
3
Log CFU
THP-1 NT
THP-1 YFP-ASC
*
1 h
24 h
B
8
4
0
Fold increase cell death
*
**
NT
Leg
NT
Leg
THP-1
Y- ASC
C
THP-1
Y-ASC
0
20
40
60
0
20
40
60
Phospho-IKKαβ
Phospho-p65
Actin

Figure 5


A
RCN
50
40
30
20
10
0
**
NT
siCtrl
siASC
ASC
Actin
B
Log CFU
5
4
3
**
NT
siCtrl
siASC
1 h
24 h
C
siCTR
siASC
0
20
40
60
0
20
40
60
P-IKKαβ
P-p65
Actin

ASC controls legionella pneumophila infection in human monocytes

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