Enhanced NLRP3, Caspase-1, and IL-1β Levels in Degenerate Human Intervertebral Disc and Their Association with the Grades of Disc Degeneration

ZHONG-HUA CHEN,1* SHEN-HUI JIN,2 MIN-YAN WANG,3 XIAO-LIANG JIN,3 CHEN LV,4 YING-FENG DENG,5 AND JUN-LU WANG2

1Department of Anesthesiology, Shaoxing People’s Hospital and Shaoxing Hospital of Zhejiang University, Shaoxing, People’s Republic of China
2Department of Anesthesiology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, People’s Republic of China
3Department of Anesthesiology, 117 Hospital of the PLA, Hangzhou, People’s Republic of China
4Department of Anesthesiology, Zhejiang Provincial Hospital of Traditional Chinese Medicine, Hangzhou, People’s Republic of China
5Department of Anesthesiology, the Affiliated Hospital of Ningbo University Medical School, Ningbo, People’s Republic of China

ABSTRACT

The NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome plays an important role in a variety of diseases. However, the role of NLRP3 in the human intervertebral disc (IVD) degeneration remains unknown. In the present study, we assessed the expression levels of the NLRP3 inflammasome and its downstream targets caspase-1 and IL-1β in 45 degenerate and seven nondegenerate IVD samples. The correlation between the degeneration scores and expression levels of NLRP3, caspase-1, and IL-1β were also analyzed. The mRNA expression levels of the three molecules (NLRP3, caspase-1, and IL-1β) were higher in the degenerate IVDs group than the controls (nondegenerate IVDs group). Immunohistochemistry showed that the expression levels of all three molecules were markedly increased in the nucleus pulposus of degenerate IVDs compared with nondegenerate IVDs. There was a positive correlation between the degeneration scores and the expression levels of the NLRP3 inflammasome as well as its downstream targets caspase-1 and IL-1β. The findings suggest that excessive activation of the NLRP3 inflammasome results in overproduction of downstream IL-1β, which participates in the pathogenesis of human IVD degeneration. Therefore, the NLRP3 inflammasome might serve as a potential therapeutic target for the prevention and treatment of IVD degeneration. Anat Rec, 298:720–726, 2015. © 2014 The Authors The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology Published by Wiley Periodicals, Inc.
Intervertebral disc (IVD) degeneration, a major cause of low back pain, is a common and debilitating disorder (Fazzalari et al., 2001; Krismer et al., 2007). About 85% of people experience low back pain at some point in their lives. This causes a considerable loss of working days and costs about $100 billion every year in the USA (Katz et al., 2006). Current treatments for IVD degeneration such as physiotherapy, anti-inflammatory medication, and spinal fusion are predominantly focused on relieving pain (Spiro et al., 2001). However, these treatments do not directly target the process of degeneration and are not capable of restoring disc mechanics or structure (Vitale et al., 2002; Mirza et al., 2006). The failure of intervention could be attributed to poor understanding of the pathogenesis of IVD degeneration.

The human IVD consists of a proteoglycan-rich nucleus pulposus (NP) and a surrounding annulus fibrosus (AF) (Boszczyk et al., 2001). Altered synthesis of components of the IVD matrix, upregulation of matrix-degrading enzyme metalloproteinases (MMPs), and a disintegrin and MMP with thrombospondin motifs (ADAMTS) have been reported to be the causes of IVD degeneration (Ritty et al., 2002; Le Maitre et al., 2007a; Niu et al., 2009; Richardson et al., 2009). Loss of IVD degeneration (Vitale et al., 2002; Mirza et al., 2006). The failure of intervention could be attributed to poor understanding of the pathogenesis of IVD degeneration.

Patients and Tissue Samples

Degenerate IVD samples were obtained from patients who were diagnosed as having IVD degeneration by magnetic resonance imaging and underwent spinal fusion or IVD replacement surgery for low back pain (N = 45). Patients with sciatica, degenerative spinal stenosis, tumors, infection, and previous lumbar surgery were not included in the study. In addition, nondegenerate IVD samples were obtained from patients who suffered spinal trauma and needed surgical intervention (N = 7 used as control samples). The study was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University (China) and written informed consent was obtained from all patients.

Materials and Methods

Key words: NLRP3 inflammasome; human intervertebral disc degeneration; caspase-1; interleukin-1 β

Quantitative Real-Time RT-PCR

Total RNA of disc tissue samples was isolated using Trizol reagent. cDNA was synthesized using the SuperScript system (Invitrogen, Carlsbad, CA). Quantitative real-time PCR was performed using an iCycler Thermal Cycler instrument (Bio-Rad Laboratories, Hercules, CA). The parameters of PCR cycling were as follows: 1 cycle at 95 °C for 3 min, 40 cycles at 95 °C for 30 s and 54 °C for 30 s, and 1 cycle at 95 °C for 1 min, followed by a melting curve initiated at 55 °C and increasing in 0.5 °C increments for 80 steps. The sequence of the primers and probes were as follows: NLRP3 forward primer 5’TGCCGACAGGATGCA-3’, reverse 5’TTCATCTTCCTCAG-3’. The human IVD consists of a proteoglycan-rich nucleus pulposus (NP) and a surrounding annulus fibrosus (AF) (Boszczyk et al., 2001). Altered synthesis of components of the IVD matrix, upregulation of matrix-degrading enzyme metalloproteinases (MMPs), and a disintegrin and MMP with thrombospondin motifs (ADAMTS) have been reported to be the causes of IVD degeneration (Ritty et al., 2002; Le Maitre et al., 2007a; Niu et al., 2009; Richardson et al., 2009). Loss of IVD degeneration (Vitale et al., 2002; Mirza et al., 2006). The failure of intervention could be attributed to poor understanding of the pathogenesis of IVD degeneration.

Tissue Processing and Degeneration Scoring

For subsequent histology and immunohistochemistry, a block of tissue incorporating the AF and NP in continuity were fixed in 10% neutral buffered formalin and decalcified (0.1 M EDTA, pH 7.4), then dehydrated and embedded in paraffin wax. Sagittal sections were cut at 5 μm and then mounted and baked on appropriate silanized glass slides, which were stained with hematoxylin and eosin (H&E) to score the degree of morphological degeneration according to published criteria (Sive et al., 2012).

Immunohistochemistry

The 5 μm sections were dewaxed and rehydrated, and hydrogen peroxide was used to block endogenous peroxidase. Sections were washed in ddH2O and then

Patients and Tissue Samples

Degenerate IVD samples were obtained from patients who were diagnosed as having IVD degeneration by magnetic resonance imaging and underwent spinal fusion or IVD replacement surgery for low back pain (N = 45). Patients with sciatica, degenerative spinal stenosis, tumors, infection, and previous lumbar surgery were not included in the study. In addition, nondegenerate IVD samples were obtained from patients who suffered spinal trauma and needed surgical intervention (N = 7 used as control samples). The study was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University (China) and written informed consent was obtained from all patients.

Materials and Methods

Key words: NLRP3 inflammasome; human intervertebral disc degeneration; caspase-1; interleukin-1 β

Quantitative Real-Time RT-PCR

Total RNA of disc tissue samples was isolated using Trizol reagent. cDNA was synthesized using the SuperScript system (Invitrogen, Carlsbad, CA). Quantitative real-time PCR was performed using an iCycler Thermal Cycler instrument (Bio-Rad Laboratories, Hercules, CA). The parameters of PCR cycling were as follows: 1 cycle at 95 °C for 3 min, 40 cycles at 95 °C for 30 s and 54 °C for 30 s, and 1 cycle at 95 °C for 1 min, followed by a melting curve initiated at 55 °C and increasing in 0.5 °C increments for 80 steps. The sequence of the primers and probes were as follows: NLRP3 forward primer 5’TGCCGACAGGATGCA-3’, reverse 5’TTCATCTTCCTCAG-3’. The human IVD consists of a proteoglycan-rich nucleus pulposus (NP) and a surrounding annulus fibrosus (AF) (Boszczyk et al., 2001). Altered synthesis of components of the IVD matrix, upregulation of matrix-degrading enzyme metalloproteinases (MMPs), and a disintegrin and MMP with thrombospondin motifs (ADAMTS) have been reported to be the causes of IVD degeneration (Ritty et al., 2002; Le Maitre et al., 2007a; Niu et al., 2009; Richardson et al., 2009). Loss of IVD degeneration (Vitale et al., 2002; Mirza et al., 2006). The failure of intervention could be attributed to poor understanding of the pathogenesis of IVD degeneration.

Tissue Processing and Degeneration Scoring

For subsequent histology and immunohistochemistry, a block of tissue incorporating the AF and NP in continuity were fixed in 10% neutral buffered formalin and decalcified (0.1 M EDTA, pH 7.4), then dehydrated and embedded in paraffin wax. Sagittal sections were cut at 5 μm and then mounted and baked on appropriate silanized glass slides, which were stained with hematoxylin and eosin (H&E) to score the degree of morphological degeneration according to published criteria (Sive et al., 2012).

Immunohistochemistry

The 5 μm sections were dewaxed and rehydrated, and hydrogen peroxide was used to block endogenous peroxidase. Sections were washed in ddH2O and then
antigen retrieval was performed in 0.01 M citrate buffer (pH 6.0, 100°C) in a microwave oven for 3 min. Nonspecific binding sites were blocked at room temperature for 60 min with 5% w/v fetal calf serum. Then the sections were incubated overnight at 4°C with the monoclonal primary antibodies against human NLRP3 (1:50) (Alexis Biochemicals, San Diego, CA), caspase-1 (1:100) (Santa Cruz Biotechnology, Santa Cruz, CA) and IL-1β (1:100) (R&D Systems, Minneapolis, MN). After washing, the sections were incubated in rabbit anti-mouse secondary antibody for 30 min at room temperature. Disclosure of secondary antibody binding was performed by the streptavidin-biotin complex technique (Dako, Carpinteria, CA) with 3, 3'-diaminobenzidine tetrahydrochloride (Sigma-Aldrich Corp., St Louis, MO). The sections were counterstained with Mayer's hematoxylin.

**Imaging and Statistical Analysis**

All slides were visualized under an Olympus IX81 microscope, and images were captured using a digital camera and analyzed with a Bio-quant Nova image analysis system. The percentage of immunopositive cells was derived by counting a minimum of 200 cells per sample. Statistical comparisons between two groups were performed by the independent-samples t-test and the correlation between degeneration scores and immunopositivity were analyzed by nonparametric linear regression using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL). The percentage of immunopositive cells were reported as mean ± SD. P < 0.05 was considered statistically significant.

**RESULTS**

**Patient Characteristics and Grades of Disc Degeneration**

Forty-five degenerate IVDs and seven nondegenerate IVDs samples were compared. The scoring system provided a histological measure of the grade: 0–3, nondegenerate; 4–6, mild degeneration; 7–9, moderate degeneration; and 10–12, severe degeneration (Fig. 1).
The clinical characteristics of patients and the degeneration scores are listed in Table 1.

### TABLE 1. Patient characteristics and grades of disc degeneration

| Laboratory number | Sex | Clinical diagnosis | Degeneration score | Age (years) |
|-------------------|-----|-------------------|--------------------|-------------|
| 1                 | M   | disc hernia       | 7                  | 59          |
| 2                 | M   | disc hernia       | 6                  | 45          |
| 3                 | F   | spondylolisthesis | 11                 | 84          |
| 4                 | M   | disc hernia       | 8                  | 29          |
| 5                 | F   | spondylolisthesis | 10                 | 47          |
| 6                 | M   | disc hernia       | 6                  | 50          |
| 7                 | F   | disc hernia       | 8                  | 45          |
| 8                 | M   | disc hernia       | 4                  | 54          |
| 9                 | F   | disc hernia       | 6                  | 40          |
| 10                | F   | spondylolisthesis | 11                 | 48          |
| 11                | F   | disc hernia       | 5                  | 28          |
| 12                | M   | disc hernia       | 6                  | 75          |
| 13                | F   | disc hernia       | 6                  | 56          |
| 14                | F   | disc hernia       | 5                  | 65          |
| 15                | F   | spondylolisthesis | 10                 | 55          |
| 16                | F   | spondylolisthesis | 11                 | 59          |
| 17                | F   | disc hernia       | 7                  | 47          |
| 18                | F   | disc hernia       | 9                  | 57          |
| 19                | M   | spondylolisthesis | 10                 | 26          |
| 20                | M   | disc hernia       | 5                  | 68          |
| 21                | M   | disc hernia       | 6                  | 46          |
| 22                | F   | spondylolisthesis | 10                 | 77          |
| 23                | F   | spondylolisthesis | 10                 | 48          |
| 24                | F   | disc hernia       | 9                  | 71          |
| 25                | M   | disc hernia       | 7                  | 49          |
| 26                | F   | spondylolisthesis | 10                 | 54          |
| 27                | F   | disc hernia       | 9                  | 41          |
| 28                | M   | spondylolisthesis | 11                 | 34          |
| 29                | M   | disc hernia       | 10                 | 39          |
| 30                | M   | disc hernia       | 9                  | 40          |
| 31                | M   | disc hernia       | 7                  | 50          |
| 32                | M   | spondylolisthesis | 10                 | 71          |
| 33                | F   | disc hernia       | 7                  | 57          |
| 34                | F   | spondylolisthesis | 9                  | 61          |
| 35                | M   | disc hernia       | 8                  | 25          |
| 36                | F   | disc hernia       | 7                  | 42          |
| 37                | M   | disc hernia       | 6                  | 51          |
| 38                | F   | spondylolisthesis | 9                  | 67          |
| 39                | M   | spondylolisthesis | 10                 | 42          |
| 40                | F   | disc hernia       | 7                  | 35          |
| 41                | M   | spondylolisthesis | 9                  | 42          |
| 42                | F   | disc hernia       | 6                  | 24          |
| 43                | M   | disc hernia       | 7                  | 46          |
| 44                | M   | spondylolisthesis | 6                  | 53          |
| 45                | M   | disc hernia       | 6                  | 46          |
| 46                | F   | spine trauma      | 1                  | 33          |
| 47                | F   | spine trauma      | 3                  | 26          |
| 48                | F   | spine trauma      | 3                  | 20          |
| 49                | M   | spine trauma      | 2                  | 35          |
| 50                | M   | spine trauma      | 3                  | 24          |
| 51                | M   | spine trauma      | 3                  | 26          |
| 52                | M   | spine trauma      | 3                  | 37          |

The clinical characteristics of patients and the degeneration scores are listed in Table 1.

**NLRP3 Inflammasome and Its Downstream Targets Caspase-1 and IL-1β mRNA Expression Levels**

Real-time RT-PCR analysis demonstrated that the mRNA expression levels of NLRP3, caspase-1, and IL-1β were higher in the degenerate IVDs group compared with the nondegenerate IVDs group (Fig. 2).

**Immunohistochemical Localization and Quantification of NLRP3, Caspase-1, and IL-1β in Degenerate IVDs**

NLRP3, caspase-1, and IL-1β were generally restricted to the cytoplasm of NP cells in the degenerate and nondegenerate IVDs (Fig. 3A). The expression levels of all three molecules were markedly higher in the degenerate IVDs group, compared to the nondegenerate IVDs group (NLRP3 immunopositive cells: 69.5 ± 10.8 vs. 21.8 ± 16.0%, *P* < 0.001; caspase-1 immunopositive cells: 75.5 ± 8.1 vs. 18.1 ± 13.0%, *P* < 0.001; IL-1β immunopositive cells: 72.8 ± 11.9 vs. 42.5 ± 10.7%, *P* < 0.05) (Fig. 3B).

**Correlation Between IVD Degeneration scores and Expression Levels of NLRP3, Caspase-1, and IL-1β**

Correlation analysis revealed a strong positive correlation between the degeneration scores and the expression levels of NLRP3 (*P* < 0.001) (Fig. 4A,B). We also evaluated the correlation between the degeneration scores and the downstream targets of NLRP3 (caspase-1 and IL-1β). As expected, increase in IL-1β (*P* < 0.001) and caspase-1 (*P* < 0.001) immunopositivity were associated with higher degeneration scores (Fig. 4C).

**DISCUSSION**

This study provides preliminary evidence for the role of NLRP3 inflammasome in human IVD degeneration. The expression levels of NLRP3, caspase-1, and IL-1β were higher in the degenerate IVDs group compared with the nondegenerate IVDs group (Fig. 2).
were significantly higher in NP cells of degenerate IVDs than the nondegenerate IVDs. Interestingly, we found a positive correlation between the degeneration scores and the NLRP3 inflammasome as well as its downstream targets caspase-1 and IL-1β. IL-1β significantly contributes to the pathogenesis of IVD degeneration (Le et al., 2005, 2007b). Two major pathogenic factors of degenerate IVDs, MMPs, and ADAMTS, have been demonstrated to be upregulated by IL-1β (Le et al., 2005; Millward-Sadler et al., 2009). Moreover, it has been demonstrated that an IL-1 receptor antagonist inhibits the degeneration of human IVDs by eliminating matrix degradation (Le Maitre et al., 2007c). In the present study, we confirmed that IL-1β was significantly increased in the NP cells of degenerate IVDs. Furthermore, we found that an increase in the expression levels of IL-1β was associated with an increase in the degeneration score.

Mature IL-1β is produced by cleavage of pro-IL-1β by caspase-1, which is activated by the NLRP3 inflammasome (Martinon et al., 2009). However, aberrant NLRP3 inflammasome activation participates in the pathogenesis of many diseases. Martinon et al. (2006) showed that uric acid crystals induced gout via NLRP3-mediated IL-1β secretion. It has also been reported that NLRP3-mediated IL-1β production contributes to the progression of chronic kidney disease (Vilaysane et al., 2010). A mutation in the NLRP3 gene causing IL-1β overproduction is strongly associated with susceptibility to various autoinflammatory diseases such as rheumatoid arthritis (Kastbom et al., 2008). In the present study, we demonstrated that the expression levels of NLRP3, caspase-1, and IL-1β were significantly higher in the NP cells of degenerate IVDs as compared to the controls. A positive correlation was found between the expression levels of NLRP3 and its downstream targets caspase-1 and IL-1β. These results suggested that the NLRP3 inflammasome participates in the pathogenesis of human IVD degeneration. Although we did not verify the relationship between IL-1β and the NLRP3 inflammasome in NP cells in vitro, the current study provides new insights into the pathogenesis of human IVD degeneration. Our future studies will focus on NP cells and experimental animals to clarify the underlying molecular mechanisms.

In summary, our results showed that the expression levels of the NLRP3 inflammasome and its downstream targets caspase-1 and IL-1β were upregulated in the degenerated IVD and the expression was correlated with the grades of degeneration. These results suggest that the NLRP3 inflammasome participates in the pathogenesis of human IVD degeneration and might serve as a...
therapeutic target for the treatment of IVD degeneration.

LITERATURE CITED

Burns K, Martinon F, Tschopp J. 2003. New insights into the mechanism of IL-1beta maturation. Curr Opin Immunol 15:26–30.

Boszczyk BM1, Boszczyk AA, Putz R. 2001. Comparative and functional anatomy of the mammalian lumbar spine. Anat Rec 262:331–9.

Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Förster I, Farlik M, Decker T, Du Pasquier RA, Romero P, Tschopp J. 2011. Type I interferon inhibits interleukin-1 production and inflammation activation. Immunity 34:215–223.

Hoyland JA, Le Maître C, Freemont AJ. 2008. Investigation of the role of IL-1 and TNF in matrix degradation in the intervertebral disc. Rheumatology (Oxford) 47:809–814.

Kastbom A, Verma D, Eriksson P, Skogh T, Wingren G, Söderkvist P. 2008. Genetic variation in proteins of the cryopyrin inflammasome influences susceptibility and severity of rheumatoid arthritis (the Swedish TIRA project). Rheumatology (Oxford) 47:415–417.

Katz JN. 2006. Lumbar disc disorders and low-back pain: socioeconomic factors and consequences. J Bone Joint Surg Am 2:21–24.

Krismer M, van Tulder M. 2007. Low back pain (non-specific). Best Pract Res Clin Rheumatol 21:77–91.

Le Maitre CL, Pockert A, Buttle DJ, Freemont AJ, Hoyland JA. 2007a. Matrix synthesis and degradation in human intervertebral disc degeneration. Biochem Soc Trans 35:652–655.

Le Maitre CL, Hoyland JA, Freemont AJ. 2007b. Catabolic cytokine expression in degenerate and herniated human intervertebral discs: IL-1β and TNF-α expression profile. Arthritis Res Ther 9:R77.

Le Maitre CL, Hoyland JA, Freemont AJ. 2007c. Interleukin-1 receptor antagonist delivered directly and by gene therapy inhibits matrix degradation in the intact degenerate human intervertebral disc: an in situ zymographic and gene therapy study. Arthritis Res Ther 9:R83.

Le Maitre CL, Freemont AJ, Hoyland JA. 2006. A preliminary in vitro study into the use of IL-1Ra gene therapy for the inhibition of intervertebral disc degeneration. Int J Exp Pathol 87:17–28.

Le Maitre CL, Freemont AJ, Hoyland JA. 2005. The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration. Arthritis Res Ther 7:R732–745.

Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. 2006. Gout-associated uric acid crystals activate the NLRP3 inflammasome. Nature 440:237–241.

Martinon F, Tschopp J. 2004. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. Cell 117:561–574.

Martinon F, Burns K, Tschopp J. 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of pro IL-1β. Mol Cell 10:417–426.

Martinon F, Mayor A, Tschopp J. 2009. The inflammasomes: guardians of the body. Annu Rev Immunol 27:229–265.

Masuda K, An HS. 2006. Prevention of disc degeneration with growth factors. Eur Spine J 3:8422–432.

Millsward-Sadler SJ, Costello PW, Freemont AJ, Hoyland JA. 2009. Regulation of catabolic gene expression in normal and degenerate human intervertebral disc cells: implications for the pathogenesis of intervertebral disc degeneration. Arthritis Res Ther 11:R65.

Mirza SK, Deyo RA. 2007. Systematic review of randomized trials comparing lumbar fusion surgery to nonoperative care for treatment of chronic back pain. Spine 32:816–823.

Niu CC, Yuan LJ, Chen LH, Lin SS, Tsai TT, Liao JC, Lai PL, Chen WJ. 2011. Beneficial effects of hyperbaric oxygen on human
degenerated intervertebral disk cells via suppression of IL-1β and p38 MAPK signal. J Orthop Res 29:14–19.

Ritty TM1, Ditsios K, Starcher BC. 2002. Distribution of the elastic fiber and associated proteins in flexor tendon reflects function. Anat Rec 268:430–440.

Richardson SM, Doyle P, Minogue BM, Gnanalingham K, Hoyland JA. 2009. Increased expression of matrix metalloproteinase-10, nerve growth factor and substance P in the painful degenerate intervertebral disc. Arthritis Res Ther 11:R126.

Sive JI, Baird P, Jeziorsk M, Watkins A, Hoyland JA, Freemont AJ. 2002. Expression of chondrocyte markers by cells of normal and degenerate intervertebral discs. Mol Pathol 55:91–97.

Spiro RC1, Thompson AY, Poser JW. 2001. Spinal fusion with recombinant human growth and differentiation factor-5 combined with a mineralized collagen matrix. Anat Rec 263:388–395.

Vilaysane A, Chun J, Seamone ME, Wang W, Chin R, Hirota S, Li Y, Clark SA, Tschopp J, Trpkov K, Hemmeling R, Beck PL, Muruve DA. 2010. The NLRP3 inflammasome promotes renal inflammation and contributes to CKD. J Am Soc Nephrol 21:1732–1744.

Vitale M1, Bassini A, Secchiero P, Mirandola P, Ponti C, Zamai L, Mariani AR, Falconi M, Azzali G. 2002. NK-active cytokines IL-2, IL-12, and IL-15 selectively modulate specific protein kinase C (PKC) isoforms in primary human NK cells. Anat Rec 266:87–92.

Watanabe H, Gaide O, Petrilli V, Martinon F, Contassot E, Roques S, Kummer JA, Tschopp J, French LE. 2007. Activation of the IL-1β-processing inflammasome is involved incontact hypersensitivity. J Invest Dermatol 127:1956–1963.

Wu J, Fernandes-Alnemri T, Alnemri ES. 2010. Involvement of the AIM2, NLRC4, and NLRP3 inflammasomes in caspase-1 activation by Listeria monocytogenes. J Clin Immunol 30:693–702.