Supplementary information, Data S1 Materials and Methods

Mice and reagents

Asc and *Nlrp3* deficient mice have been described before [1, 2]. C57BL/6J wild type (WT) mice were obtained from the Jackson Laboratory. Animal care, use and experimental procedures complied with national guidelines and were approved by the Animal Care and Use Committee at Institut Pasteur of Shanghai. All chemical reagents were from Sigma unless stated otherwise.

Identification and culture of Cryptococcus neoformans clinical isolate

Phenotypic identification of the isolate *C. neoformans* HS1101 was performed in a clinical laboratory of a teaching hospital after culture on Sabouraud's medium, biochemical test using API 20C AUX system (bioMérieux). DNA of the strain was extracted using the QIAamp DNA Mini Kit (Qiagen) per the Handbook's protocol for yeast genomic DNA extraction. Products were amplified using primer pairs comprising ITS3 (5' GCATCGATGAAGAACGCAGC 3')/ITS4 (5' TCCTCCGCTTATTGATATGC 3') [3]. ITS PCR was cycled at 98 ℃ for 30 s, followed by 35 cycles at 98 ℃ for 30 s, 56 ℃ for 30 s, and 72 ℃ for 60 s, followed by 72 ℃ for 10 min. The PCR product was sequenced by cycle sequencing with the ABI 3730 XL DNA Analyzer (Applied Biosystems/Hitachi). Pairwised sequence alignment was done in GenBank database (www.ncbi.nlm.nih.gov/BLAST/). The strain was also identified via L-Canavanine glycine bromothymol blue agar (CGB) biochemical tests [4]. In addition, the *C. neoformans* HS1101 was cultured at 30 ℃ in Yeast Extract Peptone Dextrose (YPD) broth on a shaker at 180 rpm for 48 h, then centrifuged at 3,000 rpm for 5 min, washed in sterile Phosphate Buffer Solution (PBS) and counted using a hemocytometer prior to infection.

Biofilm induction

C. neoformans cells were collected by centrifugation, washed twice with PBS, counted using a hemacytometer, and suspended at 10^6 cells per ml in YPD broth. Then, 100 µl of the *C.*

neoformans suspension or YPD as control was added into individual well of polystyrene 96 well ELISA plates (Corning) and incubated at 37 ℃ CO_2 incubator without shaking. Biofilm were formed for 48 h. Following the adhesion stage, the wells containing *C. neoformans* biofilm was washed three times with PBS to remove non-adhered fungal cells. Fungal cells that remained attached to the plastic surface were considered biofilm and were subsequently visualized using an inverted microscope fixed with a digital camera to confirm the biofilm formation.

*THP-1 cell culture, in vitro C. neoformans challenge and drug treatmen*t

THP-1 cells were maintained in RPMI 1640 media with necessary supplements at 37 ℃ with 5% CO_2. 1×10^5 THP-1 cells were pooled in plates with *C. neoformans* biofilm, MOI=1 unless stated otherwise, 12 h later the supernatants were harvested for detection of cytokine concentrations via ELISA. In some cases, supernatants and cell extracts were collected for immunoblot analysis. Cells were treated with the following drugs for 30 min before stimulation with *C. neoformans* biofilm; LPS (100 ng/ml); ATP (5 mM); AC-YVAD-CHO; DPI; CA-074 Me; Cathepsin K Inhibitor I; KCl with indicated concentrations in figure legends.

Gene silencing in THP-1 cells

shRNA vectors against NLRP3, Caspase-1, ASC, and their scramble vectors were reported [5]. shRNA vectors against Caspase-8 and AIM2 were generated by our lab. Second-generation packaging plasmids pMD2-VSVG and pCMV-R8.91 were used for lentivirus production. Targeting sequences are: *Nlrp3*, CAGGTTTGACTATCTGTTCT; *Caspase-1*, GTGAAGAGATCCTTCTGTA; *Asc*, GATGCGGAAGCTCTTCAGTTTCA; *Caspase-8*, GATGCGGAAGCTCTTCAGTTTCA; *Aim2*, GCCTGAACAGAAACAGATG.

Cytokine ELISA and immunoblotting

Supernatants were analyzed for cytokine secretion by ELISA (BD Biosciences). Antibodies for immunoblotting include: rabbit anti-human mature IL-1β (D116, Cell Signaling), goat anti-human pro-IL-1β (sc-1250, Santa Cruz), mouse anti-human NLRP3 (ALX-804-881,

Enzo Life Sciences), rabbit anti-human ASC (SC-22514-R, Santa Cruz), rabbit anti-human Caspase-1 (sc-515, Santa Cruz), mouse anti-human β-actin (KM9001, Tianjin Sungene Biotech Co., Ltd). Appropriate HRP-conjugated secondary antibodies were used for signal detection via ECL reagent (PerkinElmer).

Quantitative real-time PCR

RNA from THP-1 cells was extracted using TRIzol reagent (Invitrogen). cDNA was synthesized with TaqMan Reverse Transcription Reagents (Applied Biosystems). Quantitative PCR was performed on 7900HT fast real-time PCR system using the SYBR Green qPCR Master Mix (TOYOBO). Relative quantification of genes was normalized against β-actin as relative unit (RU) via formula [$2^{-\triangle Ct(\text{target gene-b-actin})}$]. PCR primers applied are:

IL-1β, 5'-CACGATGCACCTGTACGATCA-(forward)3',

5'-GTTGCTCCATATCCTGTCCCT-(reverse)3';

Caspase-8, 5'-AACTGTGTTTCCTACCGAAACCC-(forward)3',

5'-AGGACATCGCTCTCTCAGGC-(reverse)3';

Aim2, 5'-TGGCAAAACGTCTTCAGGAGG-(forward)3',

5'-GATGCAGCAGGACTCATTTCA-(reverse)3'.

ASC pyroptosome detection

ASC pyroptosome detection was conducted as described before [6]. Briefly, THP-1 cells or BMDCs were stimulated by *C. neoformans* biofilm for 12 h and then pelleted and resuspended in 0.5 ml of ice-cold buffer containing 20 mM HEPES- KOH, pH 7.5, 150 mM KCl, 1% Nonidet P-40, 0.1 mM phenylmethylsulfonyl fluoride, and protease inhibitors, and lysed by shearing 10 times through a 21-gauge needle. The cell lysates were centrifuged at 6,000 rpm for 10 min. The pellets were washed with PBS and then resuspended in 500 μl PBS. The resuspended pellets were cross-linked with disuccinimidyl suberate (4 mM) for 30 min and precipitated by centrifugation at 6 000 rpm for 10 min. The cross-linked pellets were resuspended in 30 μl of SDS sample buffer and fractionated on 12% SDS-PAGE, followed by immunoblotting with anti ASC antibody.

In vivo C. neoformans infection

For survival experiment, mice were challenged by intraperitoneal injection of 5×10^7 cells of *C. neoformans* in 200 μl PBS, or intranasal infected with 5×10^5 cells of *C. neoformans* in 30 μl PBS under anesthesia, mice were observed daily for survival.

Statistical analysis

Data were analyzed for statistical significance by two-tailed student's t test in Excel. Differences with P values ≤ 0.05 were considered statistically significant.

References

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