

ASC-particle-induced Peritonitis

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[Abstract] In response to pathogen infection and tissue damage, inflammasome sensors such as NLRP3 and AIM2 are activated, which triggers PYRIN domain (PYD)-mediated ASC nucleation, followed by self-perpetuating ASC polymerization, which ultimately culminates in caspase-1 activation, interleukin (IL)-1 β and IL-18 processing and release and pyroptosis (Ratsimandresy *et al.*, 2013; Cai *et al.*, 2014). Inflammasomes release not only cytokines, but also the polymeric ASC danger particles (pASC) by pyroptosis, which perpetuate and propagate inflammasome responses to bystander cells to engage cell intrinsic ASC and caspase-1 (Baroja-Mazo *et al.*, 2014; Franklin *et al.*, 2014). In this protocol we describe intraperitoneal injection of polymeric ASC particles as a danger signal and measure neutrophil infiltration and levels of the pro-inflammatory cytokine IL-1 β by ELISA in the peritoneal lavage (de Almeida *et al.*, 2015).

Materials and Reagents

1. 5 ml syringes (BD, catalog number: 309646)
2. 25 G x1 ½ needles (BD, catalog number: 305127)
3. Conical tubes (15 ml) (Corning, Falcon®, catalog number: 352095)
4. C57BL/6 mice, typically of 8-12 weeks old (male or female)
5. 1 x 10⁵ pASC-GFP particles generated from stable or transiently expressing HEK293 cells and sorted by flow cytometry as described (Fernandes-Alnemri *et al.*, 2007; Fernandes-Alnemri and Alnemri, 2008)
6. 1x Dulbecco's phosphate-buffered saline (DPBS) (Corning, catalog number: 21-030-CV)
7. HEPES
8. KOH
9. Magnesium chloride (MgCl₂)
10. EGTA
11. CHAPS
12. cOmplete protease inhibitor cocktail (Roche Diagnostics, catalog number: 11697498001)
13. IL-1 β ELISA kit (BD, catalog number: 559603)
14. Lysis buffer (see Recipes)

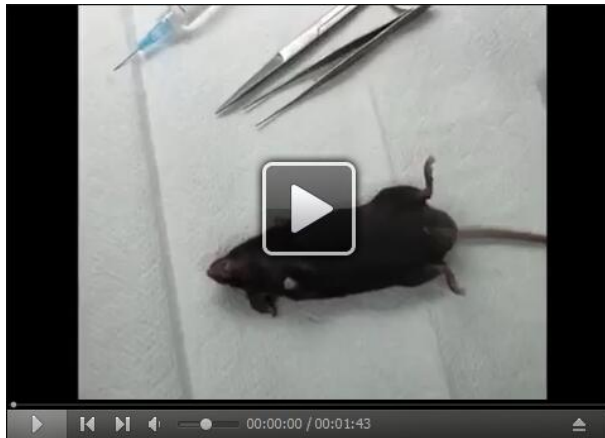
Equipment

1. Centrifuge
2. Fluorescence microscope
3. Flow cytometry
4. Surgical instruments such as tweezers and scissors
5. Biosafety cabinet

Procedure

1. Isolate pASC-GFP particles from 10^7 HEK293 cells stably or transiently transfected with ASC-GFP as described previously (Fernandes-Alnemri *et al.*, 2007; Fernandes-Alnemri and Alnemri, 2008; Martín-Sánchez *et al.*, 2015) or by flow cytometry.
2. Remove media from cells, add DPBS and gently scrape cells. Spin down cells at $1,500 \times g$ for 5 min. Discard supernatant.
3. Prepare total cell lysates by hypotonic lysis of cell pellets in 20 mM HEPES-KOH, pH 7.5, 5 mM $MgCl_2$, 0.5 mM EGTA, 0.1% CHAPS, supplemented with protease inhibitors. Use a syringe with 25 G needle to lyse the cells by syringing several times. Centrifuge cell lysates full speed to obtain cell lysates supernatant.
4. Induce aggregation of ASC-GFP by incubation of cell lysates supernatant at $37^\circ C$ for 30 min as previously described (Fernandes-Alnemri *et al.*, 2007; Fernandes-Alnemri and Alnemri, 2008). The typical number of ASC-GFP particles recovered is between 2×10^5 and 4×10^5 per million cells.
5. Add a small amount of supernatant to a microscope slide to confirm ASC polymerization into 1-3 μm aggregates by fluorescence microscopy using a standard GFP excitation/emission filter set (excitation: 484 nm; emission: 507 nm) (Figure 1).
6. Sort ASC-GFP particles (1-2 μm) by flow cytometry by gating for small particles based on forward scatter versus side scatter. Next start by gating for FITC positive particles. Later confirm ASC-GFP particles by fluorescent microscopy (Figure 1). Particles can be stored at $4^\circ C$ for one year.
7. Intraperitoneally inject mice with 1×10^5 pASC-GFP particles or the same volume DPBS for control mice (injection volume approximately 200 μl).
8. 4 h later sacrifice mice, cut the skin and expose the peritoneal wall (Video 1).

Video 1. Peritoneal lavage. The video shows how to wash peritoneal cavity after injection of pASC-GFP particles to measure IL-1 β by ELISA.



9. Inject 4 ml of DPBS into the peritoneal cavity.
10. Shake the mouse to wash the peritoneal cavity.
11. Use the syringe to recover DPBS injected by gradually pulling out the plunger.
12. Remove the needle and add content to a conical tube.
13. Spin down the cells at 1,200 x *g* for 5 min.
14. Transfer supernatant to fresh conical tubes and measure IL-1 β by ELISA. Typical IL-1 β concentrations are up to 2 ng/ml and a representative result is shown in our previous publication (de Almeida *et al.*, 2015).

Representative data

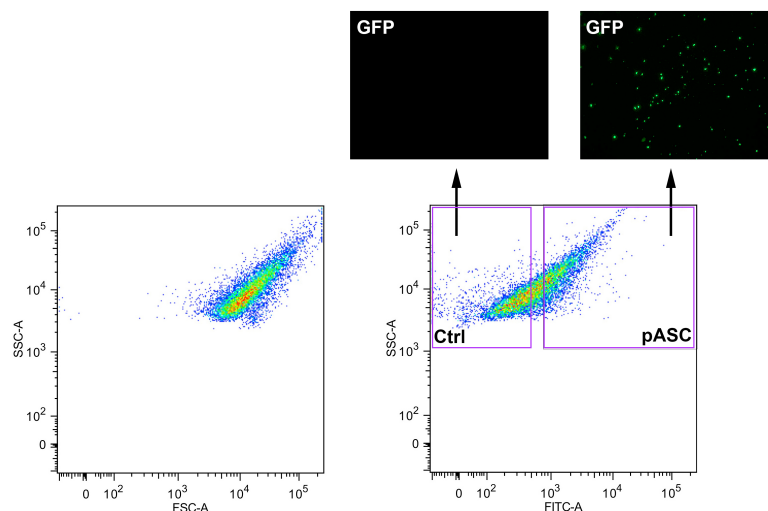


Figure 1. Total cell lysates from stable ASC-GFP-expressing HEK293 cells were GFP-sorted by flow cytometry after inducing ASC polymerization and control (Ctrl) and ASC-GFP (pASC)-containing fractions analyzed by fluorescence microscopy. HEK293 cell lysates were used as a negative control.

Notes

1. ASC-GFP particles should be protected from light until use.

Recipes

1. Lysis buffer
 - 20 mM HEPES-KOH, pH 7.5
 - 5 mM MgCl₂
 - 0.5 mM EGTA
 - 0.1% CHAPS
 - Protease inhibitors

Acknowledgments

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