β-arrestin1 Is Critical for the Full Activation of NLRP3 and NLRC4 Inflammasomes

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J Immunol published online 12 January 2015
http://www.jimmunol.org/content/early/2015/01/09/jimmunol.1401989
β-arrestin1 Is Critical for the Full Activation of NLRP3 and NLRC4 Inflammasomes

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Inflammasomes are multiprotein complexes that trigger the activation of caspase-1 and the maturation of IL-1β, which are critical for inflammation and control of pathogen infection. Although the function of inflammasomes in immune response and disease development is well studied, the molecular mechanism by which inflammasomes are activated and assembled remains largely unknown. In this study, we found that β-arrestin1, a key regulator of the G protein–coupled receptor signaling pathway, was required for nucleotide-binding domain and leucine-rich-repeat containing (NLR) family pyrin domain–containing 3 (NLRP3) and NLR family CARD domain–containing protein 4 (NLRC4) inflammasome–mediated IL-1β production and caspase-1 activation, but it had no effect on aspect oxidase in melanoma 2 (AIM2) inflammasome activation. Moreover, apoptosis-associated speck-like protein containing a CARD (ASC) pyroptosome, which is ASC aggregation mediating caspase-1 activation, was also impaired in β-arrestin1–deficient macrophages upon NLRP3 or NLRC4, but not AIM2 inflammasome activation. Mechanistic study revealed that β-arrestin1 specifically associated with NLRP3 and NLRC4 and promoted their self-oligomerization. In vivo, in a monosodium urate crystal (MSU)-induced NLRP3–dependent peritonitis model, MSU-induced IL-1β production and neutrophil flux were significantly reduced in β-arrestin1 knockout mice. Additionally, β-arrestin1 deficiency rescued the weight loss of mice upon log-phase Salmonella typhimurium infection, with less IL-1β production. Taken together, our results indicate that β-arrestin1 plays a critical role in the assembly and activation of two major canonical inflammasomes, and it may provide a new therapeutic target for inflammatory diseases. The Journal of Immunology, 2015, 194: 000–000.

P antigen recognition receptors recognize a diverse range of microbial components and environmental irritants to induce innate immune responses. Among those, some nu-

Abbreviations used in this article: AIM2, absent in melanoma 2; Arhl−/−, β-arrestin1 deficient; ASC, apoptosis-associated speck-like protein containing a CARD; BMDC, bone marrow–derived dendritic cell; e3 FA, omega-3 fatty acid; LDH, lactate dehydrogenase; MSU, monosodium urate; NLR, nucleotide-binding domain and leucine-rich-repeat containing; NLRC4, NLR family CARD domain–containing protein 4; NLRP3, NLR family pyrin domain–containing 3; NMDA, N-methyl-D-aspartate; poly(dA:dT), poly(deoxyadenylc-thymidylc) acid; WT, wild-type.

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Received for publication August 5, 2014. Accepted for publication December 5, 2014.

This work was supported by National 973 Key Project of China Grant 2013CB530504, National 863 Project of China Grants 2012AA02A0404 and 2012AA020103, National Natural Science Foundation of China Grants 31100302, 31261120409, National Science and Technology Major Projects of China Grants 2012ZX10002-007-003, 2013ZX10004-101-005, and 2013ZX10004-003-003, and Chinese Academy of Sciences Key Project Grant KJZD-EW-L09-3.

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a crosslinking assay and immunofluorescence assay, β-arrestin1 was found to promote NLRP3- and NLRC4-induced ASC pyroptosome formation. Further mechanistic study revealed that β-arrestin1 specifically interacted with NLRP3 and NLRC4 and enhanced their self-oligomerization. In vivo, β-arrestin1 was involved in a monosodium urate (MSU)–induced peritonitis model by promoting IL-1β production and neutrophil influx. During *Salmonella typhimurium* infection, which causes severe inflammation through NLRC4 inflammasome activation, β-arrestin1 deficiency played a protective role and modulated IL-1β production as well. Our results suggest the positive role of β-arrestin1 in regulating NLRP3 and NLRC4 inflammasomes, which may be a potential target in the treatment of IL-1β–associated autoinflammatory diseases.

**Materials and Methods**

**Mice and reagents**

C57BL/6 mice were obtained from Shanghai Laboratory Animal Center (Chinese Academy of Sciences). β-arrestin1–deficient (Arbr-/-) mice on a C57BL/6 background were provided by Robert J. Lefkowitz (Duke University Medical Center, Durham, NC). All mice were maintained in pathogen-free conditions. Animal care and use were in accordance with the guidelines of the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences.

All reagents were from Sigma-Aldrich unless stated otherwise. ATP was a gift from Weihuan Fang (Zhejiang University, Zhejiang, China). Flagellin of *Salmonella typhimurium* was a gift from Weihuan Fang (Zhejiang University, Zhejiang, China). Flagellin of *S. typhimurium* was a gift from Weihuan Fang (Zhejiang University, Zhejiang, China). Flagellin of *S. typhimurium* was provided by Feng Shao (National Institute of Biological Sciences, Beijing, China). IL-1β and IL-6 ELISA kits were obtained from R&D Systems. Lactate dehydrogenase (LDH) release was assessed in cell-free medium at indicated time points after the manufacturer’s instructions (cytotoxicity detection kit; Roche Diagnostics). Anti–FLAG M2 Ab was purchased from Abcam. Anti–caspase-1 Ab was obtained from Santa Cruz Biotechnology. Anti-ASC Ab was produced by immunization of rabbits with ASC expressed by Escherichia coli. Anti-NLRP3 Ab was purchased from MILLIPORE. Anti–P47TRX Ab was purchased from Abcam. MSU was prepared as previously reported (9).

**Cells**

HEK293T cells were cultured in humidified 5% CO₂ at 37°C in DMEM supplemented with 10% (v/v) FBS, penicillin (100 U/ml), and streptomycin (100 U/ml). Lipofectamine 2000 (Invitrogen) was used for transfection of HEK293T cells.

In preparation for the isolation of peritoneal macrophages, mice were infected intraperitoneally with *Salmonella typhimurium* and peritoneal exudate cells were isolated from the peritoneal cavity 4 h postinfection. The cells were then incubated at 37°C for 6 h and washed three times with HBSS. The remaining adherent cells were used as the peritoneal macrophages described in the experiments.

Bone marrow–derived dendritic cells (BMDCs) were prepared as follows: bone marrow cells were flushed from the femurs and tibias of C57BL/6 and Arbr-/- mice and depleted of red cells with ammonium chloride. Cells were cultured at 1×10⁶ cells per well in 24-well plates in DMEM supplemented with 20 mg/ml murine GM-CSF (PeproTech). Fresh medium was changed every 2 d. On day 7, cells were collected for further experiments.

**In vitro stimulation of macrophages**

Unless otherwise indicated, the macrophages were primed with 200 ng/ml LPS from *Es. coli* 0111:B4 (Sigma-Aldrich) for 4 h before stimulation with 5 mM ATP for 30 min, 20 µM nigericin for 30 min, 250 µg/ml MSU for 3 h, poly(deoxyadenylc-thymidylid) acid [poly(dA:dT)] for 6 h, flagellin for 6 h, or log-phase *S. typhimurium* strain SL3144 for 2 h. Poly(dA:dT) was transfected with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s recommendations. *S. typhimurium* was grown overnight (16–18 h) in Luria–Bertani broth at 37°C and inoculated at 1:50 in fresh Luria–Bertani broth, followed by another 2.5–3 h culture, when the bacteria came into its log phase. The bacteria were diluted in fresh macrophage medium and added to the LPS-priming macrophages at different multiplicities of infection. Cells were cultured at 37°C for 2 h before analysis.

**Immunoprecipitation and immunoblot analysis**

Immunoprecipitation and immunoblot analysis were performed as described previously (10). In short, HEK293T cells were transfected with various combinations of plasmids. At 24 h after the transfection, the cell lysates were prepared in lysis buffer and incubated with the indicated Ab together with protein A/G Plus–agarose immunoprecipitation reagent (Santa Cruz Biotechnology) at 4°C for 3 h or overnight. After three washes, the immunoprecipitates were boiled in SDS sample buffer for 10 min and analyzed by immunoblot.

For interaction of NLRs and β-arrestin1, NLRP3, NLRC4, and NLRP12 were cloned into p3XFLAG-CMV-10 expression vector (Sigma-Aldrich) to express 3xFLAG-NLRs. 3xFLAG peptide (Sigma-Aldrich) was used according to the manufacturer’s recommendations. In short, after 24 h transfection, the cell lysates were prepared in lysis buffer and incubated with anti-FLAG M2 affinity gel at 4°C overnight. The gel was washed and eluted with 100 µg/ml 3xFLAG peptide for >3 h at 4°C, and the supernatant free of gel was analyzed by immunoblot.

For endogenous coimmunoprecipitation experiments, different stimulated BMDCs were then prepared in lysis buffer and incubated with 0.5 µl anti-NLRP3 or rabbit IgG. The subsequent procedures were performed as described above.

Triton X-100 insoluble fraction was generated as previously described (11). Then 1% Nonidet P-40 buffer containing EDTA-free protease inhibitor “mixture” and phosphatase inhibitor mixture (Nacalai Tesque) was used to dissolve the insoluble part at 4°C for >1 h. After centrifugation at 5000×g for 10 min at 4°C, the supernatant was collected and applied for immunoprecipitation assay as described above.

**ASC pyroptosome detection**

ASC pyroposomes were detected as previously reported (12). Macrophages were seeded in six-well plates (2×10⁶ cells per well) and treated with different stimuli. The cells were centrifuged and resuspended in 300 µl PBS containing 20 mM HEPES-KOH (pH 7.5), 150 mM KCl, 1% Nonidet P-40, 0.1 mM PMSF, and protease inhibitor mixture, followed by shearing 10 times through a 21-gauge needle. The cell lysates were then centrifuged at 5000×g for 10 min at 4°C, and the pellets were washed twice with PBS and resuspended in 500 µl PBS. The resuspended pellets were cross-linked with disuccinimidyl suberate (4 mM) for 30 min and pelleted at 5000×g for 10 min. The cross-linked pellets were lysed in 30 µl SDS sample buffer, separated using 12% SDS-PAGE, and immunoblotted using anti-mouse ASC Abs.

**Confocal microscopy**

Peritoneal macrophages were plated overnight on coverslips. After stimulation as described above, the cells were washed and fixed with 4% PFA in PBS for 15 min, permeabilized with Triton X-100, and blocked with 1% BSA in PBS for 30 min. After incubation with FITC-conjugated anti-ASC Ab and anti-NLRP3 Ab for 2 h, cells were washed and incubated with Alexa Fluor 561 goat anti-mouse Ab (BD Biosciences) for 1 h. Finally, the cells were stained with DAPI. The confocal microscopy analyses were performed using a Leica TCS SP2.

**In vivo peritonitis model**

Mice were injected i.p. with 1 mg MSU in 200 µl PBS; control mice received PBS. At 6 h after injection, mice were sacrificed and peritoneal cells were obtained with 10 ml PBS with 1% (v/v) FBS, followed by centrifugation and total cell counting. Subsequently, cells were stained for neutrophil surface marker GR-1 and detected using flow cytometry. The number of neutrophils was calculated as total cells multiplied by the percentage of GR-1⁺ cells. The number of peritoneal macrophages was calculated as total cells multiplied by the percentage of CD11b⁺ and F4/80⁺ double-positive cells. For IL-1β and IL-6 measurements, 600 µl PBS with 1% (v/v) FBS was used and the supernatant IL-1β and IL-6 of peritoneal lavage fluid were determined.

**Animal infections**

For *S. typhimurium* infection, mice were starved for 12 h and infected i.p. with 1×10⁶ CFU log-phase bacteria (prepared as described above) in 100 µl sterile PBS, after which food and water were supplied. At day 5 postinfection, peritoneal fluid was collected for detection of IL-1β and IL-6. Spleen and liver were harvested, homogenized in PBS, and the bacteria loads were determined as previously described (13).

**Statistical analysis**

Data are presented as the means ± SD from three independent experiments. Statistical comparisons between different treatments were performed by an unpaired Student *t* test, where *p* < 0.05 was considered statistically significant and *p* < 0.01 was highly significant.
Results

β-arrestin1 regulates NLRP3 inflammasome activation

β-arrestin1 was reported to play an important role in autoimmune diseases, such as experimental autoimmune encephalomyelitis and rheumatoid arthritis (7, 8); however, the molecular mechanism remains poorly studied. Given that Th17 cells play a central role in autoimmune disease (14, 15) and IL-1, IL-6, and IL-23, produced mainly by dendritic cells and macrophages, are essential for the induction of Th17 cells (16–18), we prepared BMDCs from the wild-type (WT) and Arrb1−/− mice and stimulated these cells with LPS for different lengths of time. As shown in Supplemental Fig. 1, β-arrestin1 deficiency resulted in a dramatic decrease of IL-1β production at 48 h of stimulation, but not IL-6, TNF-α, and IL-23 production. It has been reported that IL-1β secretion involves two sequential processes: the activation of NF-κB, which contributes to the expression of pro–IL-1β and NLRP3 (19), and the second signal, the assembly of inflammasomes to generate active caspase-1 for processing pro–IL-1β (9, 20, 21). The secretion of IL-1β by LPS-stimulated BMDCs at a later time point may be due to the generation of endogenous danger signal, such as ATP, from dead dendritic cells.

To investigate whether β-arrestin1 is involved in NLRP3 inflammasome activation, we stimulated LPS-primed WT and Arrb1−/− peritoneal macrophages with ATP, nigericin, or MSU. Inconsistent with the findings in BMDCs, IL-1β production, but not IL-6, was markedly decreased in Arrb1−/− macrophages (Fig. 1A, 1B). Reduced generation of caspase-1 active form p10 was also observed in Arrb1−/− macrophages in all stimuli tested (Fig. 1C). It was interesting that β-arrestin1 could also be detected in the supernatant upon NLRP3 inflammasome activation (Supplemental Fig. 2A). Because β-arrestin1 deficiency had no effect on the LPS-induced NLRP3 and pro–IL-1β production (Supplemental Fig. 2B), the diminished caspase-1 activation in β-arrestin1-deficient macrophages was not the result of the availability of NLRP3. Given that arrestins were reported to facilitate internalization of GPCRs upon activation (22), and P2X7R, the receptor for extracellular ATP, belongs to GPCR superfamily (23), expression of P2X7R on the cell surface was analyzed. However, no significant difference was observed between WT and Arrb1−/− macrophages (Supplemental Fig. 2C), suggesting that β-arrestin1 deficiency has no effect on the internalization of P2X7R. Because pyroptosis is induced during inflammasome activation, LDH release from the stimulated macrophages was examined to determine whether cell death is affected by β-arrestin1 deficiency. As expected, similar to the pattern of IL-1β production and caspase-1 activation, LDH release was also reduced in Arrb1−/− macrophages upon various stimuli (Fig. 1D). These data suggest that β-arrestin1 deficiency results in decreased NLRP3 inflammasome-mediated caspase-1 activation and active IL-1β production.

β-arrestin1 is also required for NLRC4, but not AIM2, inflammasome activation

To define whether the effect of β-arrestin1 was confined to NLRP3 or extended to other inflammasome types, we assessed the functions of β-arrestin1 in NLRC4 and AIM2 inflammasome activation. To activate the NLRC4 inflammasome, LPS-primed WT and Arrb1−/− macrophages were infected with different multiplicities...
of infection of S. typhimurium (22). Similar to the NLRP3 inflammasome, the NLRC4 inflammasome–mediated IL-1β production and caspase-1 activation were impaired in Arrb1−/− macrophages (Fig. 2A, 2B). S. typhimurium was reported to activate the NLRP3 inflammasome as well, but the stimulation condition was different from NLRC4 (13). Therefore, to further characterize the role of β-arrestin1 in NLRC4 activation, another NLRC4 inflammasome stimulator, purified flagellin from S. typhimurium, was also tested, and the results were similar to those with S. typhimurium infection (Fig. 2C, 2D). However, upon the intracellular delivery of poly(dA:dT) (24, 25), which activates AIM2 inflammasome, β-arrestin1 deficiency had no effect on IL-1β secretion and caspase-1 activation (Fig. 2E, 2F). LDH release showed the similar trend as the IL-1β secretion during activation of these inflammasomes (Supplemental Fig. 3). Thus, β-arrestin1 is also required for NLRC4 inflammasome activation.

β-arrestin1 is critical for NLRP3- and NLRC4-mediated ASC pyroptosome formation

For NLRP3, NLRC4, and AIM2 inflammasomes, once activated, they recruit ASC to form ASC pyroptosome, a single large structure assembled by ASC, which is thought to mediate caspase-1 activation (5). We next examined ASC pyroptosome formation in WT and Arrb1−/− peritoneal macrophages. On the one hand, ASC pyroptosome was isolated and then cross-linked with noncleavable protein cross-linking agent disuccinimidyl suberate in the ATP-, S. typhimurium-, or poly(dA:dT)-stimulated macrophages. Inconsistent with IL-1β production and caspase-1 activation, deficiency of β-arrestin1 resulted in reduced NLRP3- and NLRC4-mediated ASC pyroptosome formation (Fig. 3A, 3B). In contrast, AIM2-mediated ASC pyroptosome was unaffected in Arrb1−/− macrophages (Fig. 3B). On the other hand, confocal microscopy was applied for the detection of the endogenous ASC, which showed that >78% of the WT macrophages contained the ASC pyroptosome after stimulation with LPS and ATP, compared with only 33.1% in Arrb1−/− macrophages. Flagellin-induced ASC pyroptosome formation was also impaired in Arrb1−/− macrophages, with 31.4–60.8% in WT cells, whereas AIM2 inflammasome–dependent ASC pyroptosome formation was intact with β-arrestin1 deficiency (Fig. 3C, 3D). Collectively, these data indicate that β-arrestin1 is involved in the NLRP3 and NLRC4 inflammasome assembly to activate caspase-1.

**FIGURE 3.** NLRP3- and NLRC4-, but not AIM2-mediated ASC pyroptosome formations are impaired in β-arrestin1–deficient macrophages. (A) ASC oligomerization and redistribution assay in LPS-primed peritoneal macrophages from WT mice or Arrb1−/− mice left untreated (Mock) or stimulated with ATP. Immunoblot (IB) analysis of ASC in cross-linked pellets (upper panels) and ASC, β-arrestin1, and β-actin in cell lysates (lower panels) are shown. (B) ASC oligomerization assay in LPS-primed peritoneal macrophages from WT mice or Arrb1−/− mice left untreated (Mock) or infected with S. typhimurium (S. typhi, multiplicity of infection of 50) or transfected with 1 μg poly(dA:dT). Immunoblot (IB) analysis of ASC in cross-linked pellets (upper panels) and ASC, β-arrestin1, and β-actin in cell lysates (lower panels) are shown. (C) Immunofluorescence microscopy of LPS-primed peritoneal macrophages treated with ATP, flagellin, poly(dA:dT), or left unstimulated (Mock) and then stained for ASC, NLRP3, and DNA (with DAPI). Scale bars, 10 μm. (D) Percentage of macrophages containing ASC foci from WT mice or Arrb1−/− mice treated as in (C). The quantification represents the mean of three independent experiments, with at least 200 cells counted in each experiment. The data represent at least three experiments [means ± SD in (D)]. *p < 0.05 (Student t test).
β-arrestin1 specifically interacts with NLRP3 and NLRC4 and promotes their self-oligomerization

Because β-arrestins have been reported to function as adaptors in various signaling pathways (26), the requirement of β-arrestin1 for full inflammasome activation led us to examine the potential interaction between β-arrestin1 and the NLRP3 inflammasome components. We found that β-arrestin1 specifically interacted with NLRP3, but not ASC or procaspase-1, when they were co-expressed in HEK293T cells (Fig. 4A). Given that NLRP3 has three different functional domains (PYD, NACHT, and LRR domains), we further examined the domains of NLRP3 accounting for the association with β-arrestin1. As shown in Fig. 4B, β-arrestin1 had strong association with the PYD domain and weak binding to the NACHT and LRR domains of NLRP3. Because we determined that β-arrestin1 is indispensible for not only NLRP3 inflammasome activation but also NLRC4 inflammasome activation, we sought to determine whether other inflammasome sensors would interact with β-arrestin1. Of the proteins tested, we found that NLRP3, NLRC4, and NLRP12, but not AIM2, interacted with β-arrestin1 (Fig. 4C). This is consistent with the findings that β-arrestin1 is required for NLRP3 and NLRC4 inflammasome activation but not for AIM2 inflammasome activation.

To determine the association of β-arrestin1 with NLRP3 in a more physiological setting, we immunoprecipitated endogenous NLRP3 from BMDCs and then evaluated the immunoprecipitates for the presence of β-arrestin1 upon different stimulation. In accordance with the interaction of β-arrestin1 with NLRP3 in HEK293T cells, endogenous β-arrestin1 was associated with NLRP3 under the physiological condition (Fig. 4D). Because the active inflammasome complexes are also found in the insoluble fraction (11), the NLRP3/β-arrestin1 association was also detected in this part. Upon LPS plus ATP stimulation, a fraction of β-arrestin1 sedimented in the insoluble part and showed an interaction with NLRP3, which precipitated more upon ATP stimulation compared with LPS priming alone (Fig. 4D). Taken together, our findings suggest that β-arrestin1 specifically interacts with NLRP3 and NLRC4.

It has been reported that NLRs, including NLRP3 and NLRC4, once activated, undergo self-oligomerization to form a molecular platform, which recruits the adaptor protein ASC (27). To further investigate whether β-arrestin1 plays a role in NLRP3 and NLRC4 self-oligomerization, we expressed Flag-tagged and hemagglutinin-tagged NLRP3 or NLRC4 in the presence or absence of β-arrestin1 in HEK293T cells. When immunoprecipitated with anti-Flag Ab, β-arrestin1 enhanced the interaction between different tagged NLRP3 proteins (Fig. 4E). The same effect was found on NLRC4 self-oligomerization (Fig. 4F). Thus, this assay indicates that β-arrestin1 may promote self-oligomerization of NLRP3 and NLRC4 proteins.

β-arrestin1 regulates NLRP3 and NLRC4 inflammasomes in vivo

To determine whether the effect of β-arrestin1 on NLRP3 and NLRC4 inflammasome activation was also critical in vivo, different mouse models were adopted. Under steady-state, the physiological levels of peritoneal macrophages and neutrophils were similar between WT and Arrb1−/− mice (Supplemental Fig. 4). In the MSU-induced peritoneal inflammatory mouse model, which is an NLRP3 inflammasome–induced peritonitis model (9), the production of IL-1β and IL-6 and the influx of neutrophils into the peritoneal cavity were determined. Compared with WT littermates, β-arrestin1 deficiency resulted in attenuated IL-1β production (Fig. 5A) and neutrophil infiltration (Fig. 5C), whereas IL-6 production was intact (Fig. 5B). In contrast, β-arrestin1 had no effect on zymosan-induced neutrophil influx, which is known to induce peritonitis in an IL-1β–independent manner.

Additionally, log-phase S. typhimurium–induced inflammation in vivo depends on NLRC4 inflammasome. During S. typhimurium infection, peritoneal IL-1β production was significantly reduced in Arrb1−/− mice (Fig. 5D), but not IL-6 production (Fig. 5E). β-arrestin1 deficiency also rescued the weight loss caused by S. typhimurium (Fig. 5F), with similar bacteria loads in the spleens and livers of WT and Arrb1−/− mice (Fig. 5G), suggesting that the weight loss was enhanced by excessive inflammation instead of bacteria proliferation. These results indicate the pivotal function of β-arrestin1 in NLRP3 and NLRC4 inflammasome activation in vivo.

Discussion

Although several regulators have been found involved in either NLRP3 or NLRC4 inflammasome, our present study suggests a critical role of β-arrestin1 in activation of both inflammasomes. These regulators function in diverse ways, including modification of the inflammasome components, scaffolding inflammasome assembly, or changing the localization of inflammasomes (28–30),...
For instance, deubiquitinase BRCC3, which can deubiquitinate the LRR domain of NLRC4, is essential for the full activation of NLRC4 inflammasome (30). Similarly, protein kinase C, an NLR4 kinase, is required for NLRC4 inflammasome activation via phosphorylation on NLRC4 (31). Besides, it has been reported that GBP5 is a unique activator of NLRC4-ASC complex, limited to bacterial cell wall components but not crystalline agents or dsDNA (31). With yeast two-hybrid, Tschopp and colleagues (32) found that heat shock protein 90 was required for the activity of several NLRs, including NLRP3, NLRC4, and Nod2. In this study, our results suggest that β-arrestin1 is required for the full activation of both NLRP3 and NLRC4 inflammasomes through interacting with NLR proteins and promoting their self-oligomerization. We found that β-arrestin1 could interact with three domains of NLRP3 and full-length NLRC4 in HEK293T cells. In the endogenous immunoprecipitation assay, the β-arrestin1/NLRP3 interaction was detected in both the Triton X-100–soluble and Triton X-100–insoluble fractions upon different stimulations.

During inflammasome activation, in addition to IL-1β secretion and caspase-1 activation, there is another important marker, ASC pyroptosome formation. Interestingly, only one ASC pyroptosome is formed per cell, which is crucial for the subsequent procaspase-1 recruitment and activation (5). In our study, two different strategies were adopted, and both showed a remarkable impairment of ASC pyroptosome formation in Arrb1−/− macrophages upon NLRP3 and NLRC4 inflammasome activation, whereas AIM2-induced ASC pyroptosome formation was intact. The defect of ASC pyroptosome formation in β-arrestin1 deficiency macrophages was supposed to result in subsequent attenuated IL-1β secretion.

In vivo, β-arrestin1 also played a critical role for NLRP3 and NLRC4 inflammasome–dependent disease models. MSU crystals, which cause peritonitis in a mouse model (9), were also used in our experiment, and we found that MSU-induced peritonitis was significantly reduced in Arrb1−/− mice. These findings indicate that β-arrestin1 is required for the inflammation induced by NLRP3 inflammasome.

NLRC4 is the major receptor for recognition of Gram-negative bacteria, and log-phase S. typhimurium could cause severe inflammation via NLRC4 inflammasome. Thus, in our experiment, mice were infected with S. typhimurium by i.p. injection and similar results were observed as with MSU-induced peritonitis. These results may suggest that excessive inflammation, such as undue IL-1β secretion, but not the pathogen alone, is responsible for the pathogen-induced pathogenicity.

In previous studies, it was suggested that β-arrestins may function as negative regulators to suppress NF-κB activation through direct interaction with IκBα, then preventing its phosphorylation and degradation (33), or interaction with TRAF6 and preventing its autoubiquitination and activation of NF-κB (34). Afterward, other findings indicated that the functions of β-arrestins are pleiotropic (35, 36). Nevertheless, we found that β-arrestin1 had no effect on IL-6 and pro–IL-1β production, but it regulated IL-1β secretion by regulating inflammasome activation. The contradicted effects of β-arrestins on NF-κB activation and inflammatory cytokine secretion may be explained by the different cell types and activation states adopted.

Another β-arrestin family member β-arrestin2, 78% identical to β-arrestin1 at the amino acid level, was involved in NLRP3 inflammasome inhibition induced by omega-3 fatty acids (ω-3 FAs) (37). Although endogenous β-arrestin2 could interact with NLRP3, this interaction only occurred in the presence of ω-3 FAs. When macrophages were primed with LPS together with ω-3 FAs, ω-3 FAs induced β-arrestin2/NLRP3 interaction and inhibited nigericin-induced NLRC4 inflammasome activation. However, when macrophages were stimulated with LPS and nigericin in the absence of ω-3 FAs, β-arrestin2 deficiency had no effect on NLRP3 inflammasome activation. Therefore, the suppressive effect of β-arrestin2 on NLRC4 inflammasome relied on the presence of ω-3 FAs. β-arrestin2 was also engaged in the downregulation of inflammasome by the N-methyl-D-aspartate (NMDA) receptor (38). In this work, aspartate, the stimulator of NMDA was found to downregulate transcrips of pro–IL-1β, NLRP3, and procaspase-1, which required NMDA and β-arrestin2. β-arrestin2 was also critical for dampening LPS and d-galactosamine–induced fulminant hepatitis in mice. As to the regulation of NF-κB, unlike β-arrestin2, whose effect is relatively clear, the exact role of β-arrestin1 in regulating NF-κB remains to be further investigation. At least our
results suggest β-arrestin1 has no impact on NF-κB signaling in periportal macrophages. Therefore, the role of β-arrestin1 compared with β-arrestin2 in the context of inflammasome activation could be divergent.

In conclusion, our work identifies that β-arrestin1 positively regulates NLRP3 and NLRC4 inflammasomes. Although it has been well established that β-arrestin1 has an essential role in autoimmune diseases, the molecular mechanism remains unclear. Our results provide a new mechanism by which β-arrestin1 regulates NLRP3 and NLRC4 inflammasomes activation, β-arrestin1 contributes to inflammation induced by different stimuli in vivo. These findings may provide therapeutic immune targets that would lead to extensive clinical applications and have potential protective against high morbidity or mortality associated with inflammasome-related diseases.

Acknowledgments
We thank R. Caspi (National Eye Institute, National Institutes of Health, Bethesda, MD) and Dangsheng Li (Shanghai Institutes for Biological Sciences, Shanghai, China) for helpful comments.

Disclosures
The authors have no financial conflicts of interest.

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