Fungal Zymosan and Mannan Activate the Cryopyrin Inflammasome

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Some fungal species are opportunistic pathogens that can cause infection in people with compromised immune systems. Activation of caspase-1 and the subsequent secretion of mature interleukin (IL)-1β is a major signaling pathway of the innate immune system, but how yeasts induce caspase-1 activation is unknown. We show here that stimulation of macrophages and dendritic cells with heat-killed Saccharomyces cerevisiae or the purified cell wall components zymosan and mannan induced caspase-1 activation and IL-1β secretion when combined with ATP. Macrophages deficient for the inflammasome adaptor ASC were defective in caspase-1 activation and IL-1β secretion, suggesting involvement of an ASC-dependent inflammasome. Indeed, caspase-1 activation was abrogated in macrophages lacking the NOD-like (NLR) protein Cryopyrin/Nalp3 and in wild type macrophages pretreated with the pannexin-1 inhibitor probenecid. IL-1β secretion further required the Toll-like receptor (TLR) adaptors MyD88 and TRIF, and partially relied on TLR2. We previously showed that bacterial molecules such as lipopolysaccharide (LPS) and peptidoglycan induce activation of caspase-7 through the Cryopyrin inflammasome. Similarly, Cryopyrin and ASC were required for activation of caspase-7 in macrophages stimulated with zymosan or mannan and ATP. These results demonstrate that the conserved fungal components zymosan and mannan require ASC and Cryopyrin for caspase-1 activation and IL-1β secretion and suggest an important role for the Cryopyrin inflammasome during fungal infections.

Pathogen recognition by the innate immune system relies on a limited number of fixed germline-encoded receptors, which have evolved to identify so-called pathogen-associated molecular patterns (PAMPs),2 conserved microbial structures not shared by the host and essential for their survival (1). Examples of PAMPs are LPS from Gram-negative bacteria, peptidoglycan (PGN) from Gram-positive bacteria, and zymosan and mannan from fungi. Several structurally and functionally diverse classes of pattern-recognition receptors (PRRs) exist that induce various host defense pathways, including the Toll-like receptors (TLRs) located in the plasma membrane and intracellular organelles and the more recently identified intracellular family of NOD-like receptors (NLRs) (2).

Previous studies have shown that gain-of-function mutations within the NLR protein Cryopyrin/NALP3 are associated with three autoinflammatory disorders characterized by skin rashes and prolonged episodes of fever in the absence of any apparent infection (3, 4). These hereditary periodic fever syndromes are Muckle-Wells syndrome (MWS), familial cold autoinflammatory syndrome (FACS), and neonatal-onset multisystem inflammatory disease (NOMID), and they are collectively referred to as the Cryopyrin/NALP3-associated periodic syndromes (CAPS). Subsequent studies revealed that the Cryopyrin/Nalp3 plays a crucial role in the assembly of a large (700 kDa) cytosolic protein complex termed the "inflammasome" (5–7). The bipartite adaptor protein ASC bridges the interaction between Cryopyrin/Nalp3 and caspase-1 in the inflammasome; thus allowing the recruitment and autoproteolytic activation of the cysteine protease (2). Activated caspase-1 subsequently mediates the maturation and secretion of the proinflammatory cytokines interleukin (IL)-1β and IL-18 (8–10). Interestingly, the Cryopyrin/Nalp3 inflammasome mediates caspase-1 activation in response to a variety of bacterial PAMPs such as LPS and PGN when combined with a second stimulus such as the P2X7 receptor ligand ATP (11–14). Cryopyrin/Nalp3 also mediates caspase-1 activation and IL-1β secretion in macrophages stimulated with viral RNA and ATP (15) or exposed to crystalline substances including uric acid, silica and asbestos (16–18). In contrast, the related NLR protein Ipaf is required for caspase-1 activation in macrophages infected with the intracellular pathogens Salmonella, Legionella, and Shigella (19–21).

Although the roles of specific inflammasomes in response to bacterial and viral PAMPs have been described, the inflamma- somes complexes that recognize fungal PAMPs to induce caspase-1 activation and IL-1β secretion are unknown. Here we show that heat-killed Saccharomyces cerevisiae and the purified cell wall components zymosan and mannan induced caspase-1 activation and IL-1β secretion from macrophages and dendritic cells upon co-stimulation with ATP. Macrophages deficient for the inflammasome adaptor ASC or the NLR protein Cryopyrin/

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5 The abbreviations used are: PAMP, pathogen-associated molecular pattern; BMDC, bone marrow-derived dendritic cell; BMDM, bone marrow-derived macrophage; IL, interleukin; LPS, lipopolysaccharide; NLR, NOD-like receptor; TNF, tumor necrosis factor; zymosan-Ox, NaClO-oxidized zymosan; PBS, phosphate-buffered saline; PGN, peptidoglycan; TLR, Toll-like receptor; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain.
Nalp3 were defective in zymosan- and mannan-induced caspase-1 activation and IL-1β secretion, whereas TNF-α secretion remained unaffected. Although macrophages lacking the TLR adaptors MyD88 or TRIF still activated caspase-1, zymosan- and mannan-induced secretion of IL-1β was significantly hampered. These results demonstrate that the conserved fungal cell wall components zymosan and mannan require ASC and Cryopyrin for caspase-1 activation and IL-1β secretion and suggest an important role for the Cryopyrin inflammasome during fungal infections.

**EXPERIMENTAL PROCEDURES**

**Mice, Macrophages, and Dendritic Cells—**Cryopyrin−/−, Ipaf−/−, ASC−/−, Nod2−/−, RIP2−/−, Myd88−/−, TLR2−/−, and TLR4−/− mice have been described (12, 22). Bone marrow was prepared from the leg bones of 8–20-week-old mice. The legs were dissected, and the bone marrow flushed out. Macrophages were differentiated from bone marrow cells cultured with Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 30% L929 supernatant containing 10% heat-inactivated fetal bovine serum (Invitrogen), 100 units/ml penicillin, and 100 mg/ml streptomycin at 37 °C in 5% CO₂, for 5 days. Bone marrow-derived dendritic cells (BMDCs) were cultured in RPMI supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), 100 units/ml penicillin, 100 mg/ml streptomycin, and 1000 units/ml GM-CSF at 37 °C in 5% CO₂ for 7–12 days. Bone marrow-derived macrophages and dendritic cells were then harvested with rubber scrapers and seeded.

**Microbial Ligands and Chemicals—**Zymosan, depleted zymosan, mannan, heat-killed *S. cerevisiae*, and ultrapure LPS were purchased from Invivogen and used at a concentration of 10 μg/ml. ATP was from Sigma and used at a final concentration of 3 mM. Probenecid was purchased from Sigma and used at 3 mM.

**Immunoblotting—**Cells were washed twice with phosphate-buffered saline and scraped in lysis buffer solution (150 mM NaCl, 10 mM Tris, pH 7.4, 5 mM EDTA, 1 mM EGTA, 0.1% Nonidet P-40) supplemented with a protease inhibitor mixture tablet (Roche Applied Science). Samples were clarified, denatured with SDS buffer, and boiled for 5 min. Proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were immunoblotted with primary antibodies and proteins detected with appropriate secondary anti-rabbit antibody conjugated to horseradish peroxidase followed by enhanced chemiluminescence. Rabbit anti-mouse caspase-1 was a generous gift from Dr. P. Vandenabeele (Ghent University, Belgium). Caspase-7 antibodies were from Cell Signaling Technologies.

**Measurements of Cytokines—**Mouse cytokines in culture supernatants were measured by enzyme-linked immunosorbent assay (R&D Systems) and Luminex assay (Bio-Rad). Data were analyzed with Student’s *t* test. *p* < 0.05 was considered statistically significant.

**RESULTS**

**Stimulation of Macrophages and Dendritic Cells with Heat-killed *S. cerevisiae* and ATP Triggers Caspase-1 Activation and...
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**IL-1β Secretion**—We have previously shown that heat-killed bacteria and ATP activated caspase-1 and secreted significant levels of mature IL-1β (12). To study whether heat-killed yeasts can also induce caspase-1 activation and IL-1β secretion, bone marrow derived dendritic cells (BMDCs) were stimulated with heat-killed *S. cerevisiae* for 4 h, the last 30 min of which in the presence of either vehicle control (PBS) or 5 mM ATP. As previously reported for heat-killed bacteria (12), heat-killed Saccharomyces treatment in the absence of ATP did not trigger caspase-1 activation (Fig. 1A) or IL-1β secretion (Fig. 1B) in BMDCs. Dendritic cells nevertheless responded to the heat-killed yeast preparation by inducing significant levels of the caspase-1-independent cytokines IL-6 (Fig. 1C) and TNF-α (Fig. 1D). However, caspase-1 activation and the secretion of significant levels of IL-1β were observed when BMDCs were stimulated with heat-killed yeast and ATP (Fig. 1, A and B). ATP co-stimulation was required for these responses because neither zymosan nor mannann triggered caspase-1 activation or IL-1β secretion alone (Fig. 2, A and B). However, ATP was not required for the secretion of TNF-α from zymosan- or mannan-pretreated macrophages (Fig. 2B). We sought to confirm these results with NaClO-oxidized zymosan (zymosan-Ox or depleted zymosan), which is devoid of bacterial TLR ligands. Lysates of BMDMs treated with zymosan-Ox and ATP contained levels of active caspase-1 comparable to those found in lysates of zymosan + ATP-treated macrophages (supplemental Fig. S2A). In addition, both zymosan and zymosan-Ox triggered potent IL-1β secretion when combined with ATP (supplemental Fig. S2B). These results were not restricted to macrophages because BMDCs also activated caspase-1 (Fig. 2C) and secreted IL-1β in response to zymosan + ATP or zymosan-Ox + ATP (Fig. 2D). In contrast, TNF-α secretion did not require ATP.
because it was dispensable for zymosan- and mannan-induced secretion of TNF-α (Fig. 3C); thus, suggesting a specific role for an ASC-containing inflammasome complex in zymosan- and mannan-induced activation of caspase-1. Similar to what was previously reported for heat-killed bacteria and bacterial PAMPs (2), the Nod1 and Nod2 adaptor RIP2 was not required for zymosan- or mannan-induced caspase-1 activation (Fig. 3A) or cytokine secretion (Fig. 3, B and C).

Cryopyrin Is Required for Zymosan- and Mannan-induced Caspase-1 Activation and IL-1β Secretion—Our results showed that zymosan- and mannan-induced caspase-1 activation required ASC (Fig. 3). This inflammasome adaptor protein is required in both the Cryopyrin and Ipaf inflammasomes. To determine the inflammasome mediating zymosan- and mannan-induced caspase-1 activation, BMDMs from mice deficient for Cryopyrin, Ipaf, or the related NLR family member Nod2 were stimulated with zymosan or mannan for 4 h, the last 30 min of which were in the presence of 5 mM ATP. Caspase-1 was activated in lysates of wild type macrophages stimulated with zymosan or mannan, but this activation was abolished in Cryopyrin-deficient macrophages (Fig. 4A). Similar results were obtained when the secretion of IL-1β in culture supernatants was analyzed (Fig. 4B). Cryopyrin was specifically required for caspase-1 activation and IL-1β secretion as secretion of TNF-α was unaffected in Cryopyrin-null macrophages (Fig. 4C). Unlike Cryopyrin, the NLRs Ipaf and Nod2 were not required for zymosan- or mannan-induced caspase-1 activation and IL-1β secretion (Fig. 4, A and B).

Role of TLRs and Pannexin-1 in Zymosan- and Mannan-induced Inflammasome Activation and IL-1β Secretion—TLRs are known to respond to fungal cell wall components (23, 24). To investigate the roles of TLRs in zymosan- and mannan-induced caspase-1 activation and IL-1β secretion, BMDMs deficient for TLR2, TLR4, or the TLR adaptors MyD88 and TRIF were stimulated with zymosan + ATP or mannan + ATP. Macrophages stimulated with LPS + ATP were included as controls. Both zymosan- and mannan-activated caspase-1 in TLR2−/− and TLR4−/− macrophages (Fig. 5A), although secretion of IL-1β (Fig. 5B) and TNF-α (Fig. 5C) partially depended

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**FIGURE 3. ASC is required for zymosan- and mannan-induced caspase-1 activation and IL-1β secretion.** A–C, BMDMs from wild type, ASC−/−, MyD88−/−, and RIP2−/− mice were left untreated (CTRL), or stimulated with zymosan, mannan, or LPS for 4 h, the last 30 min in the presence of 5 mM ATP. Cell extracts were immunoblotted for caspase-1 (A), and culture supernatants were analyzed for secreted IL-1β (B) and TNF-α (C). Black arrowheads indicate full-length caspase-1 (45 kDa), and white arrowheads mark the large subunits of activated caspase-1 (20 kDa). Cytokine data represent the mean ± S.E. of triplicate wells. All data are representative of three independent experiments.

co-stimulation (Fig. 2D). These results demonstrate that, comparable to bacterial cell wall components, the fungal cell wall components zymosan and mannan can induce caspase-1 activation in macrophages and dendritic cells.

The Inflammasome Adaptor ASC Is Required for Zymosan- and Mannan-induced Caspase-1 Activation and IL-1β Secretion—Macrophages deficient for the inflammasome adaptor protein ASC, or the Nod1 and Nod2 adaptor RIP2 were stimulated with zymosan + ATP or mannan + ATP to investigate the molecular mechanism by which zymosan and mannan induce caspase-1 activation and secretion of IL-1β. Macrophages stimulated with LPS + ATP were included as controls. As before, incubation of wild type macrophages with zymosan or mannan and ATP induced caspase-1 activation and secretion of elevated levels of IL-1β in the culture supernatants. Both responses were abolished in ASC-null macrophages (Fig. 3, A and B). The role of ASC was specific...
on TLR2, but not TLR4. In contrast, LPS-induced caspase-1 activation and secretion of IL-1β and TNF-α were completely dependent on TLR4, but not TLR2. We next addressed the roles of MyD88 and TRIF in zymosan- and mannan-induced caspase-1 activation and IL-1β secretion. Zymosan and mannan activated caspase-1 normally in macrophages lacking MyD88 or TRIF (Fig. 5A). However, zymosan-, mannan-, and LPS-induced IL-1β secretion all required MyD88 and partially relied on TRIF (Fig. 5B), likely for the NF-κB-dependent induction of pro-interleukin-1β. This notion was further supported by the observation that TNF-α secretion was also significantly diminished in MyD88−/− and TRIF−/− macrophages (Fig. 5C).

The hemichannel protein pannexin-1 has also been implicated in activation of the Cryopyrin inflammasome by bacterial PAMPs (12, 25, 26). We made use of the previously reported pannexin-1 inhibitor probenecid (27, 28) to assess its role in inflammasome activation by fungal PAMPs. As expected, probenecid prevented caspase-1 activation in LPS+ATP-stimulated macrophages (Fig. 5D). Interestingly, zymosan- and mannan-induced caspase-1 activation was also blocked by probenecid pretreatment (Fig. 5D), suggesting that pannexin-1 is required for activation of the Cryopyrin inflammasome by fungal PAMPs and ATP.

Cryopyrin and ASC Are Required for Zymosan- and Mannan-induced Caspase-7 Activation—We previously showed that bacterial molecules such as LPS and peptidoglycan induce activation of the executioner caspase-7 through the Cryopyrin inflammasome in macrophages co-treated with ATP (29), and this pathway was suggested to be important for LPS-induced endotoxic shock in vivo (30). To analyze whether fungal zymosan and mannan induced caspase-7 activation through the Cryopyrin inflammasome, lysates of wild type, Cryopyrin−/−, and ASC−/− macrophages were analyzed for caspase-7 processing. Similar to caspase-1 (Fig. 2A), processing of caspase-7 was observed only when zymosan- or mannan-prestimulated macrophages were treated with ATP for 30 min (Fig. 6). Caspase-7 processing was abolished in ASC−/− and Cryopyrin−/− macrophages, but not in Ipaf-deficient BMDMs (Fig. 6). These results confirm that zymosan- and mannan-induced caspase-7 activation requires components of the Cryopyrin inflammasome.

**DISCUSSION**

Some fungal species are opportunistic pathogens that can cause infection in people with compromised immune systems. Mannan and zymosan are major components of fungal cell walls and recognition of these fungal cell wall components by a variety of cell-bound and/or soluble receptors including the mannose receptor, TLRs, collectins, and C-type lectins is required for the mammalian immune response (31). However, the role of the intracellular receptors of the NLR family in the innate immune response to fungal mannan and zymosan has not been characterized.

![Figure 4](image-url)
We demonstrated that heat-killed *S. cerevisiae* and the purified cell wall components zymosan and mannan induced caspase-1 activation and IL-1β secretion from macrophages and dendritic cells when combined with ATP. Zymosan- and mannan-induced caspase-1 activation required the inflammasome components ASC and Cryopyrin, but not the Ipaf inflammasome. Although caspase-1 activation proceeded normally in MyD88−/− and TRIF−/− macrophages, zymosan- and mannan-induced generation of IL-1β required MyD88 and was partially dependent on TRIF and TLR2. In contrast, the NLR protein Nod2 and its adaptor protein RIP2 were dispensable for zymosan- and mannan-induced caspase-1 activation and IL-1β secretion.

Activation of the Cryopyrin inflammasome is believed to involve the generation of a secondary messenger, although the precise nature of this factor remains elusive. Several mechanisms have been suggested including K⁺ efflux and the generation of reactive oxygen species (ROS) (17, 18, 32, 33), lysosomal destabilization (34), and cytosolic entry of microbial PAMPs through pannexin-1 and destabilized phagosomes (35). Notably, recognition of live *Candida albicans* by the C-type lectin receptor Dectin-1 was recently proposed to induce activation of the Cryopyrin inflammasome through Syk-induced ROS production, independent of CARD9-mediated NF-κB activation (36). In addition, we demonstrated that zymosan+ATP- and mannan+ATP-induced activation of the Cryopyrin inflammasome was inhibited by the previously reported pannexin-1 inhibitor probenecid (27, 28). This result suggests a role for pannexin-1 in activation of the Cryopyrin inflammasome in cells treated with fungal PAMPs and ATP. Additional studies are required to delineate the signaling pathways that lead to activation of the Cryopyrin inflammasome.

Regardless, our results show that the NLR family member Cryopyrin/Naip3 should be added to the list of receptors able to sense, directly or indirectly, the presence of fungal cell wall components. In contrast to extracellular receptors such as the mannose receptor, TLRs, collectins, and C-type lectins (31), however, Cryopyrin/Naip3 is cytosolic and may therefore function as a sensor for the detection of the intracellular presence of fungal components. Hence, its role is specifically targeted to the assembly of caspase-1-activating inflammasome complexes to specifically trigger acti-
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FIGURE 6. Cryopyrin and ASC are required for zymosan- and mannan-induced caspase-7 activation. BMDMs were left untreated (CTRL) or stimulated with zymosan, mannan, or LPS for 4 h, the last 30 min in the presence of PBS (zymosan, mannan) or 5 mM ATP (zymosan+ATP, mannan+ATP, LPS+ATP). Cell extracts were immunoblotted for caspase-7. Black arrowheads indicate full-length caspase-7 (33 kDa), and white arrowheads mark the large subunits of activated caspase-7 (19 kDa). Data shown are representative of three separate experiments.

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REFERENCES
1. Janeway, C. A., Jr., and Medzhitov, R. (2002) Annu. Rev. Immunol. 20, 197–216
2. Kanneganti, T. D., Lamkanfi, M., and Núñez, G. (2007) Immunity 27, 549–559
3. Martinon, F., and Tschopp, J. (2004) Cell 117, 561–574
4. Pålsson-McDermott, E. M., and O’Neill, L. A. (2004) ImmunoL Immunol. 113, 153–162
5. Agostini, L., Martinon, F., Burns, K., McDermott, M. F., Hawkins, P. N., and Tschopp, J. (2004) Immunity 20, 319–325
6. Martinon, F., Burns, K., and Tschopp, J. (2002) Mol. Cell 10, 417–426
7. Lamkanfi, M., and Dixit, V. M. (2009) ImmunoL Rev. 227, 95–105
8. Ghayur, T., Banerjee, S., Hugunin, M., Butler, D., Herzog, L., Carter, A., Quintal, L., Sekut, L., Talanian, R., Paskind, M., Wong, W., Kamen, R., Tracey, D., and Allen, H. (1997) Nature 386, 619–623
9. Li, P., Allen, H., Banerjee, S., Franklin, S., Herzog, L., Johnston, C., Mc- Dowell, J., Paskind, M., Rodman, L., Selfed, J., Towne, H., Tracey, D., Wardwell, S., Wei, F. Y., Wong, W., Kamen, R., and Seshadri, T. (1995) Cell 80, 401–411
10. Kuida, K., Lippke, J. A., Ku, G., Harding, M. W., Livingston, D. J., Su, M. S., and Flavell, R. A. (1995) Science 267, 2000–2003
11. Mariathasan, S., Weiss, D. S., Newton, K., McBride, J., O’Rourke, K., Roose-Girma, M., Lee, W. P., Weinrauch, Y., Monack, D. M., and Dixit, V. M. (2006) Nature 440, 228–232
12. Kanneganti, T. D., Lamkanfi, M., Kim, Y. G., Chen, G., Park, J. H., Franchi, L., Vandenabeele, P., and Núñez, G. (2007) Immunity 26, 433–443
13. Sutterwala, F. S., Ogura, Y., Szczepanik, M., Lara-Tejero, M., Lichtenberger, G. S., Grant, E. P., Bertin, J., Coyle, A. J., Galán, J. E., Askenase, P. W., and Flavell, R. A. (2006) Immunity 24, 317–327
14. Kanneganti, T. D., Ozo¨ren, N., Body-Malapel, M., Amer, A., Park, J. H., Franchi, L., Whitfield, J., Barchet, W., Colonna, M., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., Akira, S., and Núñez, G. (2006) Nature 440, 233–236
15. Kanneganti, T. D., Body-Malapel, M., Amer, A., Park, J. H., Whitfield, J., Franchi, L., Taraporewala, Z. F., Miller, D., Patton, J. T., Inohara, N., and Núñez, G. (2006) J. Biol. Chem. 281, 36560–36568
16. Hornung, V., Bauernfeind, F., Halle, A., Samstad, E. O., Kono, H., Rock, K. L., Fitzgerald, K. A., and Latz, E. (2008) Nat. Immunol. 9, 847–856
17. Cassel, S. L., Eisenbarth, S. C., Iyer, S. S., Sadler, J. J., Colegio, O. R., Tephly, L. A., Carter, A. B., Rothman, P. B., Flavell, R. A., and Sutterwala, F. S. (2008) Proc. Natl. Acad. Sci. U.S.A. 105, 9035–9040
18. Dostert, C., Pétrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T., and Tschopp, J. (2008) Science 320, 674–677
19. Mariathasan, S., Newton, K., Monack, D. M., Vucic, D., French, D. M., Lee, W. P., Roose-Girma, M., Erickson, S., and Dixit, V. M. (2004) Nature 430, 213–218
20. Miao, E. A., Alpuche-Aranda, C. M., Dors, M., Clark, A. E., Bader, M. W., Miller, S. L., and Aderem, A. (2006) Nat. Immunol. 7, 569–575
21. Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T. D., Ozo¨ren, N., Jagirdar, R., Inohara, N., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., and Núñez, G. (2006) Nat. Immunol. 7, 576–582
22. Hoshino, K., Takeuchi, O., Kawai, T., Sanjo, H., Ogawa, T., Takeda, Y., Takeda, K., and Akira, S. (1999) J. Immunol. 162, 3749–3752
23. Underhill, D. M., Ozinsky, A., Hajjar, A. M., Stevens, A., Wilson, C. B., Bassetti, M., and Aderem, A. (1999) Nature 401, 818–815
24. Gantner, B. N., Simmons, R. M., Canavera, S. J., Akira, S., and Underhill, D. M. (2003) J. Exp. Med. 197, 1107–1117
25. Pelegrin, P., and Surprenant, A. (2006) EMBO J. 25, 5071–5082
26. Pelegrin, P., and Surprenant, A. (2007) J. Biol. Chem. 282, 2386–2394
27. Silverman, W. R., de Rivero Vaccari, I. P., Locovei, S., Qiu, F., Carlsson, S. K., Scemes, E., Keane, R. W., and Dahl, G. (2009) J. Biol. Chem. 284, 18143–18151
28. Silverman, W., Locovei, S., and Dahl, G. (2008) Am. J. Physiol. Cell Physiol. 295, C761–C767
29. Lamkanfi, M., Kanneganti, T. D., Van Damme, P., Vanden Berghe, T., Vanoverberghe, I., Vandekerckhove, J., Vandenabeele, P., Gevaert, K., and Núñez, G. (2008) Mol. Cell Proteomics 7, 2350–2363
30. Lamkanfi, M., Moreira, L. O., Makena, P., Spierings, D. C., Boyd, K., Murray, P. J., Green, D. R., and Kanneganti, T. D. (2009) Blood 113, 2742–2745
31. Brown, G. D. (2006) Nat. Rev. Immunol. 6, 33–43
32. Pétrilli, V., Papin, S., Dostert, C., Mayor, A., Martinon, F., and Tschopp, J. (2007) Cell Death Differ. 14, 1583–1589
33. Franchi, L., Kanneganti, T. D., Dubyak, G. R., and Núñez, G. (2007) J. Biol. Chem. 282, 18810–18818
34. Hornung, V., Bauernfeind, F., Halle, A., Samstad, E. O., Kono, H., Rock, K. L., Fitzgerald, K. A., and Latz, E. (2008) Nat. Immunol. 9, 847–856
35. Marina-Garcia, N., Franchi, L., Kim, Y. G., Miller, D., McDonald, C., Boons, G. J., and Núñez, G. (2008) J. Immunol. 180, 4050–4057
36. Gross, O., Poeck, H., Bscheider, M., Dostert, C., Hanneschläger, N., Endres, S., Hartmann, G., Tardivel, A., Schweighoffer, E., Tybulewicz, V., Mocsai, A., Tschopp, J., and Ruland, J. (2009) Nature 459, 433–436
37. Saijo, S., Fujikado, N., Furuta, T., Chung, S. H., Kotaki, H., Seki, K., Sudo, K., Akira, S., Adachi, Y., Ohno, N., Kinjo, T., Nakamura, K., Kawakami, K., and Iwakura, Y. (2007) Nat. Immunol. 8, 39–46
38. Taylor, P. R., Tsoni, S. V., Willment, J. A., Dennehy, K. M., Rosas, M., Findon, H., Haynes, K., Steele, C., Botto, M., Gordon, S., and Brown, G. D. (2007) Nat. Immunol. 8, 31–38