

Genetic and Molecular Basis of Inflammasome-mediated Disease*

Published, JBC Papers in Press, February 4, 2011, DOI 10.1074/jbc.R110.135491

Hal M. Hoffman^{†1} and Susannah D. Brydges⁵

From the [†]Department of Pediatrics and the ⁵Division of Rheumatology, Allergy, and Immunology, University of California at San Diego, La Jolla, California 92093

The central function of IL-1 β in fever regulation was recognized with its discovery >30 years ago (1). It was an attractive candidate as a master regulator in the pathogenesis of the hereditary fever syndromes; however, definitive proof was elusive. The mechanisms enabling maturation and release of active IL-1 β are complex, and it was difficult to detect the cytokine in patient serum. Validation of its central role in these rare auto-inflammatory conditions was achieved in the last decade with the convergence of three major developments in translational medicine research: 1) advances in the techniques used to identify human disease-causing genes, 2) innovations in genomic analysis, and 3) progress in effective targeted biologic therapeutics. Recent years have seen the explosion of exciting research elucidating the principal role of IL-1 β in fever disorders and expanding the autoinflammatory disease family beyond rare Mendelian syndromes to include common conditions such as gout and occupational lung disease (2). Concurrently, there have been significant advances in understanding the complexity of the innate immune system; the mechanisms regulating IL-1 β release; and the host response to pathogens, internal danger signals, and nonpathogenic inflammatory stimuli. With the advent of IL-1-targeted therapy, unprovoked inflammation due to excess IL-1 β can be managed and sequelae prevented. New research will continue to unravel the innate immune system's arsenal of sensory proteins and fully reveal the many pathways comprising IL-1 β function.

Genetics of Inherited Recurrent Fevers

The hereditary fever syndromes are a family of inflammatory diseases characterized by recurrent episodes of fever, joint symptoms, and rash. The clear Mendelian inheritance of these conditions combined with improved genetic mapping methods allowed the identification of the underlying genes, beginning with the *MEFV* gene for familial Mediterranean fever (FMF)² in 1997 following an international effort (3, 4). In the next 2 years, heterozygous mutations in *TNFRSF1a*, encoding the TNF

receptor, were linked to the autosomal dominant disease previously known as familial Hibernian fever, now called the TNF receptor-associated periodic syndrome (5). Homozygous mutations in the gene for mevalonate kinase were shown to cause hyperimmunoglobulinemia D with periodic fever syndrome (6, 7). Although these conditions are clinically related, a unifying underlying mechanism did not become clear until researchers mapped the genetic basis for two unusual fever syndromes, familial cold autoinflammatory syndrome (FCAS) and Muckle-Wells syndrome (MWS), in 2001 (8).

FCAS and MWS are autosomal dominant conditions; however, further similarities between the two were not immediately recognized, and initially, they were not classified as fever disorders. FCAS is characterized by day-long attacks of rash and joint pain precipitated by exposure to cold temperatures, which, although debilitating, do not generally lead to long-term morbidity (9). MWS consists of febrile episodes without the association with cold. In addition, MWS patients develop progressive hearing loss and end-stage renal disease due to amyloidosis (10). Patients with both syndromes exhibit a distinctive urticaria-like rash associated with fever, suggesting these two seemingly disparate conditions have a common root. Genetic linkage of both FCAS and MWS to chromosome 1q44 indeed indicated that these diseases are genetically related, and the final identification of heterozygous mutations in the *NLRP3* (*CIAS1*) gene in patients with both disorders was definitive proof (8, 11). As more patients were identified with phenotypes falling between FCAS and MWS, it became clear that these syndromes are part of a single disease continuum that was further enlarged by the genetic mapping of a third disorder known as neonatal onset multisystem inflammatory disease (NOMID). In addition to almost daily fevers and an urticaria-like rash, patients with NOMID exhibit a characteristic arthropathy and significant central nervous system involvement (12, 13). To reflect the cold-induced febrile episodes characteristic of FCAS, the *NLRP3* gene product was called cryopyrin ("ice fire"; also called NALP3 and NLRP3), and these diseases are collectively known as the cryopyrin-associated periodic syndromes (CAPS).

Genomics of Inflammation

The Human Genome Project provided an avalanche of raw genomic DNA and cDNA sequences that could be rapidly classified based on homology to proteins of known function. Recognition of conserved domains suggested a commonality of function among wide groups of seemingly distantly related proteins. Researchers studying inflammation and apoptosis mined databases using APAF-1, a scaffold protein that nucleates a caspase-activating complex called the apoptosome, and CIITA, the MHC Class II transactivator (14, 15). Dozens of novel intracellular protein candidates were identified, and this family became collectively known as NLR, for nucleotide-binding domain (NBD) and leucine-rich repeats (LRRs). NLR proteins have a central NBD (also known as NOD for nucleotide oligomerization domain) and, like the cell-surface Toll-like recep-

* Dr. Hoffman is a consultant for Regeneron and Novartis Pharmaceuticals Corp. Both make drugs used in cryopyrin-associated periodic syndromes. This minireview will be reprinted in the 2011 Minireview Compendium, which will be available in January, 2012.

¹ To whom correspondence should be addressed. E-mail: hahoffman@ucsd.edu.

² The abbreviations used are: FMF, familial Mediterranean fever; FCAS, familial cold autoinflammatory syndrome; MWS, Muckle-Wells syndrome; NOMID, neonatal onset multisystem inflammatory disease; CAPS, cryopyrin-associated periodic syndromes; NBD, nucleotide-binding domain; LRR, leucine-rich repeat; TLR, Toll-like receptor; CARD, caspase activation and recruitment domain; PYD, pyrin domain; ROS, reactive oxygen species.

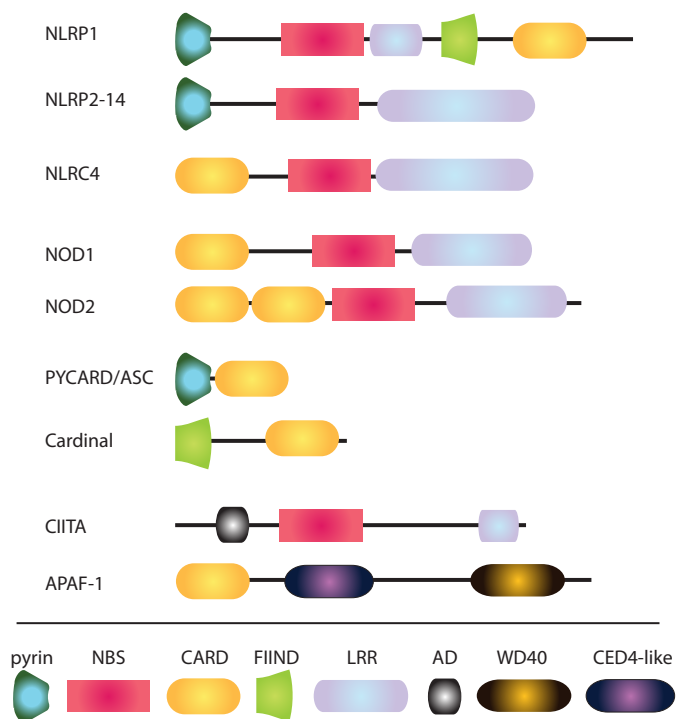


FIGURE 1. Protein domain structures of selected NLR family members, with APAF-1 and CIITA proteins as references. NBS, nucleotide-binding site; FIIND, domain with function to find; AD, activation domain.

tors (TLRs), contain variable numbers of C-terminal LRR motifs. To reflect their close analogy to TLRs, members of the NLR family are also referred to as NOD-like receptors (Fig. 1) (16).

The NLR family is further divided based on the variable N-terminal domain. NLRC proteins are an NLR subset with N-terminal caspase activation and recruitment domains (CARDs), whereas NLRP proteins, including the CAPS protein (cryopyrin/NLRP3), contain a novel death domain family motif known as the pyrin domain (PYD). The PYD was first identified in the protein pyrin, mutated in FMF, a provocative molecular connection between FMF and CAPS (3, 4). Two NLR proteins are known to contain both a PYD and a CARD, including a small adaptor protein known as PYCARD (ASC) and NLRP1, the first NLR protein demonstrated to form a multiprotein complex called the inflammasome (17).

NLR Inflammasomes: Structure and Function

The primary function of CARDs is the activation of caspases, but in addition, CARDs, as well as PYDs, NBDs, and LRRs, serve as protein-protein interaction domains in a scaffold for a complex similar to the apoptosome. However, instead of activating caspases mainly involved in apoptosis (such as caspase-3, -8, and -9), NLR-related inflammasome complexes activate caspase-1, also known as the IL-1-converting enzyme. These inflammasomes consist of NLR proteins (NLRP3, NLRP1, and NLRC4), adaptor proteins (PYCARD and possibly CARDINAL), chaperone proteins (heat shock protein 90 and SGT1), and caspases (1 and/or 5) (17–19). NLR proteins are predicted to form a hexameric or heptameric scaffold allowing adaptors or chaperones to interact based on inter- and intraprotein

domain-domain interactions (20). Multiple molecules of caspase-1 are recruited and concentrated, resulting in proximity-induced cleavage and activation. Mature caspase-1 then cleaves pro-IL-1 β and pro-IL-18. Active IL-1 β and IL-18 are secreted and initiate multiple inflammatory processes. The inflammasome therefore serves a crucial regulatory role by controlling the release of potent mediators (Fig. 2).

The NLR proteins are thought to function as innate immune sensors of intracellular pathogens that escape the extracellular or membrane-associated TLR armament. Inflammasomes have been implicated in the host response to various Gram-negative and Gram-positive bacteria, including pore-forming and toxin-producing organisms such as *Bacillus anthracis* (21), *Listeria monocytogenes* (22, 23), and *Staphylococcus aureus* (23–25), as well as *Neisseria gonorrhoeae* (26), virulence factor-producing *Shigella flexneri* (27, 28), and flagellated bacteria such as *Pseudomonas aeruginosa* (28, 29), *Salmonella typhimurium* (28), and *Legionella pneumophila* (30, 31). Studies also suggest that inflammasomes sense DNA and RNA viruses such as vaccinia and influenza (32–36), fungal products such as yeast zymosan and mannan (37) and *Candida albicans* hyphae (38–40), and hemozoin pigment produced by the malaria parasite (41–43) (see inflammasome activators in Table 1). Although there is some degree of specificity of inflammasomes for particular pathogens, clearly each NLR protein, particularly NLRP3, can recognize multiple pathogen-associated molecular pattern-activating signals. The mechanisms underlying this impressive list of microorganism targets are under active study (44).

Invading host cells to avoid the immune system is a well known tactic employed by many different microbes, thus necessitating the evolution of an arsenal of cytosolic sensory proteins such as the NLR family. Recently, however, a new class of innate immune activation signals derived from the host itself, so called “danger-associated molecular patterns,” have also been shown to activate the inflammasome. Extracellular ATP, perhaps released by dying cells, is a prerequisite to NLRP3-mediated IL-1 β release (23, 45, 46), demonstrating that bacterial products alone are not sufficient to trigger the inflammasome. Activation of the purine receptor (P2X7) and subsequent potassium efflux were shown to be necessary for this process (47). Pore-forming toxins mimic these effects, whereas incubating cells *ex vivo* in medium containing a high concentration of potassium prevents inflammasome activation (23, 48). Additional danger-associated molecular patterns include particles such as monosodium urate, the crystalline salt of endogenously produced uric acid, and calcium pyrophosphate, a metabolic byproduct (49). Components of the extracellular matrix such as hyaluronin and biglycan also signal via the inflammasome (50, 51). These host-derived activators may contribute to a sterile inflammatory response in the absence of microbial infection.

NLR Inflammasomes and Disease

Given the susceptibility of the inflammasome to minute amounts of activators encountered during normal cellular processes, it is perhaps not surprising that excess IL-1 production has been linked to several common conditions. Experimental data from recombinant mice deficient in specific inflam-

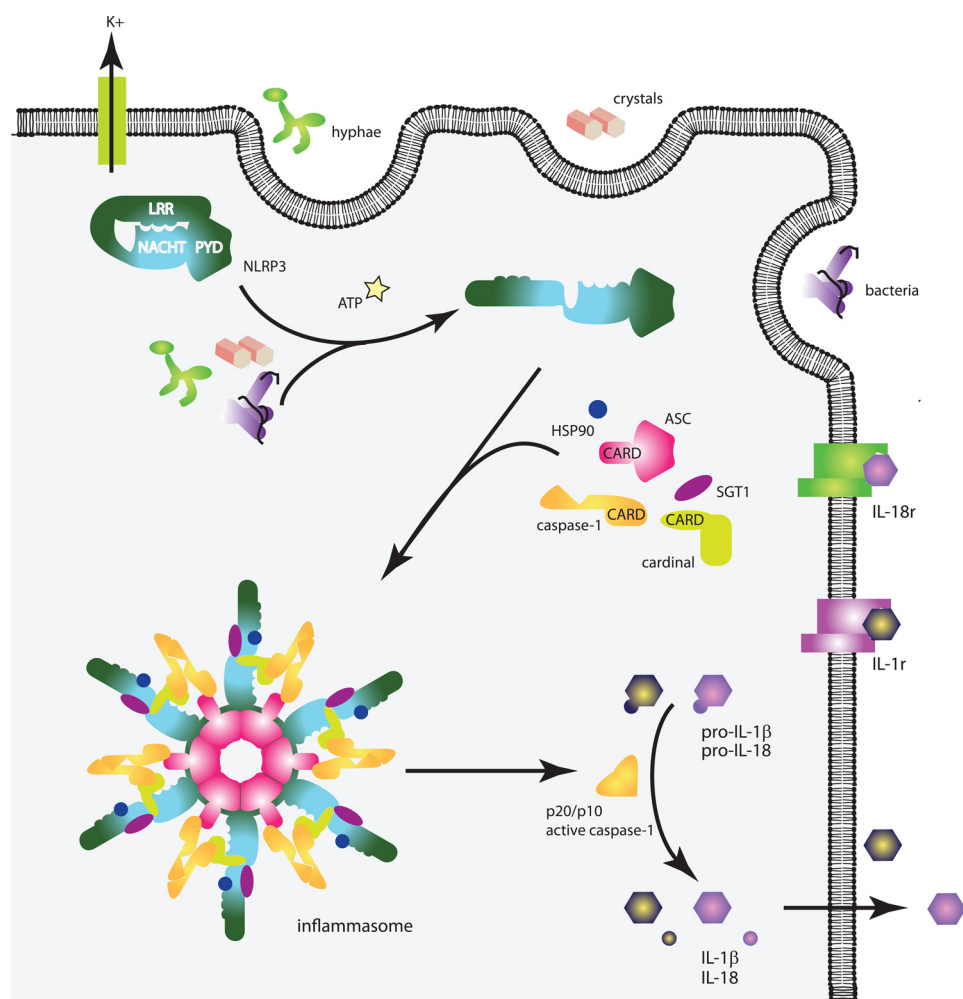


FIGURE 2. **Inflammasome structure and downstream events.** Shown is the unfolding of NLRP3 upon sensing various activators, followed by formation of a hexameric complex of NLRP3, CARDINAL, PYCARD, caspase-1, HSP90, and SGT1 proteins. Pro-IL-1 β and pro-IL-18 are cleaved, and the active cytokines exit the cell to bind to their respective receptors. IL-18r, IL-18 receptor.

masome components and from human cell lines with silenced specific gene expression support an autoinflammatory origin for the crystalline deposition disorders gout and pseudogout, triggered by inflammatory responses to monosodium urate and calcium pyrophosphate accumulation in joints (49). Occupational exposure to exogenously derived crystals such as silica and asbestos triggers lung inflammation and fibrosis in an inflammasome-dependent manner, and alum, a common vaccine adjuvant, also activates the inflammasome. Amyloid- β fibrils, thought to be pathogenic in Alzheimer disease, also trigger inflammasome activation (52–55). It is hypothesized that incomplete or “frustrated” phagocytosis of particulate antigens by macrophages leads to reactive oxygen species (ROS) formation and/or lysosomal destabilization, resulting in acidification and protease activation. It is unclear if these processes act directly on the inflammasome or through an intermediate interacting molecule, although evidence for the latter theory came from a recent study in which the thioredoxin-interacting protein TXNIP was shown to bind NLRP3 during ROS-mediated inflammasome activation (56). These studies prompted renewed interest in the use of existing and newer IL-1-targeted therapies in this expanded list of inflammasome- and IL-1 β -mediated diseases. It should be mentioned, however, that

although the mouse and *in vitro* cell line data are compelling, little evidence exists confirming similar pathways in humans or primary human cells.

Human genetic data are emerging that implicate components of the inflammasome in additional human diseases. Several studies show association of specific SNPs in or near inflammasome genes with allergic diseases such as atopic dermatitis (57), food-induced anaphylaxis, and aspirin-induced asthma (58). Data exist suggesting genetic association of *NLRP3* SNPs with Crohn disease (59, 60), susceptibility to candidiasis (61), and essential hypertension (62). Although intriguing, most of these associations have not been replicated in different populations, and identified SNPs have not been shown to impact the function of NLRP3 protein. Stronger genetic association has been described for specific SNPs in *NLRP1* in patients with vitiligo associated with multiple autoimmune diseases and other isolated autoimmune disorders; however, functional data are still limited, and it is unclear how the genetic variations influence the activation state of NLRP1 (63, 64).

CAPS and Inflammasome Activation

A wealth of genetic and functional data demonstrate direct involvement of the inflammasome in the pathogenesis of CAPS.

TABLE 1
Inflammasome activators

Unless noted otherwise, all activators pertain to the NLRP3 inflammasome.

Class/inflammasome activator	Clinical relevance	Mechanism	Refs.
G⁺			
<i>Listeria monocytogenes</i>	Food-borne disease	Listeriolysin O-induced K ⁺ efflux	22, 23
<i>Streptococcus pyogenes</i>	Skin infection, pharyngitis, rheumatic fever	Streptolysin O- and NFκB-dependent	74
<i>Staphylococcus aureus</i>	Skin infection, pneumonia, meningitis	Hemolysin-induced K ⁺ efflux	23–25
<i>Bacillus anthracis</i>	Anthrax	Lethal toxin NLRP1 inflammasome activation	21
G[−]			
<i>Legionella pneumophila</i>	Legionnaires' pneumonia	Flagellin-induced NLRC4 inflammasome activation	30, 31
<i>Shigella flexneri</i>	Food-borne disease	Type 3 secretion system protein-induced NLRC4 inflammasome activation	27, 28
<i>Salmonella typhimurium</i>	Food-borne disease	Flagellin- and type 3 secretion system protein-induced NLRC4 inflammasome activation	28
<i>Escherichia coli</i>	Food-borne disease, opportunistic and nosocomial disease, sepsis, meningitis	Flagellin- and type 3 secretion system protein-induced NLRC4 inflammasome activation	28
<i>Burkholderia pseudomallei</i>	Melioidosis	Type 3 secretion system protein-induced NLRC4 inflammasome activation	28
<i>Pseudomonas aeruginosa</i>	Opportunistic and nosocomial infections	Membrane disruption- and type 3 secretion system protein-induced NLRC4 inflammasome activation	28, 29
<i>Neisseria gonorrhoeae</i>	Sexually transmitted infection, pelvic inflammatory disease	Cathepsin B release from lysosomes	26
<i>Vibrio</i> spp.	Cholera, wound infections	Hemolysin and NFκB activation	75
<i>Porphyromonas gingivalis</i>	Periodontal disease	?	76
<i>Borrelia burgdorferi</i>	Lyme disease	?	77
<i>Mycobacterium tuberculosis</i>	Lung disease	Bacterial secretion system detection and membrane disruption	78
<i>Chlamydia trachomatis</i>	Sexually transmitted infection, pelvic inflammatory disease, eye infection	K ⁺ efflux, type 3 secretion system bacterial protein detection, ROS	79
Fungal products			
<i>Aspergillus fumigatus</i>	Opportunistic infections	K ⁺ efflux, ROS, Syk kinase activation	80
<i>Candida albicans</i>	Candidiasis, opportunistic infections	K ⁺ efflux, ROS, Syk kinase activation, hypha formation	38–40
Zymosan mannan		Pannexin-1 hemichannel protein activation	37
β-Glucan		K ⁺ efflux, ROS, Syk kinase activation	81
Viruses			
DNA/vaccinia	Vaccines	Viral endocytosis	33, 35
RNA/influenza	Influenza	RNA sensing, lysosomal acidification, ROS, RIG-1 RNA helicase activation	32, 34, 36
Parasites			
Hemozoin	Malaria	K ⁺ efflux, ROS, uric acid generation, endocytosis, Syk kinase activation	41–43
Particles			
Alum	Vaccines	K ⁺ efflux, lysosomal damage with cathepsin B release, endocytosis	54, 82–85
Asbestos	Occupational lung disease	K ⁺ efflux, ROS, endocytosis	53
Silica	Occupational lung disease	K ⁺ efflux, ROS, endocytosis, cathepsin B release, TNF	46, 52, 53, 83
Danger signals			
Amyloid β	Alzheimer disease	Lysosomal damage with cathepsin B release	55
Monosodium urate	Gout, lung fibrosis	Particle endocytosis	49, 86
Calcium pyrophosphate dehydrate	Pseudogout	Particle endocytosis	49
Biglycan	Tissue injury	ROS	50
Hyaluronin	Trauma	Endocytosis, lysosomal digestion of hyaluronin	51
Necrotic cells	Cancer therapy	Mitochondrial release of ATP from dying cells	87–89
ATP	Sterile inflammatory response	K ⁺ efflux	23, 45, 46
RNA		Double-stranded RNA sensing	90, 91
Other			
UVB light	Sun exposure	Increase in cytoplasmic Ca ²⁺	92
Implant metals	Orthopedic implants	Metal ion sensing, ROS	93

A total of 82 unique coding heterozygous mutations in *NLRP3* have been reported in patients with the three CAPS phenotypes that are not observed in matched population control samples (65). There is limited genotype-phenotype correlation, with certain mutations often associated with specific phenotypes; however, some mutations have been linked to more than one phenotype, and many CAPS patients fall between diagnoses

(20). Most mutations are missense changes in the NBD, with a few variants described in the LRR domain (66). Protein modeling of the NLRP3 NBD maps most disease variants on one surface of the hexameric protein complex, suggesting interference with inter- or intraprotein domain-domain interactions (Fig. 3). A common model proposes that CAPS mutations result in a gain-of-function phenotype leading to a constitutively active or

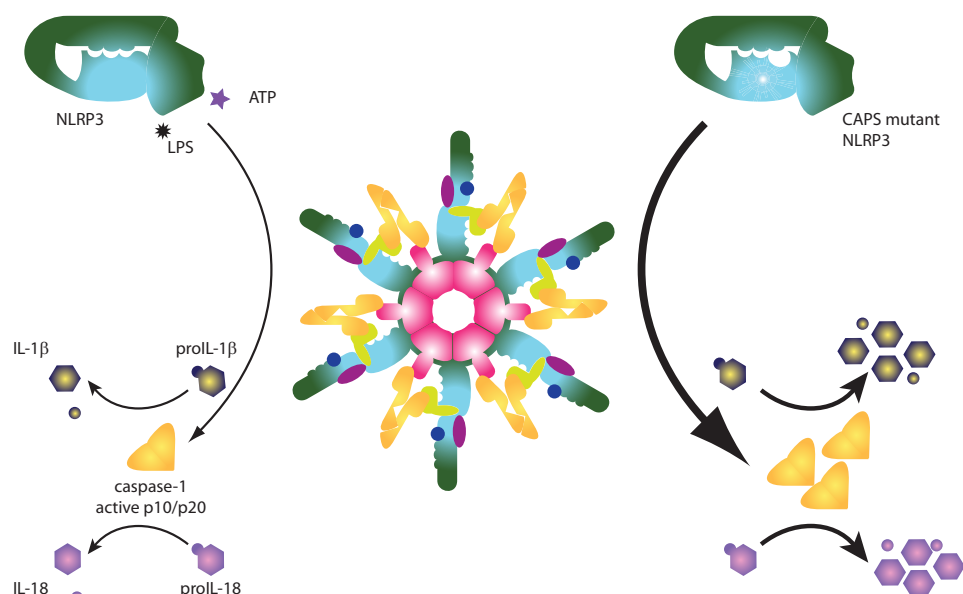


FIGURE 3. CAPS-associated mutation in the nucleotide-binding site of NLRP3 results in a labile open conformation that leads to excess caspase-1 activation and secretion of active IL-1 β and IL-18.

hyperactive inflammasome. During steady-state conditions, NLRP3 may assume an inactive or “closed” conformation with the LRR domain and NBD in a folded position. CAPS mutations may disrupt the binding between the two domains, resulting in a labile “open” conformation (20). This hyperactive state is dependent on nucleotide binding because disruption of the Walker A nucleotide-binding motifs has been shown to prevent *in vitro* IL-1 β secretion from recombinant mutant monocytic cells (45). Although the above theory is compelling, true experimental evidence is lacking, and the exact mechanism underlying the increased inflammasome activation in CAPS patients is still unclear.

Ex vivo studies of peripheral blood mononuclear cells from CAPS patients provide clear evidence of increased inflammasome activation. These cells demonstrate either constitutive IL-1 β release or increased production compared with control cells in response to various doses of a proinflammatory stimulus such as LPS (67). Low concentrations of crude LPS preparations stimulate maximal secretion of mature IL-1 β from CAPS cells, whereas control cells require much higher concentrations, likely because trace amounts of contaminating ATP are needed as a second signal for inflammasome activation. Indeed, CAPS peripheral blood mononuclear cells release IL-1 β when treated with pure preparations of LPS in the absence of ATP, whereas control cells have increased pro-IL-1 β transcription but require ATP for mature IL-1 β to be released. Pharmacologic inhibition of caspase-1 at least partially abrogates release from control and CAPS cells; thus, this process is inflammasome-dependent (68).

The development of recombinant mice with CAPS-associated mutations in the murine *Nlrp3* gene has provided further functional data to support the central role of the inflammasome in CAPS pathology. Mice expressing MWS-associated mutations devised by two independent groups show evidence of systemic inflammation, including neutrophilic infiltration and tissue cytokine expression involving the skin, lymphoid organs,

joints, muscle, and conjunctiva, that is consistent with the clinical picture of CAPS patients. In addition, significantly elevated serum levels of IL-1 β and IL-18 were observed in mutant MWS pups. Bone marrow-derived myeloid cells from these mice release increased IL-1 β in response to crude LPS and do not require ATP for IL-1 β release in response to pure LPS, similar to mononuclear cells from CAPS patients (69, 70). Interestingly, although complete phenotypic rescue occurred when the mutation was expressed on a PYCARD-null background, breeding onto an IL-1 receptor-null background only partially aborted the CAPS phenotype, and treating with high doses of a mouse form of the IL-1 Trap molecule (rilonacept) extended life by 3 days on average (69). Taken together, these findings indicate that murine CAPS is inflammasome-dependent, but other mediators besides IL-1 β play a significant role in pathology. Given patients' dramatic response to IL-1-targeted therapy, it is perhaps surprising that the mouse model had such significant disease in the absence of IL-1 signaling; however, some CAPS patients continue to have underlying inflammation despite adequate treatment, and the arthropathy characteristic of NOMID is completely nonresponsive. It is thus likely that cytokines besides IL-1 β , such as IL-18, play roles in both human and murine CAPS.

Cold-induced Inflammation in FCAS

Patients with FCAS, the mildest CAPS phenotype, consistently report development of fever, rash, and joint pain after a generalized cold exposure. Although patients show some evidence of chronic systemic inflammation, these cold-induced episodes are self-limited. Symptoms associated with these episodes can be consistently reproduced in a controlled environmental cold room challenge by exposing patients to 4 °C for ~30 min, followed by natural warming to room temperature. Within 1 h of challenge, a urticaria-like rash develops on exposed and unexposed areas, and fever and joint pain develop within the first 2–3 h. Symptoms peak at ~8 h, coinciding with

blood neutrophilia, and both resolve within 12–18 h. Serum IL-1 β levels are undetectable throughout the cold-induced episodes; however, serum IL-6, often used as a surrogate for IL-1 β , increases within 1 h and peaks at 4 h post-challenge, preceding the peak of symptoms. Tissue expression of IL-1 β and IL-6 is associated with neutrophilic infiltrate in the dermis of affected skin, but not in adjacent unaffected areas. All symptoms and laboratory features are completely abrogated by pretreatment with recombinant IL-1 receptor antagonist (anakinra), demonstrating the central role of IL-1 β in the pathogenesis of this disorder (71).

The mechanisms underlying this unusual inflammatory response to temperature are still unclear. It is known that cultured adherent monocytes from patients with FCAS, but not normal controls, release IL-1 β when exposed to temperatures similar to that of human skin (32 °C), but not at core body temperature (37 °C) (72). A similar cold-induced response is observed in bone marrow-derived dendritic cells isolated from mice with a common FCAS mutation, but not in the same cells generated from a mouse with a MWS mutation (69). This phenomenon suggests that specific inflammatory cells of myeloid origin have the ability to sense minor temperature changes. The transient receptor potential ion channels used by neurons to detect temperature change have also been identified on myeloid cells.³ Another known mechanism underlying responses to temperature involves heat and cold shock proteins, chaperones that interact with and stabilize protein complexes. It is known that HSP90 interacts with the inflammasome, but whether this protein plays a role in the response to temperature observed in FCAS remains to be seen (73). Finally, small changes in temperature can have significant effects on protein structure, so it is likely that specific FCAS-associated mutations in the gene encoding NLRP3 change the protein dynamics and affect the inter- and intraprotein domain-domain interactions. Further study is required to determine whether one or more of these mechanisms are involved in the FCAS response and whether this response plays a role in other temperature-related diseases.

Conclusion

The inflammasome is a master regulator of inflammation, translating a variety of microbe- and host-derived distress signals into IL-1 β activation. As new research elucidates the roles of NLR proteins and the autoinflammatory disease family further expands, it is likely that new clinical uses for IL-1 inhibitors will be recognized. As a clear example of the direct effects of inflammasome dysregulation, CAPS-related research will be invaluable to the study of more complex disorders and the application of targeted therapies.

REFERENCES

- Dinareello, C. A. (2009) *Annu. Rev. Immunol.* **27**, 519–550
- Masters, S. L., Simon, A., Aksentijevich, I., and Kastner, D. L. (2009) *Annu. Rev. Immunol.* **27**, 621–668
- Bernot, A., Clepet, C., Dasilva, C., Devaud, C., Petit, J. L., Caloustian, C., Cruaud, C., Samson, D., Pulcini, F., Weissenbach, J., Heilig, R., Notanicola, C., Domingo, C., Rozenbaum, M., Benchetrit, E., Topaloglu, R., Dewalle, M., Dross, C., Hadjari, P., Dupont, M., Demaille, J., Touitou, I., Smaoui, N., Nedelec, B., Méry, J. P., Chaabouni, H., Delpech, M., and Grateau, G. (1997) *Nat. Genet.* **17**, 25–31
- International FMF Consortium (1997) *Cell* **90**, 797–807
- McDermott, M. F., Aksentijevich, I., Galon, J., McDermott, E. M., Ogunkolade, B. W., Centola, M., Mansfield, E., Gadina, M., Karenko, L., Pettersson, T., McCarthy, J., Frucht, D. M., Aringer, M., Torosyan, Y., Teppo, A. M., Wilson, M., Karaarslan, H. M., Wan, Y., Todd, I., Wood, G., Schlimgen, R., Kumarajeewa, T. R., Cooper, S. M., Vella, J. P., Amos, C. I., Mulley, J., Quane, K. A., Molloy, M. G., Ranki, A., Powell, R. J., Hitman, G. A., O'Shea, J. J., and Kastner, D. L. (1999) *Cell* **97**, 133–144
- Drenth, T. P., Cuisset, L., Grateau, G., Vasseur, C., van de Velde-Visser, S. D., de Jong, J. G., Beckmann, J. S., van der Meer, J. W., and Delpech, M. (1999) *Nat. Genet.* **22**, 178–181
- Houten, S. M., Kuis, W., Duran, M., de Koning, T. J., van Royen-Kerkhof, A., Romeijn, G. J., Frenkel, J., Dorland, L., de Barse, M. M., Huijbers, W. A., Rijkers, G. T., Waterham, H. R., Wanders, R. J., and Poll-The, B. T. (1999) *Nat. Genet.* **22**, 175–177
- Hoffman, H. M., Mueller, J. L., Broide, D. H., Wanderer, A. A., and Kolodner, R. D. (2001) *Nat. Genet.* **29**, 301–305
- Hoffman, H. M., Wanderer, A. A., and Broide, D. H. (2001) *J. Allergy Clin. Immunol.* **108**, 615–620
- Shinkai, K., McCalmont, T. H., and Leslie, K. S. (2008) *Clin. Exp. Dermatol.* **33**, 1–9
- Hoffman, H. M., Wright, F. A., Broide, D. H., Wanderer, A. A., and Kolodner, R. D. (2000) *Am. J. Hum. Genet.* **66**, 1693–1698
- Aksentijevich, I., Nowak, M., Mallah, M., Chae, J. J., Watford, W. T., Hoffmann, S. R., Stein, L., Russo, R., Goldsmith, D., Dent, P., Rosenberg, H. F., Austin, F., Remmers, E. F., Balow, J. E., Jr., Rosenzweig, S., Komarow, H., Shoham, N. G., Wood, G., Jones, J., Mangra, N., Carrero, H., Adams, B. S., Moore, T. L., Schikler, K., Hoffman, H., Lovell, D. J., Lipnick, R., Barron, K., O'Shea, J. J., Kastner, D. L., and Goldbach-Mansky, R. (2002) *Arthritis Rheum.* **46**, 3340–3348
- Feldmann, J., Prieur, A. M., Quartier, P., Berquin, P., Certain, S., Cortis, E., Teillac-Hamel, D., Fischer, A., and de Saint Basile, G. (2002) *Am. J. Hum. Genet.* **71**, 198–203
- Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S. M., Ahmad, M., Alnemri, E. S., and Wang, X. (1997) *Cell* **91**, 479–489
- Steimle, V., Otten, L. A., Zufferey, M., and Mach, B. (1993) *Cell* **75**, 135–146
- Martinon, F., Mayor, A., and Tschopp, J. (2009) *Annu. Rev. Immunol.* **27**, 229–265
- Martinon, F., Burns, K., and Tschopp, J. (2002) *Mol. Cell* **10**, 417–426
- Agostini, L., Martinon, F., Burns, K., McDermott, M. F., Hawkins, P. N., and Tschopp, J. (2004) *Immunity* **20**, 319–325
- 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
- Aksentijevich, I., Putnam, C. D., Remmers, E. F., Mueller, J. L., Le, J., Kolodner, R. D., Moak, Z., Chuang, M., Austin, F., Goldbach-Mansky, R., Hoffman, H. M., and Kastner, D. L. (2007) *Arthritis Rheum.* **56**, 1273–1285
- Terra, J. K., Cote, C. K., France, B., Jenkins, A. L., Bozue, J. A., Welkos, S. L., LeVine, S. M., and Bradley, K. A. (2010) *J. Immunol.* **184**, 17–20
- Kim, S., Bauernfeind, F., Ablasser, A., Hartmann, G., Fitzgerald, K. A., Latz, E., and Hornung, V. (2010) *Eur. J. Immunol.* **40**, 1545–1551
- 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
- Craven, R. R., Gao, X., Allen, I. C., Gris, D., Bubeck Wardenburg, J., McElvania-Tekippe, E., Ting, J. P., and Duncan, J. A. (2009) *PLoS ONE* **4**, e7446
- Muñoz-Planillo, R., Franchi, L., Miller, L. S., and Núñez, G. (2009) *J. Immunol.* **183**, 3942–3948
- Duncan, J. A., Gao, X., Huang, M. T., O'Connor, B. P., Thomas, C. E., Willingham, S. B., Bergstralh, D. T., Jarvis, G. A., Sparling, P. F., and Ting, J. P. (2009) *J. Immunol.* **182**, 6460–6469
- Carneiro, L. A., Travassos, L. H., Soares, F., Tattoli, I., Magalhaes, J. G., Bozza, M. T., Plotkowski, M. C., Sansonetti, P. J., Molkentin, J. D., Philpott, D. J., and Girardin, S. E. (2009) *Cell Host Microbe* **5**, 123–136
- Miao, E. A., Mao, D. P., Yudkovsky, N., Bonneau, R., Lorang, C. G., War-

³ H. M. Hoffman and S. D. Brydges, unpublished data.

- ren, S. E., Leaf, I. A., and Aderem, A. (2010) *Proc. Natl. Acad. Sci. U.S.A.* **107**, 3076–3080
29. Sutterwala, F. S., Mijares, L. A., Li, L., Ogura, Y., Kazmierczak, B. I., and Flavell, R. A. (2007) *J. Exp. Med.* **204**, 3235–3245
 30. Akhter, A., Gavrilin, M. A., Frantz, L., Washington, S., Ditty, C., Limoli, D., Day, C., Sarkar, A., Newland, C., Butchar, J., Marsh, C. B., Wewers, M. D., Tridandapani, S., Kanneganti, T. D., and Amer, A. O. (2009) *PLoS Pathog.* **5**, e1000361
 31. Silveira, T. N., and Zamboni, D. S. (2010) *Infect. Immun.* **78**, 1403–1413
 32. Allen, I. C., Scull, M. A., Moore, C. B., Holl, E. K., McElvania-TeKippe, E., Taxman, D. J., Guthrie, E. H., Pickles, R. J., and Ting, J. P. (2009) *Immunity* **30**, 556–565
 33. Delaloye, J., Roger, T., Steiner-Tardivel, Q. G., Le Roy, D., Knaup Reymond, M., Akira, S., Petrilli, V., Gomez, C. E., Perdiguero, B., Tschopp, J., Pantaleo, G., Esteban, M., and Calandra, T. (2009) *PLoS Pathog.* **5**, e1000480
 34. Poeck, H., Bscheider, M., Gross, O., Finger, K., Roth, S., Rebsamen, M., Hanneschläger, N., Schlee, M., Rothenfusser, S., Barchet, W., Kato, H., Akira, S., Inoue, S., Endres, S., Peschel, C., Hartmann, G., Hornung, V., and Ruland, J. (2010) *Nat. Immunol.* **11**, 63–69
 35. Rathinam, V. A., Jiang, Z., Waggoner, S. N., Sharma, S., Cole, L. E., Waggoner, L., Vanaja, S. K., Monks, B. G., Ganesan, S., Latz, E., Hornung, V., Vogel, S. N., Szomolanyi-Tsuda, E., and Fitzgerald, K. A. (2010) *Nat. Immunol.* **11**, 395–402
 36. Thomas, P. G., Dash, P., Aldridge, J. R., Jr., Ellebedy, A. H., Reynolds, C., Funk, A. J., Martin, W. J., Lamkanfi, M., Webby, R. J., Boyd, K. L., Doherty, P. C., and Kanneganti, T. D. (2009) *Immunity* **30**, 566–575
 37. Lamkanfi, M., Malireddi, R. K., and Kanneganti, T. D. (2009) *J. Biol. Chem.* **284**, 20574–20581
 38. 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
 39. Hise, A. G., Tomalka, J., Ganesan, S., Patel, K., Hall, B. A., Brown, G. D., and Fitzgerald, K. A. (2009) *Cell Host Microbe* **5**, 487–497
 40. Joly, S., Ma, N., Sadler, J. J., Soll, D. R., Cassel, S. L., and Sutterwala, F. S. (2009) *J. Immunol.* **183**, 3578–3581
 41. Dostert, C., Guarda, G., Romero, J. F., Menu, P., Gross, O., Tardivel, A., Suva, M. L., Stehle, J. C., Kopf, M., Stamenkovic, I., Corradin, G., and Tschopp, J. (2009) *PLoS ONE* **4**, e6510
 42. Griffith, J. W., Sun, T., McIntosh, M. T., and Bucala, R. (2009) *J. Immunol.* **183**, 5208–5220
 43. Shio, M. T., Tiemi Shio, M., Eisenbarth, S. C., Savaria, M., Vinet, A. F., Bellemare, M. J., Harder, K. W., Sutterwala, F. S., Bohle, D. S., Descoteaux, A., Flavell, R. A., and Olivier, M. (2009) *PLoS Pathog.* **5**, e1000559
 44. Latz, E. (2010) *Curr. Opin Immunol.* **22**, 28–33
 45. Duncan, J. A., Bergstralh, D. T., Wang, Y., Willingham, S. B., Ye, Z., Zimmermann, A. G., and Ting, J. P. (2007) *Proc. Natl. Acad. Sci. U.S.A.* **104**, 8041–8046
 46. Franchi, L., Eigenbrod, T., and Núñez, G. (2009) *J. Immunol.* **183**, 792–796
 47. Ferrari, D., Chiozzi, P., Falzoni, S., Dal Susino, M., Melchiorri, L., Baricordi, O. R., and Di Virgilio, F. (1997) *J. Immunol.* **159**, 1451–1458
 48. Pétrilli, V., Papin, S., Dostert, C., Mayor, A., Martinon, F., and Tschopp, J. (2007) *Cell Death Differ.* **14**, 1583–1589
 49. Martinon, F., Pétrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006) *Nature* **440**, 237–241
 50. Babelova, A., Moreth, K., Tsalastra-Greul, W., Zeng-Brouwers, J., Eickelberg, O., Young, M. F., Bruckner, P., Pfeilschifter, J., Schaefer, R. M., Gröne, H. J., and Schaefer, L. (2009) *J. Biol. Chem.* **284**, 24035–24048
 51. Yamasaki, K., Muto, J., Taylor, K. R., Cogen, A. L., Audish, D., Bertin, J., Grant, E. P., Coyle, A. J., Misaghi, A., Hoffman, H. M., and Gallo, R. L. (2009) *J. Biol. Chem.* **284**, 12762–12771
 52. 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
 53. Dostert, C., Pétrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T., and Tschopp, J. (2008) *Science* **320**, 674–677
 54. Eisenbarth, S. C., Colegio, O. R., O'Connor, W., Sutterwala, F. S., and Flavell, R. A. (2008) *Nature* **453**, 1122–1126
 55. Halle, A., Hornung, V., Petzold, G. C., Stewart, C. R., Monks, B. G., Reinheckel, T., Fitzgerald, K. A., Latz, E., Moore, K. J., and Golenbock, D. T. (2008) *Nat. Immunol.* **9**, 857–865
 56. Zhou, R., Tardivel, A., Thorens, B., Choi, I., and Tschopp, J. (2010) *Nat. Immunol.* **11**, 136–140
 57. Macaluso, F., Nothnagel, M., Parwez, Q., Petrasch-Parwez, E., Bechara, F. G., Epplen, J. T., and Hoffjan, S. (2007) *Exp. Dermatol.* **16**, 692–698
 58. Hitomi, Y., Ebisawa, M., Tomikawa, M., Imai, T., Komata, T., Hirota, T., Harada, M., Sakashita, M., Suzuki, Y., Shimojo, N., Kohno, Y., Fujita, K., Miyatake, A., Doi, S., Enomoto, T., Taniguchi, M., Higashi, N., Nakamura, Y., and Tamari, M. (2009) *J. Allergy Clin. Immunol.* **124**, 779–785.e6
 59. Schoultz, I., Verma, D., Halfvarsson, J., Törkvist, L., Fredrikson, M., Sjöqvist, U., Lördal, M., Tysk, C., Lerm, M., Söderkvist, P., and Söderholm, J. D. (2009) *Am. J. Gastroenterol.* **104**, 1180–1188
 60. Villani, A. C., Lemire, M., Fortin, G., Louis, E., Silverberg, M. S., Collette, C., Baba, N., Libiouille, C., Belaiche, J., Bitton, A., Gaudet, D., Cohen, A., Langelier, D., Fortin, P. R., Wither, J. E., Sarfati, M., Rutgeerts, P., Rioux, J. D., Vermeire, S., Hudson, T. J., and Franchimont, D. (2009) *Nat. Genet.* **41**, 71–76
 61. Lev-Sagie, A., Prus, D., Linhares, I. M., Lavy, Y., Ledger, W. J., and Witkin, S. S. (2009) *Am. J. Obstet. Gynecol.* **200**, 301.e1–303.e6
 62. Omi, T., Kumada, M., Kamesaki, T., Okuda, H., Munkhtulga, L., Yanagisawa, Y., Utsumi, N., Gotoh, T., Hata, A., Soma, M., Umemura, S., Ogihara, T., Takahashi, N., Tabara, Y., Shimada, K., Mano, H., Kajii, E., Miki, T., and Iwamoto, S. (2006) *Eur. J. Hum. Genet.* **14**, 1295–1305
 63. Jin, Y., Birlea, S. A., Fain, P. R., and Spritz, R. A. (2007) *J. Invest. Dermatol.* **127**, 2558–2562
 64. Jin, Y., Mailloux, C. M., Gowan, K., Riccardi, S. L., LaBerge, G., Bennett, D. C., Fain, P. R., and Spritz, R. A. (2007) *N. Engl. J. Med.* **356**, 1216–1225
 65. Toutou, I., Lesage, S., McDermott, M., Cuisset, L., Hoffman, H., Dode, C., Shoham, N., Aganna, E., Hugot, J. P., Wise, C., Waterham, H., Pugnere, D., Demaille, J., and Sarrauste de Menthier, C. (2004) *Hum. Mutat.* **24**, 194–198
 66. Frenkel, J., van Kempen, M. J., Kuis, W., and van Amstel, H. K. (2004) *Arthritis Rheum.* **50**, 2719–2720
 67. Stack, J. H., Beaumont, K., Larsen, P. D., Straley, K. S., Henkel, G. W., Randle, J. C., and Hoffman, H. M. (2005) *J. Immunol.* **175**, 2630–2634
 68. Gattorno, M., Tassi, S., Carta, S., Delfino, L., Ferlito, F., Pelagatti, M. A., D'Osualdo, A., Buoncompagni, A., Alpigiani, M. G., Alessio, M., Martini, A., and Rubartelli, A. (2007) *Arthritis Rheum.* **56**, 3138–3148
 69. Brydges, S. D., Mueller, J. L., McGeough, M. D., Pena, C. A., Misaghi, A., Gandhi, C., Putnam, C. D., Boyle, D. L., Firestein, G. S., Horner, A. A., Soroosh, P., Watford, W. T., O'Shea, J. J., Kastner, D. L., and Hoffman, H. M. (2009) *Immunity* **30**, 875–887
 70. Meng, G., Zhang, F., Fuss, I., Kitani, A., and Strober, W. (2009) *Immunity* **30**, 860–874
 71. Hoffman, H. M., Rosengren, S., Boyle, D. L., Cho, J. Y., Nayar, J., Mueller, J. L., Anderson, J. P., Wanderer, A. A., and Firestein, G. S. (2004) *Lancet* **364**, 1779–1785
 72. Rosengren, S., Mueller, J. L., Anderson, J. P., Niehaus, B. L., Misaghi, A., Anderson, S., Boyle, D. L., and Hoffman, H. M. (2007) *J. Allergy Clin. Immunol.* **119**, 991–996
 73. Mayor, A., Martinon, F., De Smedt, T., Pétrilli, V., and Tschopp, J. (2007) *Nat. Immunol.* **8**, 497–503
 74. Harder, J., Franchi, L., Muñoz-Planillo, R., Park, J. H., Reimer, T., and Núñez, G. (2009) *J. Immunol.* **183**, 5823–5829
 75. Toma, C., Higa, N., Koizumi, Y., Nakasone, N., Ogura, Y., McCoy, A. J., Franchi, L., Uematsu, S., Sagara, J., Taniguchi, S., Tsutsui, H., Akira, S., Tschopp, J., Nunez, G., and Suzuki, T. (2010) *J. Immunol.* **184**, 5287–5297
 76. Yilmaz, O., Sater, A. A., Yao, L., Koutouzis, T., Pettengill, M., and Ojcius, D. M. (2010) *Cell. Microbiol.* **12**, 188–198
 77. Cruz, A. R., Moore, M. W., La Vake, C. J., Eggers, C. H., Salazar, J. C., and Radolf, J. D. (2008) *Infect. Immun.* **76**, 56–70
 78. Mishra, B. B., Moura-Alves, P., Sonawane, A., Hacohen, N., Griffiths, G., Moita, L. F., and Anes, E. (2010) *Cell. Microbiol.* **12**, 1046–1063
 79. Abdul-Sater, A. A., Koo, E., Häcker, G., and Ojcius, D. M. (2009) *J. Biol. Chem.* **284**, 26789–26796
 80. Said-Sadier, N., Padilla, E., Langsley, G., and Ojcius, D. M. (2010) *PLoS*

- ONE 5, e10008
81. Kumar, H., Kumagai, Y., Tsuchida, T., Koenig, P. A., Satoh, T., Guo, Z., Jang, M. H., Saitoh, T., Akira, S., and Kawai, T. (2009) *J. Immunol.* **183**, 8061–8067
 82. Franchi, L., and Núñez, G. (2008) *Eur. J. Immunol.* **38**, 2085–2089
 83. 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
 84. Kool, M., Pétrilli, V., De Smedt, T., Rolaz, A., Hammad, H., van Nimwegen, M., Bergen, I. M., Castillo, R., Lambrecht, B. N., and Tschopp, J. (2008) *J. Immunol.* **181**, 3755–3759
 85. Li, H., Willingham, S. B., Ting, J. P., and Re, F. (2008) *J. Immunol.* **181**, 17–21
 86. Gasse, P., Riteau, N., Charron, S., Girre, S., Fick, L., Pétrilli, V., Tschopp, J., Lagente, V., Quesniaux, V. F., Ryffel, B., and Couillin, I. (2009) *Am. J. Respir. Crit. Care Med.* **179**, 903–913
 87. Ghiringhelli, F., Apetoh, L., Tesniere, A., Aymeric, L., Ma, Y., Ortiz, C., Vermaelen, K., Panaretakis, T., Mignot, G., Ullrich, E., Perfettini, J. L., Schlemmer, F., Tasdemir, E., Uhl, M., Génin, P., Civas, A., Ryffel, B., Kanellopoulos, J., Tschopp, J., André, F., Lidereau, R., McLaughlin, N. M., Haynes, N. M., Smyth, M. J., Kroemer, G., and Zitvogel, L. (2009) *Nat. Med.* **15**, 1170–1178
 88. Iyer, S. S., Pulsikens, W. P., Sadler, J. J., Butter, L. M., Teske, G. J., Ulland, T. K., Eisenbarth, S. C., Florquin, S., Flavell, R. A., Leemans, J. C., and Sutterwala, F. S. (2009) *Proc. Natl. Acad. Sci. U.S.A.* **106**, 20388–20393
 89. Li, H., Ambade, A., and Re, F. (2009) *J. Immunol.* **183**, 1528–1532
 90. 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
 91. Kanneganti, T. D., Ozören, 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
 92. Feldmeyer, L., Keller, M., Niklaus, G., Hohl, D., Werner, S., and Beer, H. D. (2007) *Curr. Biol.* **17**, 1140–1145
 93. Caicedo, M. S., Desai, R., McAllister, K., Reddy, A., Jacobs, J. J., and Hallab, N. J. (2009) *J. Orthop. Res.* **27**, 847–854