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REVIEW

Targeting the NLRP3 inflammasome in chronic inflammatory diseases: current perspectives

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Abstract: The inflammasome is a molecular platform formed by activation of an innate immune pattern recognition receptor seed, such as NLRP3. Once activated, NLRP3 recruits the adapter ASC (apoptosis-related speck-like protein containing a caspase recruitment domain), which in turn recruits procaspase-1. Procaspase-1 autocatalyzes its cleavage and activation, resulting in maturation of the precursor forms of interleukin (IL)-1β and IL-18 into active proinflammatory cytokines and initiation of pyroptotic cell death. The NLRP3 inflammasome has been implicated in the pathogenesis of a wide variety of diseases, including genetically inherited autoinflammatory conditions as well as chronic diseases in which NLRP3 is abnormally activated. The NLRP3 inflammasome has been linked to diseases such as Alzheimer's disease, atherosclerosis, metabolic syndrome, and age-related macular degeneration. In this review, we describe the NLRP3 inflammasome complex and its activation in disease, and detail the current therapies that modulate either the NLRP3 inflammasome complex itself or the two cytokines it is responsible for activating, ie, IL-1β and IL-18.

Keywords: NLRP3, interleukin-1, interleukin-18, caspase-1, therapeutics, inflammasome

Introduction

The inflammasome was first described in 2002 by Tschopp as a large molecular platform that triggers the activation of inflammatory caspases and processing of prointerleukin-1β.[1] It is now known that these inflammasomes act as signaling platforms that can respond to a plethora of microbial products, as well as endogenous host products associated with cellular stress and damage, and that they play a vital role in innate immunity. These multiprotein complexes contain a pattern recognition receptor seed, typically a nucleotide-binding oligomerization domain-like receptor (NLR) or an absent in melanoma 2 (AIM2)-like receptor, which upon activation by pathogen-associated molecular patterns or damage-associated molecular patterns, oligomerize and recruit the adaptor protein ASC (apoptosis-related speck-like protein containing a caspase recruitment domain [CARD]) and the cysteine protease procaspase-1, leading to the autocatalysis and activation of caspase-1. Active caspase-1 ultimately cleaves the precursor proinflammatory cytokines pro-IL-1β and pro-IL-18 into their mature secreted forms. Activated caspase-1 also triggers a form of cell death known as pyroptosis.[2]

The NLRs are an evolutionarily conserved family of cytosolic receptors with a tripartite structure that share a common central nucleotide-binding and oligomerization (NACHT) domain that is usually flanked by a C-terminal leucine-rich repeat (LRR) domain and a N-terminal effector pyrin domain (PYD) or CARD. The LRR

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domain is important for ligand sensing and autoregulation, the NACHT domain is vital for forming oligomeric structures, while the N-terminal effector domain mediates downstream signal transduction.[3] So far, 23 human and 34 mouse NLR genes have been identified in humans, and have phylogenetically been grouped into three subfamilies, ie, the nucleotide-binding oligomerization domain-containing proteins (*NOD1-5* and *CIITA*), the NACHT, LRR, and PYD domain-containing proteins (*NLRP1–14*), and the IPAF family (*IPAF/NLRC4* and *NAIPs*). The AIM2-like receptor family consists of four members in humans (IFI16, IFIX, MNDA, and AIM2) that contain an N-terminal PYD domain and one or two C-terminal hematopoietic interferon-inducible nuclear antigens with 200 amino acid repeat (HIN-200) DNA-binding domains.[4]

To date, five different inflammasomes have been clearly identified, ie, NLRP1, NLRP2, NLRP3, AIM2, and IPAF/NLRC4 (see Table 1).[3,5] Each inflammasome is activated in response to different stimuli. The NLRP1 inflammasome is activated by directly binding to bacterial ligands, such as anthrax lethal toxin and muramyl dipeptide.[6] The IPAF inflammasome senses Gram-negative bacteria possessing type III or IV secretion systems, such as *Salmonella typhimurium* and *Shigella flexneri*.[7,8] The HIN-200 domain of AIM2 recognizes foreign cytoplasmic double-stranded DNA, and the AIM2 inflammasome, consisting of AIM2, ASC, and caspase-1, is activated in response to both viruses and bacteria.[9] Other members of the NLR family, namely NLRP6, NLRP7 and NLRP12, have also been described to form inflammasomes with ASC leading to caspase-1 activation, although their specific ligands are still unknown.[10,11] Herein we focus on the NLRP3 inflammasome as the best characterized inflammasome, which has been shown to have major implications in the development of chronic diseases.

Table 1 Inflammasome-forming NLRs in humans and mice

Human NLRs	**Reference**
NLRP1	1,6
NLRP2	5
NLRP3	106
NLRP6	97,107
NLRP7	108
NLRP12	109
NAIP/NLRC4	7,8,110
Mouse NLRs	**Reference**
Nalp1b	105
Nlrp3	17
Nlrp6	97
Nlrp12	109
Nlrc4	7,8

Abbreviation: NLRs, nucleotide-binding oligomerization domain-like receptors.

NLRP3 inflammasome

The NLRP3 inflammasome is by far the most studied inflammasome. It recognizes a wide variety of microbes, including *Staphylococcus aureus*, *Escherichia coli*,[12] *Influenza A* virus,[13,14] and *Candida albicans*,[15] as well as host-derived factors associated with damage, such as extracellular adenosine triphosphate (ATP),[16] uric acid crystals,[17] β-amyloid plaques,[18] islet amyloid polypeptide,[19] and drusen (Table 2).[20] NLRP3 is expressed in the cytosol of monocytes, dendritic cells, neutrophils, lymphocytes, epithelial cells, and osteoblasts.[21] The inflammasome platform is composed of NLRP3, ASC, and procaspase-1. The LRR domain of NLRP3 is likely to be involved in sensing the danger signal, which results in oligomerization of NLRP3 monomers via their NACHT domains. The PYD effector domain of NLRP3 then interacts with the PYD domain of ASC. ASC serves as an adaptor protein, recruiting procaspase-1 via its CARD domain. The NACHT domain of NLRP3 has also been shown to bind to and hydrolyze ATP. This ATPase activity of NLRP3 has been shown to be essential for NLRP3-mediated functions, and mutations in the NACHT domain reduce inflammasome oligomerization, caspase-1 activation, IL-1β and IL-18 secretion, and pyroptosis. Additionally, disruption of the NACHT domain in diseases associated with NLRP3 gain-of-function mutations greatly reduces the disease phenotype.[22]

Activation of the NLRP3 inflammasome is tightly regulated and requires two independent signals (Figure 1). Basal expression of the precursor proform of IL-1β as well as the NLRP3 protein is barely detectable, so a priming step or "signal 1" is required to drive their transcription.[23] This is usually facilitated through activation of Toll-like receptors or NOD2 to initiate the nuclear factor kappa B (NF-κB) signaling pathway. Pattern recognition receptor

Table 2 Sterile inflammatory NLRP3 activators and associated diseases

NLRP3 activator	**Disease**
Monosodium urate crystals	Gout
Beta-amyloid plaque	Alzheimer's Disease
Drusen deposits, lipofuscin, AluRNA	AMD
Free fatty acids	Type 2 diabetes
Islet amyloid polypeptide	Type 2 diabetes
Oxidized low-density lipoprotein	Atherosclerosis
Cholesterol crystals	Atherosclerosis

Abbreviation: AMD, age-related macular degeneration.

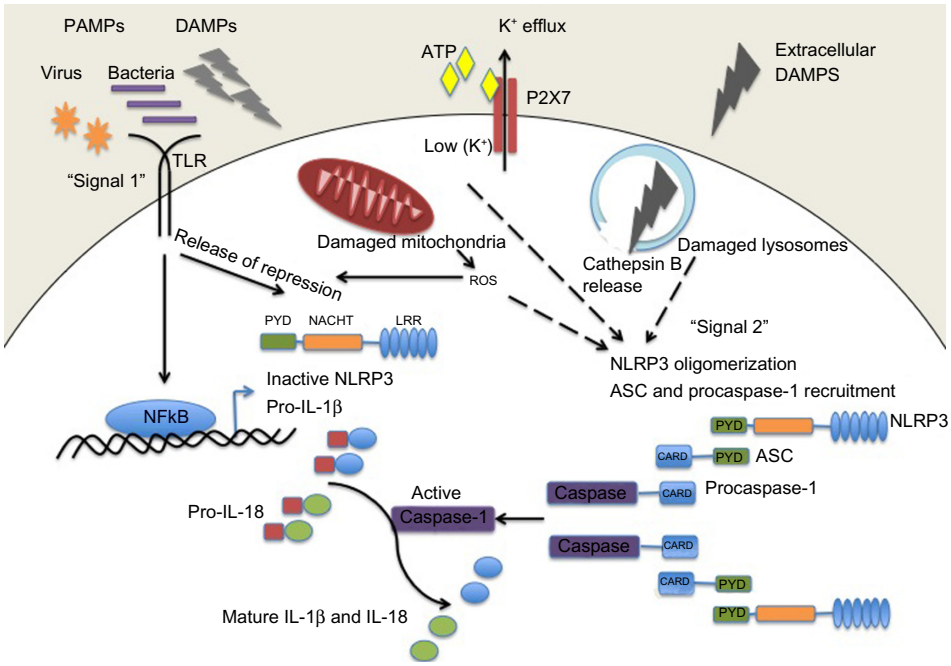

Figure 1 NLRP3 inflammasome activation. Pathogenic PAMPs from virus or bacteria, or sterile DAMPs "prime" the inflammasome by activating a TLR inducing NFκB activation and the expression of NLRP3 and pro-IL-1β (signal 1). NLRP3 oligomerizes and recruits ASC and procaspase-1 in response to an activation signal (signal 2). NLRP3 can be activated in response to potassium ion efflux through the ATP-gated P2X7 channel, in response to reactive oxygen species released from damaged mitochondria, or in response to cathepsin B release from damaged lysosomes. Once activated the NLRP3 inflammasome causes the activation of caspase-1 which cleaves the precursor proforms of IL-1β and IL-18 into their mature forms.

Abbreviations: ASC, apoptosis-related speck-like protein containing a caspase recruitment domain; ATP, adenosine triphosphate; CARD, caspase recruitment domain; DAMPS, danger or damage associated molecular patterns; IL, interleukin; LRR, leucine-rich repeat; NACHT, central nucleotide-binding and oligomerization; NF-κB, nuclear factor kappa B; P2X7, P2X purinergic receptor 7; PAMPS, pathogen associated molecular patterns; PYD, pyrin domain; ROS, reactive oxygen species; TLR, Toll-like receptor.

signaling through Toll-like receptor-4 and MyD88 was also shown to rapidly prime free cytosolic NLRP3 maintained in an inactive ubiquitinated state, through deubiquitination of its LRR domain, which was dependent on generation of reactive oxygen species (ROS).[24] A role for deubiquitination in ASC oligomerization has also been described.[25] Once primed, subsequent activation of the NLRP3 inflammasome, referred to as "signal 2", results in its oligomerization and inflammasome assembly, eventually leading to caspase-1-dependent cleavage and secretion of pro-IL-1β and pro-IL-18.

NLRP3 inflammasome activation models

The NLRP3 inflammasome is activated by a range of molecules that differ both chemically and structurally, and the exact mechanisms of NLRP3 activation remain unclear. It seems unlikely that NLRP3 binds directly with any of its agonists, and indeed no studies have yet shown a direct interaction between LRR and any NLRP3 activators. Three different models of NLRP3 activation have often been suggested, ie, potassium efflux, phagolysosomal destabilization, and mitochondrial ROS generation (Figure 1). The first model proposes that activation of P2X purinergic receptor 7 (P2X7) ATP-gated ion channels by extracellular ATP results in potassium ion efflux through the cell membrane. Supporting this model, it has been shown that potassium ion concentrations below 90 mM result in spontaneous NLRP3 inflammasome assembly, whereas this is prevented at higher potassium ion concentrations.[26] ATP-induced activation of P2X7 receptors has also been linked to a hemichannel protein, pannexin-1. Pannexin-1 associates with P2X7 post-ATP stimulation and stimulates the formation of a large nonselective pore.[27,28] These pores would allow NLRP3 agonists to gain access into the cytosol and may activate a host cellular factor that activates NLRP3. The second model proposes a mechanism by which large particulate matter, such as alum, silica, and amyloid-β, may activate the NLRP3 inflammasome. Engulfment of these particles by phagocytes and fusion with lysosomes leads to swelling and damage of the newly formed phagolysosome. As a result, lysosomal matter, such as the lysosomal protease cathepsin B, is released into the cytosol. Cathepsin B is thought to be sensed by NLRP3, leading to its activation.[29] The third model predicts involvement of ROS, as many NLRP3 agonists have been shown to promote ROS formation. Production of ROS likely activates

the inflammasome via an intermediate, and it has previously been demonstrated that an increase in ROS leads to activation of thioredoxin-interacting protein, which then binds to NLRP3, resulting in inflammasome activation.[30] The source of ROS is believed to be the mitochondria,[31] and mitochondrial DNA release by damaged mitochondria has also been shown to activate the NLRP3 inflammasome.[32] However, a recent study has suggested that ROS are only required for priming the inflammasome and not for its activation.[23]

More recently, osmotic pressure and cell volume regulation have also been implicated in activation of NLRP3.[33] In this study, macrophages subjected to hypotonic solutions underwent cellular swelling with a decrease in intracellular potassium and chloride ion concentrations. This led to a regulatory volume decrease response controlled by the transient receptor potential cation channels transient receptor potential (TRP) and transient receptor potential cation channel subfamily M member 7 (TRPM7) and the subsequent mobilization of intracellular calcium. Both low intracellular calcium concentrations and a regulatory volume decrease response controlled by TRP channels were shown to be required for activation of caspase-1. Ultimately, it is unlikely that the above mechanisms are independent, and integration of these models may explain how NLRP3 becomes activated by a diverse range of agonists.

NLRP3 inflammasome in disease

Inflammasome dysregulation has been implicated in almost every age-related condition that afflicts man. Age is one of the greatest "risk" factors for some of the most common chronic inflammatory diseases, eg, atherosclerosis, metabolic syndrome, age-related macular degeneration (AMD), Alzheimer's disease, and gout. Indeed, the term "inflamm-aging" was coined by Franceschi et al to illustrate the concept that the aging process is associated with a progressive increase in proinflammatory status due to the body's inability to manage the continuous antigenic load and stress.[35] The inflammatory response observed during aging is different from the more traditional acute inflammation; it is a low-grade, controlled, chronic systemic inflammatory state and most often asymptomatic. The NLRP3 inflammasome has been shown to be triggered by many of the metabolic byproducts associated with these diseases, eg, monosodium urate crystals associated with gout,[17] Beta-amyloid plaques associated with Alzheimer's disease,[18] drusen deposits associated with AMD,[20] islet amyloid polypeptide involved in type 2 diabetes,[19] and cholesterol crystals involved in atherosclerosis (Table 2).[36]

IL-1β is known to play a causative role in many of the NLRP3 inflammasome-related diseases. For example, in mice suffering from atherosclerosis, IL-1β plays a role in stimulating lipid plaque development and destabilizing plaques in mice, and cholesterol crystals which accumulate in this disease can activate the NLRP3 inflammasome causing increased IL-1β production. Moreover, when hypercholesterolemic mice that are deficient in the low-density lipoprotein receptor are reconstituted with bone marrow from mice deficient in various inflammasome components (NLRP3, ASC, IL-1β), fewer atherosclerotic plaques are seen to develop than in those reconstituted with wild-type bone marrow.[37] Similarly, in patients suffering from gout, monosodium urate crystals have been shown to activate the NLRP3 inflammasome, and the resulting IL-1β released can trigger the recruitment of neutrophils and peritoneal inflammation. Interestingly, in a mouse model of wet AMD, production of IL-18 has been shown to have a protective role against progression to the end-stage neovascular form of the disease.[20] IL-1 is an important mediator of cartilage destruction in rheumatic diseases, and evidence of NLRP3 inflammasome activation in rheumatoid arthritis (RA) exists due to the analysis of genetic variants in various population cohorts.[38] However, until recently, the mechanism underlying the role of the NLRP3 inflammasome in the pathogenesis of RA was unknown. Myeloid cell-specific deletion of the RA susceptibility gene A20/Tnfaip3 in mice (A20[myel-KO] mice) triggers a spontaneous erosive polyarthritis that resembles RA in patients. Deletion of *Nlrp3*, *caspase-1*, and *interleukin-1 receptor* markedly protects against RA-associated inflammation and cartilage destruction in A20 (myel-KO) mice,[39] indicating that increased NLRP3 inflammasome activation contributes to the pathology of RA in vivo, at least in mice.

Inflammatory bowel disease (IBD) has also been linked with uncontrolled inflammasome activation. A complex balance exists between the microbiota residing in the human gut and the systemic immune system of the host. Alterations in the composition of the microbiota, the gut epithelial barrier function, or inappropriate immune responses and genetic predispositions can lead to IBD or intestinal cancer. A possible role of the inflammasome in IBD arose from early studies demonstrating the association of variants in NOD2 (a pattern recognition receptor with structural similarities to NLRP3) with susceptibility to Crohn's disease.[40,41] Other NLR family members, as well as caspase-1, IL-1β, IL-18, and IL-18R, have been associated with IBD.[42] However, there has been debate as to whether

inflammasome activation, and in particular production of IL-18, is beneficial or harmful in IBD.[43–45] Recent studies have demonstrated that the location of IL-18 activation may be crucial in this regard: IL-18 activation in intestinal epithelial cells may initiate a compensatory proliferative response and preservation of the intestinal barrier, whereas activation in the lamina propria may result in a harmful proinflammatory phenotype.[46]

The inflammasome has also been implicated in various cancers, including gastrointestinal cancers, melanoma, breast cancer, and hepatitis C virus-associated hepatocellular carcinoma, with both procarcinogenic and anticarcinogenic functions attributed.[47] Anticarcinogenic functions originate from the ability of the inflammasome to promote pyroptotic cell death pathways. This enables maintenance of epithelial barrier function and wound repair processes as seen for IBD, and by stimulating anticancer immune responses during chemotherapy.[48–50] Increased cytotoxicity of natural killer cells has been observed after IL-18 stimulation, which also contributes to the inflammasome's antitumor effects.[51–53] In contrast, the inflammasome and its products, IL-1β and IL-18, have also been seen to suppress natural killer-mediated and T-cell-mediated antimetastatic actions and immunosurveillance.[54] Trophic factors of IL-1β have also been implicated, whereby IL-1β derived from myeloma was shown to induce production of IL-6 in stromal cells, which can act as a growth factor for these cells.[55]

The above conditions can loosely be grouped together and considered NLRP3 "activation" disorders. NLRP3 "genetic" disorders also exist and were previously known as periodic fever syndromes, but are now collectively referred to as cryopyrin-associated periodic syndromes (CAPS).[56] CAPS have a distinct disease phenotype compared with the more chronic NLRP3 activation disorders and result in autoinflammatory diseases. CAPS disorders are due to gain-of-function mutations in the NLRP3 gene, which leads to increased secretion of IL-1β and associated manifestation of disease (Table 3). The clinical severity of CAPS varies, with familial cold autoinflammatory syndrome (FCAS) being the mildest form, neonatal-onset multisystem inflammatory disease, or chronic infantile neurological cutaneous and articular syndrome being of intermediate severity, and Muckle-Wells syndrome (MWS) being the most severe.[57] Unlike autoimmune diseases, these autoinflammatory syndromes do not present typical characteristics of adaptive immunity, such as high-titer autoantibodies and antigen-specific T-cells.[58] Instead, they are disorders of the innate immune system and are characterized by recurrent fever, urticaria-like rashes, and systemic inflammation.[59]

Remarkable clinical outcomes have been observed for these disorders by treatment with the IL-1-blocking agents, rilonacept (Arcalyst®; Regeneron Pharmaceuticals, Tarrytown, NY, USA), canakinumab (Ilaris®; Novartis, Basel, Switzerland), and anakinra (Kineret®; Swedish Orphan Biovitrum Ltd, Waltham, MA, USA).[60–63] Given the evidence that NLRP3 is involved in a diverse range of diseases, there is considerable interest in the discovery of effective therapeutics that selectively inhibit the NLRP3 inflammasome pathway. We discuss the current therapies available and those under clinical trial and in the early stages of characterization in detail below.

Inhibiting effectors of the inflammasome

It is logical to conceive that inhibiting the final products of the inflammasome, ie, mature IL-1β and IL-18, could hold therapeutic potential. Indeed, both of these cytokines are potent immune modulators, so their expression at high levels in distinct cellular beds could have disastrous consequences if left uncontrolled. For example, prolonged expression of these cytokines in the brain calvarium could add to the malignancies observed in Alzheimer's disease and other forms of dementia.[64]

Strategies for IL-1β blockade

Inhibition of IL-1β is currently a therapeutic strategy for multiple diseases. Antibodies directed against IL-1β have therapeutic potential for the treatment of MWS and FCAS, with multiple clinical trials having been undertaken in these patients using a fully humanized IL-1β antibody, canakinumab. Canakinumab is a human immunoglobulin Gκ monoclonal antibody that specifically targets IL-1β.[65] It has been developed by Novartis as a treatment for a range of immune disorders, but predominant among these are FCAS and MWS. A fusion protein developed by Regeneron Pharmaceuticals has also been investigated in the treatment of CAPS. Their drug (rilonacept) comprises the extracellular portion of the IL-1 receptor and the Fc domain of human immunoglobulin G1; however, as rilonacept can bind to both IL-1α and IL-1β, it may have therapeutic potential over and above that of canakinumab, and its uses could extend to conditions such as diabetes and gout.[66] In addition, the IL-1 receptor antagonist, anakinra, has found utility in numerous conditions, including RA. Anakinra is a recombinant human IL-1 receptor antagonist protein that is now a commonly used subcutaneously administered drug for the treatment of RA and has been shown to prevent the cartilage degeneration associated with this condition.[67] A vaccine designed to inhibit IL-1β is

Table 3 Some common NLRP3 mutations identified with associated diseases

Gene mutation	Gene	Disease	Reference
CYS148TYR rs180177487	NLRP3	CINCA	111
ILE172THR rs180177449	NLRP3	CINCA	112
VAL198MET rs121908147	NLRP3	FCAS	113,114
CYS259TRP rs180177475	NLRP3	FCAS	115
ARG260TRP rs121908150	NLRP3	MWS/FCAS	116
VAL262ALA rs104895392	NLRP3	CINCA	115
LEU264PHE rs180177476	NLRP3	CINCA	115
LEU264HIS rs180177436	NLRP3	CINCA	115
ASP303ASN rs121908153	NLRP3	CINCA/MWS	116,117
GLU304LYS rs180177484	NLRP3	CINCA	118
LEU305PRO rs180177431	NLRP3	FCAS	59
GLN306LYS rs180177432	NLRP3	CINCA	117
PHE309SER rs121908154	NLRP3	CINCA	117
GLU311LYS rs180177470	NLRP3	MWS	119
SER331ARG rs180177451	NLRP3	CINCA	120
THR348MET rs151344629	NLRP3	MWS	116
ALA352VAL rs121908149	NLRP3	MWS	114
LEU353PRO rs28937896	NLRP3	FCAS	121
HIS358ARG rs180177434	NLRP3	CINCA	117
ALA374ASP rs180177437	NLRP3	CINCA	115
MET406ILE rs180177486	NLRP3	CINCA	118
HIS412PRO rs180177488	NLRP3	MWS	122
TYR859CYS rs180177452	NLRP3	CINCA	133
THR436ALA rs180177483	NLRP3	FCAS	123
THR436ASN rs180177433	NLRP3	CINCA	117
ALA439THR rs180177430	NLRP3	MWS	116
ALA439VAL rs121908146	NLRP3	FCAS	114,124
PHE443LEU rs180177477	NLRP3	CINCA	115
ASN477LYS rs180177485	NLRP3	CINCA	118
ILE480PHE rs180177482	NLRP3	CINCA/MWS	125
ARG488LYS rs145268073	NLRP3	FCAS	126
PHE523CYS rs180177478	NLRP3	MWS	115
PHE523LEU rs180177439	NLRP3	CINCA	115
GLU525LYS rs180177458	NLRP3	FCAS	127
TYR563ASN rs180177479	NLRP3	FCAS	115
GLU567LYS rs104895389	NLRP3	MWS	128
GLY569ARG rs121908151	NLRP3	MWS	116
TYR570CYS rs180177438	NLRP3	CINCA	129
PHE573SER rs121908152	NLRP3	CINCA	117
GLU627GLY rs121908148	NLRP3	FCAS	114,130
MET659LYS rs180177457	NLRP3	FCAS	127
MET662THR rs180177435	NLRP3	CINCA	117
GLU688LYS rs104895414	NLRP3	CINCA	118
GLY755ARG rs180177469	NLRP3	CINCA	131
GLU755ALA rs180177473	NLRP3	CINCA	115
GLY809SER rs141389711	NLRP3	CINCA	132

Abbreviations: CINCA, chronic infantile neurological cutaneous and articular syndrome; FCAS, familial cold autoinflammatory syndrome; MWS, Muckle-Wells syndrome.

also currently in development by Cytos Biopharmaceuticals (Durham, NC, USA) for the treatment of diabetes and RA;[67] however, data pertaining to its safety/efficacy are not currently available. An indepth review of strategies for IL-1 blockade is discussed in Dinarello et al.[68]

Strategies for IL-18 blockade

With regard to inhibiting IL-18 directly, numerous clinical trials have been undertaken using an antibody directed against IL-18 (termed GSK1070806) which is a clinical asset of GlaxoSmithKline (Brentford, UK). This molecule has been used for conditions as diverse as B-cell non-Hodgkin's lymphoma and IBD. Data pertaining to the safety/efficacy of using systemic IL-18 antibodies are

currently limited to information obtained from http://www.clinicaltrials.gov, but it is prudent to consider that numerous trials have been listed as "completed", which would suggest that systemic inhibition of IL-18 is at the very least tolerated in human subjects (http://www.clinicaltrials.gov).

Targeting inflammasome constituents

Currently, the best therapies for treating conditions associated with an overtly active inflammasome target the main pyogenic product of inflammasome activity, ie, IL-1β. However, alternative modes of inhibiting inflammasome activation are being actively pursued (Figure 2, Table 4). Manipulating the inflammasome therapeutically prior to activation would have the benefit of blocking the release of IL-1β and IL-18, rather than "sponging" them up, in conditions where these cytokines are deleterious. In some cases, it has been discovered that previously established pharmacological agents, in use for treatment of a variety of diseases, in fact target components of the inflammasome. The development of small-molecule inhibitors that directly target the building blocks of the inflammasome would benefit from being cheaper and less invasive modes of therapy compared with the larger biologics currently in use and aimed at sequestering IL-1β after it has been secreted.

Indirect NLRP3 inhibitors

Glyburide is a sulfonylurea drug commonly used in the treatment of type 2 diabetes. It acts by inhibiting ATP-sensitive potassium channels in pancreatic β-cells.[69] However, in addition to inhibiting IL-1β in response to ATP, glyburide can also inhibit production of IL-1β in response to multiple other NLRP3 stimuli. In fact, the inhibitory activity of glyburide is not dependent on potassium channel blockade or on the ATPase activity of NLRP3 (required for caspase-1 activity);[22] however, it does appear to be a specific inhibitor of NLRP3 because it does not block the production of IL-1β following NLRC4 or NLRP1 activation. Furthermore, although effective at inhibiting release of IL-1β upon activation of NLRP3 by pathogen-associated molecular patterns, damage-associated molecular patterns, and crystals, glyburide does not inhibit temperature-induced IL-1β release from monocytes of FCAS patients, suggesting that while it acts downstream of the P2X7 receptor, it acts upstream of NLRP3 activation.[70] These properties suggest that glyburide may be a treatment option for conditions where excessive NLRP3 activation is considered a major contributor to pathology. However, while glyburide has clear NLRP3 inhibitory activity in vitro, it requires very high doses in vivo, which are associated with hypoglycemia, limiting

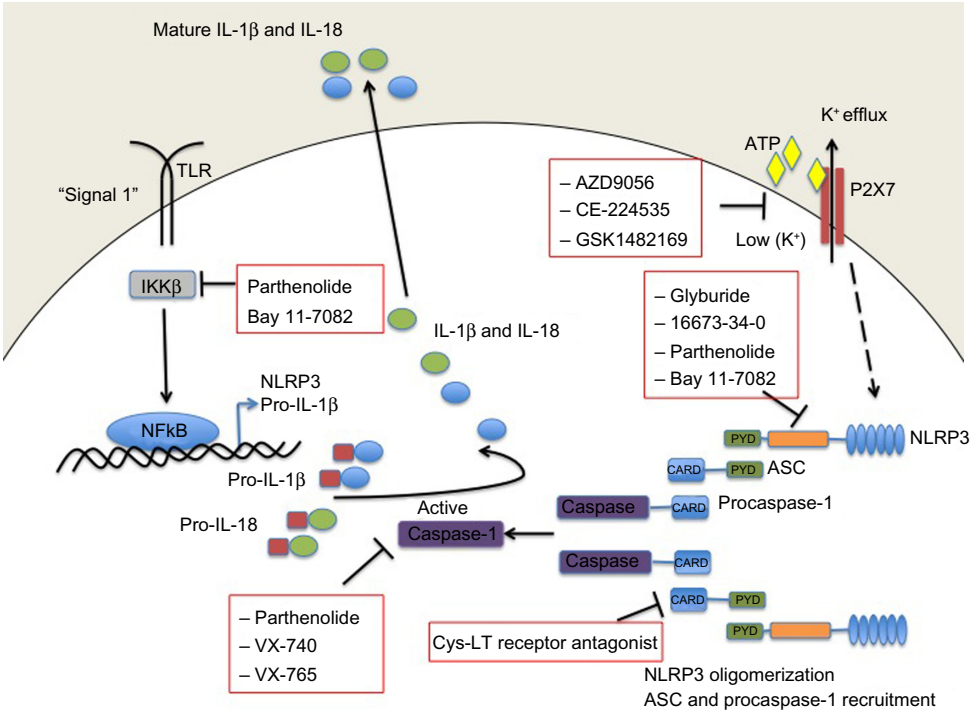

Figure 2 Small-molecule blockade of the NLRP3 inflammasome. Cartoon depicting the mode of action of the various small molecule inhibitors described in detail in the text.
Abbreviations: ASC, apoptosis-related speck-like protein containing a caspase recruitment domain; ATP, adenosine triphosphate; CARD, caspase recruitment domain; Cys-LT, cysteinyl leukotriene; IKKβ, inhibitor of κB kinase β; IL, interleukin; NF-κB, nuclear factor kappa B; P2X7, P2X purinergic receptor 7; PYD, pyrin domain; TLR, Toll-like receptor.

Table 4 Therapeutic agents that inhibit inflammasome components and their targeted diseases

Therapeutic agent	Target	Disease
Anakinra	IL-1 receptor	RA
Rilonacept	IL-1β, IL-1β	CAPS, diabetes, gout
Canakinumab	IL-1β	MWS, FCAS
GSK1070806	IL-18	B-cell non-Hodgkin's lymphoma, IBD
Glyburide	NLRP3 (indirectly)	Type 2 diabetes
16673-34-0	NLRP3 (indirectly)	Acute myocardial infarction
Pralnacasan (VX-740)	Caspase-1	RA
VX-765		MWS
Parthenolide	Caspase-1/NF-κB (IKKβ kinase activity)/ NLRP3 ATPase	Cancer
Bay 11-7082	NFκB (IKKβ kinase activity)/NLRP3 ATPase	Systemic lupus erythematosus
Cys-LT receptor antagonist	ASC oligomerization	Allergic rhinitis, asthma, nasal polyposis
AZD9056	P2X7	RA
CE-224535	P2X7	RA
GSK1482169	P2X7	RA

Abbreviations: ASC, apoptosis-related speck-like protein containing a caspase recruitment domain; ATPase, adenosine triphosphatase; CAPS, cryopyrin-associated periodic syndromes; Cys-LT, cysteinyl leukotriene; FCAS, familial cold autoinflammatory syndrome; IBD, inflammatory bowel disease; IKKβ, inhibitor of κB kinase β; IL, interleukin; MWS, Muckle-Wells syndrome; NF-κB, nuclear factor kappa B; P2X7, P2X purinergic receptor 7; RA, rheumatoid arthritis.

its use outside of type 2 diabetes. A recent publication has described 16673-34-0, an intermediate substrate free of the cyclohexylurea moiety of glyburide, as an NLRP3 inhibitor effective in a model of acute myocardial infarction that does not affect glucose metabolism.[71] It is likely that this intermediate in the glyburide synthesis pathway will prove useful as a tool to discover exactly how these sulfonyl compounds inhibit NLRP3 inflammasome activation. Further investigations will determine if it is a useful therapeutic in chronic inflammatory conditions.

Caspase-1 inhibitors

Parthenolide is a naturally occurring plant sesquiterpene lactone with multiple anti-inflammatory properties, and has been used extensively as a herbal remedy for a variety of inflammatory diseases with few and mild side effects.[72] It was thought to be an NFκB inhibitor through selective inhibition of κB kinase β (IKKβ) kinase activity; however, in addition to its effects on NFκB activation, parthenolide has now been shown to inhibit activation of caspase-1 in response to NLRP3, NLRP1, and NLRC4 stimulation. The effect of parthenolide on the spectrum of inflammasomes implies that it acts on a common component and, accordingly, it has been shown that parthenolide is a direct inhibitor of caspase-1, causing alkylation of caspase-1 on a number of cysteine residues.[73]

Two newly developed caspase 1 inhibitors, ie, pralnacasan (VX-740) and VX-765 have also been described. Pralnacasan was effective in vitro and in several animal models, and has been tested in a clinical trial in RA; however, the outcome of the trial has not been reported. VX-765 has been administered to six patients with MWS, resulting in a 40%–70% decrease in markers of inflammation as well as a significant reduction in recurrent fevers and arthritis.[68]

NLRP3 ATPase inhibitors

Interestingly, in addition to its role in inhibiting caspase-1, parthenolide also directly inhibits NLRP3 by inhibiting its ATPase activity, which is required for activation of caspase-1. Another NF-κB inhibitor, Bay 11-7082, which, like parthenolide, inhibits NFκB by blocking IKKβ kinase activity, has also been shown to inhibit the ATPase activity of NLRP3, and it appears likely that the mechanism of inhibition, at the level of NLRP3 or upstream, involves NLRP3 alkylation of nucleophilic residues.[73]

ASC inhibitors

More recently, a cysteinyl leukotriene receptor antagonist developed by Bayer Pharmaceuticals (Bayer AG, Leverkusen, Germany) (Härter et al, US Patent 7,498,460, 2009[74]) was described and found to inhibit both NLRP3 and AIM2 inflammasome induced IL-1β processing, by preventing ASC oligomerization. ASC oligomerization is essential for both NLRP3 and AIM2 inflammasome formation. In contrast, ASC appears to be dispensable for NLRC4 inflammasome formation as the CARD domain of NLRC4 can interact with caspase-1 directly. This cysteinyl leukotriene receptor antagonist does not inhibit IL-1β release following activation of the NLRC4 inflammasome. Interestingly, ASC appears to have further roles in innate immune responses separate from its role as an adaptor for inflammasome formation. Hence, this small-molecule inhibitor of ASC may hold therapeutic promise as a dual-purpose therapy in some inflammatory conditions.[74]

P2X7 antagonists

Due to the requirement for a reduction in potassium levels inside the cell prior to NLRP3-induced caspase-1 activation, regulating potassium levels presents a target for therapeutic intervention. This process is controlled by the ATP receptor P2X7 and therefore small-molecule inhibitors of P2X7 have been developed and tested in humans. AZD9056 (a P2X7

inhibitor) resulted in a significant clinical improvement in joint inflammation in patients with RA, but there was no concurrent reduction in C-reactive protein levels.[75] In fact, other P2X7 inhibitors have likewise had disappointing outcomes in relation to RA trials; CE-224535, for example, was ineffective in a Phase IIa trial in RA (ClinicalTrials.gov identifier NCT00628095).[76] Similarly, GSK1482169 disappointed in its inability to suppress IL-1β release (Phase I trial, ClinicalTrials.gov identifier NCT00849134).[77] Overall, oral inhibitors of P2X7 do not appear to be effective in diminishing RA disease symptoms.[78]

Immunotherapies

There is no doubt that the use of drugs that target IL-1β in order to block its effects has been a real success story over the last decade in tackling both autoinflammatory and chronic inflammatory disease. However, there are some conditions in which IL-1β and/or IL-18 appear to play a protective role, and support the notion that, in some cases, the use of immunotherapy in the form of administration of recombinant cytokines may be therapeutically beneficial.

Therapeutic use of IL-1β

IL-1 is a multifunctional cytokine.[79] Alongside its well known proinflammatory and fever-inducing properties, there is also an established protective effect of IL-1β in mice after irradiation or treatment with cytotoxic drugs, most likely due to the ability of IL-1β to induce levels of colony stimulating growth factors.[80–83] Administration of IL-lβ to mice has also resulted in tumor regression by either nonspecific or tumor-specific immune-mediated mechanisms. Because of these properties, IL-1β has been administered to patients in an effort to improve bone marrow function[84] and to treat a variety of solid tumors.[85–87] While the benefits of IL-1β were inconsistent in the cancer trials, levels of peripheral white blood cells did increase significantly even with administration of low doses (0.027–0.1 μg/kg) of IL-1β.[84,85] Despite the benefits associated with IL-1β therapy, at least for the improvement of bone marrow function, the acute toxicities observed in these patients were unacceptable and incompatible with use of IL-1β as a drug in practical terms. Patients experienced fever and significant hypotension, loss of appetite, generalized muscle and joint aches and pains, and headache, nausea, and fatigue, as well as gastrointestinal and sleep disturbances.[85] In fact, the signs and symptoms exhibited post-IL-1 injection into humans are practically indistinguishable from those seen with low doses of endotoxin.

Therapeutic use of IL-18

IL-18 and IL-1β are cleaved in a similar manner, ie, by caspase-1, downstream of inflammasome activation. They also share common signaling pathways and are both considered to be proinflammatory cytokines. However, there are several important differences between IL-18 and IL-1β, which caution that assumptions made for one cannot be made for the other. One striking difference is in the expression pattern of IL-18 and IL-1β. Whereas IL-1β gene expression is absent basally in all cell types tested, pro-IL-18 is constitutively present in nearly all cells, including blood monocytes, keratinocytes, endothelial cells, and epithelial cells.[88] The fact that IL-18 is constitutively expressed is consistent with a role for IL-18 in homeostasis. Furthermore, IL-18-deficient animals start to overeat at a young age, become obese, and exhibit symptoms reminiscent of the metabolic syndrome,[89] further implicating IL-18 in the regulation of homeostasis. In contrast, IL-1-deficient animals are relatively normal under specific pathogen-free conditions. As discussed above, the use of IL-1β as a treatment is not practical due to its toxicity and induction of fever. Conversely, the induction of a fever is not a significant result of IL-18 treatment, so at least in that regard the use of IL-18 as an immune stimulant is feasible.[90]

In fact, IL-18 (SB-485232, GlaxoSmithKline) has been administered to humans for the treatment of various cancers in Phase I and II trials.[91–93] IL-18 induces interferon-gamma together with either IL-12 or IL-15, promoting the differentiation of CD4+ T lymphocytes into Type 1 helper cells and inducing the generation of memory cytotoxic CD8+ T lymphocytes and natural killer cells, resulting in enhanced cellular immunity.[94] The rationale for investigating the use of recombinant IL-18 in the treatment of cancer is to harness these immune stimulatory effects of IL-18, and to increase the activity, expansion, and tumor infiltration of the immune cells. The initial trials where IL-18 was evaluated as monotherapy in patients with advanced solid tumors served the purpose to indicate that IL-18 was safe and well tolerated up to a dose of 1,000 μg/kg and has immunomodulatory activity.[95] A follow-up Phase I study has investigated the feasibility of use of IL-18 as an adjunct therapy with PEGylated liposomal doxorubicin and reported no positive drug interactions.[92] Common drug-related adverse events were grade 1 or 2 chills and nausea. However, it remains to be seen whether IL-18 administration is in fact efficacious for the treatment of cancer.

Interestingly, one of the less common symptoms experienced by patients receiving IL-18 (with PEGylated liposomal doxorubicin) was mucosal inflammation. The role of IL-18 in inflammation of the bowel appears to be a complicated one.

There are a number of studies that indicate a protective role for IL-18 in models of IBD.[96,97] These studies indicate that mice deficient for NLRP3[96] or NLRP6[97,98] are more vulnerable to dextran sodium sulfate-induced colitis and that this is due to a lack of IL-18 not IL-1β. Similarly, mice deficient in caspase-1 are also more susceptible to dextran sodium sulfate-induced colitis, and administration of IL-18 initiates mucosal healing in these mice.[99] On the other hand, blocking IL-18 with a neutralizing antibody or with IL-18 binding protein, a natural inhibitor of IL-18, reduces colitis in wild-type mice. The most likely explanation is that IL-18, which is constitutively expressed in intestinal epithelial cells, has a role in maintaining the intestinal barrier under homeostatic conditions. However, once the integrity of the barrier is lost, production of IL-18 by infiltrating macrophages causes inflammation.[46] Therefore, despite the protective role of IL-18 in barrier maintenance, there appears to be a rationale for blocking IL-18 in Crohn's disease and IBD, when the intestinal barrier is lost and chronic inflammation ensues.

AMD is the leading cause of central vision loss in industrialized nations. It is widely considered that the inflammatory response plays a role in the development of AMD, although the mechanisms involved remain unknown. A number of studies have indicated that the NLRP3 inflammasome is activated in AMD.[20,100–102] Further studies, including our own, have indicated that IL-18 acts in an antiangiogenic capacity in the context of both AMD and diabetic retinopathy,[20,103,104] suggesting that immunotherapy, in the form of recombinant IL-18, may provide some therapeutic protection in ocular conditions where neovascularization is a symptom. This hypothesis is currently being tested in preclinical trials.

Conclusion

The discoveries of IL-18 and IL-1β predate the discovery of the inflammasome by 1 and 2 decades, respectively. However, the time between the first description of the NLRP3 inflammasome and the first attempts to target this inflammasome therapeutically has been very short. There is no doubt that great leaps forward have been made in the treatment of genetically inherited autoinflammatory diseases through IL-1β blockade, which has brought untold relief to those who suffer with CAPS. It is clear that abnormal activation of the NLRP3 inflammasome is involved in many chronic inflammatory conditions, as detailed above. However, it remains to be seen whether targeting the NLRP3 inflammasome will provide therapeutic benefit in these more chronic disease states. At worst, it is possible that simply blocking IL-1β or IL-18 in these conditions may prove ineffective if the cytokines themselves are not causative of the pathology but rather resulting from it. In these cases, targeting the NLRP3 inflammasome itself, as early as possible in disease progression, may prove more beneficial, blocking not only cytokine release but also pyroptotic cell death and the ensuing tissue destruction; however, this would require reliable methods for early prediction of onset of disease. Still, even with this caveat in mind, identification of small-molecule inhibitors of the NLRP3 inflammasome offers considerable therapeutic promise, and continued research in this area is an important step in the process of developing effective medicines for the treatment of chronic diseases in which inflammation is a known component.

Disclosure

The authors report no conflicts of interest in this work.

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