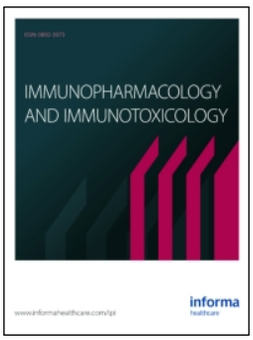

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Contribution of inflammasome complex in inflammatory-related eye disorders and its implications for anti-inflammasome therapy

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Abstract

Inflammasome complex is regarded as a major molecular regulator that exerts a significant function in caspase-1 activation and consequently, the development of cytokines like interleukin-1β (IL-1β) and interleukin-18 (IL-18). The secretion of these cytokines may induce inflammation. The role of inflammasomes in the pathologic process of eye-related illnesses like glaucoma, age-related macular degeneration (AMD), and diabetic retinopathy has been well-studied over the past decade. However, the detailed pathogenic mechanism of inflammasomes in these retinal diseases is still unknown. Therefore, further investigation and understanding various aspects of inflammasome complexes as well as their pivotal roles in the immunopathology of human ocular illnesses are essential. The present review aims to describe the significant involvement of inflammasomes in the immunopathology of important inflammatory retinal illnesses, including glaucoma, age-related macular degeneration (AMD), and diabetic retinopathy focusing on anti-inflammasome therapy as a promising approach in the treatment of inflammation-related eye diseases.

Keywords: Diabetic retinopathy; Glaucoma; IL-1β; Inflammasome; Macular degeneration.

Introduction

The interleukin-1 (IL-1) cytokine family is known as a dominant regulator of natural immunity and inflammation [1]. Cytokines of the IL-1 group are mainly associated with both the development and progression of different inflammatory illnesses. Particularly, it has been demonstrated that cytokines of the IL-1 group are related to retinal degenerative illnesses, including glaucoma, age-related macular degeneration (AMD),and diabetic retinopathy [2]. IL-1β and IL-18 are the most extensively studied cytokines of the IL-1 familywhich are linked to eye disorders that exert pro-inflammatory actions and significant biological effects [3, 4]. Production of IL-1β exclusively takes place in inactive 35 kDa pro-form following priming signals, like pathogen-or damage-associated molecular patterns (PAMPs or DAMPs), and is solely broken down to its 17 kDa active form after inflammasome complex activation in injured or abnormal conditions [5, 6]. Inflammasome, as the major molecular mediator responsible for a prominent function in inflammation, comprise a central protein, an adaptor protein ASC (apoptosis speck-like protein), and a caspase-1 protein [7]. The caspase-1 triggers the maturation process of cytokines such as IL-1β and IL-18 after activation[8, 9]. The release of these cytokines can lead to inflammation [10, 11]. Inflammasomes are considered to be involved in the pathology of a number of inflammation-related eye disorders [12, 13, 14, 15]. In the present review of the literature, we discuss the functionofinflammasomes in the most important retinal degenerative disorders including glaucoma, AMD,and diabetic retinopathy focusing on anti-inflammasome therapy for the treatment of these inflammatory-related eye disease.

Search strategies

A PubMed search was performed, and all publications from 2001 to 2019 concerning the topic “inflammatory-related eye disorders”were analyzed(Keywords: Age-related macular

degeneration (AMD), diabetic retinopathy, glaucoma, inflammasome, IL-1β, IL-18). All English studies were included for this review.

The inflammasome: A platform for caspase-1 activation and IL-1β development

Inflammasomes are cytosolic multi-protein oligomers of the natural immune system that are involved in the induction of pro-inflammatory reactions. Inflammasomes are known as molecular platformsthat areassembled by NOD-like Receptors (NLRs) following being induced by a number of stimuli.Major components of well known inflammasome complexes are also depicted in Fig. 1.However, it must be noted that not all inflammasome complexes require NLRs[16]. These complexes inducepathogen-associated molecular patterns (PAMPs), danger-associated molecular patterns (DAMPs), reactive oxygen species (ROS), and cellular stress [5, 17]. Moreover, NLRs are recognized as cellular sensors for microbial and dangersignals(Fig. 2). NLRs comprise three caspase activation and recruitment domains (CARDs) or pyrin regions at their amino-terminus, a nucleotide-binding and oligomerization region (NACHT domain) along with multiple leucine-rich repeats (LRRs) regions [18]. NLRs comprisefourteen members, including NLRP1 to 14. A total of eight proteins play a part in the assembly of inflammasomes, including one of six NLRPs (NLRP1, NLRP3, NLRC4, NLRP6, NLRP7, and NLRP12), an IFNγ-inducible protein absent in melanoma 2 (AIM2) protein, a RIG-I-like helicase, and oligomers of ASC proteins. Activation of NLRP-containing inflammasomes leads to autophosphorylation and activation of the LRRs which subsequently recruit ASC whichfinally results intherecruitment of procaspase-1. This complex of NACHT, LRRs, ASC, and procaspase-1 is collectively considered as the inflammasome. CARD regions of ASC cleave procaspase-1 to caspase-1. ASC is a central adaptor protein exerting a pivotal function in the construction of inflammasome and caspase-1 activation viaa CARD–CARD interaction[7, 19, 20, 21]. Caspase-1

is a cysteine-rich protease processing pro IL-1β to activated IL-1β and pro-IL-18 to activated IL-18. Pro-caspase-1 is a 45kDa cytoplasmic protein, active form of which includes two heterodimers that comprise 10 and 20kDa subunits. Moreover, caspase-1 activation can then result in a number of different mechanisms as a feedback to the initial inflammatory signal.For instance, cleavage of Gasdermin-D to release its N-terminal fragment is responsible for the induction of pyroptosis [5, 22]. IL-1β and IL-18 are excreted following inflammasome activation and exert a multitude of functions, including triggering IFN-γ excretion and activation of natural killer cell, cleavage and inactivation of IL-33, DNA fragmentation and cell pore formation, suppression of glycolytic enzymes, triggering lipid biosynthesis, and releasingtissue-repair mediators like pro-IL-1α [6, 23]. Also, IL-1β induces vasculogenesis and is involved in the metastasis of tumors [24]. Impaired regulation of inflammasomes has been connected to a number of inflammatory disorders, including type I and type II diabetes, inflammatory bowel disease (IBD), gouty arthritis, multiple sclerosis, polycystic ovarian syndrome and vitiligo in addition to inflammatory autoimmune illnesses [13, 25, 26, 27, 28, 29]. The aforesaid illnesses and disorders are due to excess amounts or lack of excretion of pro-inflammatory cytokines for which the inflammasome is responsible[30].

Eye disorders associated with inflammasome pathways

Glaucoma

Glaucoma is classically defined as a chronic optic neuropathy that alters the optic nerve and ultimately results in loss of ganglion cells located in the retina. This disease is also considered the leading cause of irreversible blindness [31, 32]. Senescence and increased intraocular pressure are known as main risk factors for the initiation and progression of glaucoma [7]. Despite the fact that the detailed pathways by which ganglion cell axons of the retina

degenerate in glaucoma are still unknown, an increasing body of evidence suggests a crucial role for neuroinflammation [33]. Following glaucoma-related stimuli such as optic nerve transection, ocular hypertension, and excitotoxicity, resident glial cells produce cytokines along with ILsthat might lead to detrimental effects [34]. IL-1β is a key microglia-derived cytokine that exacerbates glaucoma through triggering the formation of matrix mellanoproteinase-9, nitric oxide, and reactive oxygen species [35]. Recent investigations demonstrated that IL-1β maturation and secretion via activation of NLRP1 and 3 are highly involved in the pathogenesis of glaucoma [36, 37].Furthermore, an increase in NLRP3 inflammasome, caspase-1, and caspase-8 levels was reported in human glaucomatous eye, and both NLRP3 and NLRP1 inflammasomes are also detected in experimental study models of acute glaucoma [36, 38]. Furthermore, an animal model of acute intraocular pressure (IOP)-induced glaucoma indicated that an increase in the IOP level could activate TLR-4 and subsequently prompt caspase-8 formation. Caspase-8, as upstream of the NLRP3 inflammasome, can also activate the NLRP1 and NLRP3 inflammasomes. In this study, the formation of NLRP1 and NLRP3, ASC, caspase-1, and IL-1β was significantly decreased by suppression of caspase-8, while levels of IL-1β were slightly reduced as a result of caspase-1 blockade [36].Also, it has beenreportedthat rapid elevation of IOP can trigger the secretion of high-mobility group box 1 (HMGB1) protein. This protein that is released by necrotic cells can activate TLR-2 and TLR-4 as a DAMP [39, 40, 41]. Inhibition of HMGB1 resulted in a significant reduction of NLRP3, caspase-8, and IL-1β levels.In conclusion,this results in decreased diseaseseverity[42]. Moreover, intravitreal administration of caspase-1 and caspase-8 suppressors inhibits the activation of the NLRP1 and 3 inflammasomes while improving acute glaucoma [36]. More extensive researches are essential in human models to explore the exact function of inflammasomes in glaucoma and answer the question of how

inflammasome inhibition may stop disease progression. Reasonable answers to such questions open new ways for novel anti-inflammasome therapies for the treatment of glaucoma.

Age-related macular degeneration (AMD)

Age-related macular degeneration (AMD) is a neurodegenerative disease and the most common cause of blindnessin the elderly in the western area of the globe,which is specified by an elevation in the number and size of drusen and extracellular deposits which accumulate in the space separating the retinal pigment epithelium (RPE) and Bruch's membrane (BM) intheouter retina [43]. AMD is a multifactorial disease. Indeed, it is caused by hereditary as well as environmental risk factors [44]. Recent evidence demonstrated crucial involvement of the pro-inflammatory reactions in the pathogenesis as well as the progression of this disease [45]. Inflammasome activation, as a powerful arm of the natural immune system, has been discovered in RPE cells and suggested as a crucial player in RPE dysfunction and degradation. Deposition of drusen leads to cell destruction in the RPE layer and triggers the NLRP3 inflammasome [46, 47, 48]. Interestingly, research has not determined which pathway (e.g., apoptosis, necrosis or pyroptosis) is being responsible for RPE cell death [49]. Recent investigations indicated that drusen derived elements like carboxyethylpyrrole and complement protein C1q could trigger the NLRP3 inflammasome in macrophages extracted from bone marrow [50]. Moreover, NLRP3 inflammasome could be induced by nucleic acids (e.g., *Alu* RNA) during RPE degeneration. [51]. Moreover, photo-oxidative damage due to blue light can trigger NLRP3 inflammasome in RPE cells. In brief, phototoxic cell death results in the rupture of lysosomal membranes, ultimately leading to the release of their ingredients into cytosol. Lysosomal rupture then induces NLRP3 as well as triggering caspase-1 and IL1-β and IL-18 excretion [52]. Research has shown that the NLRP3 inflammasome was induced in geographic atrophy in response to repetitive

transposable subtypes of non-coding RNA, termed *Alu* RNA, which aggregated in RPE because of a deficiency in miR processing enzyme DICER1. Lately, it was reported that clinical grade IL-18 does not exert a any influence on retinal or RPE integrity if injected intra-vitreally in cynomolgus monkeys (at a dosage of 10,000 ng) [53]. In addition, IL-18 was found to act as anti-angiogenic and anti-permeability via mediating VEGF synthesis and suppressing VEGF-induced RPE barrier disintegration [50, 54, 55]. On the other hand, *Alu* RNA aggregation as a result of DICER1 deficiency was shown to higher the excretion of IL-18, but not IL-1β, in human RPE cells after NLRP3 inflammasome induction, indicating that IL-18 was the compound responsible for the *Alu* RNA-mediated RPE cytotoxicity. Research has discovered that neutralizing IL-18, but not IL-1β, enhances*Alu* RNA-triggered RPE degeneration[46].

Diabetic retinopathy

Diabetic retinopathy (DR) is a microvascular disease of the eye retina recognized by vasculogenesis and neuroinflammation of the retina [56]. DR involves dysfunction in multiple pathways triggering cellular and microvascular damage in the retina. Current studies introduce chronic low-grade inflammation as a main culprit in the pathogenesis of DR [57, 58]. Activated microglia/macrophages control the secretion of inflammatory cytokines (e.g., IL-1β), dysfunction of the blood-retinal barrier (BRB), vascular leakage, and neovascularization [59]. Additionally, increased serum and vitreous quantities of pro-inflammatory cytokines IL-1β was observed in clinical researches [60, 61]. The DAMPs, including oxidized lipoproteins, glycated proteins, uric acid, DNA, and RNA released by necrotic cells inducethe assemblyof NLRP3inflammasome, ASC, and Caspase-1, and are involved in the pathogenesis of retinal diseases [62, 63, 64]. Although the expression NLRP3 in the vitreous of proliferative diabetic retinopathy (PDR) patients suggests the role of this multi-protein complex in retinal

inflammation, the involvement of the NLRP3 inflammasome has not been fully understood. Clinical studies have indicated that secretion of IL-1β through induction of NLRP3 inflammasome in diabetic patients can be neutralized using IL-1β antagonist [65, 66]. In addition, deficiency in NLRP3 components has been reported to dampen diabetic symptoms in rodent models [67]. Furthermore, a significant rise in caspase-1 activity was observed intheretinas of diabetic mice, galactose-fed mice, diabetic humans, as well as retinal Müller cells that were administered a high dose of glucose [68]. More evidence suggested that hyperglycemia during diabetes can upregulate Thioredoxin-interacting protein (TXNIP) which is the major mediator of retinal inflammation. Increased levels of TXNIP induce the NLRP3 expression and ultimately IL-1β formation [69]. The role of inflammasomes and their mechanism of action in diabetic retinopathy is yet to be investigated, and further surveys are required to explore the exact role of inflammasomes in diabetic retinopathy.

Anti-inflammasome therapy for the treatment of inflammation-related eye disorders

Based on the evidence mentioned above, the NLRP1 and 3 inflammasomes exert a pivotal function in the pathologiesof the retina, comprising glaucoma, AMD, and diabetic retinopathy. The treatments that aim at inflammasome pathways to treat inflammatory diseases are currently being experimented at the stage of preclinical trials (Table 1) [70]. Although specific compounds that inhibit inflammasome pathway are under investigation, numerous basic and clinical studies have demonstrated the potential of several inhibitors in stopping up- and down-stream inflammasome pathways (Fig. 3) [71]. An increasing number of previous researches indicated that antioxidants are potent inhibitors of NLRP3 inflammasome activity. The ROS scavenger N-acetyl cysteine (NAC) is among the most commonly administered antioxidants that inhibit the triggering of NLRP3 inflammasome. The inhibitory effect of NAC is

dependent on its dosage. Notably, its concentration must be > 10 mM to effectively block the inflammasome [72]. Glyburide is known as a second-generation sulphonylurea drug that is generally used for the treatment of T2DM. Recent investigations demonstrated that Glyburide exerts some anti-inflammatory effects through inhibiting neutrophil chemotaxis and NLRP3 inflammasome, but the suppression of NLRP3 inflammasome by glyburide should be further tested in clinical and preclinical studies [73]. The induction of NLRP3 inflammasome via extracellular ATP through binding to P2X7R has been implicated in many pathological inflammatory conditions like contact hypersensitivity, graft-versus-host disease, the development of rheumatoid arthritis, lung inflammation and fibrosis, and irritable bowel syndrome [74, 75, 76, 77]. Recent investigations have indicated that the blockade of the NLRP3 inflammasome and IL-1β release by P2X7R antagonists had positive effects on the treatment of different inflammatory pathologies [78, 79]. The AZD9056, as a known P2X7R antagonist, resulted in improving outcomes of some inflammatory diseases, including rheumatoid arthritis and Crohn's disease in Phase II clinical trials [80]. Furthermore, caspase-1 blockers such as VX-765 are considered the most important inhibitors of the inflammasome pathway. These inhibitors are available orally as pro-drug and are hydrolyzed rapidly into their active form and decrease the secretion of IL-1β and IL-18 in individuals with CAPS [81]. Utilizing caspase-1 blockers has revealed encouraging therapeutic outcomes in certain ocular diseases [82]. Although many inflammasome blockers have been vastly researched, they are not yet to be investigated in clinical trials or *in vivo* studies of ocular diseases. The mentioned discovered inflammasome inhibitors, if verified in related animal models of ocular disorder, may result in the discovery of novel promising curatives for inflammatory or autoimmune eye diseases. Recent advances regarding anti-inflammasome therapies in inflammatory eye diseases are now being translated at the stage of clinical trials for

illnesses like AMD. Nevertheless, further research is required to exactly uncover the signaling pathway of inflammasome and its regulators as well as stimulators for introducing anti-inflammasome agents as new and powerful approaches.

Conclusion and future directions

Inflammasomes are highly involved in inflammation-related ocular illnesses. The latest investigations regarding the contribution of inflammasomes to eye function clarify several new mechanisms. Still, the definite role of inflammasomes and their related ligands in human ocular illnesses need further exploration. Although the recently developed understanding of the way inflammasomes are activated in eye-related disease raises new questions in this field, this mechanistic insight also brings about new opportunities to create novel curatives for individuals with inflammatory diseases of the retina. Inhibition of caspase-1 has demonstrated encouraging outcomes in preventing or reversing the progression of several ocular disorders. Acquiring a more in-depth insight on the detailed role of inflammasomes in human eye diseases has the potential of paving the path to the development of effective and novel therapeutic agents for clinical practice. Nowadays, there are many inhibitors of inflammasomes that require further testing in human or animal models of ocular illnesses before any clinical applications. These novel inflammasome inhibitors may result in the development of therapeutic approaches for treatments of various inflammatory, autoimmune, and infectious ocular illnesses. Mature IL-18 and IL-1β can be synthesized via both the RPE and systemic or resident myeloid derived cells, but it has not been perfectly understood how theses cytokines can specifically trigger RPE apoptosis or other suggested mechanisms. More research aiming at uncovering of such pathways may develop the knowledge in the field and suggest novel potential for new treatments.

Declaration

Ethics approval and consent to participate

Not Applicable

Consent for publication

All authors agreed to publish this manuscript on the BMC Eye and Vision

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

Seyed Ahmad Rasoulinejad, Ahmad Karkhah, Kiarash Saleki, Marzieh Pirzadeh, and Alireza Paniridrafted the manuscript. Ahmad Karkhah, Kiarash Saleki, Marzieh Pirzadeh and Alireza Paniri collected the relevant literature. Hamid Reza Nouri collected the relevant literature and revised the manuscript. All authors have read and approved the finalized manuscript.

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Table 1. Therapeutic agents inhibiting inflammasome pathway and their related diseases

Inhibitors	Mechanism of action	Related diseases
Anakinra	Blocks IL-1 receptor	RA
Rilonacept	Neutralize circulating IL-1β	CAPS, diabetes, gout
Canakinumab	Block IL-1β	MWS, FCAS
GSK1070806	Binds to IL-18 and inhibits signaling through the IL-18 receptor	B-cell non-Hodgkin's lymphoma, IBD
Gevokizumab	Prevents the binding to the IL-1RI and co-receptor	Non-necrotizing anterior scleritis
Glyburide	Block NLRP3 inflammasome activation	Type 2 diabetes
16673-34-0	Block NLRP3 inflammasome activation	Acute myocardial infarction
NAC	Block NLRP3 inflammasome activation	Septic shock
MCC950	Inhibit the activation of the NLRP3	IBD, CAPS
Pralnacasan (VX-740)	Inhibit caspase-1 activity	RA
VX-765	Inhibit caspase-1 activity	MWS
Thalidomide	Inhibit caspase-1 activity	Cancer
Parthenolide	Inhibitor of Caspase-1/NF-κB (IKKβ kinase activity)/NLRP3 ATPase	Cancer
Bay 11-7082	Inhibitor of NFκB (IKKβ kinase activity)/NLRP3 ATPase	Systemic lupus erythematosus
Cys-LT receptor antagonist	Inhibit ASC oligomerization	Allergic rhinitis, Asthma, Nasal polyposis
AZD9056	Inhibitor of P2X7	RA
CE-224535	Inhibitor of P2X7	RA
GSK1482169	Inhibitor of P2X7	RA
probenecid	Blocking of pannexin-1	Gout

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 auto-inflammatory syndrome; IBD: inflammatory bowel disease; NAC: N-acetyl cysteine; 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.

Figure legends

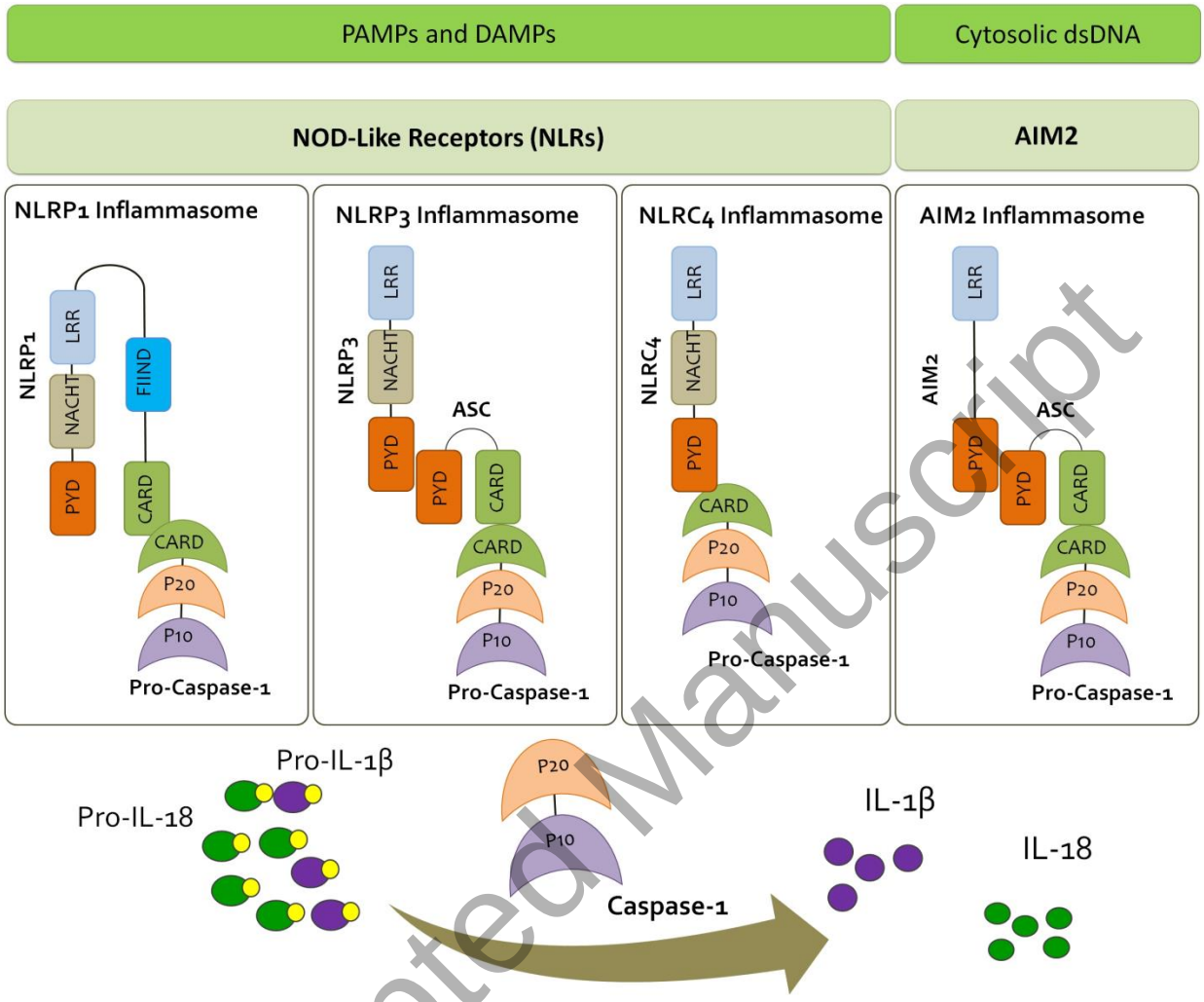

Fig. 1 Schematic representation of inflammasome complexes. All inflammasomes are composed of NACHT, LRR, FIIND and CARD domains. Four well known inflammasome including NLRP1, NLRP3, nlrc4 and AIM2 inflammasome. ASC; apoptosis associated with Speck-like protein, **CARD;**caspase activation and recruitment domain, FIIND; function to find domain, LRR; leucine rich repeat, NACHT; nucleotide-binding and oligomerization domain, PYD; pyrin domain.

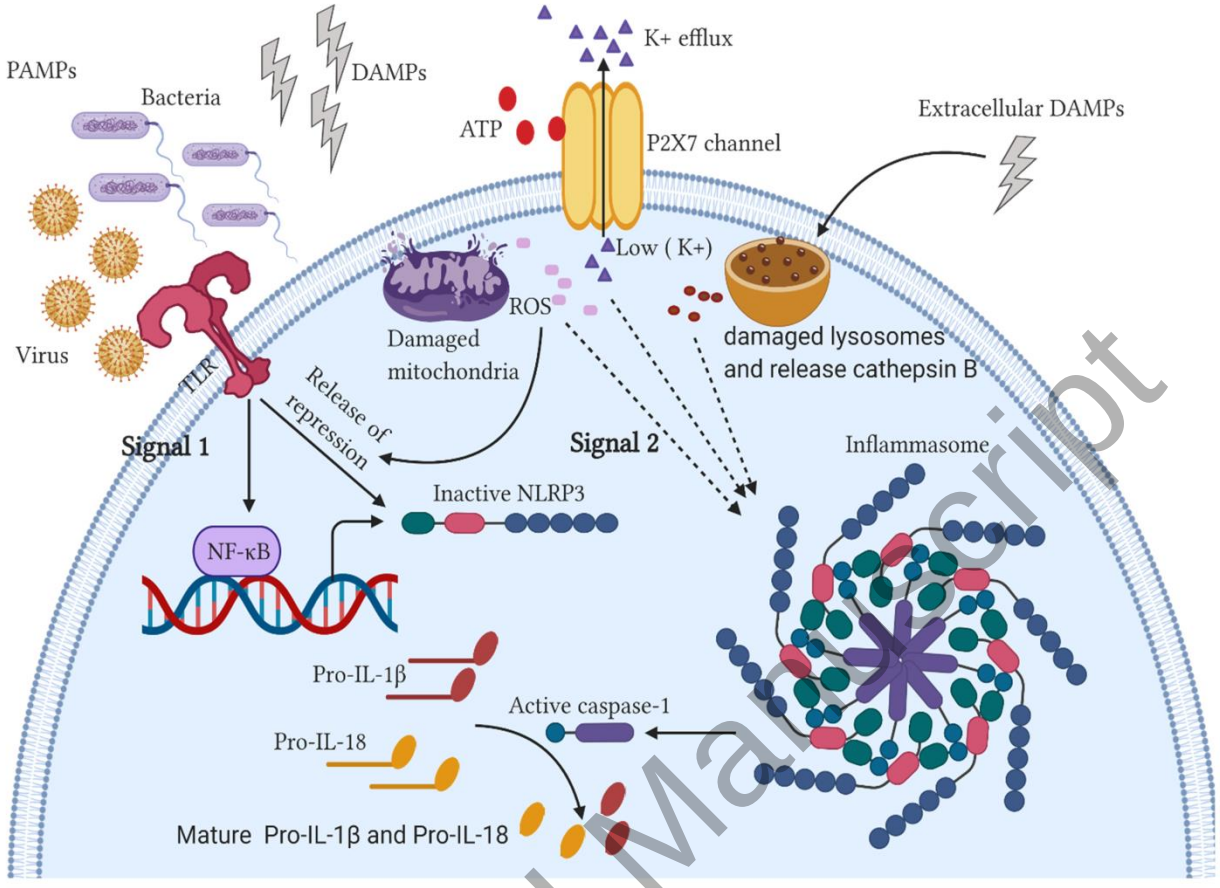

Fig. 2NLRP3 inflammasome activation in response to different stimuli. First, recognition of a PAMP or DAMP mediates the activation of NF-κB and also induces elevated expression of NLRP3. NF-κB activation induces the production of pro-IL-1β and pro-IL-18. After priming, a second signal stimulatesNLRP3 protein to interact with ASC and caspase-1,forming a multi-protein complex known as NLRP3 inflammasome. The NLRP3 inflammasome activation leads to the cleavage of caspase-1 and then, active caspase-1 mediatesthematuration and secretion of IL-1β and IL-18. The release of these cytokines can lead to NLRP3 inflammasome-dependent inflammation in retinal disorders.

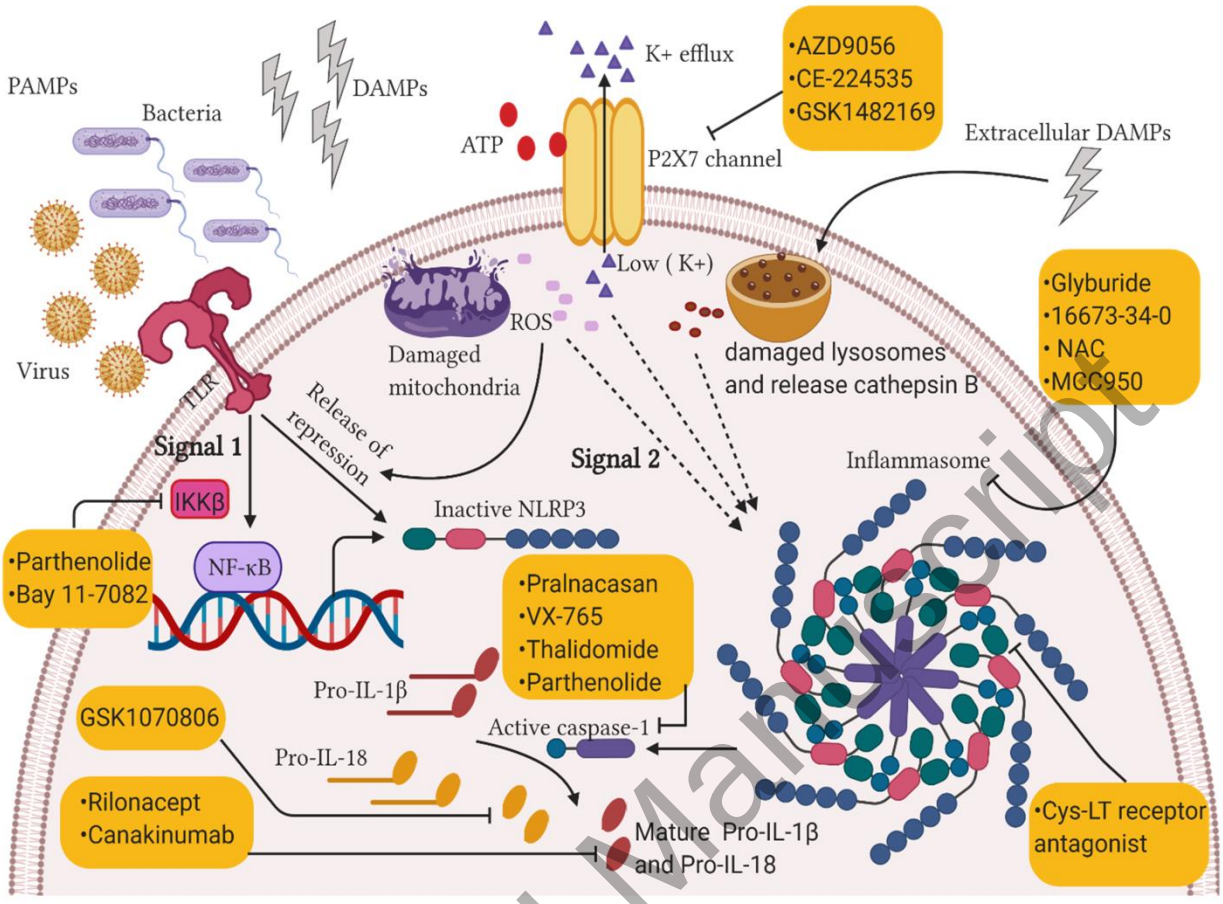

Fig. 3 Compounds currently tested in preclinical studies that target inflammasome activation.