Review

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Mechanistic Inhibition of IL-1β with a Focus on the P2X7 Receptor

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Inflammatory mediators are important molecules that adjust the inflammatory response and prevent tissue damage. Cytokines are relevant mediators involved in inflammation. The interleukin-1 (IL-1) family is a well-known cytokine group that regulates inflammatory responses, in which IL-1β plays a pivotal role in the promotion of inflammation. Although there are several underlying mechanisms, the nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3) pathway is the most studied pathway for the secretion of IL-1β. The NLRP3 inflammasome is a protein complex formed after extracellular adenosine triphosphate (eATP) binds to the P2X family purinergic receptor 7 (P2X7R), and NLRP3 inflammasome activation results in IL-1β release. The P2X7 receptor plays a crucial role in the immune response, and its modulation may trigger the development of pathological conditions characterized by inflammation. Therefore, it is important to highlight the P2X7 receptor as a potential therapeutic target in diseases in which an inflammatory profile is observed due to high concentrations of secreted IL-1β. This study aimed to elucidate the mechanism by which the P2X7 receptor may affect IL-1β cytokine release.

Keywords: IL-1β; inflammation; IL-1β antagonism; P2X7 receptor

Introduction

Inflammation is a vital physiological process that combats pathogens and facilitates tissue repair through pathogen destruction, dilution, or neutralization. As a result, inflammation plays a pivotal role in maintaining bodily homeostasis. Nonetheless, under certain circumstances, inflammation may exhibit adverse effects. Excessive activation of the inflammatory cascade can lead to dysregulation of proinflammatory cytokines, thereby precipitating the onset of chronic inflammatory conditions and tissue injury [1].

Cytokines serve as pivotal mediators of cellular communication and constitute a crucial component of the body's response against infections and cellular injury. Both proinflammatory and anti-inflammatory cytokines collaborate to maintain physiological equilibrium [2].

Within the interleukin-1 (IL-1) family, a group of important cytokines orchestrates either anti-inflammatory or proinflammatory reactions. Notably, IL-1β is a well-recognized proinflammatory cytokine involved in diverse pathways wherein the organism mobilizes and amplifies defence mechanisms [3].

In the context of inflammation, IL-1β activates immune cells, triggering CD4+ T-cell polarization into T helper type (Th1) and Th17 cells. It also promotes leukocyte infiltration at infection sites by increasing the expression of adhesion receptors on immune system cells and endothelial cells. However, elevated IL-1β signalling aggravates inflammatory diseases such as Alzheimer's disease, stroke, and other neurological disorders, in which neuronal cell death is observed. Additionally, certain hereditary gain-of-function mutations in the inflammasome may lead to IL-1β overproduction, contributing to the severity of some autoimmune syndromes [4].

The P2X family purinergic receptor 7 (P2X7R) initiates the activation of a pathway through which IL-1β is released. Once activated by adenosine triphosphate (ATP), the receptor induces the activation of different signalling cascades, contributing to the inflammatory response. The ionic current induced by P2X7R activation leads to nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3) assembly, which triggers IL-1β secretion after cleavage [5]. In this scenario, inhibiting P2X7R represents a promising strategy for reducing the inflammatory process regulated by IL-1β. However, current pharmacological therapies for inflammatory conditions often lack selectivity in targeting the response and, in many cases, have serious side effects [6]. Therefore, this work sought to elucidate the interplay between the P2X7 receptor and the IL-1β cytokine release mechanism, underscoring its pivotal role in inflammatory disease management.

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P2X7-Induced IL-1β Release

The P2X7 receptor, part of the P2 receptor family activated by purines and pyrimidines, functions as an ion channel with distinct characteristics from other P2X receptors. Structurally, P2X7R forms a trimer with a distinct carboxy-terminal domain that is crucial for sustained current and pore opening. While activated by ATP, similar to other P2X receptors, P2X7 receptors exhibit unique permeability to large molecules and evoke two responses to ATP: transient cation flux and sustained current through a large pore upon prolonged stimulation [7].

After agonist binding and receptor gating, P2X7 promotes the flux of mono- and divalent cations, such as Na^+, K^+, and Ca^{2+}, causing membrane depolarization and modulating excitatory synaptic transmission [8].

Macrophages, among other immune cells, express the P2X7 receptor and can release proinflammatory molecules, particularly in response to infection and tissue damage. Within the inflammatory cascade, IL-1 cytokines play a crucial role in both initiating and sustaining the inflammatory response [9].

ATP, a key molecule in cellular energy transfer, is tightly regulated by ecto-ATPases. However, under conditions of cellular damage, ATP can be released from damaged cells at millimolar concentrations. This extracellular ATP acts as a damage-associated molecular pattern (DAMP), serving to signal and amplify the immune response [10]. Activation of P2X7R induces potassium efflux, precipitating ionic alterations that trigger a conformational shift in the NLRP3 inflammasome complex, which is composed of apoptosis-associated speck-like protein containing a CARD (ASC), an adapter protein, and a pro-caspase-1 effector protein [11]. This conformational change renders the inflammasome active, initiating a cascade of events culminating in the activation of caspase-1. Caspase-1, in turn, cleaves and facilitates the maturation of pro-IL-1β into its biologically active form, IL-1β, which can then be secreted. Consequently, P2X7R functions as a secondary signal, enabling the conversion of immature cytosolic IL-1β into its active state [12]. Fig. 1 illustrates this pathway and elucidates the mechanism underlying IL-1β release via this pathway.

A recent study has suggested that P2X7R may trigger IL-1β release via an NLRP3-independent pathway in human macrophages. Pharmacological inhibition of P2X7R or genetic deletion of NLRP3 in the THP-1 cell line resulted in partial IL-1β secretion, and P2X7R cleavage was not mediated by caspase-1 activity. These findings underscore the importance of investigating different cell types due to species heterogeneity and the potential variability in responses to disease treatment [13].

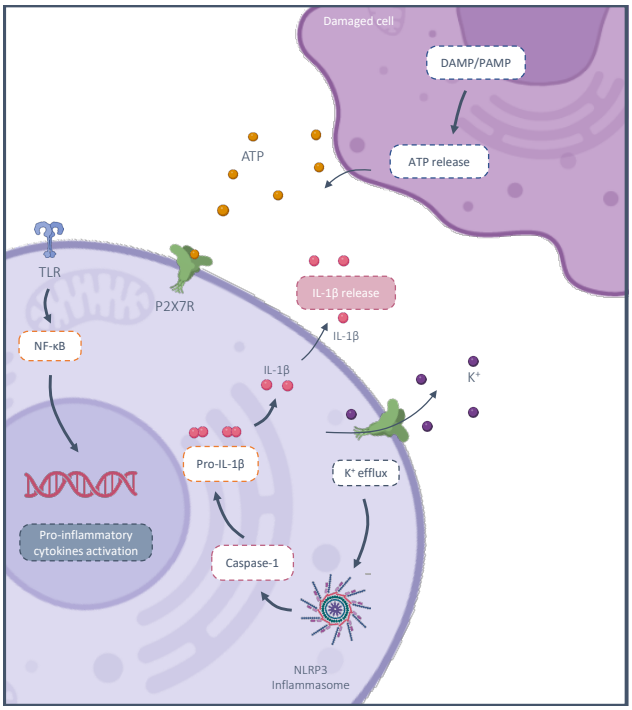

Fig. 1. P2X7R-mediated IL-1β release. Damaged cells release ATP through pannexin-1 channels, which in turn, activates P2X7R. P2X7R induces ion flux, leading to K^+ efflux. A decrease in K^+ is necessary for NLRP3 assembly, which activates pro-caspase 1 to caspase-1 and thus triggers pro-IL-1β cleavage. Once active, IL-1β is secreted into the cytosol through exocytosis. Figure created via https://www.biorender.com/. P2X7R, P2X family purinergic receptor 7; IL-1β, interleukin-1β; ATP, adenosine triphosphate; DAMP, damage-associated molecular pattern; NLRP3, nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3.

Role of IL-1β and P2X7R Modulation in Inflammatory Conditions

High expression of IL-1β has been identified as a biomarker in some inflammatory diseases, such as rheumatoid arthritis (RA) and periodic syndrome associated with cryopyrin (CAPS). CAPS is a group of inflammatory diseases caused by a mutation in the *CIAS-1/NLRP3* gene, which leads to increased activity. This gain of function leads to an increase in caspase-1 activity and IL-1β secretion [14]. The role of IL-1β in RA has been widely studied, and some authors have determined the role of the highly activated NLRP3 inflammasome in the synovial activity of patients with RA. Blocking with an NLRP3 inhibitor leads to less bone degradation in animal models [15]. Thus, the usual drugs used to treat these pathologies aim to inhibit or block IL-1R.

The role of IL-1β in the pathogenesis of neurological diseases such as depression has already been discussed.

Through chronic stress, numerous mechanisms are activated, including the activation of the immune response in the central and peripheral nervous systems, triggering the activation of caspase-1 by the NLRP3 inflammasome and resulting in a large-scale inflammatory response [16]. Increasing evidence indicates that increased IL-1β secretion in the hippocampus can lead to depressive symptoms [17].

In addition, the authors reported a close association between IL-1β and the development of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced hyperinflammation and inflammatory cell death. The virus can rapidly lead to NLRP3 assembly, which in turn promotes IL-1β processing via caspase-1 and secretion. This response is abolished when NLRP3 is silenced or caspase-1 is inhibited. Interestingly, IL-1β is released through a nonclassical pathway that depends on caspase-1, caspase-8, autophagy, and K^+ efflux [18]. Other research groups have correlated this nonclassical pathway with the activation of the purinergic receptor P2X7, which requires extracellular K^+ to ensure NLRP3 assembly [19]. IL-1β plays an important role in the development of a wide range of inflammatory diseases. Thus, its inhibition is a fundamental strategy for the treatment of inflammation-associated diseases. Table 1 (Ref. [15,20–31]) summarizes IL-1β-related diseases. Table 1 summarizes the role of IL-β in some inflammatory conditions (Table 1). The involvement of the P2X7 receptor in inflammatory conditions has garnered increasing attention due to its ability to promote and enhance inflammation. The P2X7 receptor has been shown to serve as a cancer driver and is implicated in tumour aggressiveness and the induction of proinflammatory cytokines in various cancer types. In bone cancer, P2X7 receptor activation stimulates pathways associated with primary bone tumours and osteoblastic metastasis [32]. P2X7 receptor antagonists are effective at alleviating cancer-induced bone pain by suppressing the release of proinflammatory cytokines in the tumour microenvironment. This receptor is implicated in both inflammatory and neuropathic bone pain [33,34]. In addition, the P2X7 receptor has been implicated in several autoimmune disorders, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis. The activation of the P2X7 receptor by extracellular ATP triggers the production of inflammatory mediators such as cytokines (IL-1β, IL-6, IL-18, and tumor necrosis factor-alpha (TNF-α)) and the shedding of the lymphocyte homing receptor CD62L, disrupting the immune system balance [35]. Oxidized ATP (oxATP), an inhibitor of the P2X7 receptor, can suppress T-cell activity, potentially mitigating autoimmune responses [36]. In SLE, P2X7 receptor activity regulates the production of autoantibodies by Tfh cells and is influenced by genetic loci associated with lupus susceptibility. Restoring P2X7 receptor activity in SLE patients could control Tfh cell generation and autoantibody production [37]. In rheumatoid arthritis, P2X7 receptor blockade reduces cytokine release and intracellular calcium concentration in synoviocytes, inhibiting Th17 cell differentiation and alleviating inflammatory cytokine release, thereby easing RA symptoms [38].

Table 1. Inflammatory diseases mediated by the cytokine IL-1β.

IL-1β-mediated diseases	Reference
Rheumatoid arthritis	[15]
Periodic fever, aphthous stomatitis, pharyngitis, adenitis syndrome	[20]
Type 2 diabetes	[21]
Postmyocardial infarction heart failure	[22]
Osteoarthritis	[23]
Familial Mediterranean fever	[24]
Cryopyrin-associated periodic syndromes	[25]
Depression	[26]
Crohn's disease	[27]
Atherosclerosis	[28,29]
Smouldering myeloma	[30,31]

Therapeutic Approaches Targeting IL-1β and P2X7R

Therapeutic approaches targeting IL-1β release and P2X7R modulation hold great promise for the treatment of inflammatory conditions and offer potential alternatives or complements to existing therapies. Continued research into the mechanisms underlying IL-1β regulation and P2X7 receptor function will further inform the development of novel therapeutic strategies.

Preclinical and Clinical Trials Targeting P2X7R

Due to the importance of P2X7R signalling in pathophysiological processes, research groups are investigating the effects of P2X7R hyperactivation and the consequences of P2X7R inhibition. During the lipopolysaccharide (LPS) challenge, P2X7R is overexpressed and affects leukocyte function by inducing an inflammatory response in wild-type mice, which does not occur in P2X7R knockout mice [39]. Its inhibition has been shown to exhibit an anti-inflammatory effect, not only by acting on the release of IL-1β but also by considerably decreasing the expression of NLRP3, caspase-1, ROS, and NO [40–43]. Cicko *et al.* (2018) [44] demonstrated that prophylactic inhibition of P2X7R by its antagonist KN62 resulted in a significant improvement, as demonstrated through reduced levels of proinflammatory mediators, such as IL-1β, ATP and immune system cells.

Several P2X7 receptor antagonists have been developed to minimize the damage caused by IL-1β release and the sustained inflammatory response. JNJ-54175446 (ClinicalTrials.gov Identifier: NCT04116606) inhibited IL-1β production in a given patient group in a dose-dependent manner [45]. Additionally, in healthy volun-

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Table 2. Commercial IL-1β inhibitors.

Name	Classification	Application	Reference
Pralnacasan (VX-740)	Caspase-1 inhibitor	Osteoarthritis	[55]
Anakinra	IL-1 receptor antagonist (IL-1Ra)	Rheumatoid arthritis	[56]
Belnacasan (VX-765)	Caspase-1 inhibitor	Rheumatoid arthritis and skin inflammation	[57]
Rilonacept	Soluble IL-1 decoy receptors	Cryopyrin-associated periodic syndrome	[58]
Canakinumab	Anti-IL-1β antibodies	Cryopyrin-associated periodic syndrome	[59]
Gevokizumab	Anti-IL-1β antibodies	Familial Cold Autoinflammatory Syndrome (FCAS) and Muckle-Wells Syndrome (MWS)	[60]
MEDI-78998 (AMG108)	IL-1 receptor-blocking antibody	Osteoarthritis	[61]
MABp1	Anti-IL-1α antibodies	Type 2 diabetes, advanced cancer, cancer cachexia, leukaemia, severe psoriasis, occlusive vascular disease, and scarring acne vulgaris	[3]
CYT-013	Vaccine targeting IL-1β	Type 2 diabetes	[3]

teers, it suppressed peripheral IL-1β release and mitigated dexamphetamine-induced improvements in mood and (visual) motor performance. In depression, P2X7R inhibition may mitigate immune-related mood dysregulation. One study investigated the effects of JNJ-54175446 in patients with major depressive disorder (MDD) undergoing total sleep deprivation (TSD). While JNJ-54175446 was well tolerated and had mild to moderate adverse effects, it reduced IL-1β release from white blood cells and blunted the acute reduction in anhedonia induced by TSD without significantly affecting overall mood. These findings suggest that P2X7R inhibition may be particularly effective in situations where mood regulation is disrupted, such as acute emotional perturbations such as TSD-induced anhedonia [46].

Tests with the antagonist AZD9056 demonstrated improvement in Crohn's disease symptoms, a disease with high levels of IL-1β, IL-6, and TNF-α in the gut and bloodstream [47]. AZD9056 (ClinicalTrials.gov Identifier: NCT00520572) also exhibited promising results in alleviating rheumatoid arthritis, in which improvements were detected against a placebo. A decrease in joint pain and swelling was reported as one of the most striking features of the study [48]. The compound CE-224,535 (ClinicalTrials.gov Identifier: NCT00628095) proved to be a potent P2X7 receptor antagonist. This drug was selected for a phase II study due to its ability to reduce LPS-induced IL-1β and IL-18 secretion by leukocytes [49]. Tests with the compound GSK1482160 (ClinicalTrials.gov Identifier: NCT00849134), which acts as an antagonist of P2X7, proved highly effective in neuropathic pain tests. The study reported decreased IL-1β concentrations in a dose-dependent manner [50]. These findings underscore the therapeutic potential of targeting P2X7R for mitigating inflammatory responses and associated pathologies via IL-1β.

IL-1β Inhibitors

The pharmaceutical market has effective anti-inflammatory drugs. However, finding new therapies is challenging in terms of effectiveness, low cost, and few or no adverse effects. Nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are the most common classes used for chronic inflammatory disease treatment. Despite this, they have many side effects, including gastric irritation and skin manifestations; promotion of opportunistic infections, metabolic disorders, and bone loss; and increased risk of cardiovascular events once these enzymes catalyse prostaglandin biosynthesis [51–54]. Therefore, drugs for new targets are being developed to reduce these adverse effects. Thus, compounds targeting IL-1β have been the focus of several scientific groups (Table 2, Ref. [3,55–61]).

Two inhibitors of IL-1 are currently approved: anakinra, an IL-1 receptor antagonist (IL-1Ra) receptor antagonist, and canakinumab, a monoclonal cytokine IL-1β antagonist [62]. Nevertheless, long-term treatment generates body toxicity, especially at the injection site. This effect must be related to the treatment time and medication dosage [63].

Although some details concerning IL-1β biosynthesis have already been elucidated, some of the underlying secretion mechanisms remain unknown. In the presence of external stimuli, cytokines are produced. However, in the absence of an aseptic stimulus, a portion of IL-1β ceases to be secreted, remains in the intracellular environment, and is subsequently degraded. Extracellular adenosine triphosphate (eATP) is considered an essential secondary stimulant for rapid cytokine secretion [64,65]. ATP, which is a physiological agonist of the P2X7 receptor, can be released from damaged membrane proteins or cells [5,66]. When activated by eATP, the P2X7 receptor activates the NLRP3 inflammasome, eliciting IL-1β maturation and secretion. Therefore, study has focused on P2X7 receptor inhibition as a potential therapeutic target to decrease the inflammatory response, as observed in a carrageenan-induced acute inflammatory pain mouse model [67]. At the same time, many studied antagonists fail to elicit an effective response due to the lack of selectivity for the P2X7 receptor or, com-

pared to conventional drugs already used in clinical trials, lack of clinical efficacy, revealing a need to find new effective blockers. Thus, inhibiting this signalling pathway would be a valuable strategy for decreasing IL-1β secretion and levels in the inflammatory microenvironment.

Conclusions

IL-1β is a pivotal cytokine that is critical for orchestrating the intricate processes of an effective immune response. Its multifaceted roles span from initiating inflammation to modulating immune cell activities. However, the unbridled release of IL-1β can instigate a cascade of detrimental effects, exacerbating the severity of chronic inflammatory and autoimmune diseases. This finding underscores the urgent need for stringent regulation of IL-1β production and secretion.

Among the components of these intricate inflammatory pathways, the P2X7 receptor has emerged as a central player. This receptor, known for its involvement in ATP-mediated signalling, is involved in the synthesis, maturation, and secretion of cytokines, including IL-1β. Through its intricate signalling cascades, P2X7R influences the activation of inflammasomes, which are pivotal for regulating the release of mature IL-1β. Thus, targeting P2X7R represents a promising pathway for therapeutic intervention aimed at modulating IL-1β levels and mitigating inflammatory responses.

In light of these insights, it has become increasingly imperative to delve deeper into the complexities surrounding IL-1β regulation. Advances in our understanding of the mechanisms governing IL-1β production and release hold immense potential for the development of novel therapeutic strategies. By revealing the intricate interplay between IL-1β and P2X7R, researchers can pave the way for the discovery of innovative blockers capable of finely tuning the immune response, thereby offering hope for more effective management of inflammatory and autoimmune conditions.

Availability of Data and Materials

Not applicable.

Author Contributions

JVF conceived the original idea and wrote the manuscript. JPS contributed with ideas and wrote the manuscript. RXF designed the work and contributed with new insights, supervised, reviewed and gave the final approval for the manuscript to be published. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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