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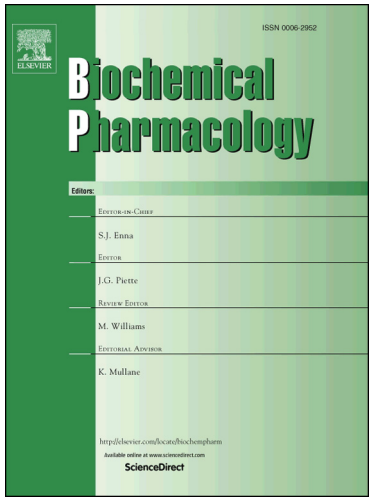

Review

The P2X7 receptor: a main player in inflammation

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The P2X7 receptor: a main player in inflammation

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Abstract

Damage associated molecular patterns (DAMPs) are intracellular molecules released from infected or injured cells to activate inflammatory and reparatory responses. One of the most ancient and conserved DAMPs is extracellular ATP (eATP) that exerts its phlogistic activity mainly through activation of the P2X7 receptor (P2X7R). The P2X7 is an ATP gated ion channel, expressed by most immune cells, including the monocyte-derived cell lineages, T and B lymphocytes an their precursors. Here we give an overview of recent and established literature on the role of P2X7R in septic and sterile inflammation. P2X7R ability in restraining intracellular bacteria and parasite infection by modulation of the immune response is described, with particular focus on *Mycobacteria* and *Plasmodium*. Emerging literature on the role of P2X7 in viral infections such as HIV-1 is also briefly covered. Finally, we describe the numerous intracellular pathways related to inflammation and activated by the P2X7R, including the NLRP3 inflammasome, NF-kB, NFAT, GSK3β and VEGF, and discuss the involvement of P2X7R in chronic diseases. The possible therapeutic applications of P2X7R antagonists is also described.

Key words:

P2X7, ATP, Inflammation, pathogens, immune response.

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1. Introduction

Adenosine triphosphate (ATP) plays a central role in cellular energy homeostasis, being the main product of reactions such as photophosphorylation, respiration and fermentation, and in turn acting as an energy donor in most endergonic biosynthetic processes that support cell survival, proliferation and motility. The cytoplasm of most mammalian cells contains an ATP concentration in the 5-10 mM range, and even higher ATP levels are stored within neuronal synaptic vesicles. ATP can be released from injured or dying cells following plasma membrane damage, as well as from intact cells upon mechanical deformation, hypoxia, stimulation of vesicular release, or via membrane proteins such as ABC cassettes, pannexins, connexins and the P2X7R [1, 2]. Considering that the concentration of ATP in the extracellular milieu is in the nanomolar range under physiological conditions (albeit the synaptic cleft might be an exception), even a small ATP release can generate a very strong signal against the basically negligible background, thus characterizing ATP-based purinergic transmission as an extracellular messenger system with a remarkably high signal-to-noise ratio. Once in the extracellular space ATP undergoes a rapid enzymatic degradation mediated by ectonucleotidases, which control not only the half-life of ATP as agonist, but also generate ligands for additional purinergic receptors such as ADP-activated P2Y1, P2Y12 or P2Y13, or the adenosine-selective A1, A2A, A2B and A3 receptors [3].

During evolution, multicellular organisms ranging from plants to higher mammals developed complex signalling systems allowing the exploitation of ATP as an extracellular messenger. Interestingly, although evolved from different protein families in plant or animal [4, 5], receptors for extracellular ATP are widely expressed almost in all *phila*, where they mediate similar protective and life-supporting responses such as recognition of potentially life-threatening events [6, 7], stimulation of inflammation and promotion of wound healing and cell growth [7-9]. In mammals, ATP binds and activate two subfamilies of P2 receptors: G-protein coupled P2Y receptors (P2YRs), and P2X ligand-gated ion channels (P2X receptors, P2XRs). Among the eight

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human P2Y receptors subtypes, only P2Y11 is ATP selective, while all the others show higher affinity for other nucleotide ligands such as ADP, UTP, UDP and UDP-glucose [9]. Nevertheless, P2Y receptors have been variably implicated in several inflammation-related, ATP-associated responses, such as chemotaxis, release of pro-inflammatory cytokines and stimulation of platelet granule release [10-12].

With nearly 600 studies reported on Medline as of December 2017, the P2X7 receptor (P2X7R) is the P2 receptor (P2R) unanimously associated to inflammation [13, 14]. This is probably due, in the first place, to the merely contingent reason that P2X7R was the first P2R to be thoroughly investigated in a well-defined immune cell population [15, 16] (although by no means the first P2R to be implicated in immune cell responses [17, 18]). In the second place, the P2X7R is the first P2R to which a clear-cut and very relevant immune-related response, i.e. NLRP3 inflammasome activation and IL-1β release, could be assigned [19, 20]. Intriguingly, P2X7R also participates in other processes activated in response to damage and aimed at restoring homeostatic conditions such as wound healing and cell growth [9, 21].

Here we wish to summarize recent advances on the role of P2X7 in septic (pathogen-dependent) and sterile (pathogen-independent) inflammation, and to explore the potential of this receptor as therapeutic target for inflammatory diseases.

2. Structural P2X7R features

The P2X7R is an ATP-gated ion channel supporting Na^+ and Ca^{2+} influx into and K^+ efflux out of the cell cytoplasm [13, 22]. P2X7R stimulation with high (0.5 to 1 mM and above) concentrations of ATP causes in as yet poorly understood fashion the opening of a non-selective pore permeable to hydrophylic solutes of MW up to 900 Da, such as ethidium bromide, YO-Pro, or Lucifer yellow [22-25]. Large pore formation, which requires an intact C-terminal tail, has been associated to P2X7 cytotoxicity [22, 26]. The *P2RX7* gene localizes to human chromosome 12 (12q24.31) close to the *P2RX4* gene (12q24.32) [27]. Several single nucleotide polymorphisms (SNPs) were identified

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(≈1500), including 10 loss- and 3 gain-of-function (recently reviewed in refs [28, 29]). Selection of mutated alleles might have been due to environmental pressure by infectious agents, or by prevailing chronic inflammatory diseases.

Recent studies describing the crystal structures of zebra fish P2X4R, human P2X3R and panda P2X7R [30-33] allowed the resolution by homology modelling of the tertiary structure of all the protomers of the P2XR subfamily, including mouse and human P2X7Rs [13, 28, 34], and the definition of the 3D structure resulting from subunit assembly into the functional homotrimeric P2X7R. The P2X7 subunit consists of a large extracellular loop that participates in the formation of the agonist and antagonist binding pouches, two alpha-helical transmembrane regions, and cytoplasmic N- and C- terminal domains. Typical of P2X7R is the extended C-terminal tail [35].

The tri-dimensional rendering of P2X3, P2X4 and P2X7 subunits resembles a dolphin in shape, and accordingly subunit domains have been named after the corresponding dolphin anatomical parts: the head, the body, the dorsal fin and the flippers are all parts of the extracellular region, while the transmembrane helices make the flukes [30, 31]. The β sandwich structure making the dolphin's upper body is the area of interaction among the three monomers to form the active P2X7 trimeric receptor [31, 34]. The ATP-binding site is provided by each of two adjacent monomers. Crystallographic studies confirmed that binding of three ATP molecules is needed for gating. Insights about the interaction of P2X receptors with their ligands, the conformational changes accompanying channel opening and the consequent desensitization were further provided by the recent publication of the X-ray structures of the human P2X3R and of a truncated form of the panda P2X7R [32, 33]. Crystallographic data by clarifying the spatial conformation of the receptors in different states, including the agonist-bound open, the desensitized/closed and the antagonist-bound states, provide a new basis for the development of P2X7R-targeting drugs [32]. Moreover, resolution of the panda P2X7R 3D structure allowed to identify a common putative allosteric binding pocket, located between two neighbouring subunits and different from the ATP binding site. This putative allosteric binding site was shown to accommodate five small inhibitor drugs of

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diverse chemical structure [32]. Unfortunately, crystallization of the full-length P2X7 subunit has not yet been possible, therefore we have no structural information related to the long C-terminal tail, possibly the most intriguing P2X7 domain, responsible for the opening of the large conductance pore and putatively interacting with other intracellular signalling molecules [36-40].

3. P2X7 agonists and antagonists

3.1. P2X7R agonists

Compared to the other receptors of the P2X sub-family, P2X7R shows low affinity for ATP (i.e. in the mM range), therefore it has become a standard experimental procedure to use as a more potent, although not selective, agonist the synthetic ATP derivative 2'(3')-O-(4-benzoylbenzoyl) adenosine-5'-triphosphate (Bz-ATP) [22]. However, while often considered a selective P2X7 ligand Bz-ATP activates also P2X1, P2X4 and P2Y11 [28, 41]. Interestingly, the antibacterial peptide cathelicidin LL37 [42, 43] and the Alzheimer β amyloid peptide [44] have also been reported to activate the P2X7R, either as *bona fide* agonists or as positive allosteric modulators. A similar role has also been suggested for other compounds including drugs, antibiotics and plant derivatives, such as tenidap [45], polymixin B [46] and ginsenosides [47].

3.2. P2X7R antagonists

Over the years, several P2X7R antagonists or negative allosteric modulators were also identified, ranging from the prototypical covalent blocker oxidized-ATP [16], to brilliant blue G (BBG) [48] and KN62 [49]. However, these molecules are poorly selective as they also block other cellular targets besides the P2X7R, such as CAMK-II (KN62), or voltage-gated sodium channels (BBG), and or even inhibit immune cell functions in the absence of P2X7R [50]. Moreover, KN62 is preferentially active at the human, while BBG at the rat P2X7 [51, 52]. Three widely used and selective antagonists, A-740003 and A-438079 and AZ10606120, are very effective blockers of the

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human, rat and mouse P2X7R [52, 53]. Other antagonists active at both human and rodent P2X7R are GW791343, JNJ-47865567, JNJ-42253432, JNJ-54166060 and JNJ47965567 [41, 54-56]. Compounds AZ11645373 and GSK1482160 have very good affinity for the human but slightly lower affinity for the rat receptor [57, 58]. In silico studies have helped highlight some of the structural features underlying species-selectivity of some P2X7 antagonists. Model docking studies have suggested that presence of a phenylalanine at position 95 (present in the human but absent in the rat receptor) is responsible for higher affinity of KN62 or AZ11645373 for the human versus the rat P2X7R [57]. Some high affinity P2X7 antagonists have been used to develop radioligands for the investigation of neuroinflammation and possibly other inflammatory conditions [59-61].

The mode of action of several P2X7R blockers is still a matter of debate. Some of the most frequently used antagonists, such as A740003 and JNJ47965567, that were originally described as competitive antagonists [56, 62], are now suggested to be negative allosteric modulators binding to a common site shared with other allosteric compounds such as AZ10606120 [33, 63]. However, further studies are needed to properly define the mechanism of action of these and other P2X7 blockers.

Small drug-like P2X7 antagonists have been taken to Phase I and Phase II/III clinical trials in selected chronic inflammatory diseases, raising no safety and tolerability concerns, but with rather disappointing results as to clinical efficacy [13, 28, 64] (Table I). These unsatisfactory data may prompt the use of P2X7R-targeted biologics. Two Companies are currently developing anti-P2X7R antibodies that are at present under extensive pre-clinical test [65], or have undergone Phase I clinical study [66]. Biologics may prove better P2X7R modulators than small drug antagonists.

4. P2X7 in the response against pathogens.

Inflammation, the fundamental homeostatic response that protects the organism against exogenous (pathogens) or endogenous sources of danger, develops through the coordinated activity of specialized cells of the myeloid and lymphoid lineage responsible for innate and adaptive immunity.

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The P2X7 receptor is expressed by virtually all immune and inflammatory cells, and is up-regulated during inflammation. Cells of the monocyte/macrophage axis, including dendritic cells, microglia and osteoclasts, are by far the immune cell lineage where P2X7 activity has been best characterized, nonetheless this receptor is also expressed by T and B lymphocytes, mast cells and natural killer cells [8].

4.1. P2X7 in the response against intracellular parasites

Early experiments showed that P2X7 activation potentiated killing of intracellular pathogens such as mycobacteria [67], *Chlamydia* [68], *Toxoplasma* [69, 70], and *Leishmania* [71] mainly through facilitation of phago-lysosome fusion and acceleration of acidification of parasitophorus vacuole, thus leading to elimination of the microbial load [72]. However, translation of in vitro observations to the in vivo disease models produced contrasting results. In fact, while in the case of *Chlamydia* and *Leishmania* in vivo experimental models confirmed the in vitro data, i.e. *Chlamydia* [73] and *Leishmania* [74] caused a more severe disease in P2X7R genetically-deleted mice, infection with highly aggressive mycobacterial strains caused a less severe disease in the P2X7R-null host [75]. In particular, tissue damage was largely reduced in the absence of P2X7R. These apparently paradoxical results are not surprising based on the role of the P2X7 receptor in sustaining and propagating chronic inflammation: in fact the cytotoxic activity of P2X7R is well established, as well as its role in promoting multinucleated giant cell formation [76]. Therefore it is likely that during tuberculosis infection P2X7R promotes the local tissue reactions associated to type IV hypersensitivity that eventually end up in granuloma formation, caseous necrosis and extensive tissue damage [77, 78]. The picture becomes even more complicated in humans as several genetic studies and following meta-analyses failed to show a clear association between P2X7 SNPs and the susceptibility to extra-pulmonary or pulmonary tuberculosis (recently reviewed in ref [28]).

A recent study by Machado de Salles and colleagues, demonstrated a key role of P2X7R in the response against the parasite *Plasmodium chabaudi* [79], showing that P2X7R null mice are

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more susceptible to malaria infection due to altered Th1 differentiation. During malaria blood stage P2X7R on $CD4^+$ lymphocytes is activated promoting the development of protective immunity against *Plasmodium chabaudi* via t-bet expression and INFγ/ IL 2 secretion. P2X7R deficiency in the $CD4^+$ T lymphocyte subset causes a shift in the differentiation of this population, thus promoting the expression of Bcl6 and the consequent differentiation into T follicular helper cells (Tfh), which in turn drive the production of anti-plasmodium antibodies. However, despite the increase in antibody titer, lack of P2X7 and the associated Th1/Tfh unbalance lead to more severe parasitemia in chronically infected mice, strongly suggesting that P2X7R is required for the development of cell-mediated acquired immunity against *Plasmodium chabaudi* [79]. Plasmodium parasites aggressiveness is related to their ability to survive host immunity, therefore recent studies concentrated on the manipulation of immune response to eliminate the infection. Data reported by Machado de Salles and colleagues prompt to the use of P2X7R-targeted molecules to induce protective immunity as a novel therapeutic approach or to improve vaccine strategies [79].

4.2. P2X7 in the response against viruses

A small number of studies also investigated P2X7R expression and function in viral infections with contrasting results as both host-protective and detrimental, infection-promoting, roles depending upon the given infectious agent were reported. The P2X7R was shown to help contain Dengue virus-2 infection by decreasing viral load via nitric oxide release [80], or to exacerbate adenoviral infection, leading to accelerated host damage [81], and even to facilitate hepatocyte infection by hepatitis B virus [82]. Recent studies raised the possibility that the P2X7R could be central in the pathogenesis of HIV and that some of the effects mediated by nucleotide reverse transcriptase inhibitors (NRTIs), currently a main-stake in AIDS treatment, might be dependent upon P2X7R blockade [83-85]. In fact, NRTIs, which are part of the combined antiretroviral therapy (ART) that drastically increased the life expectancy of HIV patients, were shown to efficaciously antagonize P2X7R both in vitro and in in vivo models of inflammation [84].

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These data open the way to the design of new P2X7R antagonist based on NRTIs structure and suggest NRTI repurposing to treat P2X7R-related diseases. In addition, they also support the hypothesis that P2X7 is implicated in HIV pathogenesis and that ART is so effective also because of the inhibition of P2X7R-activated pathways. In support of this hypothesis, it has been recently demonstrated that ATP dependent P2X7R activation causes release of HIV-1 virions from macrophage of infected individuals, where they are stored within vacuoles acting as infectious reservoirs. Accordingly, administration of the P2X7R antagonist A-438079 prevented virion release. P2X7R-stimulated release of HIV-1 virions was not due to macrophage cell death but rather to exocytosis of the intracellular vacuoles [85]. Further proof of the potential of P2X7R blockade in HIV therapy is provided by the demonstration that P2X7R antagonist administration restores $CD4^+$ differentiation cells from their stem cell precursors.

The subset of HIV patients that lack ability to generate sufficient numbers of Th cells following ART administration are defined as immunological non-responders and are generally plagued with worse prognosis. $CD34^+$ hematopoietic progenitor cells from these patients overexpress P2X7R, which has been shown to block differentiation into the T lymphocyte lineage. P2X7R blockade might therefore prove efficacious in restoring $CD4^+$ levels and extend the life expectancy of immunological non-responders [86].

5. P2X7R as sensor of cell damage and a trigger of the NLRP3 inflammasome

ATP plays a widely acknowledged role in host defence, from plants to mammals, as the paradigmatic example of danger associated molecular pattern (DAMP), i.e. a cellular component that is released following tissue injury or stress and is able to activate a protective/regenerative immune response. DAMPs include different class of molecules that are normally sequestered intracellularly, only to be released upon cell damage. Once in the extracellular space, DAMPs activate the immune system by interaction with specific receptors. The P2X7R is unanimously recognized as the main sensor for ATP during inflammation and a main trigger of the early phases

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of the immune response by mediating the maturation and secretion of interleukin-1β (IL-1β) [87-89]. Due to its potent local and systemic pro-inflammatory effects maturation of IL-1β is tightly controlled and requires the activation of a multiprotein complex called the inflammasome [90].

Production of IL-1β represents a multistep process involving synthesis of the immature pro-IL-1β, proteolytic cleavage to mature IL-1β and, finally, release into the extracellular environment. The synthesis of immature pro-IL-1β is induced through a process requiring the activation of the nuclear factor NF-κB. Maturation of the 31 kDa form to the bioactive 17 kDa protein requires cleavage by caspase-1 (casp-1), without this conversion, pro-IL-1β is quickly degraded by the proteasome [91]. In turn, casp-1 is matured and activated via formation of the inflammasome complex [92-94]. The inflammasome is itself made by the assembly of casp-1 plus a sensor and an adaptor molecule. Different inflammasome subtypes are known, whether they are based on nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs), or on AIM-2-, RIG-1, IFI-16 or pyrin proteins [95, 96]. ATP activates the NLRP3 inflammasome, which can also assemble in response to molecules derived from infectious agents (PAMPs, pathogen-associated molecular patterns), other DAMPs or chemical and physical agents causing cell damage [97]. Cleavage of casp-1 and maturation of IL-1β were first associated to ATP-dependent K^+ efflux in the late 1990's [19, 98, 99]. In the following decades, several papers demonstrated P2X7R-dependent inflammasome aggregation almost in all immune cell types [88, 100-103]. The pro-inflammatory cytokines secreted following P2X7 activation include not only IL-1β and IL-18 but also IL-6 and IL-1α, albeit via an inflammasome-independent route [100, 104].

Intracellular K^+ drop is probably the best-established mechanism to explain P2X7R-dependent NLRP3 inflammasome formation [105-107]. Recent reports support this view by identifying NEK7 as the cytoplasmic kinase able to translate the ATP-triggered changes in intracellular K^+ into NLRP3 inflammasome activation [108, 109]. Reactive oxygen species (ROS) formation may also play a role in inducing inflammasome aggregation and IL-1β secretion downstream to P2X7R activation [110-113]. Furthermore, protein-protein interaction has been

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hypothesized to occur between P2X7R and the inflammasome scaffold proteins NLRP2 and NLRP3 [114-116]. The NLRP2/P2X7R protein complex includes pannexin-1 and is present in astrocytes where it is reported to mediate IL-1β maturation and release [114], while NLRP3/P2X7R interaction has been shown to occur at restricted sub-plasma membrane sites in microglia and macrophages [115].

The P2X7R/NLRP3 axis has been also associated to pyroptosis a form of cell death occurring in immune cells via activation of inflammatory caspases including casp-1 and casp-11 in mice and casp-1, casp-4 and casp-5 in humans [117]. During pyroptosis cells undergo swelling and increase of plasma membrane permeability, and at the same time promote inflammation by secreting IL-1β and IL-18 [118]. A recent report identified a pivotal role of P2X7R in LPS-stimulated pyroptosis, showing that LPS triggers pyroptosis via maturation of casp-11, that in turn cleaves pannexin-1 that finally mediates ATP release and autocrine/paracrine P2X7R activation [119]. To confirm the central role of P2X7 in LPS-dependent pyroptosis, P2X7 null mice are resistant to endotoxic shock [119].

6. Other inflammatory pathways activated by the P2X7R

The P2X7R promotes inflammation also via activation of intracellular pathways different, although often linked, to the inflammasomes. Possibly one of the best characterized is the activation of the nuclear factor NF-κB, a transcription factor controlling expression of several inflammatory genes, including TNFα, COX-2 and IL-1β itself. The seminal studies demonstrating P2X7R-dependent activation of NF-κB in microglia, osteoclasts and osteoblasts [120-122] were further confirmed by an increasing number of reports linking P2X7 pro-inflammatory activity to NF-κB nuclear translocation [123-126]. Liu and colleagues suggested that Toll-like receptors and P2X7R share a common NF-kB activation pathway via the MyD88 adaptor protein, which was proposed to directly interact with the C terminal domain of P2X7 subunits [127]. NF-κB positively modulates innate as

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well as adaptive immunity contributing with the nuclear factor of activated T cells (NFAT) to T cell proliferation downstream to T lymphocyte activation. Interestingly, P2X7R is long known to increase T cell proliferation and activate NFAT [128, 129]. Accordingly, its inhibitor oxidized ATP is an efficient immune suppressor preventing transplant rejection [130, 131]. P2X7R dependent T-cell proliferation proceeds through NFAT and focal adhesion kinase (FAK) activation finally leading to IL-2 secretion [132, 133]. P2X7R-dependent NFAT nuclear translocation has a central role in proliferation of lymphocytes, osteoblasts and tumor cells [134-139]. An additional mechanism by which P2X7R might promote NFAT function is the negative regulation of glycogen synthase kinase (GSK) 3β activity [140, 141]. GSK3β modulates adaptive immunity promoting lymphocyte proliferation and survival. In fact, expression of constitutively active GSK3β decreases T cell proliferation, differentiation and survival via inhibition of different factors, NFAT included [142, 143].

Other classical mediators of inflammation released upon P2X7R activation include eicosanoids such as prostaglandin E2, leukotriene B4 and thromboxane A2. Interestingly, the production of these arachidonic acid derivatives is initiated not only by ATP but also by interaction of P2X7 with the cathelicidin peptide LL-37 [144-148]. P2X7R activation induces cyclo-oxygenase (COX)-1 activation and COX-2 expression leading to fever [144] and inflammatory pain [149, 150], via classical signalling pathways such as ERK/MAPK kinases and phospholipase A2 [146-148, 151]. This potent pro-inflammatory activity makes P2X7R an attractive target for the development of new drugs alternative to aspirin or other COX inhibitors.

Tissue injury causes ATP release in virtually all multicellular organisms, from plants to mammals [7], and consequently activates the healing process. Although platelet clot formation is mainly dependent on P2Y receptor stimulation [152], P2X7 has been proposed to participate in healing- and regeneration-associated events such as neo-vascularization [153]. Bertics and collaborators firstly reported release of VEGF following monocyte P2X7R stimulation [154]. These seminal data were confirmed by several later studies demonstrating that P2X7R pharmacological

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blockade inhibits angiogenesis in different preclinical models [138, 140, 155, 156]. Interestingly, P2X7 is also linked to HIF-1α, the master regulator of VEGF expression, by a bi-univocal relationship in that P2X7R stimulation drives HIF-1α expression, but in turn, HIF-1α activation up-modulates P2X7R expression [140, 157-159].

7. P2X7R in chronic diseases

7.1. The P2X7R in neurodegenerative diseases

The P2X7R has been putatively associated to various chronic inflammatory neurological disorders. The P2X7R is expressed by several cell types in the nervous system, including astrocytes, oligodendrocytes, microglia, and Schwann cells [160]. Whether it is also expressed by neurons is currently a matter of debate [161-163]. Many reports associated P2X7R activation to neurological disorders, such as multiple sclerosis (MS), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (AML). Inflammatory processes causing tissue damage are relevant in MS pathogenesis [164]. Increased microglial activation and P2X7R expression have been found in both MS and ALS [165]. Moreover, MS-associated inflammation can be down modulated by Glatiramer acetate, a drug currently used to reduce the frequency of MS relapses which also reduces P2X7R activity [166]. The P2X7R has been shown to be up-regulated and associated to an increased pro-inflammatory response in in vitro ALS microglia [165, 167]. In an ALS mice model (SOD1-G93A), blockade of P2X7 by BBG delayed the course of the disease, decreased microglia proliferation and motor neuron loss, thus improving motor performance and inflammatory parameters [168]. On the other hand, and contrary wise, genetic ablation of *P2RX7* in the same SOD1-G93A ALS mice model produced worsening of gliosis and motor neuron death [167]. Therefore, the role of P2X7R in the etio-pathology of ALS is rather ambiguous at this stage, and further investigation is clearly needed [168]. A role for P2X7R has been hypothesized in AD: soluble amyloid peptide stimulates mouse microglia via P2X7R, and P2X7R-null mice are resistant to the injurious effect of

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intracerebral amyloid β inoculation AD [44]. In a mouse model of AD, microglia was shown to promote neuronal damage by P2X7R-dependent stimulation of ROS production [169].

7.2 The P2X7R in liver fibrosis and auto-immune diseases

Liver fibrosis is another condition in which P2X7R-targeting may prove beneficial. Administration of the P2X7R blocker A438079 helped reducing CCl4-induced P2X7R expression and liver injury and fibrosis [124]. P2X7R antagonism was also effective in reducing splanchnic hyperemia, portal-systemic shunt and liver fibrosis in common bile duct-ligated cirrhotic rats [155], indicating that P2X7R blockade might be a viable approach in the therapy of cirrhosis and its complications.

Rheumatologic diseases represent another chronic inflammatory condition where P2X7R targeting may offer therapeutic opportunities. The P2X7R has been implicated in the pathogenesis of systemic lupus erythematosus (SLE), one of the human diseases more heavily characterized by inflammation and immune-mediated tissue damage [170]. P2X7R inhibition via BBG or via siRNA-mediated knockdown was shown to have beneficial effects in lupus nephritis in two mouse models of SLE, MRL/lpr and NZM 2328 mice, by reducing anti-dsDNA antibodies production, immune complex deposition, and renal inflammation [171]. In addition, increased glomerular and tubular expression of P2X7R was detected in renal biopsies from patients with immune-related glomerulonephritis [172]. The P2X7R might also have a role in rheumatoid arthritis (RA) as LPS-activated mononuclear cells from RA patients showed higher levels of ATP-induced IL-1β production compared to cells from healthy subjects [173]. Reason for enhanced P2X7R-dependent IL-1β release is unclear since P2X7R expression was comparable in the two cohorts, and therefore difference in IL-1β release was putatively ascribed to genetic polymorphisms in the P2X7R gene [173]. Following studies found an association between RA and the gain of function SNPs 489C>T and 1068G>A [174, 175]. The P2X7R might also have a role in primary Sjögren's syndrome (pSS), as its expression is significantly higher in salivary glands from pSS patients versus control subjects

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[176]. Gene expression levels of NLRP3, ASC and caspase-1 were also significantly higher in pSS gland specimens, in accordance with increased level of mature IL-18 in pSS saliva samples [176]. Table II gives an overview on preclinical models used to study P2X7 function in infection and inflammation.

8. Conclusions

The cradle of purinergic signalling was neurotransmission, where ATP was long known to have a well-defined role as neuromediator. However, the more we learn about purinergic receptors, the more we realize that purinergic signalling extends well beyond the central or peripheral nervous systems, and in fact, its participation to inflammation and immunity might be even more relevant and pervasive. In this scenario, the P2X7 receptor is standing out as a main player and a promising target for innovative therapeutics.

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Disclosures

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References

[1] P. Pellegatti, S. Falzoni, P. Pinton, R. Rizzuto, F. Di Virgilio, A novel recombinant plasma membrane-targeted luciferase reveals a new pathway for ATP secretion, Mol Biol Cell 16(8) (2005) 3659-65.

[2] E.R. Lazarowski, Vesicular and conductive mechanisms of nucleotide release, Purinergic Signal 8(3) (2012) 359-73.

[3] G.G. Yegutkin, Enzymes involved in metabolism of extracellular nucleotides and nucleosides: functional implications and measurement of activities, Crit Rev Biochem Mol Biol 49(6) (2014) 473-97.

[4] J. Choi, K. Tanaka, Y. Cao, Y. Qi, J. Qiu, Y. Liang, S.Y. Lee, G. Stacey, Identification of a plant receptor for extracellular ATP, Science 343(6168) (2014) 290-4.

[5] Z. Hou, J. Cao, Comparative study of the P2X gene family in animals and plants, Purinergic Signal 12(2) (2016) 269-81.

[6] F. Di Virgilio, Purinergic mechanism in the immune system: A signal of danger for dendritic cells, Purinergic Signal 1(3) (2005) 205-9.

[7] K. Tanaka, J. Choi, Y. Cao, G. Stacey, Extracellular ATP acts as a damage-associated molecular pattern (DAMP) signal in plants, Front Plant Sci 5 (2014) 446.

[8] F. Di Virgilio, P2X receptors and inflammation, Curr Med Chem 22(7) (2015) 866-77.

[9] F. Di Virgilio, E. Adinolfi, Extracellular purines, purinergic receptors and tumor growth, Oncogene (2016).

[10] W.G. Junger, Immune cell regulation by autocrine purinergic signalling, Nat Rev Immunol 11(3) (2011) 201-12.

[11] M. Idzko, D. Ferrari, H.K. Eltzschig, Nucleotide signalling during inflammation, Nature 509(7500) (2014) 310-7.

ACCEPTED MANUSCRIPT

[12] M.R. Thomas, R.F. Storey, Effect of P2Y12 inhibitors on inflammation and immunity, Thromb Haemost 114(3) (2015) 490-7.

[13] F. Di Virgilio, D. Dal Ben, A.C. Sarti, A.L. Giuliani, S. Falzoni, The P2X7 Receptor in Infection and Inflammation, Immunity 47(1) (2017) 15-31.

[14] E. Orioli, E. De Marchi, A.L. Giuliani, E. Adinolfi, P2X7 Receptor Orchestrates Multiple Signalling Pathways Triggering Inflammation, Autophagy and Metabolic/Trophic Responses, Curr Med Chem 24(21) (2017) 2261-2275.

[15] C. el-Moatassim, G.R. Dubyak, A novel pathway for the activation of phospholipase D by P2z purinergic receptors in BAC1.2F5 macrophages, J Biol Chem 267(33) (1992) 23664-73.

[16] M. Murgia, S. Hanau, P. Pizzo, M. Rippa, F. Di Virgilio, Oxidized ATP. An irreversible inhibitor of the macrophage purinergic P2Z receptor, J Biol Chem 268(11) (1993) 8199-203.

[17] J. Lin, R. Krishnaraj, R.G. Kemp, Exogenous ATP enhances calcium influx in intact thymocytes, J Immunol 135(5) (1985) 3403-10.

[18] G.R. Dubyak, D.S. Cowen, H.M. Lazarus, Activation of the inositol phospholipid signaling system by receptors for extracellular ATP in human neutrophils, monocytes, and neutrophil/monocyte progenitor cells, Ann N Y Acad Sci 551 (1988) 218-37; discussion 237-8.

[19] D. Ferrari, M. Villalba, P. Chiozzi, S. Falzoni, P. Ricciardi-Castagnoli, F. Di Virgilio, Mouse microglial cells express a plasma membrane pore gated by extracellular ATP, J Immunol 156(4) (1996) 1531-9.

[20] D. Ferrari, P. Chiozzi, S. Falzoni, M. Dal Susino, L. Melchiorri, O.R. Baricordi, F. Di Virgilio, Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages, J Immunol 159(3) (1997) 1451-8.

[21] F. Di Virgilio, S. Falzoni, A.L. Giuliani, E. Adinolfi, P2 receptors in cancer progression and metastatic spreading, Curr Opin Pharmacol 29 (2016) 17-25.

[22] A. Surprenant, F. Rassendren, E. Kawashima, R.A. North, G. Buell, The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7), Science 272(5262) (1996) 735-8.

ACCEPTED MANUSCRIPT

[23] S. Falzoni, M. Munerati, D. Ferrari, S. Spisani, S. Moretti, F. Di Virgilio, The purinergic P2Z receptor of human macrophage cells. Characterization and possible physiological role, J Clin Invest 95(3) (1995) 1207-16.

[24] C. Virginio, A. MacKenzie, F.A. Rassendren, R.A. North, A. Surprenant, Pore dilation of neuronal P2X receptor channels, Nat Neurosci 2(4) (1999) 315-21.

[25] T.H. Steinberg, A.S. Newman, J.A. Swanson, S.C. Silverstein, ATP4- permeabilizes the plasma membrane of mouse macrophages to fluorescent dyes, J Biol Chem 262(18) (1987) 8884-8.

[26] F. Di Virgilio, V. Bronte, D. Collavo, P. Zanovello, Responses of mouse lymphocytes to extracellular adenosine 5'-triphosphate (ATP). Lymphocytes with cytotoxic activity are resistant to the permeabilizing effects of ATP, J Immunol 143(6) (1989) 1955-60.

[27] G.N. Buell, F. Talabot, A. Gos, J. Lorenz, E. Lai, M.A. Morris, S.E. Antonarakis, Gene structure and chromosomal localization of the human P2X7 receptor, Receptors Channels 5(6) (1998) 347-54.

[28] E. De Marchi, E. Orioli, D. Dal Ben, E. Adinolfi, P2X7 Receptor as a Therapeutic Target, Adv Protein Chem Struct Biol 104 (2016) 39-79.

[29] R. Sluyter, The P2X7 Receptor, Adv Exp Med Biol (2017).

[30] T. Kawate, J.C. Michel, W.T. Birdsong, E. Gouaux, Crystal structure of the ATP-gated P2X(4) ion channel in the closed state, Nature 460(7255) (2009) 592-8.

[31] M. Hattori, E. Gouaux, Molecular mechanism of ATP binding and ion channel activation in P2X receptors, Nature 485(7397) (2012) 207-12.

[32] S.E. Mansoor, W. Lu, W. Oosterheert, M. Shekhar, E. Tajkhorshid, E. Gouaux, X-ray structures define human P2X3 receptor gating cycle and antagonist action, Nature 538(7623) (2016) 66-71.

[33] A. Karasawa, T. Kawate, Structural basis for subtype-specific inhibition of the P2X7 receptor, Elife 5 (2016).

ACCEPTED MANUSCRIPT

[34] L. Grimes, M.T. Young, Purinergic P2X receptors: structural and functional features depicted by X-ray and molecular modelling studies, Curr Med Chem 22(7) (2015) 783-98.

[35] R.A. North, M.F. Jarvis, P2X receptors as drug targets, Mol Pharmacol 83(4) (2013) 759-69.

[36] M. Kim, L.H. Jiang, H.L. Wilson, R.A. North, A. Surprenant, Proteomic and functional evidence for a P2X7 receptor signalling complex, EMBO J 20(22) (2001) 6347-58.

[37] E. Adinolfi, M. Kim, M.T. Young, F. Di Virgilio, A. Surprenant, Tyrosine phosphorylation of HSP90 within the P2X7 receptor complex negatively regulates P2X7 receptors, J Biol Chem 278(39) (2003) 37344-51.

[38] P. Pelegrin, A. Surprenant, Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor, EMBO J 25(21) (2006) 5071-82.

[39] C. Guo, M. Masin, O.S. Qureshi, R.D. Murrell-Lagnado, Evidence for functional P2X4/P2X7 heteromeric receptors, Mol Pharmacol 72(6) (2007) 1447-56.

[40] H.L. Wilson, S.A. Wilson, A. Surprenant, R.A. North, Epithelial membrane proteins induce membrane blebbing and interact with the P2X7 receptor C terminus, J Biol Chem 277(37) (2002) 34017-23.

[41] K.A. Jacobson, C.E. Muller, Medicinal chemistry of adenosine, P2Y and P2X receptors, Neuropharmacology 104 (2016) 31-49.

[42] A. Elssner, M. Duncan, M. Gavrilin, M.D. Wewers, A novel P2X7 receptor activator, the human cathelicidin-derived peptide LL37, induces IL-1 beta processing and release, J Immunol 172(8) (2004) 4987-94.

[43] L. Tomasinsig, C. Pizzirani, B. Skerlavaj, P. Pellegatti, S. Gulinelli, A. Tossi, F. Di Virgilio, M. Zanetti, The human cathelicidin LL-37 modulates the activities of the P2X7 receptor in a structure-dependent manner, J Biol Chem 283(45) (2008) 30471-81.

[44] J.M. Sanz, P. Chiozzi, D. Ferrari, M. Colaianna, M. Idzko, S. Falzoni, R. Fellin, L. Trabace, F. Di Virgilio, Activation of microglia by amyloid {beta} requires P2X7 receptor expression, J Immunol 182(7) (2009) 4378-85.

ACCEPTED MANUSCRIPT

[45] J.M. Sanz, P. Chiozzi, F. Di Virgilio, Tenidap enhances P2Z/P2X7 receptor signalling in macrophages, Eur J Pharmacol 355(2-3) (1998) 235-44.

[46] D. Ferrari, C. Pizzirani, E. Adinolfi, S. Forchap, B. Sitta, L. Turchet, S. Falzoni, M. Minelli, R. Baricordi, F. Di Virgilio, The antibiotic polymyxin B modulates P2X7 receptor function, J Immunol 173(7) (2004) 4652-60.

[47] R.M. Helliwell, C.O. ShioukHuey, K. Dhuna, J.C. Molero, J.M. Ye, C.C. Xue, L. Stokes, Selected ginsenosides of the protopanaxdiol series are novel positive allosteric modulators of P2X7 receptors, Br J Pharmacol 172(13) (2015) 3326-40.

[48] L.H. Jiang, A.B. Mackenzie, R.A. North, A. Surprenant, Brilliant blue G selectively blocks ATP-gated rat P2X(7) receptors, Mol Pharmacol 58(1) (2000) 82-8.

[49] C.E. Gargett, J.S. Wiley, The isoquinoline derivative KN-62 a potent antagonist of the P2Z-receptor of human lymphocytes, Br J Pharmacol 120(8) (1997) 1483-90.

[50] R.D. Beigi, S.B. Kertesy, G. Aquilina, G.R. Dubyak, Oxidized ATP (oATP) attenuates proinflammatory signaling via P2 receptor-independent mechanisms, Br J Pharmacol 140(3) (2003) 507-19.

[51] P. Dauch, H. Barelli, J.P. Vincent, F. Checler, Fluorimetric assay of the neurotensin-degrading metalloendopeptidase, endopeptidase 24.16, Biochem J 280 (Pt 2) (1991) 421-6.

[52] D.L. Donnelly-Roberts, M.T. Namovic, P. Han, M.F. Jarvis, Mammalian P2X7 receptor pharmacology: comparison of recombinant mouse, rat and human P2X7 receptors, Br J Pharmacol 157(7) (2009) 1203-14.

[53] A. Brandao-Burch, M.L. Key, J.J. Patel, T.R. Arnett, I.R. Orriss, The P2X7 Receptor is an Important Regulator of Extracellular ATP Levels, Front Endocrinol (Lausanne) 3 (2012) 41.

[54] A. Bhattacharya, Q. Wang, H. Ao, J.R. Shoblock, B. Lord, L. Aluisio, I. Fraser, D. Nepomuceno, R.A. Neff, N. Welty, T.W. Lovenberg, P. Bonaventure, A.D. Wickenden, M.A. Letavic, Pharmacological characterization of a novel centrally permeable P2X7 receptor antagonist: JNJ-47965567, Br J Pharmacol 170(3) (2013) 624-40.

ACCEPTED MANUSCRIPT

[55] B. Lord, L. Aluisio, J.R. Shoblock, R.A. Neff, E.I. Varlinskaya, M. Ceusters, T.W. Lovenberg, N. Carruthers, P. Bonaventure, M.A. Letavic, T. Deak, W. Drinkenburg, A. Bhattacharya, Pharmacology of a novel central nervous system-penetrant P2X7 antagonist JNJ-42253432, J Pharmacol Exp Ther 351(3) (2014) 628-41.

[56] D.M. Swanson, B.M. Savall, K.J. Coe, F. Schoetens, T. Koudriakova, J. Skaptason, J. Wall, J. Rech, X. Deng, M. De Angelis, A. Everson, B. Lord, Q. Wang, H. Ao, B. Scott, K. Sepassi, T.W. Lovenberg, N.I. Carruthers, A. Bhattacharya, M.A. Letavic, Identification of (R)-(2-Chloro-3-(trifluoromethyl)phenyl)(1-(5-fluoropyridin-2-yl)-4-methyl-6,7-di hydro-1H-imidazo[4,5-c]pyridin-5(4H)-yl)methanone (JNJ 54166060), a Small Molecule Antagonist of the P2X7 receptor, J Med Chem 59(18) (2016) 8535-48.

[57] E.A. Caseley, S.P. Muench, S.A. Baldwin, K. Simmons, C.W. Fishwick, L.H. Jiang, Docking of competitive inhibitors to the P2X7 receptor family reveals key differences responsible for changes in response between rat and human, Bioorg Med Chem Lett 25(16) (2015) 3164-7.

[58] M.H. Abdi, P.J. Beswick, A. Billinton, L.J. Chambers, A. Charlton, S.D. Collins, K.L. Collis, D.K. Dean, E. Fonfria, R.J. Gleave, C.L. Lejeune, D.G. Livermore, S.J. Medhurst, A.D. Michel, A.P. Moses, L. Page, S. Patel, S.A. Roman, S. Senger, B. Slingsby, J.G. Steadman, A.J. Stevens, D.S. Walter, Discovery and structure-activity relationships of a series of pyroglutamic acid amide antagonists of the P2X7 receptor, Bioorg Med Chem Lett 20(17) (2010) 5080-4.

[59] D. Ory, S. Celen, R. Gijsbers, C. Van Den Haute, A. Postnov, M. Koole, C. Vandeputte, J.I. Andres, J. Alcazar, M. De Angelis, X. Langlois, A. Bhattacharya, M. Schmidt, M.A. Letavic, W. Vanduffel, K. Van Laere, A. Verbruggen, Z. Debyser, G. Bormans, Preclinical Evaluation of a P2X7 Receptor-Selective Radiotracer: PET Studies in a Rat Model with Local Overexpression of the Human P2X7 Receptor and in Nonhuman Primates, J Nucl Med 57(9) (2016) 1436-41.

[60] P.R. Territo, J.A. Meyer, J.S. Peters, A.A. Riley, B.P. McCarthy, M. Gao, M. Wang, M.A. Green, Q.H. Zheng, G.D. Hutchins, Characterization of (11)C-GSK1482160 for Targeting the P2X7 Receptor as a Biomarker for Neuroinflammation, J Nucl Med 58(3) (2017) 458-465.

ACCEPTED MANUSCRIPT

[61] E.R. Fantoni, D. Dal Ben, S. Falzoni, F. Di Virgilio, S. Lovestone, A. Gee, Design, synthesis and evaluation in an LPS rodent model of neuroinflammation of a novel (18)F-labelled PET tracer targeting P2X7, EJNMMI Res 7(1) (2017) 31.

[62] P. Honore, D. Donnelly-Roberts, M.T. Namovic, G. Hsieh, C.Z. Zhu, J.P. Mikusa, G. Hernandez, C. Zhong, D.M. Gauvin, P. Chandran, R. Harris, A.P. Medrano, W. Carroll, K. Marsh, J.P. Sullivan, C.R. Faltynek, M.F. Jarvis, A-740003 [N-(1-{[(cyanoimino)(5-quinolinylamino) methyl]amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide], a novel and selective P2X7 receptor antagonist, dose-dependently reduces neuropathic pain in the rat, J Pharmacol Exp Ther 319(3) (2006) 1376-85.

[63] R.C. Allsopp, S. Dayl, R. Schmid, R.J. Evans, Unique residues in the ATP gated human P2X7 receptor define a novel allosteric binding pocket for the selective antagonist AZ10606120, Sci Rep 7(1) (2017) 725.

[64] R.A. North, P2X receptors, Philos Trans R Soc Lond B Biol Sci 371(1700) (2016).

[65] W. Danquah, C. Meyer-Schwesinger, B. Rissiek, C. Pinto, A. Serracant-Prat, M. Amadi, D. Iacenda, J.H. Knop, A. Hammel, P. Bergmann, N. Schwarz, J. Assuncao, W. Rotthier, F. Haag, E. Tolosa, P. Bannas, E. Boue-Grabot, T. Magnus, T. Laeremans, C. Stortelers, F. Koch-Nolte, Nanobodies that block gating of the P2X7 ion channel ameliorate inflammation, Sci Transl Med 8(366) (2016) 366ra162.

[66] S.M. Gilbert, A. Gidley Baird, S. Glazer, J.A. Barden, A. Glazer, L.C. Teh, J. King, A phase I clinical trial demonstrates that nfP2X7 -targeted antibodies provide a novel, safe and tolerable topical therapy for basal cell carcinoma, Br J Dermatol 177(1) (2017) 117-124.

[67] A. Molloy, P. Laochumroonvorapong, G. Kaplan, Apoptosis, but not necrosis, of infected monocytes is coupled with killing of intracellular bacillus Calmette-Guerin, J Exp Med 180(4) (1994) 1499-509.

ACCEPTED MANUSCRIPT

[68] R. Coutinho-Silva, L. Stahl, M.N. Raymond, T. Jungas, P. Verbeke, G. Burnstock, T. Darville, D.M. Ojcius, Inhibition of chlamydial infectious activity due to P2X7R-dependent phospholipase D activation, Immunity 19(3) (2003) 403-12.

[69] G. Correa, C. Marques da Silva, A.C. de Abreu Moreira-Souza, R.C. Vommaro, R. Coutinho-Silva, Activation of the P2X(7) receptor triggers the elimination of Toxoplasma gondii tachyzoites from infected macrophages, Microbes Infect 12(6) (2010) 497-504.

[70] M.P. Lees, S.J. Fuller, R. McLeod, N.R. Boulter, C.M. Miller, A.M. Zakrzewski, E.J. Mui, W.H. Witola, J.J. Coyne, A.C. Hargrave, S.E. Jamieson, J.M. Blackwell, J.S. Wiley, N.C. Smith, P2X7 receptor-mediated killing of an intracellular parasite, Toxoplasma gondii, by human and murine macrophages, J Immunol 184(12) (2010) 7040-6.

[71] S.P. Chaves, E.C. Torres-Santos, C. Marques, V.R. Figliuolo, P.M. Persechini, R. Coutinho-Silva, B. Rossi-Bergmann, Modulation of P2X(7) purinergic receptor in macrophages by Leishmania amazonensis and its role in parasite elimination, Microbes Infect 11(10-11) (2009) 842-9.

[72] A.C. Morandini, L.E. Savio, R. Coutinho-Silva, The role of P2X7 receptor in infectious inflammatory diseases and the influence of ectonucleotidases, Biomed J 37(4) (2014) 169-77.

[73] T. Darville, L. Welter-Stahl, C. Cruz, A.A. Sater, C.W. Andrews, Jr., D.M. Ojcius, Effect of the purinergic receptor P2X7 on Chlamydia infection in cervical epithelial cells and vaginally infected mice, J Immunol 179(6) (2007) 3707-14.

[74] V.R. Figliuolo, S.P. Chaves, L.E. Savio, M.L. Thorstenberg, E. Machado Salles, C.M. Takiya, M.R. D'Imperio-Lima, H.L. de Matos Guedes, B. Rossi-Bergmann, R. Coutinho-Silva, The role of the P2X7 receptor in murine cutaneous leishmaniasis: aspects of inflammation and parasite control, Purinergic Signal (2016).

[75] E.P. Amaral, S.C. Ribeiro, V.R. Lanes, F.M. Almeida, M.R. de Andrade, C.C. Bomfim, E.M. Salles, K.R. Bortoluci, R. Coutinho-Silva, M.H. Hirata, J.M. Alvarez, E.B. Lasunskaia, M.R.

ACCEPTED MANUSCRIPT

D'Imperio-Lima, Pulmonary infection with hypervirulent Mycobacteria reveals a crucial role for the P2X7 receptor in aggressive forms of tuberculosis, PLoS Pathog 10(7) (2014) e1004188.

[76] F. Di Virgilio, The P2Z purinoceptor: an intriguing role in immunity, inflammation and cell death, Immunol Today 16(11) (1995) 524-8.

[77] I. Lemaire, S. Falzoni, N. Leduc, B. Zhang, P. Pellegatti, E. Adinolfi, P. Chiozzi, F. Di Virgilio, Involvement of the purinergic P2X7 receptor in the formation of multinucleated giant cells, J Immunol 177(10) (2006) 7257-65.

[78] I. Lemaire, S. Falzoni, E. Adinolfi, Purinergic signaling in giant cell formation, Front Biosci (Elite Ed) 4 (2012) 41-55.

[79] E.M. Salles, M.N. Menezes, R. Siqueira, H. Borges da Silva, E.P. Amaral, S.I. Castillo-Mendez, I. Cunha, A.D.A. Cassado, F.S. Vieira, D.N. Olivieri, C.E. Tadokoro, J.M. Alvarez, R. Coutinho-Silva, M.R. D'Imperio-Lima, P2X7 receptor drives Th1 cell differentiation and controls the follicular helper T cell population to protect against Plasmodium chabaudi malaria, PLoS Pathog 13(8) (2017) e1006595.

[80] G. Correa, A.L.C. de, C. Fernandes-Santos, M. Gandini, F. Petitinga Paiva, R. Coutinho-Silva, F.K. C, The purinergic receptor P2X7 role in control of Dengue virus-2 infection and cytokine/chemokine production in infected human monocytes, Immunobiology 221(7) (2016) 794-802.

[81] B.H. Lee, D.M. Hwang, N. Palaniyar, S. Grinstein, D.J. Philpott, J. Hu, Activation of P2X(7) receptor by ATP plays an important role in regulating inflammatory responses during acute viral infection, PLoS One 7(4) (2012) e35812.

[82] J.M. Taylor, Z. Han, Purinergic receptor functionality is necessary for infection of human hepatocytes by hepatitis delta virus and hepatitis B virus, PLoS One 5(12) (2010) e15784.

[83] T.H. Swartz, G.R. Dubyak, B.K. Chen, Purinergic Receptors: Key Mediators of HIV-1 Infection and Inflammation, Front Immunol 6 (2015) 585.

ACCEPTED MANUSCRIPT

[84] B.J. Fowler, B.D. Gelfand, Y. Kim, N. Kerur, V. Tarallo, Y. Hirano, S. Amarnath, D.H. Fowler, M. Radwan, M.T. Young, K. Pittman, P. Kubes, H.K. Agarwal, K. Parang, D.R. Hinton, A. Bastos-Carvalho, S. Li, T. Yasuma, T. Mizutani, R. Yasuma, C. Wright, J. Ambati, Nucleoside reverse transcriptase inhibitors possess intrinsic anti-inflammatory activity, Science 346(6212) (2014) 1000-3.

[85] F. Graziano, M. Desdouits, L. Garzetti, P. Podini, M. Alfano, A. Rubartelli, R. Furlan, P. Benaroch, G. Poli, Extracellular ATP induces the rapid release of HIV-1 from virus containing compartments of human macrophages, Proc Natl Acad Sci U S A 112(25) (2015) E3265-73.

[86] I. Menkova-Garnier, H. Hocini, E. Foucat, P. Tisserand, L. Bourdery, C. Delaugerre, C. Benne, Y. Levy, J.D. Lelievre, P2X7 Receptor Inhibition Improves CD34 T-Cell Differentiation in HIV-Infected Immunological Nonresponders on c-ART, PLoS Pathog 12(4) (2016) e1005571.

[87] D. Ferrari, C. Pizzirani, E. Adinolfi, R.M. Lemoli, A. Curti, M. Idzko, E. Panther, F. Di Virgilio, The P2X7 receptor: a key player in IL-1 processing and release, J Immunol 176(7) (2006) 3877-83.

[88] A. Piccini, S. Carta, S. Tassi, D. Lasiglie, G. Fossati, A. Rubartelli, ATP is released by monocytes stimulated with pathogen-sensing receptor ligands and induces IL-1beta and IL-18 secretion in an autocrine way, Proc Natl Acad Sci U S A 105(23) (2008) 8067-72.

[89] C.A. Dinarello, Immunological and inflammatory functions of the interleukin-1 family, Annu Rev Immunol 27 (2009) 519-50.

[90] F. Di Virgilio, The therapeutic potential of modifying inflammasomes and NOD-like receptors, Pharmacol Rev 65(3) (2013) 872-905.

[91] J.S. Ainscough, G. Frank Gerberick, M. Zahedi-Nejad, G. Lopez-Castejon, D. Brough, I. Kimber, R.J. Dearman, Dendritic cell IL-1alpha and IL-1beta are polyubiquitinated and degraded by the proteasome, J Biol Chem 289(51) (2014) 35582-92.

ACCEPTED MANUSCRIPT

[92] N.A. Thornberry, H.G. Bull, J.R. Calaycay, K.T. Chapman, A.D. Howard, M.J. Kostura, D.K. Miller, S.M. Molineaux, J.R. Weidner, J. Aunins, et al., A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes, Nature 356(6372) (1992) 768-74.

[93] F. Martinon, K. Burns, J. Tschopp, The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta, Mol Cell 10(2) (2002) 417-26.

[94] Y. Ogura, F.S. Sutterwala, R.A. Flavell, The inflammasome: first line of the immune response to cell stress, Cell 126(4) (2006) 659-62.

[95] S. Benko, D.J. Philpott, S.E. Girardin, The microbial and danger signals that activate Nod-like receptors, Cytokine 43(3) (2008) 368-73.

[96] T. Prochnicki, M.S. Mangan, E. Latz, Recent insights into the molecular mechanisms of the NLRP3 inflammasome activation, F1000Res 5 (2016).

[97] Y.K. Kim, J.S. Shin, M.H. Nahm, NOD-Like Receptors in Infection, Immunity, and Diseases, Yonsei Med J 57(1) (2016) 5-14.

[98] D. Ferrari, P. Chiozzi, S. Falzoni, S. Hanau, F. Di Virgilio, Purinergic modulation of interleukin-1 beta release from microglial cells stimulated with bacterial endotoxin, J Exp Med 185(3) (1997) 579-82.

[99] D. Perregaux, C.A. Gabel, Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity, J Biol Chem 269(21) (1994) 15195-203.

[100] P.C. Englezou, S.W. Rothwell, J.S. Ainscough, D. Brough, R. Landsiedel, A. Verkhratsky, I. Kimber, R.J. Dearman, P2X7R activation drives distinct IL-1 responses in dendritic cells compared to macrophages, Cytokine 74(2) (2015) 293-304.

[101] C. Pizzirani, S. Falzoni, M. Govoni, R. La Corte, S. Donadei, F. Di Virgilio, F. Trotta, A. Lo Monaco, Dysfunctional inflammasome in Schnitzler's syndrome, Rheumatology (Oxford) 48(10) (2009) 1304-8.

ACCEPTED MANUSCRIPT

[102] M. Karmakar, M.A. Katsnelson, G.R. Dubyak, E. Pearlman, Neutrophil P2X7 receptors mediate NLRP3 inflammasome-dependent IL-1beta secretion in response to ATP, Nat Commun 7 (2016) 10555.

[103] A. Gustin, M. Kirchmeyer, E. Koncina, P. Felten, S. Losciuto, T. Heurtaux, A. Tardivel, P. Heuschling, C. Dostert, NLRP3 Inflammasome Is Expressed and Functional in Mouse Brain Microglia but Not in Astrocytes, PLoS One 10(6) (2015) e0130624.

[104] T. Gicquel, S. Robert, P. Loyer, T. Victoni, A. Bodin, C. Ribault, F. Gleonnec, I. Couillin, E. Boichot, V. Lagente, IL-1beta production is dependent on the activation of purinergic receptors and NLRP3 pathway in human macrophages, FASEB J 29(10) (2015) 4162-73.

[105] V. Petrilli, S. Papin, C. Dostert, A. Mayor, F. Martinon, J. Tschopp, Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration, Cell Death Differ 14(9) (2007) 1583-9.

[106] O. Gross, C.J. Thomas, G. Guarda, J. Tschopp, The inflammasome: an integrated view, Immunol Rev 243(1) (2011) 136-51.

[107] R. Munoz-Planillo, P. Kuffa, G. Martinez-Colon, B.L. Smith, T.M. Rajendiran, G. Nunez, K(+) efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter, Immunity 38(6) (2013) 1142-53.

[108] Y. He, M.Y. Zeng, D. Yang, B. Motro, G. Nunez, NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux, Nature 530(7590) (2016) 354-7.

[109] H. Shi, Y. Wang, X. Li, X. Zhan, M. Tang, M. Fina, L. Su, D. Pratt, C.H. Bu, S. Hildebrand, S. Lyon, L. Scott, J. Quan, Q. Sun, J. Russell, S. Arnett, P. Jurek, D. Chen, V.V. Kravchenko, J.C. Mathison, E.M. Moresco, N.L. Monson, R.J. Ulevitch, B. Beutler, NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component, Nat Immunol 17(3) (2016) 250-8.

ACCEPTED MANUSCRIPT

[110] J.R. Yaron, S. Gangaraju, M.Y. Rao, X. Kong, L. Zhang, F. Su, Y. Tian, H.L. Glenn, D.R. Meldrum, K(+) regulates Ca(2+) to drive inflammasome signaling: dynamic visualization of ion flux in live cells, Cell Death Dis 6 (2015) e1954.

[111] A.S. Rothmeier, P. Marchese, B.G. Petrich, C. Furlan-Freguia, M.H. Ginsberg, Z.M. Ruggeri, W. Ruf, Caspase-1-mediated pathway promotes generation of thromboinflammatory microparticles, J Clin Invest 125(4) (2015) 1471-84.

[112] Y.H. Liao, Y.C. Lin, S.T. Tsao, Y.C. Lin, A.J. Yang, C.T. Huang, K.C. Huang, W.W. Lin, HMG-CoA reductase inhibitors activate caspase-1 in human monocytes depending on ATP release and P2X7 activation, J Leukoc Biol 93(2) (2013) 289-99.

[113] S.C. Hung, C.H. Choi, N. Said-Sadier, L. Johnson, K.R. Atanasova, H. Sellami, O. Yilmaz, D.M. Ojcius, P2X4 assembles with P2X7 and pannexin-1 in gingival epithelial cells and modulates ATP-induced reactive oxygen species production and inflammasome activation, PLoS One 8(7) (2013) e70210.

[114] J. Minkiewicz, J.P. de Rivero Vaccari, R.W. Keane, Human astrocytes express a novel NLRP2 inflammasome, Glia 61(7) (2013) 1113-21.

[115] A. Franceschini, M. Capece, P. Chiozzi, S. Falzoni, J.M. Sanz, A.C. Sarti, M. Bonora, P. Pinton, F. Di Virgilio, The P2X7 receptor directly interacts with the NLRP3 inflammasome scaffold protein, FASEB J 29(6) (2015) 2450-61.

[116] E. Salaro, A. Rambaldi, S. Falzoni, F.S. Amoroso, A. Franceschini, A.C. Sarti, M. Bonora, F. Cavazzini, G.M. Rigolin, M. Ciccone, V. Audrito, S. Deaglio, P. Pelegrin, P. Pinton, A. Cuneo, F. Di Virgilio, Involvement of the P2X7-NLRP3 axis in leukemic cell proliferation and death, Sci Rep 6 (2016) 26280.

[117] A. de Gassart, F. Martinon, Pyroptosis: Caspase-11 Unlocks the Gates of Death, Immunity 43(5) (2015) 835-7.

[118] T. Bergsbaken, S.L. Fink, B.T. Cookson, Pyroptosis: host cell death and inflammation, Nat Rev Microbiol 7(2) (2009) 99-109.

ACCEPTED MANUSCRIPT

[119] D. Yang, Y. He, R. Munoz-Planillo, Q. Liu, G. Nunez, Caspase-11 Requires the Pannexin-1 Channel and the Purinergic P2X7 Pore to Mediate Pyroptosis and Endotoxic Shock, Immunity 43(5) (2015) 923-32.

[120] D. Ferrari, S. Wesselborg, M.K. Bauer, K. Schulze-Osthoff, Extracellular ATP activates transcription factor NF-kappaB through the P2Z purinoreceptor by selectively targeting NF-kappaB p65, J Cell Biol 139(7) (1997) 1635-43.

[121] J. Korcok, L.N. Raimundo, H.Z. Ke, S.M. Sims, S.J. Dixon, Extracellular nucleotides act through P2X7 receptors to activate NF-kappaB in osteoclasts, J Bone Miner Res 19(4) (2004) 642-51.

[122] D.C. Genetos, N.J. Karin, D.J. Geist, H.J. Donahue, R.L. Duncan, Purinergic signaling is required for fluid shear stress-induced NF-kappaB translocation in osteoblasts, Exp Cell Res 317(6) (2011) 737-44.

[123] C.C. Marques, M.T. Castelo-Branco, R.G. Pacheco, F. Buongusto, A. do Rosario, Jr., A. Schanaider, R. Coutinho-Silva, H.S. de Souza, Prophylactic systemic P2X7 receptor blockade prevents experimental colitis, Biochim Biophys Acta 1842(1) (2014) 65-78.

[124] C. Huang, W. Yu, H. Cui, Y. Wang, L. Zhang, F. Han, T. Huang, P2X7 blockade attenuates mouse liver fibrosis, Mol Med Rep 9(1) (2014) 57-62.

[125] X. Chang, H. He, L. Zhu, J. Gao, T. Wei, Z. Ma, T. Yan, Protective effect of apigenin on Freund's complete adjuvant-induced arthritis in rats via inhibiting P2X7/NF-kappaB pathway, Chem Biol Interact 236 (2015) 41-6.

[126] K. Zhang, J. Liu, X. You, P. Kong, Y. Song, L. Cao, S. Yang, W. Wang, Q. Fu, Z. Ma, P2X7 as a new target for chrysophanol to treat lipopolysaccharide-induced depression in mice, Neurosci Lett 613 (2016) 60-5.

[127] Y. Liu, Y. Xiao, Z. Li, P2X7 receptor positively regulates MyD88-dependent NF-kappaB activation, Cytokine 55(2) (2011) 229-36.

ACCEPTED MANUSCRIPT

[128] O.R. Baricordi, D. Ferrari, L. Melchiorri, P. Chiozzi, S. Hanau, E. Chiari, M. Rubini, F. Di Virgilio, An ATP-activated channel is involved in mitogenic stimulation of human T lymphocytes, Blood 87(2) (1996) 682-90.

[129] D. Ferrari, C. Stroh, K. Schulze-Osthoff, P2X7/P2Z purinoreceptor-mediated activation of transcription factor NFAT in microglial cells, J Biol Chem 274(19) (1999) 13205-10.

[130] A. Vergani, S. Tezza, F. D'Addio, C. Fotino, K. Liu, M. Niewczas, R. Bassi, R.D. Molano, S. Kleffel, A. Petrelli, A. Soleti, E. Ammirati, M. Frigerio, G. Visner, F. Grassi, M.E. Ferrero, D. Corradi, R. Abdi, C. Ricordi, M.H. Sayegh, A. Pileggi, P. Fiorina, Long-term heart transplant survival by targeting the ionotropic purinergic receptor P2X7, Circulation 127(4) (2013) 463-75.

[131] A. Vergani, C. Fotino, F. D'Addio, S. Tezza, M. Podetta, F. Gatti, M. Chin, R. Bassi, R.D. Molano, D. Corradi, R. Gatti, M.E. Ferrero, A. Secchi, F. Grassi, C. Ricordi, M.H. Sayegh, P. Maffi, A. Pileggi, P. Fiorina, Effect of the purinergic inhibitor oxidized ATP in a model of islet allograft rejection, Diabetes 62(5) (2013) 1665-75.

[132] T. Yu, W.G. Junger, C. Yuan, A. Jin, Y. Zhao, X. Zheng, Y. Zeng, J. Liu, Shockwaves increase T-cell proliferation and IL-2 expression through ATP release, P2X7 receptors, and FAK activation, Am J Physiol Cell Physiol 298(3) (2010) C457-64.

[133] L. Yip, T. Woehrle, R. Corriden, M. Hirsh, Y. Chen, Y. Inoue, V. Ferrari, P.A. Insel, W.G. Junger, Autocrine regulation of T-cell activation by ATP release and P2X7 receptors, FASEB J 23(6) (2009) 1685-93.

[134] E. Adinolfi, M.G. Callegari, M. Cirillo, P. Pinton, C. Giorgi, D. Cavagna, R. Rizzuto, F. Di Virgilio, Expression of the P2X7 receptor increases the Ca2+ content of the endoplasmic reticulum, activates NFATc1, and protects from apoptosis, J Biol Chem 284(15) (2009) 10120-8.

[135] E. Adinolfi, M. Cirillo, R. Woltersdorf, S. Falzoni, P. Chiozzi, P. Pellegatti, M.G. Callegari, D. Sandona, F. Markwardt, G. Schmalzing, F. Di Virgilio, Trophic activity of a naturally occurring truncated isoform of the P2X7 receptor, FASEB J 24(9) (2010) 3393-404.

ACCEPTED MANUSCRIPT

[136] M.W. Grol, A. Pereverzev, S.M. Sims, S.J. Dixon, P2 receptor networks regulate signaling duration over a wide dynamic range of ATP concentrations, J Cell Sci 126(Pt 16) (2013) 3615-26.

[137] A.L. Giuliani, D. Colognesi, T. Ricco, C. Roncato, M. Capece, F. Amoroso, Q.G. Wang, E. De Marchi, A. Gartland, F. Di Virgilio, E. Adinolfi, Trophic activity of human P2X7 receptor isoforms A and B in osteosarcoma, PLoS One 9(9) (2014) e107224.

[138] E. Adinolfi, L. Raffaghello, A.L. Giuliani, L. Cavazzini, M. Capece, P. Chiozzi, G. Bianchi, G. Kroemer, V. Pistoia, F. Di Virgilio, Expression of P2X7 receptor increases in vivo tumor growth, Cancer Res 72(12) (2012) 2957-69.

[139] E. Adinolfi, F. Amoroso, A.L. Giuliani, P2X7 Receptor Function in Bone-Related Cancer, J Osteoporos 2012 (2012) 637863.

[140] F. Amoroso, M. Capece, A. Rotondo, D. Cangelosi, M. Ferracin, A. Franceschini, L. Raffaghello, V. Pistoia, L. Varesio, E. Adinolfi, The P2X7 receptor is a key modulator of the PI3K/GSK3beta/VEGF signaling network: evidence in experimental neuroblastoma, Oncogene 34(41) (2015) 5240-51.

[141] R. Gomez-Villafuertes, A. del Puerto, M. Diaz-Hernandez, D. Bustillo, J.I. Diaz-Hernandez, P.G. Huerta, A.R. Artalejo, J.J. Garrido, M.T. Miras-Portugal, Ca2+/calmodulin-dependent kinase II signalling cascade mediates P2X7 receptor-dependent inhibition of neuritogenesis in neuroblastoma cells, FEBS J 276(18) (2009) 5307-25.

[142] M. Martin, K. Rehani, R.S. Jope, S.M. Michalek, Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3, Nat Immunol 6(8) (2005) 777-84.

[143] E. Beurel, S.M. Michalek, R.S. Jope, Innate and adaptive immune responses regulated by glycogen synthase kinase-3 (GSK3), Trends Immunol 31(1) (2010) 24-31.

[144] M. Barbera-Cremades, A. Baroja-Mazo, A.I. Gomez, F. Machado, F. Di Virgilio, P. Pelegrin, P2X7 receptor-stimulation causes fever via PGE2 and IL-1beta release, FASEB J 26(7) (2012) 2951-62.

ACCEPTED MANUSCRIPT

[145] J. Anrather, E.F. Gallo, T. Kawano, M. Orio, T. Abe, C. Gooden, P. Zhou, C. Iadecola, Purinergic signaling induces cyclooxygenase-1-dependent prostanoid synthesis in microglia: roles in the outcome of excitotoxic brain injury, PLoS One 6(10) (2011) e25916.

[146] P. Chotjumlong, J.G. Bolscher, K. Nazmi, V. Reutrakul, C. Supanchart, W. Buranaphatthana, S. Krisanaprakornkit, Involvement of the P2X7 purinergic receptor and c-Jun N-terminal and extracellular signal-regulated kinases in cyclooxygenase-2 and prostaglandin E2 induction by LL-37, J Innate Immun 5(1) (2013) 72-83.

[147] Y. Toki, T. Takenouchi, H. Harada, S. Tanuma, H. Kitani, S. Kojima, M. Tsukimoto, Extracellular ATP induces P2X7 receptor activation in mouse Kupffer cells, leading to release of IL-1beta, HMGB1, and PGE2, decreased MHC class I expression and necrotic cell death, Biochem Biophys Res Commun 458(4) (2015) 771-6.

[148] H.Y. Lin, C.H. Tsai, C. Lin, W.L. Yeh, C.F. Tsai, P.C. Chang, L.H. Wu, D.Y. Lu, Cobalt Protoporphyrin Upregulates Cyclooxygenase-2 Expression Through a Heme Oxygenase-Independent Mechanism, Mol Neurobiol 53(7) (2016) 4497-508.

[149] B.F. King, Novel P2X7 receptor antagonists ease the pain, Br J Pharmacol 151(5) (2007) 565-7.

[150] L.A. Alves, R.J. Bezerra, R.X. Faria, L.G. Ferreira, V. da Silva Frutuoso, Physiological roles and potential therapeutic applications of the P2X7 receptor in inflammation and pain, Molecules 18(9) (2013) 10953-72.

[151] M. Wan, O. Soehnlein, X. Tang, A.M. van der Does, E. Smedler, P. Uhlen, L. Lindbom, B. Agerberth, J.Z. Haeggstrom, Cathelicidin LL-37 induces time-resolved release of LTB4 and TXA2 by human macrophages and triggers eicosanoid generation in vivo, FASEB J 28(8) (2014) 3456-67.

[152] S. Nylander, R. Schulz, Effects of P2Y12 receptor antagonists beyond platelet inhibition--comparison of ticagrelor with thienopyridines, Br J Pharmacol 173(7) (2016) 1163-78.

[153] E. Adinolfi, M. Capece, F. Amoroso, E. De Marchi, A. Franceschini, Emerging roles of P2X receptors in cancer, Curr Med Chem 22(7) (2015) 878-90.

ACCEPTED MANUSCRIPT

[154] L.M. Hill, M.L. Gavala, L.Y. Lenertz, P.J. Bertics, Extracellular ATP may contribute to tissue repair by rapidly stimulating purinergic receptor X7-dependent vascular endothelial growth factor release from primary human monocytes, J Immunol 185(5) (2010) 3028-34.

[155] H.C. Tung, F.Y. Lee, S.S. Wang, M.H. Tsai, J.Y. Lee, T.I. Huo, H.C. Huang, C.L. Chuang, H.C. Lin, S.D. Lee, The Beneficial Effects of P2X7 Antagonism in Rats with Bile Duct Ligation-induced Cirrhosis, PLoS One 10(5) (2015) e0124654.

[156] T. Mizutani, B.J. Fowler, Y. Kim, R. Yasuma, L.A. Krueger, B.D. Gelfand, J. Ambati, Nucleoside Reverse Transcriptase Inhibitors Suppress Laser-Induced Choroidal Neovascularization in Mice, Invest Ophthalmol Vis Sci 56(12) (2015) 7122-9.

[157] F. Amoroso, S. Falzoni, E. Adinolfi, D. Ferrari, F. Di Virgilio, The P2X7 receptor is a key modulator of aerobic glycolysis, Cell Death Dis 3 (2012) e370.

[158] M. Tafani, L. Schito, L. Pellegrini, L. Villanova, G. Marfe, T. Anwar, R. Rosa, M. Indelicato, M. Fini, B. Pucci, M.A. Russo, Hypoxia-increased RAGE and P2X7R expression regulates tumor cell invasion through phosphorylation of Erk1/2 and Akt and nuclear translocation of NF-{kappa}B, Carcinogenesis 32(8) (2011) 1167-75.

[159] M. Tafani, E. De Santis, L. Coppola, G.A. Perrone, I. Carnevale, A. Russo, B. Pucci, A. Carpi, M. Bizzarri, M.A. Russo, Bridging hypoxia, inflammation and estrogen receptors in thyroid cancer progression, Biomed Pharmacother 68(1) (2014) 1-5.

[160] B. Sperlagh, E.S. Vizi, K. Wirkner, P. Illes, P2X7 receptors in the nervous system, Prog Neurobiol 78(6) (2006) 327-46.

[161] L.Y. Lenertz, M.L. Gavala, Y. Zhu, P.J. Bertics, Transcriptional control mechanisms associated with the nucleotide receptor P2X7, a critical regulator of immunologic, osteogenic, and neurologic functions, Immunol Res 50(1) (2011) 22-38.

[162] M.T. Miras-Portugal, A. Sebastian-Serrano, L. de Diego Garcia, M. Diaz-Hernandez, Neuronal P2X7 Receptor: Involvement in Neuronal Physiology and Pathology, J Neurosci 37(30) (2017) 7063-7072.

ACCEPTED MANUSCRIPT

[163] P. Illes, T.M. Khan, P. Rubini, Neuronal P2X7 Receptors Revisited: Do They Really Exist?, J Neurosci 37(30) (2017) 7049-7062.

[164] L. Steinman, Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system, Cell 85(3) (1996) 299-302.

[165] Y. Yiangou, P. Facer, P. Durrenberger, I.P. Chessell, A. Naylor, C. Bountra, R.R. Banati, P. Anand, COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord, BMC Neurol 6 (2006) 12.

[166] M. Caragnano, P. Tortorella, A. Bergami, M. Ruggieri, P. Livrea, L.M. Specchio, G. Martino, M. Trojano, R. Furlan, C. Avolio, Monocytes P2X7 purinergic receptor is modulated by glatiramer acetate in multiple sclerosis, J Neuroimmunol 245(1-2) (2012) 93-7.

[167] S. Apolloni, S. Amadio, C. Montilli, C. Volonte, N. D'Ambrosi, Ablation of P2X7 receptor exacerbates gliosis and motoneuron death in the SOD1-G93A mouse model of amyotrophic lateral sclerosis, Hum Mol Genet 22(20) (2013) 4102-16.

[168] S. Apolloni, S. Amadio, C. Parisi, A. Matteucci, R.L. Potenza, M. Armida, P. Popoli, N. D'Ambrosi, C. Volonte, Spinal cord pathology is ameliorated by P2X7 antagonism in a SOD1-mutant mouse model of amyotrophic lateral sclerosis, Dis Model Mech 7(9) (2014) 1101-9.

[169] H.G. Lee, S.M. Won, B.J. Gwag, Y.B. Lee, Microglial P2X(7) receptor expression is accompanied by neuronal damage in the cerebral cortex of the APPswe/PS1dE9 mouse model of Alzheimer's disease, Exp Mol Med 43(1) (2011) 7-14.

[170] F. Di Virgilio, A.L. Giuliani, Purinergic signalling in autoimmunity: A role for the P2X7R in systemic lupus erythematosus?, Biomed J 39(5) (2016) 326-338.

[171] J. Zhao, H. Wang, C. Dai, H. Wang, H. Zhang, Y. Huang, S. Wang, F. Gaskin, N. Yang, S.M. Fu, P2X7 blockade attenuates murine lupus nephritis by inhibiting activation of the NLRP3/ASC/caspase 1 pathway, Arthritis Rheum 65(12) (2013) 3176-85.

ACCEPTED MANUSCRIPT

[172] C.M. Turner, F.W. Tam, P.C. Lai, R.M. Tarzi, G. Burnstock, C.D. Pusey, H.T. Cook, R.J. Unwin, Increased expression of the pro-apoptotic ATP-sensitive P2X7 receptor in experimental and human glomerulonephritis, Nephrol Dial Transplant 22(2) (2007) 386-95.

[173] A. Al-Shukaili, J. Al-Kaabi, B. Hassan, A comparative study of interleukin-1beta production and p2x7 expression after ATP stimulation by peripheral blood mononuclear cells isolated from rheumatoid arthritis patients and normal healthy controls, Inflammation 31(2) (2008) 84-90.

[174] L. Portales-Cervantes, P. Nino-Moreno, M. Salgado-Bustamante, M.H. Garcia-Hernandez, L. Baranda-Candido, E. Reynaga-Hernandez, C. Barajas-Lopez, R. Gonzalez-Amaro, D.P. Portales-Perez, The His155Tyr (489C>T) single nucleotide polymorphism of P2RX7 gene confers an enhanced function of P2X7 receptor in immune cells from patients with rheumatoid arthritis, Cell Immunol 276(1-2) (2012) 168-75.

[175] A. Al-Shukaili, J. Al-Kaabi, B. Hassan, T. Al-Araimi, M. Al-Tobi, M. Al-Kindi, A. Al-Maniri, A. Al-Gheilani, A. Al-Ansari, P2X7 receptor gene polymorphism analysis in rheumatoid arthritis, Int J Immunogenet 38(5) (2011) 389-96.

[176] C. Baldini, C. Rossi, F. Ferro, E. Santini, V. Seccia, V. Donati, A. Solini, The P2X7 receptor-inflammasome complex has a role in modulating the inflammatory response in primary Sjogren's syndrome, J Intern Med 274(5) (2013) 480-9.

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Figure legends

Figure 1.

Role of P2X7R in infectious diseases. P2X7 exerts both infection-promoting and protective functions. On one hand, P2X7R facilitates hepatitis B virus entry into hepatocytes and the release of HIV-1 virions stored into macrophage vacuoles. On the other hand, P2X7R is protective against Dengue-virus (DENV) infection via nitric oxide (NO) production, thus reducing the viral load. P2X7R expressed on immune cells such as lymphocytes and macrophages also plays a pro-inflammatory role, helping containing parasite dissemination and bacterial infections. The causative agent of murine malaria, *Plasmodium chabaudi*, triggers ATP release from infected erythrocytes via the P2X7R of Th1 lymphocytes, thus promoting t-bet expression followed by INFγ and IL-2 release. Finally, ATP drives macrophage activation via facilitation of phagolysosome fusion, reactive ROS production and IL-1β secretion.

Figure 2.

Inflammatory pathways activated by the P2X7R. Following P2X7R activation, K^+ is released from the cell causing NLRP3 inflammasome assembly, likely via NEK7 recruitment. Activated inflammasome cleaves pro-casp-1 into casp-1, which causes maturation of pro- IL-1β and pro-IL-18. In addition, the P2X7R promotes cytokine gene expression via NF-κB nuclear translocation and triggers Ca^{2+}-dependent NFAT activation, which in turn causes IL-2 secretion, lymphocyte proliferation and GSK3β down-modulation. Finally, the P2X7R promotes HIF-1α activation and the associated VEGF release.

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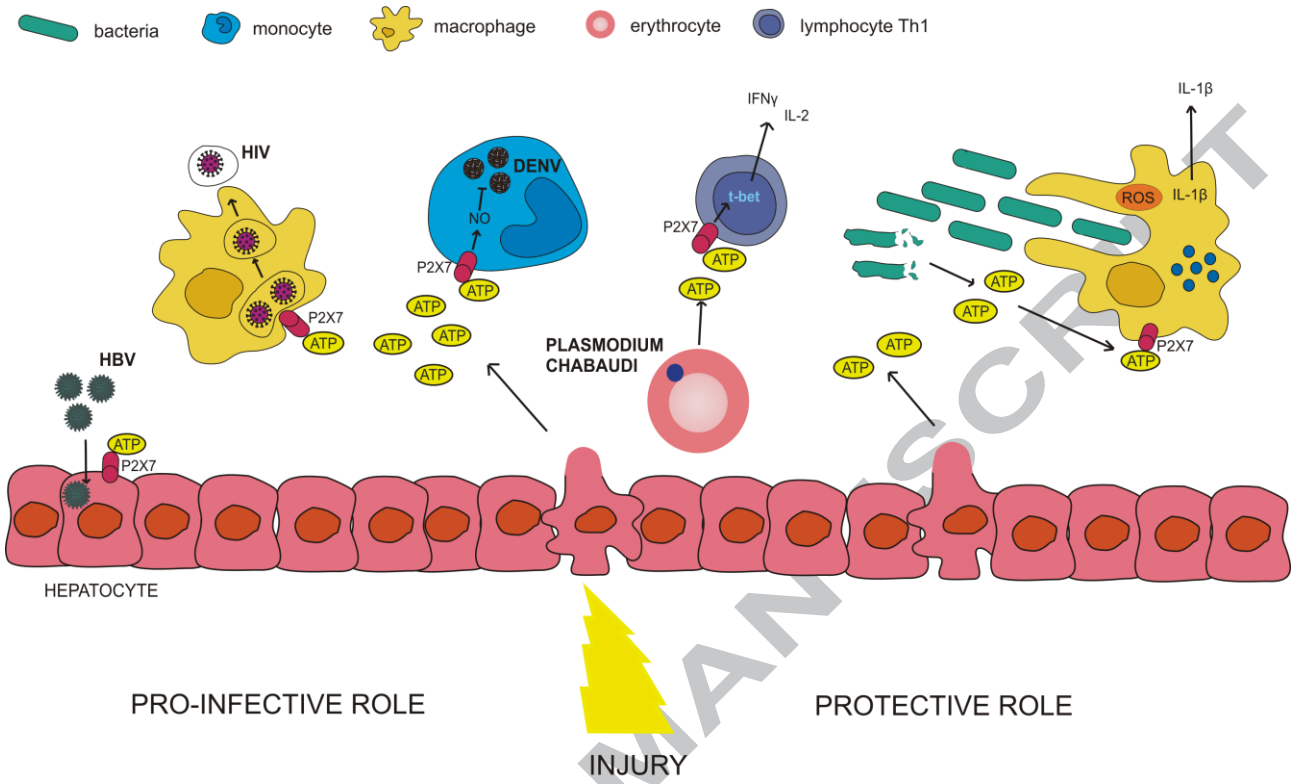

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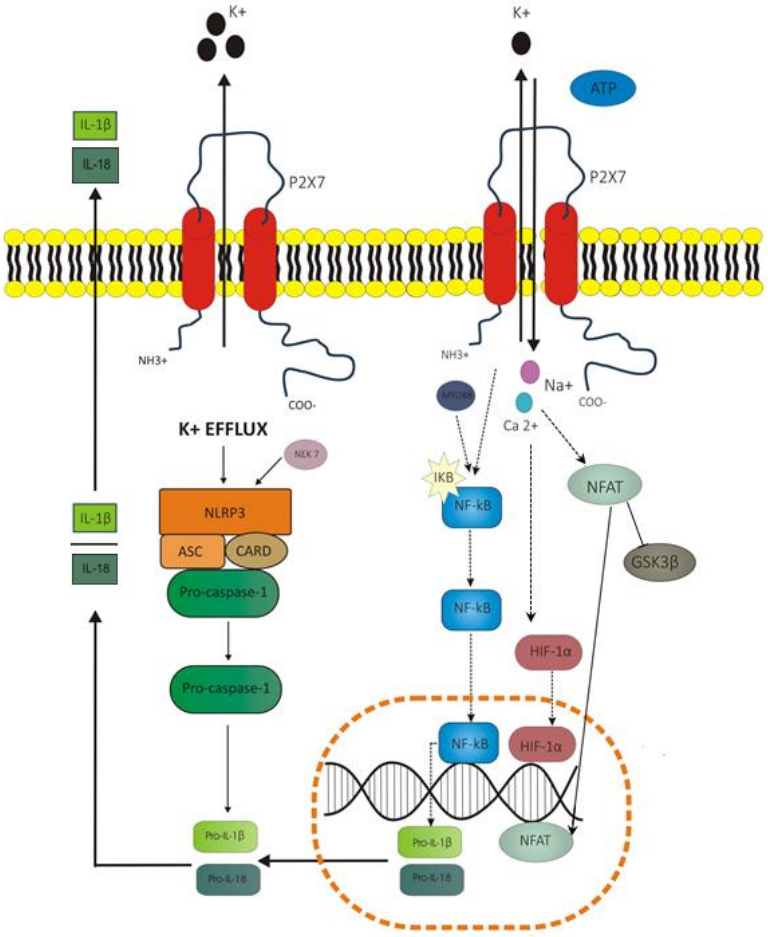
K+
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IL-1β
IL-18
P2X7
P2X7
NH3+
NH3+
COO-
COO-
Na+
Ca 2+
K+ EFFLUX
NEK 7
IKB
NF-kB
NFAT
GSK3β
NLRP3
ASC
CARD
Pro-caspase-1
NF-kB
HIF-1α
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Table I

P2X7R clinical trials

Title	Status	ID	Conditions
Investigation of the Safety and Tolerability of BSCT (Anti-nf-P2X7) 10% Ointment	Completed, has results	NCT02587819	Basal Cell Carcinoma (BBC)
Feasibility Study: Accuracy of Biomarker in Detection of Endometrial Cancer	Terminated, no results available	NCT00471120	Uterine Cancer, Endometrial Cancer
Gene-Polymorphisms in the P2X7 Gene in Patients With Osteoporotic Fractures	Unknown	NCT00293189	Hip fracture
A P2X7R Single Nucleotide Mutation Promotes Chronic Allograft Vasculopathy	Active, not recruiting, no results available	NCT02082821	Cardiac allograft vasculopathy
A Study to Investigate P2X7 Receptor Occupancy by JNJ-54175446 With the Newly Developed P2X7 Receptor Positron Emission Tomography (PET) Tracer 18F-JNJ-64413739	Active, not recruiting, no results available	NCT03088644	Healthy
Study of CE-224,535 A Twice Daily Pill To Control Rheumatoid Arthritis In Patients Who Have Not Totally Improved With Methotrexate	Completed, no results available	NCT00628095	Rheumatoid arthritis
Decoding of the Expression of Tumor Suppressor P2RX7 in Inflammatory and Malignant Colonic Mucosa	Unknown, no results available	NCT02293811	Crohn Disease-Associated Colorectal Adenocarcinoma
Cohort Study on Associations Between Purinergic Receptor SNPs and Osteoporosis Risk	Completed, no results available	NCT00697983	Osteoporosis
First Time in Human Study Evaluating the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics and the Effect of Food of Single Assending Doses of GSK1482160.	Completed, no results available	NCT00849134	Inflammatory pain

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Table II

Main animal/cellular models used to investigate P2X7R in inflammation and immunity

Disease or pathogen	Animal models	Cellular models	References
Tubercolosis	C57BL/6 Wild Type and P2X7R$^{-/-}$ mice	Macrophages	[63]
Chlamidia	C57BL/6 Wild Type and P2X7R$^{-/-}$ mice	Cervical epithelial cells	[61]
Leishmania	C57BL/6 Wild Type and P2X7R$^{-/-}$ mice	Macrophages	[62]
Plasmodium chabaudi	C57BL/6 Wild Type, B6.SJL-Ptprca Pepcb/BoyJ (CD45.1$^{+/+}$), B6.129P2-P2X7R tm1Gab/J (P2X7R$^{-/-}$), B6.Cg-Fosn1nu/J (nude) and B6.129S2-Cd4tm1Mak/J (Cd4$^{-/-}$) mice	Blood and spleen cells	[67]
Dengue virus-2	C57BL/6 Wild Type and caspase-1$^{-/-}$, P2X7R$^{-/-}$ mice	Monocyte Lung epithelial Macrophages	[68, 69]
Hepatitis B virus	---	Hepatocytes	[70]
HIV virus	C57BL/6 Wild Type, C57BL/6 (B6, H2Kb), BALB/c (H2Kd), LysM-eGFP$^{+/+}$ mice	Retinal pigment epithelium, monocytes, macrophages	[72-74]
Inflammation	C57BL/6 Wild Type, Casp1$^{-/-}$/Casp11$^{-/-}$, Casp11$^{-/-}$, Nlrp3$^{-/-}$, Nlrp6$^{-/-}$, Nlrp12$^{-/-}$, Nlrc4$^{-/-}$, Pycard$^{-/-}$, P2X7R$^{-/-}$, Panx1$^{-/-}$, Aim2$^{-/-}$, Asc$^{-/-}$,Cd18$^{-/-}$, Rag1$^{-/-}$ BALB/c, B6.C-H2^{bm12} (bm12) mice	Astrocytes, embryonic kidney, macrophages, monocytes, dendritic cells, neutrophils, microglia, osteoclasts, osteoblasts, splenocytes	[76, 88, 90, 91, 102, 107, 109, 110, 118]
Amyotrophic lateral sclerosis	C57BL/6 SOD1-G93A mice	Microglia	[153, 155, 156]
Alzheimer's disease	C57BL/6 Wild Type and P2X7R$^{-/-}$, APPswe/PS1dE9 mice	Microglia	[42, 157]
Liver fibrosis	C57BL/6 Wild Type mice, bile duct-ligated cirrhotic rats	---	[112, 143]
Lupus erythematosus	MRL/lpr, NZM 2410 mice	---	[159]
Rheumatoid arthritis	---	Leukocytes	[161-163]
Sjögren's syndrome	---	Monocytes	[164]

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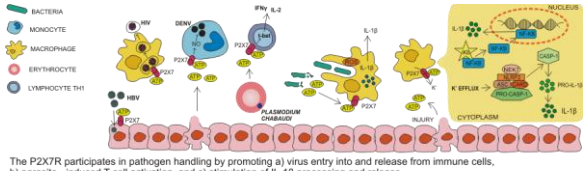

The P2X7R participates in pathogen handling by promoting a) virus entry into and release from immune cells, b) parasite - induced T cell activation, and c) stimulation of IL-1β processing and release.

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