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Review

The bumpy road of purinergic inhibitors to clinical application in immune-mediated diseases

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From the Contents

Abstract

Purinergic signaling plays important roles throughout the body in the regulation of organ functions during and following the disruption of homeostasis. This is also reflected by the widespread expression of two families of purinergic receptors (P1 and P2) with numerous subtypes. In the last few decades, there has been increasing evidence that purinergic signaling plays an important role in the regulation of immune functions. Mainly, signals mediated by P2 receptors have been shown to contribute to immune system-mediated pathologies. Thus, interference with P2 receptors may be a promising strategy for the modulation of immune responses. Although only a few clinical studies have been conducted in isolated entities with limited success, preclinical work suggests that the use of P2 receptor inhibitors may bear some promise in various autoimmune diseases. Despite the association of P2 receptors with several disorders from this field, the use of P2 receptor antagonists in clinical therapy is still very scarce. In this narrative review, we briefly review the involvement of the purinergic system in immunological responses and clinical studies on the effect of purinergic inhibition on autoimmune processes. We then open the aperture a bit and show some preclinical studies demonstrating a potential effect of purinergic blockade on autoimmune events. Using suramin, a non-specific purinergic inhibitor, as an example, we further show that off-target effects could be responsible for observed effects in immunological settings, which may have interesting implications. Overall, we believe that it is worthwhile to further investigate this hitherto underexplored area.

Key Words: autoimmune diseases; neurological disorders; purinergic system; P2 receptor inhibitors; suramin

Introduction

The concept of a purinergic signaling system using purine nucleotides and nucleosides as messengers was first proposed over 50 years ago and slightly later, two families of cell-surface purine receptors were proposed, implicit for the concept of purinergic signaling (Burnstock, 2020). The two families of purine receptors are adenosine or P1 receptors and P2 receptors (P2Rs) recognizing primarily adenosine-5′-triphosphate (ATP), adenosine diphosphate, uridine-5′-triphosphate, and uridine diphosphate. While P1 receptors all couple to G proteins, P2Rs divide naturally into a family of ligand-gated ion channels and G protein-coupled receptors termed P2X receptors (P2XRs) and P2Y receptors (P2YRs), respectively (Burnstock, 2018b). Currently, seven P2X subunits ($P2X_1$ to $P2X_7$) are recognized. Their activation leads to an influx of cations such as sodium and calcium (Sheng and Hattori, 2022). In P2YRs, eight subtypes are recognized. The P2YRs consist of $P2Y_1$-like and $P2Y_{12}$-like receptors. These two subfamilies constitute pharmacologically distinct groups. The first subfamily ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors) couples to G_q protein ($P2Y_{11}$ can also couple with G_s proteins) and activates phospholipase C, which promotes the generation of the second messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol, while the second subfamily ($P2Y_{12-14}$) is coupled to $G_{i/o}$ proteins to inhibit adenylate cyclase (Jacobson et al., 2012; Jacobson et al., 2020).

Only about 20 years after the postulation of the purinergic hypothesis, purinergic signaling was also reported in the central nervous system (CNS). First, it was described in the medial habenula (Edwards et al., 1992) but has since been supplemented with observations in various other areas such as the spinal cord, hippocampus, and somatosensory cortex (Bardoni et al., 1997; Shibuya et al., 1999; Mori et al., 2001; Pankratov et al., 2002).

Purinergic pathways contribute to the maintenance of homeostasis in the healthy organism and regulate several organ systems such as the cardiovascular, renal, gastrointestinal, and central nervous system (Antonioli et al., 2013; Bele and Fabbretti, 2015). Besides others, especially immune homeostasis seems to strongly rely on an equilibrium of low levels of extracellular ATP and its degradation product adenosine diphosphate surrounding immune cells (Antonioli et al., 2019). The extracellular ATP, released in inflammatory conditions by activated or dying cells, initiates the purinergic signaling cascade. Extracellular ATP functions as a pro-inflammatory metabolite and leads to the influx of cations via P2XRs or to the activation of secondary messengers by P2YRs (Eberhardt et al., 2022).

The aim of this review is to provide a synopsis of the potential involvement of P2Rs in immunological processes and give an overview of the described effects of purinergic inhibitors in the context of autoimmune diseases with a special focus on neuroautoimmune diseases.

This article is not intended to be an exhaustive list of all substances available to date that inhibits the purinergic system, but rather an up-to-date compilation of described effects of purinergic inhibitors observed in autoimmune-related contexts or models of autoimmune conditions. Finally, this review provides a perspective about potential clinical applications of purinergic inhibitors taking into account pharmacological considerations.

Search Strategy

This article is a narrative review summarizing the findings of previous research. The articles considered in this article related to the following keywords: purinergic signaling, purinergic system, suramin, immune-mediated disease, immune system, autoimmune diseases, neurological disorders, P2R inhibitors. Various combinations of them were used and search results were electronically retrieved from the PubMed database, Google Scholar, and clinicaltrials.gov. All years were chosen in the search. This review did not follow a strict pre-established protocol with explicit inclusion and exclusion criteria. Articles were screened by title and abstract and articles that were not within our topic were excluded from further analysis. If they were considered relevant, we accessed the whole paper. Only articles published in English were included.

Role of Purinergic Signaling in Immunological Responses

Nowadays, purinergic signaling is considered to represent an important messenger system in the modulation and development of immune responses and immune cell communication. The immune system has emerged as one

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of the major research areas in purinergic signaling (for an extensive review about purinergic signaling in modulating and controlling immune responses see (Antonioli et al., 2019; Eberhardt et al., 2022). Indeed, extracellular ATP, which is usually almost exclusively present inside cells, is an ideal reporter of cellular distress, and P2Rs responding to changes in ATP levels are a vigilant warning system.

P2YRs are widely expressed in virtually all types of immune cells (Idzko et al., 2014) mediating important functions such as cell proliferation, chemoattraction, and release of inflammatory cytokines. These receptors are on the one hand essential to launch the immune response. On the other hand, overshooting activation can have detrimental effects on the organism (Cekic and Linden, 2016). The finding that a deficiency in the ATP-degrading ectoenzyme CD39 also sustains a pro-inflammatory state (Cohen et al., 2013), further supports the pro-inflammatory nature of P2R activation.

In accordance, studies on knock-out mice have also shown an involvement of P2YR in immune responses. Mice lacking $P2Y_2$ show a decreased susceptibility to mount an autoimmune response, mainly based on a hampered lymphocyte activation with reduced cytokine secretion (Relvas et al., 2015).

Besides $P2Y_2$, $P2Y_6$, $P2Y_{11}$, and $P2Y_{12}$ have been shown to be involved in immune responses and partly in the pathophysiology of neuroinflammatory diseases (Chadet et al., 2015; Calovi et al., 2019). Interestingly, $P2Y_{14}$ seems to be involved in immune processes especially in the brain as it plays a role in the control of microglia (Curet and Watters, 2018) and its blockade increases the release of tumor necrosis factor-alpha from astrocytes (Kinoshita et al., 2013).

Equally, the ionotropic purinergic receptors (P2XRs), which mainly convey ATP-mediated Ca^{2+} signaling, play essential roles in various inflammatory and immunological processes. $P2X_4$ and $P2X_7$ have been demonstrated to be involved in immune disorders and inflammation (Di Virgilio et al., 2017; Zabala et al., 2018; Freire et al., 2019). Besides others, $P2X_7$ induces NLRP3-mediated inflammasome assembly, finally leading to cytokine release, e.g., interleukin (IL)-1β (Wang et al., 2020; Kong et al., 2022). $P2X_4$ activation inhibits the development of T helper 17 cells and the production of IL-17 (Hamoudi et al., 2022), and interfering with $P2X_4$ signaling modulates microglia polarization (Zabala et al., 2018).

Due to the widespread distribution and function of P2R in various pathophysiological conditions, selective purinergic receptor antagonism is regarded as a promising approach for potential therapeutic interventions (summarized in various review articles (Burnstock, 2018a; da Silva Ferreira et al., 2019)).

Despite a growing understanding of purinergic signaling in regulating immune functions and controlling immune-mediated inflammatory diseases as well as its pro-inflammatory nature, antagonism of P2R as a potential treatment of inflammatory conditions has not yet been widely incorporated into clinical studies regarding their efficacy and usefulness in autoimmune diseases.

P2 Receptor Antagonism and Potential Clinical Merits

During the last years, several substances targeting P2Rs have been identified and described [broadly reviewed elsewhere e.g., for P2YR (Jacobson et al., 2020) and for P2XR (Illes et al., 2021)]. While receptor-targeting substances can be divided into agonists and antagonists, here we focus on inhibitors due to the pro-inflammatory nature of P2R activation.

Besides low-affinity P2YR antagonists such as suramin, PPADS, and reactive blue 2, different more specific and potent substances blocking P2Rs have been developed.

Among P2YRs, $P2Y_2$ is a promising candidate in autoimmune diseases. The effects of $P2Y_2$ antagonists have been studied in various pre-clinical disease models such as Sjögren's disease (Jasmer et al., 2021), inflammatory bowel disease, and atopic dermatitis (Jasmer et al., 2022). Selective $P2Y_2$ antagonists are e.g., Ap4A, Up4U, MRS2768, PSB1114, and AR-C118925 with the latter being the most promising candidate because of its pharmacokinetic and pharmacodynamic properties (Jasmer et al., 2022; Neumann et al., 2022).

While another metabotropic receptor $P2Y_6$ and its antagonist MRS2578 seem to be involved in the inflammatory regulation of microglia (Timmerman et al., 2022; Ju et al., 2023), the involvement of $P2Y_{12}$ is well established in the regulation of thrombus formation and thromboinflammatory reactions (Mansour et al., 2020). Many antagonists for $P2Y_{12}$ have been developed and are in clinical use (e.g., clopidogrel, prasugrel), however, so far only for their antithrombotic activity despite the fact that $P2Y_{12}$ is also involved in immune responses and the pathophysiology of neuroinflammatory diseases (Liverani et al., 2014; Calovi et al., 2019; Gomez Morillas et al., 2021). In contrast, the role of $P2Y_{13}$ is less well-established. There is evidence that MRS2211-induced inhibition of $P2Y_{13}$ reduces the effects of dextran sulfate sodium-induced colitis (Wu et al., 2022). But also in this case, the application of receptor antagonists has not yet progressed beyond the status of experimental studies.

Regarding ionotropic purinergic P2XR, besides the moderately potent and nonselective antagonists suramin, reactive blue 2, and PPADs, more specific antagonists for different subtypes have been developed over the last decades, e.g., NF-279 and NF-449 for $P2X_1$, A740003 and A804598 for $P2X_7$, NF770 for $P2X_2$ (Antonioli et al., 2019). For instance, $P2X_4$ antagonism may be a potential target for the treatment of multiple sclerosis, as P2X4 is expressed in microglia (Beggs et al., 2012). However, among P2XRs, $P2X_7$ seems to be the most important ionotropic receptor subtype in inflammatory conditions, playing a potential role in diverse autoimmune diseases (Cao et al., 2019). $P2X_7$ hyperactivation has been reported to be a key driver of inflammation and NLRP3 inflammasome activation in several inflammatory conditions (Franciosi et al., 2021; Ribeiro et al., 2021; Simoes and Bagatini, 2021). In multiple sclerosis and its animal model experimental autoimmune encephalomyelitis (EAE), $P2X_7$ activation has been shown to promote the activation and differentiation of T helper 17 cells, which are involved in the pathogenesis of EAE lesions (Domercq and Matute, 2019). In line with this observation, $P2X_7$ deficiency suppressed the development of EAE (Sharp et al., 2008). Further support for the involvement of $P2X_7$ in the pathogenesis of multiple sclerosis comes from the finding that in EAE rats, inhibition of these receptors improved neurological symptoms accompanied by reduced astrogliosis and demyelination (Grygorowicz et al., 2016). In other autoimmune diseases outside the CNS, such as rheumatoid arthritis, $P2X_7$ activation has been shown to induce the production of pro-inflammatory cytokines, such as IL-1β, tumor necrosis factor-α, and IL-6, by macrophages and synovial fibroblasts (McInnes et al., 2014; Liu et al., 2020). In addition, $P2X_7$ blockade has been shown to reduce joint inflammation and bone destruction in a mouse model of rheumatoid arthritis. In systemic lupus erythematosus, $P2X_7$ activation has been shown to promote the activation and differentiation of autoreactive B cells and the production of autoantibodies (Collison, 2019; Faliti et al., 2019). Moreover, $P2X_7$ blockade reduced disease activity and improved survival in a mouse model of systemic lupus erythematosus (Zhao et al., 2013). Due to the growing body of evidence of $P2X_7$ involvement in inflammation, a multitude of $P2X_7$ antagonists has been developed. However, they struggle with different pharmacological hurdles. As some substances exert anti-inflammatory effects independently of their target or miss specificity for their target and interact with other P2R receptors, results must be analyzed critically (Savio et al., 2018). Despite the widespread use of the developed $P2X_7$ antagonists in preclinical models of various autoimmune diseases, so far clinical studies remained rare and mostly unsuccessful (**Table 1**).

Despite a clear association of purinergic signaling pathways with processes of the immune system, drugs targeting nucleotide- and nucleoside-mediated signaling did not enter broad clinical application yet also reflected by the low number of clinical studies testing antipurinergic drugs in autoimmune disorders. At present, exceptions are still the rule, and examples of clinical situations, where purinergic inhibition is the direct target such as in chronic cough where Gefapixant, a $P2X_3$ antagonist has recently been licensed (McGarvey et al., 2022), are still rare.

In summary, various P2R antagonists and their effects have been studied in the context of immune-mediated diseases. However, despite the use of purinergic antagonists for the treatment of a relatively broad range of inflammatory diseases (Eltzschig et al., 2012; Wiedemar et al., 2020), there are no P2R antagonists in clinical use for autoimmune diseases so far.

Pleiotropic Effects of P2 Receptor Inhibitors

Accumulating evidence from extensive preclinical investigation of purinergic signaling, suggests that the pharmacological effects of substances classified as purinergic antagonists are not necessarily because of the blockade of specific P2R-mediated effects, but can be attributed to other off-target mechanisms. Examples are suramin, reactive blue 2 as well as PPADS which were first developed for other applications and the anti-purinergic activity has only been observed later. These substances are still broadly applied in preclinical studies due to their ready availability and manyfold demonstrated efficacy in different inflammatory conditions such as glomerulonephritis (Piao et al., 2016) or arthritis (Sahu et al., 2017). Besides their anti-purinergic effects, suramin, PPADS, NF449, and reactive blue 2 are thought to interact with various other targets such as proteins and enzymes as exemplified in **Figure 1** for suramin leading to off-target effects (Jacobson et al., 2021).

While multiple targets could significantly increase the risk of side effects, at the same time they might also significantly expand the field of applications for such substances. Here, suramin is an example worth mentioning. Suramin is effective as a non-selective P2R inhibitor. For a century, it has been used clinically for the treatment of African Sleeping Sickness (Trypanosomiasis). In 1988, suramin was examined for its anti-purinergic actions and was found to be an effective inhibitor of ATP-mediated P2X and P2Y signaling (Dunn and Blakeley, 1988). Suramin had and still has a model character for the development of other purinergic antagonists (Brockmann et al., 2019). While it is still in use for the treatment of first-stage infection with Trypanosoma brucei rhodiense, since its introduction it has been tested in a broad spectrum of other inflammatory and/or immune-mediated diseases, such as viral or parasitic infections (other than African trypanosomiasis), but also in autoimmune diseases, cancer, and autism (Wiedemar et al., 2020; **Table 2**). Only recently, it has been even shown that suramin is a potent inhibitor of the SARS-CoV-2-RNA polymerase (Yin et al., 2021).

Suramin shares similarities with other multifunctional molecules with modulating effects on purinergic receptors in clinical use such as ivermectin (Hariyanto et al., 2022) and moxidectin (Milton et al., 2020). In infectious diseases, direct effects on the invader's enzymes involved in metabolism, on the cytoskeleton, on cell entry, or proliferative machinery are the predominant actions.

Table 1 | **Clinical studies on purinergic inhibitors in immune-mediated disease**

Drug	Purinoceptor Subtype	Disease	Study type	Result	Reference
Gefapixant	$P2X_3$	Chronic cough	Phase 2b, randomized, placebo-controlled	Improvement in 24-hour cough frequency	Smith et al., 2020
Eliapixant	$P2X_3$	Chronic cough	Phase 2a, double-blind, crossover	Reduction of cough frequency and severity	Morice et al., 2021
AZD9056	$P2X_7$	Rheumatoid arthritis	Placebo-controlled, double-blind	No significant efficacy	Keystone et al., 2012
AZD9056	$P2X_7$	Crohn's disease	Phase 2a, randomized, placebo-controlled, double-blind	Probable potential to reduce pain and general well-being, questionable antiinflammatoy activity	Eser et al., 2015
CE-224535	$P2X_7$	Rheumatoid arthritis	Phase 2a, placebo-controlled	No efficacy for the treatment of rheumatoid arthritis	Stock et al., 2012

Table 2 | **Examples for studies testing the effectiveness of suramin in various infectious and immune-mediated conditions**

	Organism	Main effect	Mechanism	Reference
Parasites				
Malaria	Human erythrocytes suspension	Inhibition of erythrocyte invasion by merozoites	Binding of merozoite MSP1 prevents cleavage and invasion	Fleck et al., 2003
Malaria	Human erythrocyte suspension	Inibition of hemolysis of parasitized human RBCs	Antagonism of $P2Y_1$ receptors	Tanneur et al., 2006
Malaria	Mice	Delay of P. falciparum and P. berghei growth	Antagonism of $P2Y_1$ receptors	Tanneur et al., 2006
Onchocerciasis	Human patients	Elimination of microfliariae and adult worms	NA	Duke, 1968
Viruses				
SARS-CoV-2	Cell culture (Vero E6 cells, Calu-3 cells, primary human airway epithelial cells)	Protection against virus-induced cell death, inhibition of viral replication	Likely by prevention of virus entry	Salgado-Benvindo et al., 2020
SARS-CoV-2	Cell culture	Blockade of viral replication	Inhibition of SARS-CoV-2 RNA-dependent RNA polymerase	Yin et al., 2021
SARS-CoV-2	*In vitro*	Stop of SARS-CoV-2 replication	Inibition of SARS-CoV-2 main protease (3CLpro, in combination with quinacrine)	Eberle et al., 2021
SARS-CoV-2	*In vitro*		Inhibition of binding to nsp12 (nsp12 is a modulator of the RNA-dependent RNA polymerase activity)	Dey et al., 2022
Zika virus	Cell culture	Inhibition of Zika virus replication	Inhibition of virus binding to cells by interaction with E2 envelope	Albulescu et al., 2017
Zika virus	Cell culture	Inhibition of Zika virus replication	Strong interaction with ZIKV helicase	Tan et al., 2017
HIV (former HTLV.lll)	Cell culture	Reduction of viral infectivity and cytopathic effect	Inhibition of reverse transcriptase	Mitsuya et al., 1984
HIV (former HTLV.lll)	Human patients	Reduction in viral replication	Inhibition of reverse transcriptase	Broder et al., 1985
Bacteria				
Mycobacterium tuberculosis	*In vitro*	Inhibitory effect on mycobacterial RecA protein	Disassembling the RecA-ssDNA filament	Nautiyal et al., 2014
Mycobacterium tuberculosis	*In vitro*		Inhibition of c-di-AMP synthase	Opoku-Temeng and Sintim, 2016
Systemic autoimmune diseases				
Glycerol-induced acute kidney injury	Rat	Acceleration of renal function recovery	Decrease in proinflammatory IL-1β and NF-κB and growth inhibitory TGF-β1 mediators	Korrapati et al., 2012
Glomerulonephritis	Rat glomerular mesangial cells	Blockade of complement-dependent cell lysis	Inhibition of antibody binding and suppression of p38 activation	Piao et al., 2016
Glomerulonephritis	Mouse	Attenuated kidney damage and survival benefit	Blockade of $P2Y_2R$	Rennert et al., 2018
Cll induced experimental arthritis	Mouse	Attenuation of Cll-mediated joint damage	Inhibition of $P2X_7R$ signaling	Fan et al., 2016
Collagen induced arthritis	Rat	Reduction of inflammation and joint destruction	Unclear, reduction of pro-inflammatory cytokine levels in plasma	Sahu et al., 2012
Collagen induced arthritis	Rat	Significant amelioration of arthritis	Modulation of oxidative stress	Sahu et al., 2017
Autoimmune diseases of the nervous system				
Autoimmune uveoretinitis (EAU)	Mouse and rat	Suppression of EAU	Inhibition of antigen priming and T cell proliferation	Sartani et al., 1995
Neuromyelitis optica	Mouse	Prevention of astrocytic death	Unfolding of IgG	Kalluri et al., 2022
Experimental allergic encephalomyelitis (EAE)	Mouse	Blockade of clinical manifestation	inhibition of IFN-gamma production and T-cell proliferation	Novales-Li, 1996
EAE	Rat	Reduction of EAE severity	unclear	van der Veen et al., 1985
Psychiatric disorders				
Autism spectrum disorder	Mouse	Correction of multisystem abnormalities defining the Autism spectrum disorder-like phenotype	Blockade of purinergic signaling	Naviaux et al., 2013
Autism spectrum disorder	Human patients	Improvement of Autism related symptoms	Unclear	Naviaux et al., 2017

c-di-AMP: Cyclic di-adenosine monophosphate; Cll: type ll collagen; EAE: experimental autoimmune encephalomyelitis; EAU: experimental autoimmune uveitis; HIV: human immunodeficiency virus; IFN-gamma: interferon gamma; IgG: immunoglobulin G; IL-1β: interleukin 1β; MSP1: merozoite surface protein-1; NA: not available; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; P. falciparum/berghei: plasmodium falciparum/berghei; RBCs: red blood cells; RecA-ssDNA: RecA single-stranded deoxyribonucleic acid; RNA: ribonucleic acid; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TGF-β1: transforming growth factor β1.

In parallel, the potential of suramin in autoimmune diseases has been investigated. In glomerulonephritis, suramin has been shown to be protective in glycerol-induced nephritis (Korrapati et al., 2012). It does inhibit antibody-triggered cell lysis in antibody plus complement-induced glomerulonephritis (Piao et al., 2016) and is protective in antibody-mediated glomerulonephritis via its effect on P2Y2 (Rennert et al., 2018). In arthritis, P2X7 mediated effects (Fan et al., 2016), as well as potential antioxidant effects (Sahu et al., 2017) of suramin, are being discussed as the main mediator of its effects. Besides purinergic effects, suramin has been suggested to interact with various other inflammatory signaling molecules such as IL-6 (Strassmann et al., 1993), nuclear factor kappa-B (Liu et al., 2021), and cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS) (Li et al., 2021).

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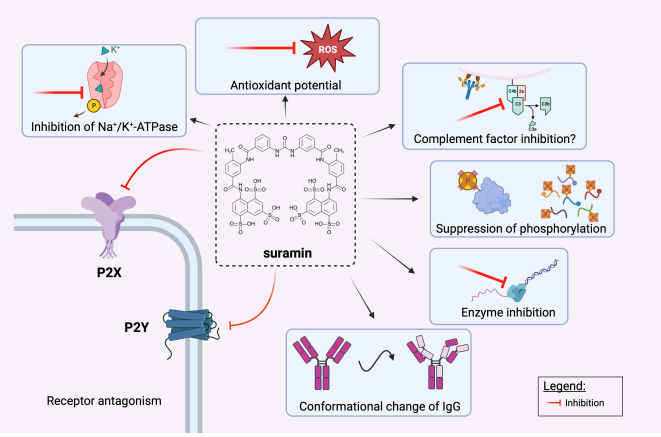

Figure 1 | **Postulated pleiotropic effects of the purinergic inhibitor suramin.**
Data are also listed in **Table 2**. Created with BioRender.com. C2a, C3, C3a, C3b, C4b: Complement system components; IgG: immunoglobulin G; K^+: potassium; P: phosphate; P2X: purinergic receptor (ligand-gated ion channels); P2Y: purinergic receptor (G protein-coupled receptors); ROS: reactive oxygen species.

We have recently identified a new mode of action that could partly explain the anti-inflammatory effects of suramin in autoimmunity. When testing suramin and several other commonly used P2R inhibitors in a model of autoimmune-mediated neuroinflammation, we unexpectedly discovered a concentration-dependent unfolding of autoantibodies, preventing complement-mediated immune responses (Kalluri et al., 2022). Using circular dichroism spectroscopy, we have demonstrated that suramin binds to autoantibodies from the IgG subtype, leading to a conformational change. This partial unfolding of IgGs inhibits complement binding and thus activation of the complement cascade. This interaction does not involve purinergic signaling and was already visible at concentration ranges between 50–100 µM. This result is intriguing because it shows that purinergic inhibitors potentially could have effects on various antibody-mediated disease entities by direct interaction with autoantibodies. Moreover, the effects of purinergic inhibitors in previous studies on autoimmune conditions that have been ascribed to the modulation of P2R inhibition, could in fact have originated from direct conformational change of respective antibodies. In an acute mouse model of Neuromyelitis optica, the application of P2R inhibitors completely prevented astrocytes from aquaporin-4-IgG-mediated cell death, highlighting their efficacy in classical autoimmune pathophysiology.

The interaction with autoantibodies seems not to be specific for AQP4-IgG but seems to apply for different other IgG subtypes and even in different species (Kalluri et al., 2022). This new mode of action could therefore also be potentially relevant in different systemic autoimmune disorders, like Goodpasture Syndrome or rheumatologic disorders. This newly discovered IgG unfolding mechanism presumably serves as an explanation for the long-lasting observation that suramin inhibits antibody-mediated complement activation (Fletcher and Lin, 1980; Sartani et al., 1995) and probably by this route suppresses the induction of experimental autoimmune uveoretinitis (Sartani et al., 1995) as well as EAE (van der Veen et al., 1985).

Pharmacological Considerations

Even after the successful identification of promising P2R inhibitor candidates, the question of whether a P2R antagonist may enter clinical trials with a reasonable probability of a positive outcome remains open. Until a drug enters clinical trials, it often takes a long journey. Several reasons attribute to this, ranging from a partially poor knowledge of the structures of the targeted P2YRs aggravating the proper investigation to poor pharmacokinetic properties. When targeting neuroautoimmune diseases, the ability of compounds to cross the blood-brain barrier (BBB) has to be considered, what is often a major obstacle for efficient drug delivery to the CNS. Even if promising CNS-penetrant candidates have been identified, they are further required to exhibit an adequate brain-tissue half-life.

In general, drug candidates should exert pharmacokinetic properties that allow their systemic (optimally oral) application and have a large therapeutic window. Considering P2Y receptors for therapeutic reasons, it is also critical to be aware of whether the affected functions are the result of homomeric P2YR activations or the product of heteromeric interactions between different P2YR subtypes. It has been shown that for example, $P2Y_1$ can form a complex with $P2Y_{11}$ which can change the agonist/antagonist profile compared to individual receptor subtypes (Ecke et al., 2008).

For the development of P2R antagonists, one additional aspect should not be overlooked which may complicate the development of suitable P2R inhibitor candidates. While purinergic receptors show a wide distribution throughout the body in men and mice, it is important to consider species-differences in the pattern and pharmacology of P2R expression. This has important implications and may form a significant hurdle for the interpretation and direct translatability of preclinical findings to the human situation. Therefore, not only the selectivity towards specific subtypes of the manifold purinergic receptors but also species selectivity (human, rats, mice, etc.) is a further concern for the development of new P2R ligands.

Lastly, biased agonism/antagonism (an emerging concept in G-protein coupled receptor pharmacology) - provides the possibility to preferentially activate or inactivate only a subset of signaling pathways initiated by the cognate receptor (Pupo et al., 2016). In contrast to partial agonists which have a lower intrinsic activity at receptors compared to full agonists but still activate all signaling pathways activated by a full agonist, biased agonists allow a more targeted modulation of cell function. This may maximize drug effectiveness by reducing adverse effects, if they are mediated by signaling pathways distinct from those that induce the therapeutic effect. Integrating this concept into the identification of novel P2R inhibitors may even increase the safe and viable application of pharmacological purinergic blockade and may provide additional beneficial clinical characteristics compared to traditional substances in the future.

Limitations of P2 Receptor Antagonists for Clinical Application

Despite promising results in preclinical studies, many P2R antagonists have not reached clinical approval in inflammatory and autoimmune diseases so far. That can be partly explained by several limitations that P2R inhibitors show in their clinical application.

One limitation that dampens the excitement about these different newly discovered modes of action, is that P2R inhibitors cannot cross the BBB in their current form. None of them is so far CNS-penetrant and thus cannot directly eliminate pathogenic antibodies from cerebrospinal fluid, which would be decisive, especially in neuroautoimmune diseases. Without being able to cross the BBB, P2R inhibitors do not have access to the resident immune system, where they potentially could exert their beneficial pleiotropic effects. This caveat limits their usage in autoimmune conditions with intrathecal antibody production.

However, many antibodies in the neuroautoimmune context (such as AQP4-IgG) are produced in the periphery and are circulating in the blood before they reach the CNS upon BBB breakdown. Therefore, a clear effect in peripheral blood could also represent a promising strategy. In general, classical ways to render antibodies in autoimmune diseases are (1) the elimination of antibodies from patient blood by plasmapheresis or (2) by extracting the IgG fraction by infusion of immunoglobulins, by binding to the body's own IgGs and following the elimination of such IgG-complexes via the kidney. The unfolding mechanisms of purinergic inhibitors described above represent a third way to make autoantibodies ineffective. Here, the conformation of antibodies is modulated by small molecules, impairing their functionality.

Second, their clinical application is also limited by the fact that P2R inhibitors such as suramin are not well tolerated, having a substantial number of side effects. Among nausea, abdominal pain, and peripheral neuropathy, suramin can lead to severe nephrotoxicity probably via complex formation, anemia, and hypotonia, especially when higher doses are needed to achieve a therapeutic effect (Ahe et al., 2018; Wiedemar et al., 2020).

Third, while multiple mechanisms of action can potentially expand the areas of application as mentioned above, the pleiotropic effects of P2R antagonists also pose risks of a higher number of unpredictable side effects. Altogether, the prospect of potential candidates should be incentive enough to develop new drugs or discover new mechanisms of action.

Given that anti-purinergic drugs do not suppress antibody production, their usage seems to be more reasonable in a short-term treatment regime, whereas for long-term treatment immunomodulatory strategies (such as B cell depleting therapies) are more effective.

Future Therapeutic Perspectives

For several decades, the purinergic system has been studied as a possible therapeutic target for inflammatory diseases. In comparison, the field of possible use of purinergic blockers in autoimmune diseases is still new. Here, controlling P2R signaling may be fundamental in controlling damage and maintaining healthy tissue.

However, concentrating only on the inhibition of P2R signaling as a way to modulate purinergic signaling would not do justice to the complexity of the purinergic system and would neglect numerous other promising starting points for therapy. For instance, besides inhibition of purinergic receptors, depletion of extracellular nucleotides may also represent effective strategies to positively influence the course of autoimmune diseases. Moreover, given that the purinergic system with its numerous pieces (mediators, receptors, purine metabolizing enzymes) is already a large puzzle, newly identified mechanisms of action of purinergic inhibitors may further make the image richer in nuances. On the one hand, the pleiotropic effects may complicate the consideration of anti-purinergic inhibitors for therapeutic purposes, but at the same time may open new trajectories for potenital unexpected therapeutic benefits.

Overall, the accumulating understanding of the role of purinergic signaling in autoimmune diseases will likely pave the way for new possible indications

and therapeutic interventions. More research is needed to close further gaps in the puzzle and to estimate the real potential of P2R inhibitors in the management of patients suffering from neuroautoimmune disorders.

Conclusion

Anti-purinergic therapy may offer a fresh new direction for research on the pathogenesis and development of new drug candidates for the treatment of systemic and neurologic immune-mediated disorders. Generally, P2R inhibitors will likely be a beneficial add-on therapy offering the possibility to gain time for more targeted interventions or to synergistically increase the effect of a second treatment. Although some pieces of the puzzle have been added over the last years, there are still very large gaps in the picture of P2R inhibition and its potential utility in the management of immune-mediated diseases.

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