Purinergic Signalling
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REVIEW ARTICLE

Purinergic modulation of the immune response to infections

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Abstract

Infectious diseases are caused by the invasion of pathogenic microorganisms such as fungi, bacteria, viruses, and parasites. After infection, disease progression relies on the complex interplay between the host immune response and the microorganism evasion strategies. The host's survival depends on its ability to mount an efficient protective anti-microbial response to accomplish pathogen clearance while simultaneously preventing tissue injury by keeping under control the excessive inflammatory process. The purinergic system has the dual function of regulating the immune response and triggering effector antimicrobial mechanisms. This review provides an overview of the current knowledge of the modulation of innate and adaptive immunity driven by the purinergic system during parasitic, bacterial and viral infections.

Keywords Purinergic system · Infectious diseases · Immune response · ATP · Adenosine

Abbreviations

ADA	Adenosine deaminase
ADO	Adenosine
ADORA	Adenosine receptors
ADP	Adenosine diphosphate
AIDS	Acquired immunodeficiency syndrome
AMP	Adenosine monophosphate
ART	Antiretroviral therapies
ATP	Adenosine triphosphate
BM	Bone marrow
CK-MB	Creatine kinase-myocardial band
CLP	Cecal ligation and puncture
CMV	Cytomegalovirus
CRS	Cytokines release syndrome
DENV	Dengue virus
EBV	Epstein-Barr virus
E-NTPDases	Ectonucleoside triphosphate diphosphohydrolases
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
IECs	Intestinal epithelial cells
IFN	Interferon
IL	Interleukin
iNOS	Inducible nitric oxide synthase
KO	Knock-out
MDSCs	Myeloid-derived suppressor cells
NECA	5′-N-ethylcarboxamide adenosine
NETs	Neutrophil extracellular traps
NO	Nitric oxide
PAMPs	Pathogen-associated molecular patterns
Tfh	T follicular helper cells
TLR	Toll-like receptors
TNF	Tumor necrosis factor
Tr1	Type-1 regulatory T cells
Treg	Regulatory T cell

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VL Visceral leishmaniasis
WT Wild-type

Introduction

The immune response is a biological process to protect the host from infections. A healthy immune system can recognize and differentiate self-antigens and antigens belonging to microorganisms such as parasites, fungi, viruses and bacteria. As such, immunity pursues pathogen clearance and preserves tissue homeostasis through a wide range of complex networks that involve cellular and humoral components with regulatory feedback systems [1]. A variety of inflammatory and suppressive responses are generated following infection or injury to restore the homeostatic conditions. The host exerts intrinsic control between both responses to achieve pathogen elimination while simultaneously preventing collateral damage.

The purinergic system has the dual function of modulating the host immune response and triggering effector mechanisms [2–4]. Adenosine triphosphate (ATP), adenosine (ADO) and other purine metabolites are the major representative mediators of purinergic signaling cascades (Fig. 1) [5]. The release of intracellular ATP in response to hypoxic or inflamed conditions by intact, activated or dying cells is the first step of purinergic signaling [6]. Extracellular ATP can activate and initiate a purinergic signaling cascade through purinergic receptors, a concept introduced by Geoffrey Burnstock in the early 1970 [7]. Extracellular ATP triggers multiple immune effector functions to inhibit infections and is primarily a pro-inflammatory metabolite [8], which can act at both paracrine and autocrine level activating two types of P2 receptors [9]. P2X receptors are plasma membrane channels that mediate influx or efflux of various cations, such as Ca^{+2}. P2Y are G-protein-coupled receptors that modulate signaling events of secondary messengers [10]. The life span of extracellular ATP is determined by purinergic ectoenzymes that coordinate a two-step enzymatic process of ATP hydrolysis into the potent anti-inflammatory ADO. The family of ectonucleoside triphosphate diphosphohydrolases (*E-NTPDases*), the largest family of ectonucleotidases consisting of eight E-NTPDases, hydrolyze nucleotide triphosphates (e.g., ATP) and diphosphates (e.g., adenosine diphosphate ADP) to generate nucleotide monophosphates (e.g., AMP). The most studied is the ENTPD1, also known as ecto-apyrase CD39 [11]. The second and limiting enzymatic step for ADO production involves converting AMP mediated by 5'-ectonucleotidase (*NT5E*), commonly known as CD73. Extracellular ADO exerts a central role in restricting tissue inflammation, as it is considered a retaliatory metabolite that inhibits ATP-driven pro-inflammatory effects due to its recognition by P1 receptors [12]. There are 4 types of purinergic G-coupled receptors ADO receptors (ADORA): ADORA1, ADORA2a, ADORA2b and ADORA3, which bind ADO with different affinity and exert various functions. It should be noted that ADO half-life in the extracellular compartment is extremely short, as it is metabolized by adenosine deaminase (ADA) between 15 seconds to a couple of minutes, depending on the host [13].

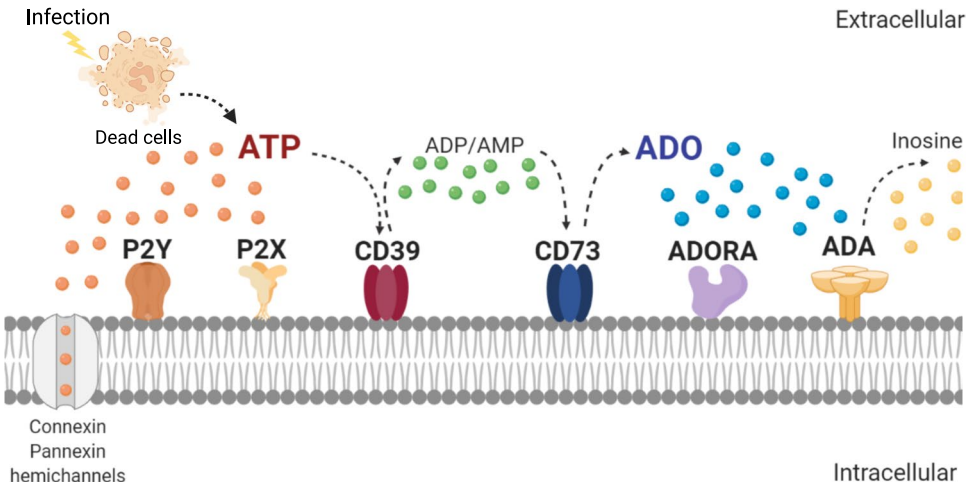

Fig. 1 Components of the purinergic signaling pathway. Stimulation of specific surface immune cell receptors that recognize pathogens, antigens, cytokines or chemokines triggers the release of ATP through connexin or pannexin hemichannels. ATP in the extracellular space can stimulate the P2X or P2Y receptors to trigger an inflammatory response. The ecto-apyrase (CD39) catalyzes the hydrolysis of ATP to ADP/AMP, and consecutively, the enzyme 5'-ectonucleotidase (CD73) catalyzes the limiting step of conversion to adenosine (ADO), which is the ligand for P1 receptors promoting anti-inflammatory responses. ADO can be removed by nucleoside transporters, which facilitates its cellular absorption, or by adenosine deaminase (ADA), which converts ADO to inosine

Although dampening inflammation is beneficial for preventing tissue damage ensuring host survival, it may also be detrimental for pathogen clearance after infection. The failure in the fine control of the immune response balance can alter pro-inflammatory/regulatory microenvironments, which eventually lead to pathophysiological conditions, such as chronic infectious diseases. In this regard, protective immune responses in some instances are the countermeasures that prevent disease, but not necessarily infection. Besides immune cells, several pathogens also utilize or possess purinergic machinery to foster their persistence or disrupt the host immune response.

Purinergic modulation of immune response

Purinergic signaling involves various mechanisms used by innate and adaptive immune cells to modulate their functions and adapt to local microenvironment [14]. During inflammatory conditions, the purinergic signaling pathways are activated to initiate immune cell chemotaxis toward inflamed tissue and fine-tuned control local inflammation. Initially, ATP release to the extracellular environment is mediated by pannexin or connexin hemichannels to regulate cell functions in an autocrine or paracrine manner [8]. The first cell to arrive at the inflammation site is the neutrophil, which employ an extracellular ATP-dependent mechanism to generate a chemotactic gradient and orientate its migration [15, 16]. Remarkably, the purinergic system regulates many effector functions of neutrophils, such as phagocytosis, oxidative burst, degranulation and neutrophil extracellular traps (NETs) formation [17]. After its death, apoptotic neutrophils release ATP to stimulate mononuclear phagocytic cell influx and promote engulfment and clearance functions [18]. The local microenvironment composed by ATP and the consequent metabolization to ADO by CD39 and CD73 ectoenzymes influence host–pathogen interaction by metabolic reprogramming of the innate and adaptive immune cells [19, 20]. Among myeloid cells, macrophages are the most studied cells in terms of phenotype and function modified by the purinergic system [21]. Several studies reported that ADO can reprogram monocyte and macrophages from a microbicidal and pro-inflammatory profile (classically activated—M1-like phenotype) to an alternatively activated and anti-inflammatory (M2-like) phenotype via ADORA receptors [22]. Macrophages have high plasticity capacity and can undergo profound metabolic modifications when recognizing pathogen-associated molecular patterns (PAMPs) mediated by Toll-like receptors (TLR)-stimulation. Indeed, macrophages induce the production of ATP and rapid extracellular degradation to control their activation state in an autoregulatory mechanism [23]. CD39 deficiency in macrophages promotes a sustained inflammatory activation state and inhibits the switch to an immunosuppressive phenotype [24]. ADO stimuli of ADORA2a and ADORA2b receptors in macrophages increase the expression of M2 macrophage markers and diminish the expression of inflammatory genes [25, 26]. In addition, ADO has been reported to induce the production of anti-inflammatory cytokines such as IL-10 and VEGF and suppress the production of pro-inflammatory cytokines (TNF-α and IL-6) by macrophage [27]. ADORA2b-inhibited and IFN-γ-treated macrophages extend their microbicidal and pro-inflammatory activities with increased IL-12 and TNF-α production in response to TLR stimulation [28].

Regarding adaptive immunity, the purinergic system has been reported as a modulator of the induction of different T helper profiles. T cell antigen receptor (TCR) ligation initiates T cell activation by increasing cytosolic calcium and the release of ATP via pannexin-1 mechanism. Extracellular ATP acts in an autocrine manner via P2X receptors to induce IL-2 production and cell proliferation. The absence of this purinergic pathway leads to T cell anergy with increased expression of anergy-associated genes 2 and 3 (*Erg2* and *Erg3)* [29]. In line with this, ATP-dependent activation of P2X7R inhibits suppressive functions of T regulatory (Treg) cells, inducing the Th17 differentiation [30]. Furthermore, P2X7R plays a role in generating long-lived memory CD8 + T cells, regulating their mitochondrial fitness and promoting the differentiation and survival of resident memory subsets via TGF-β signaling [31, 32]. On the other hand, ADORA2a receptor signal inhibits Th1 cell generation and IFN-γ production, triggering the induction of FoxP3 + Treg cell subset and the production of TGF-β [33, 34]. ATP catabolism and generation of retaliatory metabolite ADO is a typical suppression mechanism of regulatory cells involving Treg, type-1 regulatory (Tr1) T cells and myeloid-derived suppressor cells (MDSCs) [35, 36]. These regulatory cells express CD39 and CD73 to abrogate ATP-related effects and enable the inhibitory properties [37–40].

Relevance of the purinergic immune modulation in infectious diseases

The purinergic system has acquired a fundamental role in cellular and tissue microenvironment modulation upon damage to promote system regeneration and homeostasis [2, 3]. Remarkably, immune cell regulation mediated by purinergic signaling pathways has taken great therapeutic relevance in carcinogenic and inflammatory processes. The role of purinergic signaling in the immunomodulation in these contexts was reviewed elsewhere [41, 42]. Several recent studies suggest that a precisely orchestrated purinergic mechanism defines effector functions on immune cells and, thus, the result of host–microbe interaction [3, 11, 14,

43, 44]. However, a clear understanding of its relevance in the pathogenesis of infectious diseases and the possible therapeutic approaches still requires future research. Here, we focused on understanding the regulation of immune cell effector functions driven by purinergic signaling in the context of parasitic, viral and bacterial infections and their effects on pathogen clearance. This review aims to comprehensively summarize the current literature regarding the purinergic modulation of the immune response to pathogens and the impact on the development and progression of infectious diseases.

Parasitic infections

Leishmaniases comprise a wide range of diseases caused by the infection with the protozoan parasite *Leishmania* transmitted by the bite of infected female phlebotomine sandflies. Infection clinical presentation may be asymptomatic or may manifest as cutaneous or muco-cutaneous disease, which if left untreated may result in disfiguring scars; or the visceral form that may be lethal if untreated. The lack of a vaccine against human leishmaniasis and the diverse response to treatment make this disease a significant worldwide health problem. Several studies suggest a potential contribution of purinergic nucleotidases in *Leishmania* immune responses [45]. Indeed, *Leishmania* parasites augment ectonucleotidases activity to increase ADO production and consequently establish a chronic infection through purinergic immunoregulatory mechanisms [46]. Patients with visceral leishmaniasis (VL) present increased levels of ADA and ADO [47]. Increased ADO levels were further confirmed and associated with disease's pathogenicity, diminishing with the successful treatment. Moreover, the frequency of CD73 + CD39 + double-positive cells is augmented in VL active disease compared with treated VL patients and healthy controls[48]. Additionally, circulating monocytes exhibit upregulated levels of ADORA2b receptor, which shows direct association with parasite load. ADO signaling in *L. donovani*-infected monocytes inhibits IL-10 production and nitric oxide (NO) release, promoting immune downmodulation and parasite survival [49]. Besides, bone marrow (BM) from VL patients shows increased ADA levels compared to the non-infected BM, which inversely correlated with the local parasite load [50]. Recently, it was demonstrated that murine macrophages release ATP and increase CD39 and CD73 expression upon in vitro *L. donovani* infection. Thus, the resulting extracellular ADO promotes an anti-inflammatory microenvironment crucial for parasite persistence through ADORA2a and ADORA2b signaling [51]. On the other hand, *L. infantum* parasites utilize ADORA2a signaling pathway to successfully colonize the host and promote parasite surveillance. Indeed, ADORA2a-deficient mice present robust Th1 adaptive immune response in infected organs with augmented neutrophil infiltration and decreased Treg cells and IL-10 production. In accordance, infected ADORA2aKO mice show reduced parasite burdens in liver and spleen compared to infected wild-type (WT) mice [52]. Furthermore, after *L. amazonensis* infection, murine macrophages are significantly more sensitive to extracellular ATP-induced pore opening and show augmented P2X7R expression. ATP treatment inhibits parasite growth in an apoptosis-dependent mechanism [53]. Interestingly, *L. amazonensis* elimination mediated by P2X7R and LBT4 is dependent on non-canonical NLRP3 inflammasome and IL-1β signaling activation, as well as ROS production [54]. A crosstalk between P2Y2R and P2X7R is evidenced by the fact that both receptors cooperate to trigger a potent protective response against *L. amazonensis* infection [55]. However, Countinho-Silva and colleagues proposed another protective role for P2X7R since infected P2X7R-deficient mice show increased IFN-γ and reduced TGF-β production suggesting that an exacerbated pro-inflammatory response could increase the susceptibility to infection. In fact, P2X7R abrogation results in increased *L. amazonensis* parasite load and lesion size [56]. Altogether, these studies suggest that purinergic signaling plays a critical role in host immune response and parasite interaction; however, the fine balance of those signals would determine the outcome of infectious diseases.

Notably, several reports established a close relationship between the E-NTPDase and ecto-5'-nucleotidase activities to the virulence in different species of Leishmania genus [57]. The virulent parasite *L. amazonensis* hydrolyzes higher amounts of purinergic nucleotides compared to other species. Addition of ADO at the time of infection results in augmented skin lesion size and parasite load. In the same line, ADORA2b receptor inhibition decreases injury size and parasitism. Thus, ADO immunomodulatory properties contribute to the establishment of Leishmania infections [58]. Previous studies reported that *L. amazonensis* employs ADORA2b receptor signaling to suppress dendritic cell activation as an evasion mechanism. Blockade of ADORA2b receptor by PSB1115 treatment in vivo reduces lesion size and tissue parasitism, as well as increases IFN-γ levels [59].

Toxoplasmosis is caused by the infection with the protozoan parasite *Toxoplasma gondii* and is one of the most common human zoonoses, infecting about a third of the world's population. The disease is generally benign and often goes unnoticed in immunocompetent individuals; however, the infection also has health-threatening and fatal implications in immunocompromised individuals. In immunodeficiency conditions, reactivation of a latent infection might result in encephalitis, which is potentially fatal if not treated properly. Susceptibility to this infection is determined by the inability of the host immune response to control parasite replication.

Toxoplasma gondii-infected Wistar rats present increased E-NTPDase and E-ADA activities in circulating lymphocytes at early days post-infection, showing a positive correlation with the number of lymphocytes [60]. Likewise, the infection causes increment in the purine levels in the brain with the exception of inosine, which was diminished due to diminished ADA activity [61]. Treatment of *T. gondii*-infected Swiss mice with resveratrol, a non-flavonoid polyphenol, decreases ATP and AMP hydrolysis by NTPDase and CD73 and increases ADA activity in the cerebral cortex compared with non-treated infected groups [62]. Nevertheless, several studies assign a protective role to the purinergic ectoenzymes against *T. gondii* infection. CD73 genetic deficiency in mice infected with *T. gondii* increases their susceptibility to acute toxoplasmosis, with augmented neutrophils and T cell infiltration into the peritoneal cavity and increased levels of pro-inflammatory cytokines. Because of this, the ADO receptor agonist (NECA) treatment protects CD73-deficient mice against *T. gondii*-induced immunopathology [63]. In the same line, CD73-dependent ADO production and ADORA2a/2b activation protect the host from developing lethal pathology. Acute *T. gondii* infection induces the reduction of CD73 + cells in several infected tissues and increases immune-mediated pathology in the gastrointestinal tract and mucosa [64].

In contrast to the acute infection, during chronic toxoplasmosis CD73-generated ADO has a detrimental role because it is essential for *T. gondii* bradyzoite differentiation and cyst formation in the central nervous system. Indeed, CD73-deficient mice are more resistant to chronic infection and present reduced parasite burden in the brain compared to WT mice [65]. Accordingly, it is of ultimate importance to consider the radical differences between host immune response during the acute or chronic phases of the infection to therapeutically promote parasite eradication. Alternatively, the P2X7R was widely studied as a host mechanism of resistance to infection. P2X7R deficiency in oral *T. gondii*-infected mice leads to higher susceptibility to cerebral toxoplasmosis, with an increased number of cysts, less inflammatory infiltrates and reduced pro-inflammatory cytokine levels [66]. In this sense, it was demonstrated that P2X7R activation inhibits *T. gondii* replication in macrophages by canonical NLRP3 inflammasome activation, which in turn produced IL-1β leading to increased mitochondrial ROS production [67]. These findings are consistent with other reports confirming that P2X7R activation in macrophages mediates the killing of tachyzoites [68, 69]. Furthermore, P2X7R signaling in epithelial cells mediates CCL5 chemokine responses to promote the recruitment of CD11c + CD103 + dendritic cell in the intestinal epithelium promoting the initiation of the immune response [70]. Besides, activation of P2Y receptors in infected macrophages mediated by UTP and UDP induces early egress of tachyzoites and these parasites have reduced infectivity in subsequent infections [71]. The role of purinergic signaling in *T. gondii* infection was recently summarized in [72].

Chagas disease is caused by the infection with the protozoan parasite *Trypanosoma cruzi.* About 6 million people are estimated to be infected worldwide, mostly in Latin America. Chronic cardiomyopathy represents the most frequent and serious complication of this infectious disease. To date, there is no effective chemotherapy or prophylactic vaccine. Our group has reported that Chagas patients in the indeterminate stage show diminished frequencies of circulating CD39 + and CD73 + lymphocytes and increased plasma ATP levels [73]. In line with this, peripheral monocytes exhibit long-lasting functional phenotypic changes evidenced by increased expression of HIF-1α (the master regulator of metabolic adaptation to hypoxia), pro-inflammatory cytokines (IL-1β, IL-6), and pro-oxidative capacity. Strikingly, Chagas patients show augmented frequency of CD39 + monocytes and increased expression of CD73 per cell compared to healthy donors [74]. Thus, it is plausible that circulating monocytes promote ATP degradation to dampen the inflammatory microenvironment. Besides, leukocytes within cardiac explant from terminal Chagas disease patients exhibit a strong expression of CD73 and HIF-1α, which positively correlates with the myocarditis degree and the local parasite burden (Fig. 2B) [75]. Regarding ATP receptors, the gene and protein expression of P2X7R are unaltered in circulating lymphocytes from patients with indeterminate form of Chagas disease [76].

In experimental models of Chagas disease, infected Swiss mice increase NTPDase and E-ADA activities in circulating lymphocytes and augment E-ADA activity in cardiac homogenates, although a reduction on tissue CD73 activity is found in the acute phase compared to uninfected controls [77]. Furthermore, E-NTPDase and E-ADA activities are higher in splenic lymphocytes of acutely *T. cruzi*-infected mice compared to uninfected controls. The prophylactic treatment with avian IgY (polyclonal immunoglobulins against *T. cruzi* parasite) prevents these alterations in ectoenzyme activities and reduces the parasitemia [78]. Similarly, our group has previously reported that transient pharmacological specific inhibition of CD73 in the murine acute phase of infection enhances the microbicidal M1-like macrophage subset, promotes a pro-inflammatory environment and diminishes cardiac parasite load. Likewise, *T. cruzi* infection of CD73KO mice leads to an enhanced cardiac antimicrobial immune response as it augments the frequency of M1-like macrophages with enhanced production of IL-12 and the microbicidal metabolite NO. Additionally, C73-deficient mice presented increased CD8 + T cell effector functions which induces protection as observed by the reduced cardiac parasite burden compared to their WT counterpart (Fig. 2A) [79]. Thus, CD73 inhibition ameliorates the

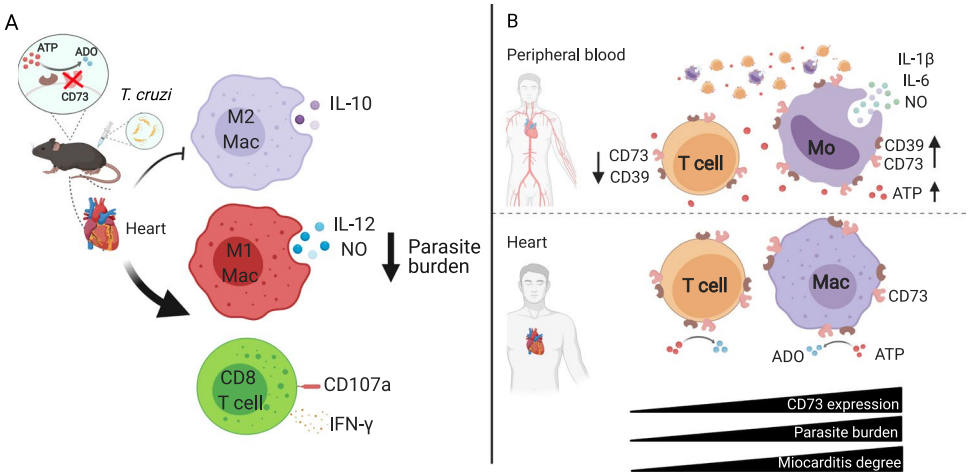

Fig. 2 Ectonucleotidases regulation of immune response against *Trypanosoma cruzi* infection. *T. cruzi* infection manipulates the expression of purinergic enzymes. **(A)** *T. cruzi*-infected CD73KO mice have an enhanced cardiac microbicidal immune response. CD73 abrogation diminishes IL-10 production of M2-like macrophages (*M2 Mac*) and augments the effector response (IL-12 and nitric oxide; NO) of M1-like macrophages (*M1 Mac*). CD73KO mice present augmented cytotoxic and pro-inflammatory (CD107a and IFN-γ) CD8 + T cell response, which induces protection as observed by the reduced parasite burden. **(B)** Patients with chronic asymptomatic Chagas disease show diminished ectonucleotidases expression (CD73 and CD39) in circulating lymphocytes and increased expression in monocytes. In human cardiac tissue, the augmented expression of CD73 positively correlates with the myocarditis severity and the cardiac parasite burden

outcome of chronic Chagas cardiomyopathy as it diminishes biochemical myocardial-specific injury marker (CK-MB) and improves the electrocardiographic characteristics [80].

It must be taken into account that parasites have catabolic ATP machinery which can affect the host's immune response favoring their virulence and persistence. In fact, different strains of *T. cruzi* parasites exhibit distinct levels of ATPDase hydrolytic activities. After treatment with ATPDase inhibitors, a marked reduction of trypomastigotes infectivity is observed in in vitro culture. Likewise, infection with Ecto-NTPDase-inhibited trypomastigotes leads to lower levels of parasitemia and higher mouse survival than infection with non-inhibited parasites [81]. Analogue products of purines (3'deoxyadenosine and deoxycoformycin) were used as antiparasitic treatments, and the observed therapeutic effect is due to an inability of trypanosomes to engage in new purine synthesis [82]. Hence, more studies are needed to elucidate whether it is possible to promote anti-*T. cruzi* immune response and at the same time inhibit parasite virulence factors.

Malaria is caused by five species of eukaryotic *Plasmodium* parasites transmitted by the bite of Anopheles spp. mosquitoes to humans. Malaria remains one of the most severe infectious diseases; it is responsible for hundreds of thousands of deaths predominantly among children in Africa. Purine signaling modulation seems to have relevant importance for *Plasmodium* parasites infectivity and malaria blood stage. Several studies have shown E-NTPDase to be involved in the infectivity of *P. falciparum* due to its relevance for parasite life cycle [83]. Inhibition of purinergic signaling by apyrase inhibitors impairs *P. falciparum* replication and reduces red blood cells (RBC) infection [84]. Indeed, mammalian RBCs express ADORA2b and several P2Y and P2X receptors on their surface [85]. In experimental cerebral malaria the expression of several P2X and P2Y receptors is altered after *P. berghei* infection [86]. However, to date little is known about purinergic modulation of malaria-specific immune response. Recent studies suggest that the expression of P2X7R in CD4 + T cells could be critical for Th1 profile development and IL-2 and IFN-γ production during chronic *P. chabaudi* infection, while increasing the T follicular helper (Tfh) cell population [87]. P2X7R-deficient mice present increased susceptibility to infection, which is associated with impaired Th1 response. Furthermore, infected children with severe malaria show augmented expression of CTLA-4 and PD-1, while children with uncomplicated malaria show increased CD39 + and Granzyme B + CD4 + T cells, indicating that distinctive regulatory mechanisms are triggered and may influence the clinical outcome of acute malaria [88].

Helminthiases are heterogeneous diseases caused by helminths, which are large multicellular pathogens that affect one-quarter of the human population. On the other hand, helminths are mainly controlled and eradicated by a canonical type 2 immune response [89]. The mammalian immune system needs to initiate rapid and effective anti-helminth mechanisms while simultaneously organizing the repair of inflicted mechanical damage to re-establish homeostasis

and prevent immunopathology. Although two antiparasitic drugs (moxidectin in 2018 and triclabendazole in 2019) have been recently approved by the FDA to treat helminthiases, these infectious diseases continue to be an important health problem, mainly among the poorest population[90]. Regarding purinergic modulation of anti-helminth type 2 immune responses, ADO is reported to play a protective role. Patel and colleagues reported that ADO signaling through ADORA2a induces *Heligmosomoides polygyrus* worm expulsion and a robust associated development of type-2 immune response. Mice genetically deficient in ADORA2a receptor exhibit increased *H. polygyrus* parasite load, decreased M2 macrophages and eosinophils recruitment and reduced IL-4 and IL-13 production against the tissue-dwelling parasites [91]. On the other hand, CD39 ectoenzyme activity has a controversial role in filariasis, a chronic helminth infection. Patients infected with filarial nematodes, such as *Mansonella ozzardi,* exhibit augmented frequency of circulating CD39 + CD4 + T cells compared to control donors, which correlates with the filarial parasite load. Infected patients also present decreased plasma TNF, IL-8 and IL-6 levels than their non-infected counterparts. Besides, CD39 inhibition decreases lymphocyte proliferation in vitro and pro-inflammatory cytokine response [92]. Moreover, during murine *Schistosoma mansoni* infection, mesenteric endothelial cells increase the expression of NTPDases 2 and 3 favoring local ATP hydrolysis and mononuclear cell adhesion via P2Y1R. This mechanism may contribute to mesenteric inflammation and infection morbidity [93]. Some authors propose the purinergic signaling as a potential target to reduce schistosomiasis morbidity [94]. In fascioliasis, an infectious disease caused by *Fasciola hepatica*, the purinergic pathway modulation depends on the phase of infection. In the acute phase, a decrease in NTPDase activity in serum and an increase in the ectoenzyme activity in the liver are observed. In contrast, after the establishment of chronic infection, an increase in NTPDase and 5′nucleotidase activities is found in both periods of infection. The modulation of purinergic environment may generate high ADO levels, which promotes host protection due to tissue injury caused by chronic infection [95]. Altogether, these findings reveal the direct involvement of purinergic pathways in immune response modulation in several parasitic infections. Given the current unmet medical need for novel pharmacological approaches, further studies are needed to deepen the knowledge in the molecular mechanisms and provide supporting evidence to evaluate purinergic target candidates for the treatment of neglected parasitic disease.

Bacterial infections

Tuberculosis (TB) is one of the most widely spread infectious disease and one of the major causes of mortality, since about two million people die annually because of this disease. It is caused by *Mycobacterium tuberculosis* and, less frequently, *M. bovis*. The lung is the portal of *M. tuberculosis*, as transmission occurs upon inhalation of aerosolized bacilli coughed from the lungs of infected individuals, so TB is primarily a pulmonary disease. However, it has many other manifestations, affecting bones, the central nervous system, among many other systems. The immune response against TB infection has been reported to be modulated by the purinergic pathway. TB patients present an increased Treg cell population ($CD4+CD25^{high}$ CD39 +) with regulatory properties. Depletion of CD39 + Treg cells significantly augments anti-TB T cell responses in vitro [96]. Likewise, CD39 mediates suppressive functions in CD8 + Treg cells in patients with active TB [97]. These reports suggest that CD39 plays an important role in the immunosuppression exerted by human Treg cell populations, possibly contributing to the balance between immune-mediated suppression and immunopathology in patients with TB. The CD73 ectoenzyme has also been described to modulate the anti-mycobacterial immune response. Indeed, CD73 activity restricts the early influx of neutrophils to the infected lungs without affecting bacterial load and spreading [98].

Purinergic receptors also have a role in determining the outcome of TB. P2X7R signaling in macrophages had a detrimental role in severe TB infection. Intracellular proliferation of high-virulent mycobacteria results in massive macrophage damage, ATP release and activation of P2X7R augment macrophage necrosis and spread the bacilli. Therefore, infected WT mice exhibit wide lung leukocyte infiltration and high mortality, in contrast to infected P2X7RKO mice, which showed moderate leukocyte infiltrates with ameliorated lung pathology [99]. Similarly, P2X7R signaling blockade with the antagonist BBG during experimental advanced pulmonary TB prevents the development of severe form of TB pathology in mice [100]. Additionally, chimeric WT mice with adoptive transfer of P2X7RKO hematopoietic cells show lower lung *M. bovis* bacterial burden and reduced pneumonia compared to WT mice. Lung necrosis and bacterial spreading to the spleen and liver are also diminished, suggesting that P2X7R in hematopoietic cells play a central role in the progression to severe TB [101]. In contrast to the results observed with high-virulence *M. tuberculosis and M. bovis* strains [99, 101], another report showed that P2X7RKO mice infected with less virulent *M. tuberculosis* exhibit augmented bacillary load in the lungs and increased Treg population but decreased B cells compared to WT mice [102]. Additionally, the potentiation of P2X7R augments inflammasome activation, resulting in the limitation of mycobacterial proliferation [103]. Indeed, patients with polymorphism in 1513C allele from P2X7R gene have increase susceptibility to extrapulmonary TB [104]. Given these results, it is plausible to speculate that immune response downstream P2X7R activation depends

on the mycobacterial virulence. ATP/P2X7R axis seems to be essential in the inflammasome-dependent IL-1β production and pathogen clearance in vivo in different bacterial infections (Fig. 3) [105, 106]. On the other hand, a critical modulatory function was assigned to the ADORA2a receptor during severe TB. AMP regulates macrophage state through ADORA2a receptor signaling. After ATP stimulation, it was quickly hydrolyzed to AMP, which downregulates inflammatory response and chemokine family genes, as well as strongly upregulates genes related to tissue repair, wound healing and angiogenesis [107]. In the same way, caffeine treatment, an ADORA2a receptor antagonist, enhances accumulation of CD4 + T cells with increased IFN-γ production in the lungs and promotes mouse survival during severe TB [108].

Streptococcus pneumoniae infection causes different illnesses, such as pneumonia, bacteremia and meningitis. *S. pneumoniae* interferes with the purinergic signaling by the induction of P2Y2R internalization in pulmonary epithelial cells in vitro, suggesting that bacteria may exert purinergic-dependent evasion mechanisms to promote infection [109]. Nevertheless, the host creates an adenosinergic microenvironment to be protected from exacerbated inflammation during infection. ADA-inhibited mice exhibit increased resistance to infection and ten-thousand-fold diminished bacterial load in the lungs and undetectable bacteremia. In contrast, CD73 pharmacologic inhibition induces increased susceptibility with higher pulmonary bacterial burden and increased bacteremia compared to mock-treated mice. The mechanism underlying this effect seems to be executed by ADO since it downregulates the neutrophil infiltration and their bactericidal ability, and the absence of these regulatory mechanisms is reported to be detrimental to the host [110]. Confirming this speculation, the authors found that CD73 augments neutrophil bactericidal activity by inhibiting IL-10 production, which is essential for ROS secretion [111]. On the other hand, ADORA1R signaling was reported to inhibit bacterial binding to human lung epithelial cells increasing host resistance to pulmonary infection [112]. Likewise, CD73-dependent ADO production signaling via ADORA2a receptor on B cells promotes isotype switch and regulates antibody response against *S. pneumoniae* infection. Stimulation of ADORA2a receptor with CGS21680 agonist augments IgG3 antibody protection in vaccinated young mice and enhanced survival after challenge [113]. On the other hand, ADORA2bR deficiency promotes extracellular bactericidal activity in neutrophils, enhancing NETs production. Indeed, ADORA2bR-deficient mice show diminished lung bacterial load and improved survival [114].

It has been also well-documented that purinergic signaling modulates immunity against enteropathogenic infections[115]. CD73 is highly expressed in the colonic epithelium, more specifically in the apical surface of intestinal epithelial cells (IECs). CD73 knockdown (KD) in IECs protects against *Salmonella* colitis as demonstrated by less marked weight loss and decreased colon shortening. CD73KD mice also show reduced bacterial translocation to the lumen and decreased *Salmonella* load in mesenteric lymph nodes, spleen and liver, suggesting that ADO promotes initial invasion and systemic dissemination [116]. *Salmonella* infection also induces downregulation of CD39 and CD73 ectoenzymes in CD4 + T cell population. Inhibition

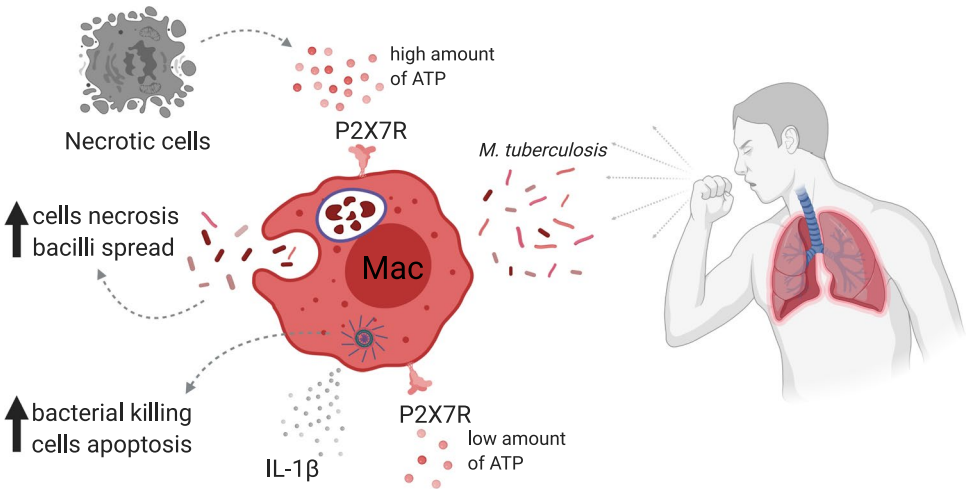

Fig. 3 P2X7R dual function in *Mycobacterium tuberculosis* infection. P2X7R activation plays a crucial role in control of infectious diseases. ATP-induced P2X7R activation may participate in the control of *M. tuberculosis* by improving intracellular pathogen killing and triggering the assembly and activation of the inflammasome and the consequent release of pro-inflammatory cytokines, such as IL-1β. However, its role in the host defense against *M. tuberculosis* infection is still controversial, due to its strong pro-inflammatory and cytotoxic activity; it may potentiate tuberculosis progression and the associated tissue damage. High concentrations of ATP can activate the P2X7R and induce the necrosis of infected cells leading to the spread of the bacilli and worsening the disease

of CD73 enzymatic activity in *Salmonella* whole cell lysate (WCL)-treated cells increases pro-inflammatory cytokine production, such as IFN-γ, TNF and IL-1β and inducible nitric oxide synthase (iNOS), leading to a more efficiently elimination of *Salmonella* bacteria compared to infected WT cells [117]. In addition, CD73 inhibition of Salmonella WCL-treated macrophages enhances the production of NO, pro-inflammatory cytokines IL-1β and TNF. Phagocytosis and intracellular killing are significantly higher in the APCP (CD73 inhibitor)-treated macrophages and in CD73KO peritoneal macrophages than untreated and CD73-competent macrophages [118]. Altogether, these results suggest that inhibiting ADO formation promotes pro-inflammatory and bactericidal functions in macrophage and improves the host's resistance to *Salmonella* infection. On the other hand, it has been also reported that P2X7R restricts Tfh cell expansion and germinal center reaction in the Peyer's patches in the setting of this infection. Depleting extracellular ATP, via administration of exogenous apyrase, increases the induction of specific IgA in response to *Salmonella* infection or an inactivated oral vaccine providing better protection from secondary infection [119]. Thus, preventing ATP accumulation could also be favorable for the improvement of the immune response against *Salmonella* infection.

Sepsis is a severe health condition caused by the exacerbated immune response to a microbial systemic infection. Extracellular ATP levels are augmented in the mouse model of polymicrobial sepsis induced by the cecal ligation and puncture (CLP). In this sense, several works have described the protective function of macrophages in septic conditions. ATP signaling in macrophages via P2X4R mediates a stimulatory effect on *E. coli* bacterial killing through augmented ROS production. P2X4R stimulation improves mouse survival by decreasing bacterial burden, pro-inflammatory cytokines, chemokine levels and spleen, liver and kidney injury in CLP-induced sepsis. As a logical consequence, pharmacological stimulation with ivermectin, a P2X4R agonist, protects the host against bacterial dissemination and mortality in sepsis [120]. Indeed, ADORA2b-deficient macrophages exhibit increased bacteria phagocytosis capacity, while ADORA2bKO mice show decreased peritoneal bacterial load and improved survival in a CLP model of sepsis [121]. Likewise, accumulation of adenosine by expanded CD39 + plasmablast subpopulation impairs macrophage bacterial killing and promotes IL-10 production via ADORA2a signaling in septic mice [122]. Besides, employing this mouse model of sepsis, Haskó and colleagues describe a protective role of P2X7R in macrophages by diminishing bacterial load and production of pro-inflammatory cytokines and chemokines, as well as improving mouse survival [123].

Purinergic signaling is also essential for TLR4-induced mitochondrial ATP synthesis and autocrine signaling, required for monocyte/macrophage activation, inflammasome assembly and IL-1β production[124]. ADP protects mice from *E. coli*-induced peritonitis by enhancing MCP-1-dependent recruitment of macrophages to the infected tissues. Both P2Y12R and P2Y13R deficiency inhibit the ADP-mediated immune response and promotes *E. coli* persistence in infected mice [125]. In the same way, P2X4R- and P2X7R-deficient mice have enhanced susceptibility to sepsis induced by uropathogenic *E. coli* and are more prone to die from a severe infection compared to WT mice. Higher systemic bacterial burden, plasmatic pro-inflammatory cytokines levels and activated coagulation cascade with increased levels of thrombin–antithrombin complexes are associated with their increased mortality [126]. Similarly, constant intravenous infusion with P2X1R antagonist markedly accelerates development of a septic response to *E. coli*-induced bacteremia. P2X1R antagonist-treated mice died at early times post-infection with high bacteremia and hematuria, substantially elevated plasmatic pro-inflammatory cytokines levels, massive intravascular coagulation and a concomitant reduction in circulating platelets [127]. Conversely, ATP-induced P2XR activation in vitro has a key role in the cytotoxic effects of α-hemolysin and leukotoxin A on THP-1 human monocytes. Inhibition of P2X1R, P2X4R or P2X7R significantly reduce THP-1 cytolysis protecting macrophage/monocyte phagocytosis function [128]. In vitro experiments also revealed that ATP signaling via P2X7R inhibits J774 macrophage phagocytosis of bio-particles coated with *S. aureus* or E. coli. A438079, an antagonist of P2X7R, partially reverts the effect of ATP on bacterial phagocytosis [129].

During *Staphylococcus aureus* infection, the signaling via P2X7R has been described as favorable for the elimination of the bacterial infection. Human macrophages internalize antimicrobial peptide LL-37 derived from neutrophils via a P2X7R-dependent mechanism, primarily involving a clathrin-dependent endocytosis pathway. P2X7R signaling in macrophages enhances intracellular ROS production and accumulation of lysosomes, which promote intracellular *S. aureus* elimination [130]. Similarly, P2XR inhibitors interfere with binding and oligomerization of hemolysin A from *S. aureus* (Hla) with cell membranes. The P2XR antagonists restrict the function of a membrane pore-forming toxin through non-canonical mechanisms. The data suggest the need for a critical revision of the notion that P2XRs are enhancers of membrane pore-forming toxin-dependent hemolysis. The role of ATP release and P2XR signaling in destabilizing the membrane stability and induction of pore formation is still controversial [131]. In line with this, several selective P2X7R antagonists inhibited *Clostridium perfringens* beta-toxin-induced cytotoxicity in THP-1 cells [132]. Finally, it has been shown that several bacteria exploit the production of ADO to dampen inflammation and support their survival during infection [133]. Summing up, the

findings described clearly illustrate that different pathogenic bacteria may exert their evasion mechanisms based on the production of purinergic metabolites, promoting an immunosuppressive microenvironment to persist in the host and establish a chronic disease. On the other hand, microbiota also can manage the purinergic system to its favor. Further investigations are needed to completely visualize the role of purinergic signaling in the host–bacteria interplay.

Viral infections

One of the most serious public health problems globally is HIV type 1 (HIV-1) infection since 38 million people are infected worldwide, and almost two million new infections occur each year. Antiretroviral therapies (ART) have allowed infected people to benefit from a better general state of health by significantly increasing their life expectancy thanks to the sustained suppression of viremia. Nevertheless, infected patients suffer from co-morbidities due to chronic inflammation derived from coinfections and immune dysregulation. Several studies emphasize the role of the purinergic pathway in the sustained inflammation and in different cellular processes of the acquired immunodeficiency syndrome (AIDS), usually associates with HIV-1 infection [134, 135]. HIV-1 infects several immune cells such as CD4 + T cells, myeloid dendritic cells and monocyte macrophages, all populations sharing the expression of CD4 receptor along with the chemokines receptors CCR5 and/or CXCR4. Indeed, untreated infected patients present increasing immunodeficiency with decreasing CD4 + T cell counts and a reversed CD4 + /CD8 + T cells ratio.

Although it is not fully addressed, ADO contributes to the suppression of CD4 + T cell responses during HIV infection. Numerous authors had reported that the expression of CD39 is upregulated in Treg cells compared to activated CD4 + T cells in HIV + patients. Regulatory functions of Treg cells are diminished by blocking with anti-CD39 antibody in a co-culture of Treg and CD8 + T cells. Furthermore, it was also shown that the expansion of CD39 + Treg cells inversely correlates with CD4 + T cells counts in treated patients [136]. In line with this, IL-2 production by activated CD4 + T cells is suppressed by CD39 + Treg cells via ADO/cAMP enzymatic pathway. Increased expression of ADORA2a receptor and intracellular cAMP levels in CD4 + T cells of HIV-infected patients diminishes IL-2 production and inhibits T cell proliferation [137]. Treg cells serve as viral reservoirs during chronic infection. Patients with advanced AIDS exhibit increased CD39 expression, and the CD39 + naïve Treg frequency directly correlates with HIV DNA levels [138]. Altogether, these findings suggest that CD39 pathway in Treg cells may be playing a deleterious role in the progression of HIV infection by suppressing CD4 + and CD8 + T cell responses and contributing to chronic inflammation and T cell dysfunction. Another mechanism that may be responsible for the AIDS pathogenesis is the significantly augmented frequency of CD39 + CD56^{bright} NK cells, observed in untreated HIV patients. This NK cell subpopulation is associated with an increased viral load, lower CD4 + T cell count and disease progression. Indeed, the authors propose CD39 as a marker of a regulatory NK subpopulation, since it exhibits increased AhR expression and IL-10 production [139]. Moreover, CD73 is also involved in the regulation of T cell activation. Contrasting to CD39 + Treg subset, CD73 + cells do not express FoxP3 and CD25 at high levels; thus, CD73 + CD4 + T cells represent a phenotypically and functionally different subpopulation [140]. CD73 + CD4 + T cell subset is preferentially depleted in HIV + patients compared to healthy controls, regardless of viral load and treatment. This subset depletion suggests the existence of an ADO-diminished microenvironment that may be unable to prevent persistent immune activation. Supporting this idea, an inverse correlation was shown between the CD73 + CD4 + T cell count and T cell activation, as well as plasma C reactive protein levels [141]. Additionally, HIV-infected patients show diminished expression of CD73 on CD8 + T cells, which is correlated with disease progression and partially reverts after effective ART [142]. Besides, CD73 is involved in the expansion of HIV-specific CD8 + T cells, whereas CD73 expression is higher in memory CD8 + T cell subset. The frequency of CD73 + CD8 + T cells is inversely associated with cell activation and plasma viral load. Interestingly, HIV controllers express high levels of CD73 [143]. Besides, untreated chronic patients present lower frequency of CD39 + CD73 + B cells compared to ART-treated patients and healthy controls. Downregulation of CD73 on B cells is associated with augmented expression Ki67 + and PD-1. In addition, CD73 loss also correlates with low CD4 + T cell counts, higher expression of markers of cellular proliferation and exhaustion, such as Ki-67 and PD-1, and decreased in vitro AMP catabolic activity and IgG switch ability dysregulation [144]. All these results in HIV + patients are depicted in Fig. 4.

Another component of the purinergic system that is associated with increased immune activation is ADA due to its potential to reverse immunosuppression driven by extracellular ADO. It has been shown that in both healthy and HIV-infected individuals, ADA is associated with increased expression of costimulatory molecules CD80, CD83, CD86 and CD40, promoting the maturation of DCs regardless of its enzymatic activity as shown after desalting and treatment with $HgCl_2$. ADA treatment enhances DCs immunogenicity and promotes their capacity to stimulate allogenic T cell subpopulations to proliferate [145]. In line with this, it has also been shown that in autologous co-cultures of T cells with HIV-1 peptide-pulsed DCs, the addition of ADA leads to a

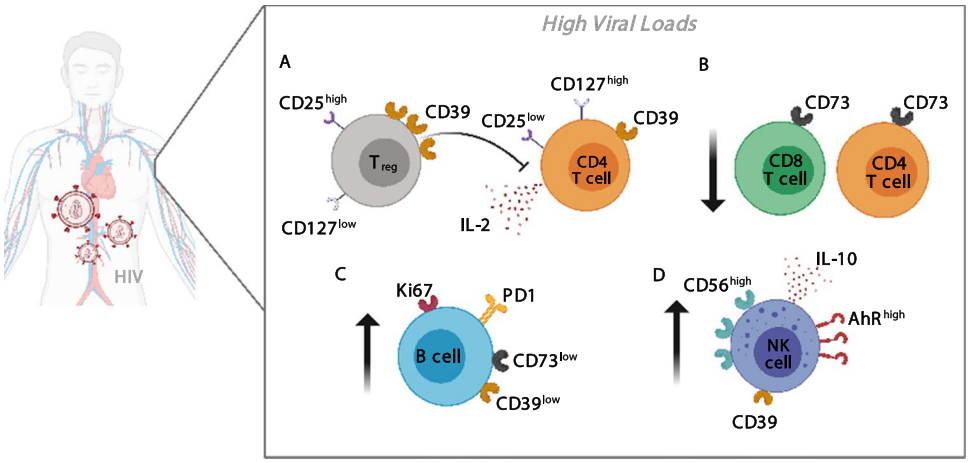

Fig. 4 Purinergic dysregulation in untreated HIV + patients with high viral loads. **(A)** CD39 + Tregs suppress in vitro IL-2 production by activated CD4 + T cells via adenosine/cAMP enzymatic pathway. **(B)** CD4+ and CD8+ T cells subsets expressing CD73 ectoenzyme are preferentially depleted in HIV + patients compared to healthy controls, regardless of viral load and treatment. **(C)** Untreated chronic patients present diminished frequency of CD39 + CD73 + B cells. In fact, the downregulation of CD73 expression on B cells is associated with augmented cellular proliferation (Ki67 +) and exhaustion phenotype (PD-1 +). **(D)** CD39 + CD56^{bright} NK cell subset presents increased AhR expression and IL-10 production and is associated with lower CD4+ T cell counts, higher viral loads and disease progression.

reduction in Treg frequency and to a significant enhancement of HIV-1-specific effector CD4 + T cells in a dose-dependent manner. At the same time, ADA addition increases the secretion of Th1 inflammatory cytokines and proliferation of CD8 + T cells, demonstrating that ADA treatment could improve the immune subpopulations involved in the control of HIV-1 infection [146]. These results are in agreement with previous reports showing that the addition of extracellular ATP to the co-cultures also inhibits the propagation of HIV-1 from the immature DCs to autologous CD4 + T lymphocytes [147]. ADA-1 modulates the program of circulating and germinal center Tfh cells, enhancing their function. This mechanism promotes effective collaboration between T and B cells and the generation of antibodies, by influencing the production of IL6/IL-2 and controlling the expression of CD26 and the ADORA signaling. The authors suggest that ADA represents a potential target for the development of Tfh-targeted vaccine strategy [148].

It should be noted that purinergic signaling has been associated with both a protective and pathological role in different viral infections. The role of adenosine pathway signaling in HIV infection has been addressed in[149]. For example, HIV infection of CD4 + T cell induces Panx1 hemichannels opening, required for effective HIV replication in CD4 + T lymphocytes [150]. Furthermore, ATP activates P2Y2R and induces activation tyrosine kinase 2 and transient depolarization of the plasma membrane, which stimulates fusion between the envelope protein and CD4-containing membranes [151]. On the other hand, P2X1R inhibition has been reported to block HIV-1 entry and replication within target cells [152]. Inhibition of P2X1R, P2X7R and P2Y1 significantly reduces viral replication within human macrophages, whereas P2X1R antagonists block viral entry to the cell. The data also indicate that binding gp120 protein results in the rapid ATP release from macrophages and allows autocrine P2R activation, facilitating HIV infection [153]. In the same way, the ecto-ATPase activity increases in in vitro HIV-1-infected human macrophages and its blockade prevents HIV-1 replication [154]. In fact, it has been proven that P2X1R and P2X7R selective antagonists abrogate both HIV-1 infection and the subsequent inflammation. The purinergic P2X1R and P2X7R antagonists inhibit HIV-1 infection, but only P2X1R antagonists downregulate IL-1β and IL-10 production in ex vivo infection of human tonsil [155]. It has been reported that P2X1R antagonist (NF279) inhibits the fusion with the HIV-1 cell membrane by blocking the virus binding and entrance via CXCR4 and CCR5l [156]. Moreover, blockade of P2XR signaling inhibits HIV-1 membrane fusion [157].

Extracellular ATP is capable of reducing the replication of DNA and RNA viruses through activation of P2X7R, enhancing the production and release of IFN-β, which is crucial in promoting antiviral immunity [158]. This is also an essential antiviral mechanism against respiratory viruses. Adenovirus and Influenza viruses increase ATP and ADO levels in the respiratory tract. The induction of purinergic P2X7R signaling plays a key role in generating a robust inflammatory response capable of controlling these infections. If the infection persists, a systemic inflammatory response can be triggered, which is associated with higher mortality. Disruption of the ATP/P2X7R interaction avoids symptoms of acute respiratory distress syndrome (ARDS)

associated with less infiltration of neutrophils and macrophages and lower levels of pro-inflammatory cytokines, associated with an enhanced survival of mice after infection with Adenovirus [159] or Influenza virus [160]. In turn, a marked increase in morbidity and mortality is observed in P2Y2R-deficient mice in response to lung infection with the human respiratory syncytial virus. The lower survival of the P2Y2KO mice could be associated with the defective infiltration and response of Th1 cells and higher viral titers, evidencing the protective role of P2Y2 receptor in lung infections [161]. On the other hand, ADORA1R activation significantly contributes to the generation ARDS. Nevertheless, the activity of CD73 is not required to generate ARDS [162]; rather, ADO generation is due to the enzymatic action of tissue non-specific alkaline phosphatase, an enzyme present in the lung parenchyma with low affinity but high capacity to metabolize ATP/AMP in a non-specific way [163].

In December 2019, the new ARDS-related to Coronavirus 2 (SARS-CoV-2) infection that causes COVID-19 disease was reported for the first time in China. As already reported, severe cases are associated with hyper-activation of the immune system and an excessive release of cytokines called cytokines release syndrome (CRS). So far, Ahmadi and colleagues showed that circulating cytotoxic cells (CD8 + T cells, NK cells and NKT cells) express lower levels of CD73 ectoenzyme in COVID-19 patients compared to healthy donors. Furthermore, CD73 + and CD73- subpopulations of CD8 + T cells and NKT cells with different functionality are observed in COVID-19 patients. CD8 + T cells and NKT cells lacking CD73 exhibit increased cytotoxic effector capacity compared to their counterpart CD73 + [164]. P2X7R hyperactivation has been reported in COVID-19 as a key driver of inflammation and NLRP3 inflammasome activation [165, 166], potentially driving central nervous system diseases [165, 167]

Purinergic pathway also has been studied in endemic mosquito-borne viral diseases, such as dengue virus (DENV) infection. This represents the most common arboviral infection in humans and can generate a wide spectrum of clinical manifestations. In severe cases, higher mortality is associated with hyper-activation of the immune system. Through the addition of specific P2X7R inhibitors, several authors demonstrated that P2X7R signaling generates an increase in antiviral responses, either by augmenting NO and cytokine production in human monocytes [168] or by improving human T cell function, which provide an early source of IFN-γ and killing DENV-infected cells [169]. Dengue hemorrhagic fever is a systemic endothelial dysfunction resulting from the prolonged and exacerbated immune response. Many proteins and signaling pathways are involved in maintaining the integrity of the endothelial barrier, including the CD73/ADO axis. Probably, the endothelial infection with DENV decreases expression of CD73 and this could be associated with a recovery of endothelial barrier function [170].

Viral infections are the major causes of acute and chronic liver diseases such as hepatitis. Hepatotropic virus, such as hepatitis B virus (HBV) and hepatitis delta virus (HDV), requires the activity of P2XR to infect primary human hepatocytes [171]. In chronic hepatitis C virus (HCV)-infected patients, the expression of P2X1R, P2X4R and P2X7R in peripheral blood mononuclear cells increases compared to control patients. However, it remains to be elucidated whether the expression of purinergic receptors is associated with an antiviral immunity or participates in the pathophysiology of the disease [172, 173]. Furthermore, in patients that develop hepatocellular carcinoma (HCC) induced by a HCV infection, the P2X4R expression is increased compared to patients with HCC from non-viral causes [174]. Regarding the ectoenzymes, recently a population of CD39 + Treg cells was identified to be associated with potent immunosuppressive activity and the progression of viral infections. Tang and colleagues showed that the proportion of circulating CD39 + FoxP3 + Treg cells correlates positively with HBV viremia levels and, conversely, negatively correlates with markers of liver damage [175].

A prominent role of the purinergic system in intestinal function was demonstrated under physiological and pathological conditions. ADO plays a central role in intestinal functions, by modulating the interaction between enteric nervous system (ENS), smooth muscle and epithelial barrier function. Viral-induced ENS neurodysfunction is associated with a markedly altered ADO metabolism, with a spatial and functional reorganization of all ADORA receptors and an increased expression of ADA and CD73 ectoenzyme in the gut [176]. On the other hand, the treatment of different human cell lines with imiquimod, an antagonist of the ADORA1 receptor, suppresses the replication of HSV-1 [177]. Within the family of herpes viruses, the human cytomegalovirus (CMV) is a β-herpes virus that infects a large proportion of the population worldwide. Unlike what happens in immunocompetent people, it can cause severe diseases in immunosuppressed patients. In vitro studies show that several components of the purinergic signaling system play critical roles within CMV-infected cells. CMV infection induces P2Y2R and P2X5R expression in human fibroblasts. Whereas P2Y2R enhances CMV performance due to its importance for efficient virus gene expression, DNA synthesis and production of infectious progeny, the P2X5R reduces CMV production [178]. On the other hand, the purinergic system may be involved in the carcinogenesis of different chronic viral infections, such as Epstein-Barr virus (EBV)-associated and human papillomavirus (HPV)-related carcinomas [179, 180].

Conclusions and therapeutic perspectives

For several decades, the purinergic system has been extensively studied as possible therapeutic target for inflammatory, autoimmune and oncological diseases [181–186]. Inflammation drives the expression of purinergic components and subsequent, activation of purinergic signaling to prevent tissue collateral damage. After infection, extracellular ATP supports the establishment of a set point of various signaling pathways and affects the responses of immune cells. Therapies targeting increased ATP and P2 receptor signaling may be fundamental in controlling the collateral injury and maintaining healthy tissues for the survival of the host in the acute phase of infection. In particular, ATP-P2X7R pathway inhibition may be potentially effective for therapeutic development [44]. ATP catabolism to ADO remarkably increases as a result of the activities of extracellular ecto-apyrases, ecto-ATPases and ectonucleotidases. Rapid uptake and metabolism of ADO maintains its low levels in healthy and unstressed cells or tissues, but during inflammatory conditions this equilibrium breaks down and extracellular ADO level rises substantially. The imbalance of the purinergic microenvironment and its signaling mechanism can trigger immune exacerbation or suppression. Resulting purinergic microenvironment and receptor signaling certainly have a fundamental role as mediators of host immune response and pathogen interaction. Indeed, adenosinergic pathway inhibitors may reinvigorate the immune cell effector functions and improve immune response fitness [187]. Better understanding of the effect of ADO on both host innate and adaptive immune system function in chronic infectious diseases is required to avoid the persistence of the microbe and the progression of the disease [188].

Ectonucleotidases from the microorganisms are major tools to escape from innate and adaptive immunity and should be taken into account as potential targets for new therapeutic approaches, and also, vaccines development [189, 190]. In some cases, the host's own organism generates an anti-inflammatory microenvironment to prevent vital organs damage and promote survival, thus allowing the microorganism to replicate without having to hide or fight a robust immune response against it. The persistence of numerous microorganisms is improved by their ability to modulate the release of nucleotides and nucleosides and signal host parenchymal and immune cells. Depletion of extracellular nucleotides or pharmacological inhibition of purinergic receptors during certain infections may represent effective strategies to prevent infectious disease. Further investigations will precisely define the molecular actors of these processes and hence might identify a series of novel druggable targets for the treatment of microbial infections.

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Declarations

Conflicts of interest The authors declare that there is no conflict of interest.

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Consent to participate Not applicable.

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