Abstract

Human immunodeficiency virus (HIV) infection is a serious condition associated to severe immune dysfunction and immunodeficiency. Mechanisms involved in HIV-associated immune activation, inflammation and loss of CD4+ T cells have been extensively studied, including those concerning purinergic signaling pathways. Purinergic signaling components are involved in viral entry and replication and disease progression. Research involving the participation of purinergic signaling in HIV infection has been not only important to elucidate disease mechanisms but also to introduce new approaches to therapy. The involvement of purinergic signaling in the pathogenesis of HIV infection and its implications in the control of the HIV infection are reviewed in this paper.

Key words: Human immunodeficiency virus; Purinergic signaling; Immune activation; Inflammation

Core tip: This paper reviews the latest findings regarding the involvement of the purinergic signaling system and human immunodeficiency virus (HIV) infection. On the last 10 years, several studies have been published on the participation of purinergic signaling in HIV infection. The findings helped to elucidate disease mechanisms and proposed new targets and approaches to therapy. We have found that basic and clinical research on this field are very promising and must be further pursued.

Daniela F Passos, Maria Rosa C Schetinger, Daniela BR Leal

Correspondence to: Daniela BR Leal, PhD, Departamento de Microbiologia e Parasitologia, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Av. Roraima, Santa Maria, RS 97105-900, Brazil. daniela.leal@ufsm.br
Telephone: + 55-55-32209581
Fax: + 55-55-32208242

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INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is a pandemic disorder caused by the human immunodeficiency virus (HIV). HIV infection is characterized by persistent immune activation, inflammation and loss of CD4+ T cells, which altogether lead to immunodeficiency[1-3]. The pathological mechanisms involving these dysfunctions along with disease progression markers and prospective ways of halting disease progression are targets of extensive research. Although a great number of pathological mechanisms have been proposed, a lot remain unclear.

The connection between purinergic signaling and HIV infection is not consistently in favour of the host or the pathogen. Purinergic receptors may favour viral entry[4,5] whilst its enzymes may help to halt disease progression[6] and boost immune response against the virus[7]. However, the increasing knowledge of purinergic signaling components and their connection to HIV infection has been remarkably valuable in the understanding of the disease mechanisms. Furthermore, this knowledge has raised the possibility of using purinergic receptors antagonists, purinergic signaling mediators and their analogs in HIV therapy[8-10].

The aim of this paper is to review purinergic signaling and its involvement, through its components (enzymes, receptors) and mediators (adenosine triphosphate (ATP) and adenosine), in HIV virus entry and replication, disease progression, and potential therapeutic strategies.

BACKGROUND ON PURINERGIC SIGNALING

Following the identification of ATP, along with purinergic co-transmission and the P1 and P2 receptors in the 70’s, the purinergic signaling system has been intensely studied[11,12]. The receptors were characterized and the ATP mechanisms of release and breakdown have been described. Consequently, the involvement of the purinergic system in the pathophysiology of several human disorders has been uncovered and the possibility of using these pathways as targets for therapy has been raised[12].

ATP, adenosine diphosphate (ADP), Adenosine monophosphate (AMP) and adenosine are extracellular purines that mediate a series of physiological and pathological processes[12]. Receptors to purines are specific cell surface molecules called purinergic receptors. Two distinct purinergic receptor families have been indentified: P1 and P2 receptors. P1 are specific to adenosine and comprise 4 subfamilies, while P2 receptors are selective to ATP and AMP and contain two subfamilies, P2X and P2Y, based on their chemical properties. Additionally, P2X is subdivided into 7 subtypes and P2Y into 8[12,14].

Purinergic receptors families, subfamilies and subtypes are shown on Table 1.

Extracellular nucleotide concentrations are regulated by ectoenzymes that hydrolyse these nucleotides; the NTPDase1 (CD39) cleaves ATP to AMP, NTPDase2 (CD39L1) cleaves ATP to ADP, 5′-ectonucleotidase (CD73) produces adenosine from AMP, ectonucleotide pyrophosphatase/phosphodiesterase (E-NPP) breaks down ATP into AMP, and alkaline phosphatases (AP) which dephosphorylates nucleotides[13]. Adenosine, which has its physiological effects mediated by P1 receptors, can be either transported into the cell or inactivated by adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP)[15]. Figure 1 shows a schematic representation of the purinergic pathway. Although each purinergic signalling component has its own function they do not operate independently. In physiological conditions they act in cooperation interfering with the function of other elements. When considering pathogenic conditions these cross-talks and networks must be taken into consideration[16].

In viral infection, purinergic extracellular nucleotides and receptors not only participate in the innate and adaptive responses but also modulate the immune responses[17]. The release of ATP by damaged cells generates a danger signal acting as a DAMP, it also stimulates the NOD-like receptor mediated inflammasome and the activation of the caspase-1 pathway[18]. Extracellular ATP also acts as a costimulatory signal to T cells and drives the differentiation of gut T helper 17 (Th17) cells[19].

The purinergic signaling system is involved in a series of processes including neurotransmission, neuro and immune modulation, secretion, cell proliferation, differentiation, apoptosis, cell death, phagocytosis, chemotaxis and embryonic development[11,17,20,21]. Purinergic signaling has been linked to a series of acute and chronic inflammatory diseases including inflammatory bowel disease[22], cancer[12], ischemia[23], acute lung injury[24] and osteoporosis[25]. Therapeutic approaches using the components of the purinergic system are being developed for a number of diseases[12] such as cancer[20,26,27], diabetes[28], osteoporosis[20] and neurodegenerative diseases[29] as well as HIV[8-10]. Consequently, the involvement of the purinergic system in the pathophysiology of several human disorders has been uncovered and the possibility of using these pathways as targets for therapy has been raised[12].

The first study linking the purinergic signaling system and HIV infection was published in 2005, Leal et al[6] identified an increased NTPDase activity in CD39-positive lymphocytes of HIV-infected patients. Further studies associating CD39 and HIV disease progression were published in 2011 and 2013[21-23]. In 2007, a study highlighted the protective effect of adenosine receptors against neuronal damage in primary murine cultured brain cells[26], followed by later studies correlating dementia and other HIV-associated neurocognitive disorders to ATP release and P2X in primary neuron-glia co-cultures from mouse striatum[25] and adenosine receptors in HIV-infected macrophages[30]. Studies on pannexin hemichannels and purinergic receptors and HIV infection have been
As with other viruses, the first line of defence against HIV is the innate response. Germ line-encoded Pattern-Recognition Receptors (PRRs) are essential players in the innate response. PRRs include toll-like receptors (TLRs), membrane-bound C-type lectin receptors (CLRs), NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs), and unidentified proteins capable of recognizing DNA or RNA[43]. These PRRs recognize pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). Although the knowledge of HIV-specific PRRs and PAMPs remains scarce, RIG-I was recently described as being involved in recognizing cytosolic HIV genomic RNA[44]. The recognition of PAMPs and DAMPs by the PRRs triggers a cascade of signaling pathways on the surface of antigen-presenting cells (APCs) and dendritic cells (DCs), which initiate host inflammatory and immune responses. When the APCs and the DCs are stimulated, the CD4+ T cells and the natural killer (NK) cells are activated in the lymph nodes and the adaptive response is initiated[45,46].

DCs are not only important APCs along with macrophages and monocytes in both innate and adaptive responses[3], but they also modulate the adaptive response together with NK cells[46]. Once the DCs are activated, they produce cytokines and induce T helper 1 (Th 1) responses and consequently cytotoxic T lymphocyte (CTL) responses[46]. However, HIV specifically targets CD4+ T cells along with macrophages and DCs which are essential for the antiviral response[2].

### Table 1 Purinergic receptors families, subfamilies and subtypes, subdivisions

| Family | Subfamily | Subtype | Ligand | Cell type expression |
|--------|-----------|---------|--------|----------------------|
| P1     | A1        | NA      | Adenosine | Neutrophils, monocytes, macrophages, and DCs |
|        | A2A       | NA      | Adenosine | Neutrophils, monocytes, macrophages, DCs, T, B and NK cells |
|        | A2B       | NA      | Adenosine | Neutrophils, monocytes, macrophages, DCs, T and NK cells |
|        | A3        | NA      | Adenosine | Neutrophils, monocytes, macrophages, DCs, T and NK cells |
| P2     | P2Y       | P2Y1    | ADP     | Neutrophils, monocytes, macrophages, DCs, T, B and NK cells |
|        |           | P2Y2    | ATP, UTP| Neutrophils, monocytes, macrophages, DCs, T and NK cells |
|        |           | P2Y4    | UTP (ATP, UDP) | Monocytes, macrophages, DCs, T and B cells |
|        |           | P2Y6    | UDP, UTP| Neutrophils, monocytes, macrophages, DCs, T and B cells |
| P2Y11/P2Y8 | P2Y11 | ATP | Monocytes, macrophages, DCs, T and B cells |
|        | P2Y12     | ADP     | Neutrophils, monocytes, macrophages, T and B cells |
|        | P2Y13     | ADP, ATP| Neutrophils, monocytes, DCs, T and B cells |
|        | P2Y14     | UDP, glucose | Neutrophils, DCs, T, and B and NK cells |
| P2X    | P2X1      | ATP     | Neutrophils, monocytes, macrophages, DCs, T, B and NK cells |
|        | P2X2      | ATP     | B and NK cells |
|        | P2X3      | ATP     | B and NK cells |
|        | P2X4      | ATP     | Neutrophils, monocytes, macrophages, DCs, T, B and NK cells |
|        | P2X5      | ATP     | Neutrophils, monocytes, macrophages, DCs, T and B cells |
|        | P2X6      | ATP     | B and NK cells |
|        | P2X7      | ATP     | Neutrophils, monocytes, macrophages, DCs, T and NK cells |

NA: Not applicable; DC: Dendritic cells; NK: Natural killer cells. Adapted from Junger WC, 2011[54].

### HIV INFECTION AND THE IMMUNE SYSTEM

Host defence against HIV depends on a combination of adaptive and innate responses[39]. Despite the ability of these responses to briefly control disease progression, they are not capable of eliminating the virus. The complex interaction between the host response and HIV virus has been extensively studied and the HIV has been shown to take advantage of host metabolic pathways and proteins, known as host permissive factors, allowing the virus to take advantage of host metabolic pathways and proteins, which results in activation of purinergic receptors[40-42].

As with other viruses, the first line of defence against HIV is the innate response. Germ line-encoded Pattern-Recognition Receptors (PRRs) are essential players in the innate response. PRRs include toll-like receptors (TLRs), membrane-bound C-type lectin receptors (CLRs), NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs), and unidentified proteins capable of recognizing DNA or RNA[43]. These PRRs recognize pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). Although the knowledge of HIV-specific PRRs and PAMPs remains scarce, RIG-I was recently described as being involved in recognizing cytosolic HIV genomic RNA[44]. The recognition of PAMPs and DAMPs by the PRRs triggers a cascade of signaling pathways on the surface of antigen-presenting cells (APCs) and dendritic cells (DCs), which initiate host inflammatory and immune responses. When the APCs and the DCs are stimulated, the CD4+ T cells and the adaptive response is initiated[45,46].

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### PURINERGIC SIGNALING AND THE IMMUNE SYSTEM

In physiological conditions, the cells are able to maintain a balance in the levels of ATP[47]. In pathological conditions, on the other hand, injured, necrotic and activated cells release ATP into the extracellular environment, where it interacts with P2 receptors or is degraded by ectoenzymes. Purinergic signaling seems to be an important regulator of the activation of NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome. P2 receptors control the potassium efflux contributing to the activation of the inflammasomes[48,49]. The release of ATP from necrotic cells activates the NLRP3 inflammasome via the P2X7 receptor[50]. HIV-1 acts as primary signal to activate the NLRP3 inflammasome[51], since local release of ATP is stimulated by the binding of HIV gp120 to its receptor which results in activation of purinergic receptors[52].

The activation of the inflammasome is important in the antiviral response since it may lead to the elimination of the infected cells by pyroptosis[53-55]. Figure 2 illustrates the activation of NLRP3 inflammasomes in a HIV infected CD4 T cell. In the specific case of HIV infection pyroptosis might not be a protective method since it does not...
eliminate the infection, instead it creates a pathogenic cycle in which the death of CD4+ T cells results in the release of inflammatory signals that attract more CD4+ T cells which subsequently die creating a state of chronic inflammation\cite{54}.

Recruitment of inflammatory cells to the sites of infection as part of an antiviral response involves the release of nucleotides and an autocrine purinergic signaling pathway\cite{42,56}. The release of nucleotides triggers the polarization of purinergic proteins and receptors contributing to migration of phagocytes to the sites of inflammation and infection\cite{60}. P2 purinergic receptors are also involved in chemotaxis\cite{42}. Purinergic receptors are not only involved in chemotaxis but also in the modulation of immune responses\cite{56}.

The multiple and complex processes leading to inflammation and immune activation are not fully understood. Appay and Sauce\cite{2} (2008) proposed a simplified model for immune activation and inflammation in HIV infection in which three well-known major events, depletion of CD4+ T cells, immune activation and exhaustion of regenerative capacity, all contribute to inefficient immune response and loss of T cell homeostatic regulation\cite{2}. During acute infection, the depletion of gut mucosal CD4+ T cells triggers the loss of protective mechanisms such as the epithelial barrier and immune cells that otherwise would block translocation of microbial products from the gut into the circulation\cite{57-59}. These microbial products such as bacterial DNA and lipopolysaccharides activate the innate response receptors and a signaling cascade, consequently boosting the production of inflammatory cytokines\cite{60}. These events prompt a systemic immune activation that characterizes the chronic phase of HIV infection and consequent loss of peripheral CD4+ T cells\cite{60}. Chronic immune activation and inflammation increases cell turnover and causes the accelerated aging of the immune system driving HIV-specific CD8+ T cells to exhaustion\cite{2}.

DCs represent an important link between the innate and adaptive responses. ATP enhances the antiviral response by activating DCs which then migrate to the lymph nodes. Extracellular ATP was found to interfere with the transfer of HIV-1 from immature DCs to CD4+ T cells thereby controlling the spread of the virus by halting viral replication\cite{53}.

ATP RELEASE AND PURINERGIC RECEPTORS IN HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS

ATP release and purinergic receptors are involved in pathologies of the central nervous system (CNS) in neurodegenerative, neuropsychiatric and neurocognitive diseases\cite{62}. Regarding the toxic effects of extracellular purines in HIV infection, a connection between HIV-associated neurocognitive disorders (HAND) and the pathological release of purines by HIV-infected macrophages was found. High concentrations of ATP, ADP, AMP and small amounts of adenosine were found in HIV-infected macrophages along with glutamate, suggesting that ATP release from these cells might be involved in neuronal damage in HIV-infected patients\cite{53}. These purinergic molecules are thought to mediate calcium influxes through activation of calcium receptors, causing damage...
Several mechanisms are involved in the neurocognitive impairment that affects HIV infected patients. The HIV transactivator of transcription Tat induces the release of cytokines and chemokines from microglia, macrophages, neurons, and astrocytes in the CNS and causes disruption of the blood-brain barrier with resulting neurotoxicity. Recently it was suggested the P2X receptors are involved in HIV and opioid neuropathogenesis. The use of TNP-ATP, a non-selective P2X antagonist, prevented the neurotoxic damage caused by exposure to morphine and/or HIV Tat or ATP in striatal neurons. P2X4 receptors are expressed by subpopulations of striatal neurons and glia and are activated by excess levels of extracellular ATP caused by morphine and/or HIV-1 Tat. The involvement of P2X4 in HIV and opioid neuropathogenesis was confirmed by the fact that selective blockade of P2X1, P2X3, or P2X7 receptors, not P2X4, were not able to prevent Tat or morphine induced neurotoxicity, suggesting this particular receptor might be a potential new target for prevention of HAND.

HIV-associated dementia is linked to inflammation and consequent production of proinflammatory cytokines. The adenosine receptor A1AR was shown to inhibit Tat-mediated proapoptosis by attenuating intracellular Ca²⁺ and production of nitric oxide. More recently, it was demonstrated that the activation of the A2A adenosine receptor inhibits Tat-induced TNF-α production in monocytes thereby suggesting that adenosine is an important regulator of cytokine production and its pathway a possible therapeutic target for CNS inflammatory disorders.

**PANNEXIN HEMICHANNELS AND PURINERGIC RECEPTORS ARE INVOLVED IN THE PROCESS OF HIV VIRAL ENTRY AND IMMUNE ACTIVATION**

ATP is transported mainly through a combination of vesicular release, connexin and pannexin hemichannels, but also includes P2X7 receptors and maxi-ion channels. The role of pannexin hemichannels have been studied in a number of pathophysiological events including calcium signaling, cellular differentiation, cellular migration, inflammation, cell death, innate and adaptive immune responses.
ECTOENZYMES AND HIV DISEASE PROGRESSION

Whilst ATP may promote inflammation, adenosine is considered a mostly anti-inflammatory molecule and a crucial regulator in innate and adaptive immune responses[70]. Ectoenzymes CD39 (NTPDase-1) and CD73 (5'-ecto-nucleotidase) are known to dephosphorylate extracellular ATP to adenosine, playing an important role in immune modulation[33,71,72]. The role of the ectoenzymes in physiological processes and diseases has been extensively studied, specially the NTPDase and 5'-ecto-nucleotidase activities[73]. Co-expression of CD39 and CD73 plays an important part in keeping the balance between activation and regulation of the immune response against HIV and, subsequently, in the halting of disease progression[31,74].

CD39 cleaves ATP into AMP while CD73 converts AMP into adenosine, increasing the expression of A2AR agonist and cyclic AMP (cAMP). CD39 is a marker of lymphoid activation which requires ATP as an energy source. This enzyme hydrolyses ATP into AMP to maintain the ATP levels, preserve cellular integrity and modulate the immune response[6]. Our group has found that the NTPDase-1 activity is increased in lymphocytes of HIV-positive patients suggesting that it might be due to apoptosis and the need to reduce the toxic effects of ATP release[6]. In the last few years a series of studies have investigated the role of T regulatory (Treg) cells expressing CD39 in HIV infection[31-33]. Nikolova et al[23] (2011) have established that the expansion of Treg CD39+ cells in HIV-infected individuals might contribute to disease progression, since they inhibit T cell proliferation. Another study also describes the increased expression of CD39 in Treg cells and its association with disease progression and immune activation[32]. In addition, CD39 was found to be involved in the inefficiency of the CD4+ T cell response during chronic HIV infection by inhibition of important cytokine production[33]. In support of this finding, Jenabian et al[31] (2013) show that the expansion of Treg CD39+ cells inhibits IL-2 production thereby suppressing the function of CD4+ T cells; this occurs through demethylation of an essential CpG site in the IL-2 gene promoter. CD39+ Treg cells were shown to inhibit HIV replication mediated by cAMP in conventional T cells, suggesting they have a protective effect against HIV infection[75].

Unlike CD39, that is overexpressed in HIV-infected individuals, CD73 has shown to be depleted, which leads to decreased adenosine suppression and failure to control persistent cell activation contributing to disease progression[76,77]. Another interesting finding is that once the pool of CD4+CD73+ cells is depleted they can no longer be expanded, even if the CD4+ cell levels are recovered[70].

ADA is a purinergic signaling enzyme responsible for catalyzing the deamination of adenosine and also involved in modulating the T cell response against HIV[7,78-80]. ADA boosts both the CD4+ and CD8+ memory cell response
to HIV and also reduces the suppression mediated by Treg cells\(^7,78-80\). In DCs, ADA has been shown to be capable of boosting immunogenicity and increasing the secretion of pro-inflammatory cytokines\(^7,78,80\). All this data taken together suggests that ADA would be a strong candidate target for therapeutic and vaccine approaches\(^7,78,79\).

**ADA AS DISEASE PROGRESSION AND SENESCENCE BIOMARKER**

HIV infected individuals are subject to accelerated aging, this might be due not only to the chronic inflammation and immune activation inherent of HIV infection\(^81\), but also as a consequence of highly active antiretroviral therapy (HAART)\(^82\). In fact, immune activation has been a major cause of morbidity and mortality from AIDS-defining and non-AIDS defining diseases in patients undergoing antiretroviral treatment\(^83\). The increased production of inflammatory cytokines arising from the chronic inflammatory status enhances a process called “inflammaging”\(^84\). The term “inflammage” was employed to define the extensive activation of innate and adaptive immunity during HIV infection, which resembles the process of inflammaging\(^85,86\). The HIV infection per se predisposes infected patients to premature aging, however the interplay between these pathological changes and HAART makes the situation even more complex\(^82\).

The age-associated disorders that affect HIV infected patients include neurological and metabolic diseases and immunosenescence; the premature aging also has an impact on disease progression markers such as CD4+ and CD8+ T cell counts\(^87\). Since immune activation is a strong predictor of disease progression and consequent immunosenescence and premature aging, several biomarkers have been identified\(^37,38,88\). ADA has been shown to be not only a suitable marker of immune activation and disease progression\(^37\) but also a suitable biomarker of senescent human CD8+ T cells\(^38\).

**PURINERGIC RECEPTORS, ATP/ADENOSINE AND ATP ANALOGS AS POTENTIAL THERAPEUTIC APPROACHES**

The study of the implications of ATP and purinergic signaling in HIV infection has highlighted its potential use in therapeutic approaches\(^4,8,9,89\).

As discussed earlier in this paper, studies have shown that purinergic receptors are essential for viral entry and replication\(^4,5\). A recent study has demonstrated that the inhibition of purinergic signaling blocks HIV-1 membrane fusion\(^10\). This study reveals that PPADS, a nonselective purinergic antagonist, is capable of inhibiting cell-to-cell and cell-free HIV-1 infection in both X4- and R5-tropic virus infections\(^10\). This finding highlights the potential use of P2X-selective purinergic antagonists to inhibit HIV-1 fusion.

Wagner\(^8\) (2011) proposes the use of ATP combined with HAART to eliminate infected cells, exploiting the ability of ATP to modulate the immune response\(^37\).

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**Figure 3** Schematic representation of purinergic receptors involvement in human immunodeficiency virus infection. Pannexin hemichannels (Panx-1) are open in response to ATP release and activation of purinergic receptors, facilitating viral entry, fusion and replication. The blockage of viral entry by P2X1 antagonists suggests it is involved in this stage of infection. P2Y2 increases cell membrane depolarization facilitating fusion. P2X7 and P2Y1 are involved in later steps of viral cycle.
Recently, the use of ATP analogs was proposed to inhibit HIV-1 transcription by preventing cyclin-dependent kinases (Cdks) binding to Tat thus inhibiting Tat-dependent transcription[9].

CONCLUSION

HIV infection is a serious condition with a huge impact on global health. Despite all efforts to contain the spread of the virus, prolonging the life span and quality of life of infected individuals, prevalence[90], morbidity and mortality[91] rates are still high. Much is known about HIV infection but there are still critical areas of need both basic and clinical research[92,93].

The understanding of pathogenic mechanisms involved in HIV infection and progression to AIDS, in which metabolic pathways such as purinergic signaling are involved, is crucial to achieve important objectives in controlling this condition.

Metabolic pathways have been the subject of investigation in the search for new therapies[22] and even a cure for HIV[94]. Even though the involvement of the purinergic signaling pathway in viral infections, including HIV, are multiple and ambiguous, it deserves further attention[22]. Studies on the subject have helped to elucidate disease mechanisms and propose new targets and approaches to therapy. The identification of prognostic biomarkers and the recognition of candidate target genes or pathways to improve therapy are high priority areas where HIV infection is concerned. Purinergic signaling system components are relevant topics of study both for development of biomarkers as well as potential therapeutic targets.

The data reviewed in this paper reveals that this research field must be encouraged. We believe further studies targeting purinergic signaling enzymes and receptors must be particularly pursued due to its relevance in the process of understanding of the pathogenetic mechanisms and the promise of new therapies in the future.

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