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REVIEW ARTICLE

The Adenosine Pathway and Human Immunodeficiency Virus-Associated Inflammation

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Human immunodeficiency virus (HIV) is associated with an increased risk of age-associated comorbidities and mortality compared to people without HIV. This has been attributed to HIV-associated chronic inflammation and immune activation despite viral suppression. The adenosine pathway is an established mechanism by which the body regulates persistent inflammation to limit tissue damage associated with inflammatory conditions. However, HIV infection is associated with derangements in the adenosine pathway that limits its ability to control HIV-associated inflammation. This article reviews the function of purinergic signaling and the role of the adenosine signaling pathway in HIV-associated chronic inflammation. This review also discusses the beneficial and potential detrimental effects of pharmacotherapeutic strategies targeting this pathway among people with HIV.

Keywords. adenosine; HIV; inflammation, non-AIDS comorbidities; purinergic signaling.

Despite viral suppression on antiretroviral therapy (ART), human immunodeficiency virus (HIV) has been associated with elevated levels of systemic inflammation and immune activation, which accompanies an increased risk of morbidity and mortality from non-acquired immunodeficiency syndrome (AIDS)-associated chronic diseases [1, 2]. People with HIV (PWH) experience accelerated immunologic aging and develop cardiovascular disease (CVD), liver disease, and non-AIDS-defining cancers earlier than people without HIV (PWOH) of similar age [3, 4].

In a recent study, purinergic signaling has been implicated in regulating the immunopathogenesis of HIV [5, 6]. Purinergic receptor activation impacts phagocytosis, antigen presentation, cytotoxicity, chemotaxis, chemokine and cytokine release, and T lymphocyte differentiation [7, 8]. Therefore, tailoring pharmacotherapeutic interventions to target purinergic signaling could be an important strategy in regulating chronic inflammation and persistent immune activation associated with chronic HIV. This review highlights (1) the immunologic importance of adenosine triphosphate (ATP) and its nucleoside, adenosine, in modulating the immunologic response and inflammation in chronic HIV infection and (2) the potential role of purinergic receptor-targeted therapies in the prevention and treatment of the chronic disease events seen in PWH.

THE IMMUNOLOGIC FUNCTION OF ADENOSINE

The relationship between extracellular ATP to adenosine is a major local signal of immunoactivation versus immunosurveillance, which is governed by extracellular and intracellular purinergic metabolism (Figure 1). In response to stress, large amounts of ATP are actively and passively released into the extracellular space. Adenosine triphosphate acts as a damage-associated molecular pattern (DAMP) and activates type 2 purinergic (P2) receptors [9]. The P2 receptors consist of 2 main subtypes, P2X and P2Y receptors. The P2X receptors are ATP-gated ionotropic channels that are generally involved in proinflammatory processes. In particular, $P2X_7$ stimulation causes further ATP release that triggers a positive feedback loop to amplify the ATP signal while recruiting appropriate cells to the area [10]. The P2Y receptors are G-protein-coupled receptors that are implicated in a broad range of functions, including facilitating platelet aggregation, vasodilation, cell migration, and immune responses [11].

Extracellular ATP is catabolized into adenosine 5'-monophosphate (5'-AMP) by a family of enzyme ectonucleotidases, the most important being dephosphorylase-1 (CD39). Subsequently, 5'-AMP is converted to adenosine mainly by ecto-5'-nucleotidase (CD73) and other tissue nonspecific alkaline phosphatases [12]. Extracellular adenosine can be further metabolized to a proinflammatory substrate, inosine, via adenosine deaminase or be transported intracellularly

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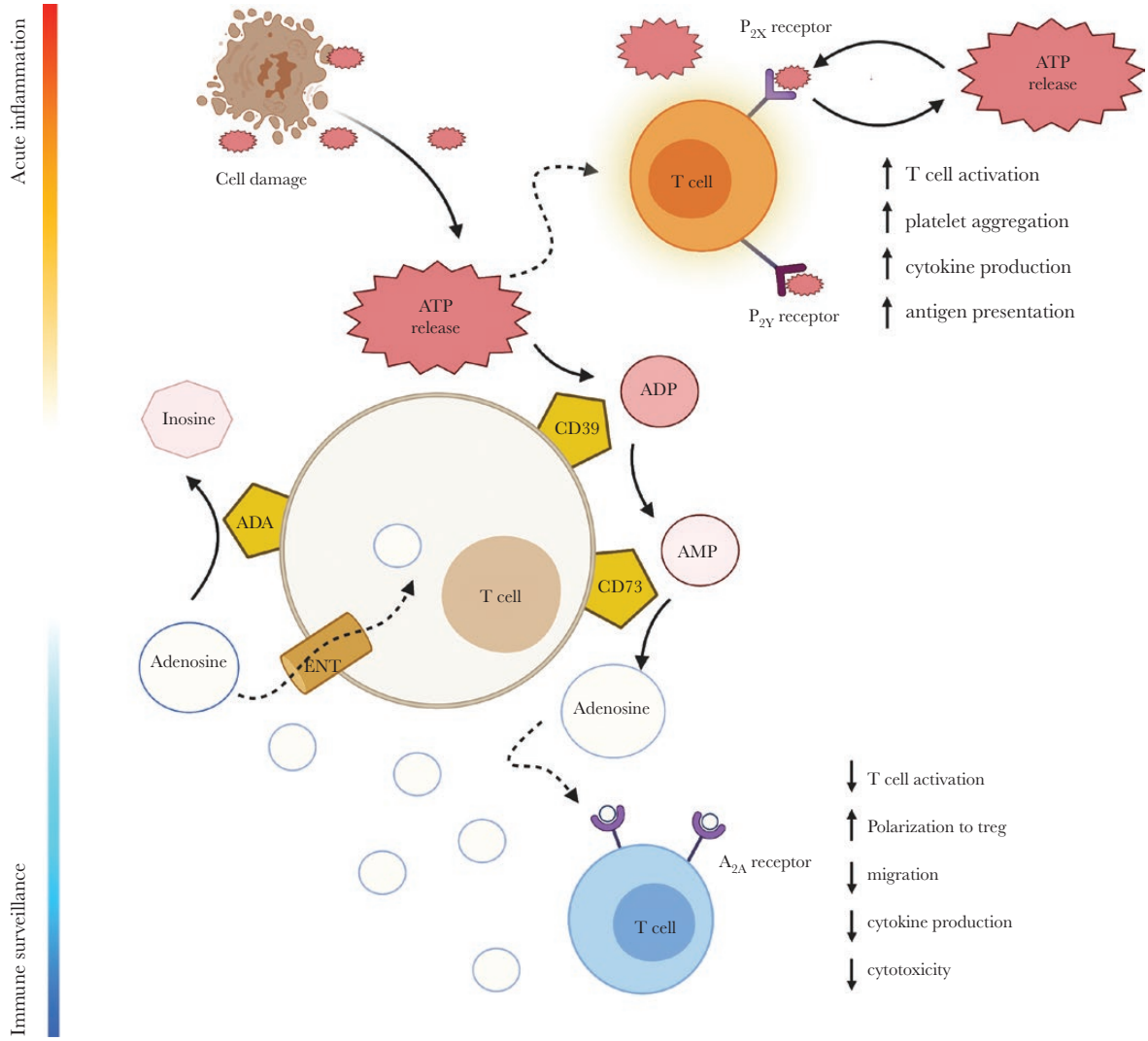

Figure 1. Schematic representation of transport pathways and extracellular enzymes involved in purinergic metabolism. The ratio of extracellular adenosine triphosphate (ATP) to adenosine signal for local immunoactivation (ATP-rich environment) versus immunosurveillance (adenosine-rich environment). The ATP is released into the extracellular space as a sign of cell damage, which promotes immune activation via purinergic 2X (P_{2X}) and purinergic 2Y (P_{2Y}) receptor activation. Extracellular ATP is broken down to adenosine diphosphate (ADP), adenosine monophosphate (AMP), and adenosine via CD39 and CD73 ectonucleotidases. Extracellular adenosine promotes local immunosuppression via activation of A_{2A} receptor. Extracelullar adenosine concentration is regulated by transport into the cell via equilibrative nucleoside transporter (ENT) or conversion to inosine via adenosine deaminase (ADA).

via equilibrative nucleoside transporters (ENTs) [13, 14]. Extracellular adenosine activates type 1 purinergic (P1) receptors, which consists of subtypes A_1, A_{2A}, A_{2B}, and A_3, that modulate adenylyl cyclase and the 3'5'-cyclic monophosphate pathway [15]. The A_1 receptor is ubiquitous throughout the human body and typically has a proinflammatory effect [16]. However, A_2 receptors exhibit an anti-inflammatory role [17]. Specifically, activating the A_{2A} receptor suppresses neutrophil responses [18, 19], monocyte and macrophage recruitment as well as macrophage phagocytic function [20], and proinflammatory cytokine secretion [21, 22]. A_{2A} receptor agonists interfere with T-cell receptor signaling and suppress T-cell proliferation and effector function [23], ultimately producing anergic T cells [24, 25].

Constitutively low levels of extracellular ATP and adenosine are maintained by nucleoside and purine transporters under normal physiological conditions [26]. On most cells, including lymphocytes and endothelial cells, the surface expression of CD39 and CD73 is regulated by external stimuli that ultimately influence the concentration of adenosine in the local environment to mediate paracrine signaling [27]. The concentrations of extracellular ATP and adenosine are intrinsically regulated during inflammation and immune responses, which modulates the functions of myeloid and lymphoid cells [28]. During acute inflammatory responses, high levels of extracellular ATP act as a DAMP and trigger proinflammatory effector functions in a setting of low extracellular adenosine levels, including T lymphocyte migration and proliferation [29]. In the ATP-rich,

adenosine-poor environment, A_1 receptors work synergistically with P2 receptors to promote cell migration, cytotoxicity, apoptosis, and proinflammatory cytokine secretion in neutrophils [30], monocytes [31, 32], and macrophages [33, 34].

As inflammation persists, the concentration of extracellular adenosine increases as a result of the breakdown of ATP [35, 36]. Immune cells in the most injured areas produce an adenosine-rich environment to inhibit themselves, and other local immune cells, while allowing neighboring cells to continue eliminating the pathogen [37]. The protective increase in extracellular adenosine inhibits effector functions of neutrophils, macrophages, dendritic cells, and T lymphocytes [28]. Thus, a rise in extracellular adenosine and A_{2A} receptor expression provides a negative feedback mechanism to prevent further tissue damage [38]. These characteristics, combined with the short half-life of adenosine in vivo, allow for efficient paracrine and autocrine adenosine signaling among immune cells [39].

THE ADENOSINE PATHWAY IN HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Given the established ability of the adenosine pathway in modulating immune function, multiple studies have examined the relationship between adenosine and HIV (Table 1). Specifically, antagonism of the P2X receptor inhibits HIV infection of $CD4^+$ T lymphocytes [52]. For example, $CD34^+$ hematopoietic progenitor cells from PWH who are immunologic nonresponders (INRs) overexpress $P2X_7R$, and inhibition of these receptors promotes maturation of $CD4^+$ T cells [51]. Moreover, macrophages, which can act as HIV reservoirs, release HIV-1 virions from stored vacuoles when stimulated with $P2X_7R$ agonists [53]. Human immunodeficiency virus infection directly impairs purinergic metabolism on inflammatory immune cells, which skews towards an adenosine-poor local environment, thereby promoting chronic immune activation [6]. In our study comparing CD39 and CD73 expression on Tregs, we found higher frequencies of ectonucleotidase coexpression as well as higher levels of adenosine in gut mucosal tissue in the nonprogressive model of simian immunodeficiency virus (SIV) infection in African green monkeys (AGM) compared with the progressive model in pigtailed macaques (PTM) [5]. This finding suggests a potential role for adenosine in AGM through the control of immune activation and inflammation, despite SIV infection, that prevents them from progressing to AIDS. When examining the functionality of adenosine ex vivo, we found that adenosine significantly suppressed cytokine production of $CD4^+$ and $CD8^+$ T cells in both AGM and PTM [5].

In addition to chronic immune activation, changes to the purinergic pathway during HIV infection are associated with T-cell exhaustion, immunosenescence, and immunosuppression, which mitigates the ability of the immune system to effectively manage chronic viral infection or cancer [54–56]. Human immunodeficiency virus infection is associated with the downregulation of $CD73^+$ and upregulation of $CD39^+$ on $CD8^+$ T cells, the latter of which has been identified as a marker of terminal exhaustion [50, 57]. Among viremic PWH, there is greater CD39, but not CD73, expression in natural killer cells, which correlates with viral load and markers of systemic inflammation [58].

Although the adenosine pathway could be protective in HIV-associated chronic inflammation and immune activation, its immunosuppressive function could have important implications in HIV persistence. Regulatory T cells (Tregs) represent important viral reservoirs during chronic HIV infection, and the frequency of $CD39^+$ Tregs is correlated with Treg HIV deoxyribonucleic acid levels [59]. The Treg cells coexpress CD39 and CD73, which make them highly efficient in generating adenosine [60]. Because CD39 expression is upregulated in Tregs for PWH, this creates an adenosine-Treg positive feedback loop to promote a local adenosine-rich, immunosuppressant environment [48]. In addition, A_{2A} receptor activation increases Treg suppressive activity [25, 61, 62]. There is evidence in vitro that $CD4^+CD25^+$ Tregs in ART-treated PWH diminishes $CD4^+$ and $CD8^+$ T-cell function and proinflammatory cytokine production [63, 64]. Specifically, the adenosine/Treg-mediated suppression of $CD4^+$ T cells inhibits interleukin (IL)-2 and interferon-γ release as well as gag-stimulated $CD8^+$ T-cell cytotoxic activity [65–67]. Antibodies that block CD39 activity inhibit Treg-mediated suppression of $CD8^+$ T-cell cytokine production, suggesting that adenosine metabolism is integral in the suppressive effects of Tregs in HIV infection [48]. Therefore, although HIV infection is associated with a persistent immunoactivated state, local enhanced production of adenosine by Treg cells may mediate inappropriate immune tolerance.

IMPACT OF ADENOSINE SIGNALING ON INFLAMMATION AND COMORBIDITIES IN TREATED HUMAN IMMUNODEFICIENCY VIRUS

Despite the extended survival of PWH on ART, virally suppressed individuals experience a greater rate of age-associated non-AIDS events compared with PWOH, and this is a believed to be due to higher levels of inflammation [3]. Multiple factors contribute to this persistent inflammation. We have previously shown that PWH, regardless of viremia level or $CD4^+$ T-cell reconstitution, have lower frequencies of $CD4^+$ T cells expressing the rate-limiting enzyme CD73 and that $CD4^+CD73^+$ T-cell frequencies are associated with lower T-cell activation and C-reactive protein levels [49]. Likewise, Tóth et al [50] showed decreased frequencies of $CD8^+$ T cells expressing CD73, and this correlated with immune activation and T-cell exhaustion. These findings suggest that alterations in the adenosine pathway are playing an important role in chronic HIV-associated inflammation.

An important factor contributing to the persistent inflammation in PWH is microbial translocation resulting from the

Table 1. Summary of Findings on the Adenosine Pathway and HIV Infection

Reference	Study Population	Endpoint	Major Findings
Blanco et al [40]	In vitro; murine clones expressing human CD26, CD4, and CXCR4	Investigation of gp120-induced inhibition of ADA binding to human CD26	• Soluble gp120 and HIV particles able to inhibit ADA-CD26 binding • CXCR4 cells enhanced gp120 inhibitory effects • CXCR4 cells dependent on CD4 expression for inhibition
Fotheringham et al [41]	In vitro; primary human monocytes	Adenosine receptor influence on HIV Tat-induced intracellular calcium and TNF-α production	• A_{2A}R activation inhibited Tat-induced calcium release, and reduction of intracellular calcium inhibited TNF-α production in monocytes • Inhibitory actions of adenosine receptors relied on protein phosphatase activity
Pingle et al [42]	In vitro; PC12 cells and rat cerebellar granule neuron cultures	Neuroprotective potential of A_1 receptor activation against HIV Tat-induced toxicity	• A_{1A}R activation suppressed the increase in calcium and nitric oxide mediated by HIV Tat protein • A_{1A}R inhibition of inducible nitric-oxide synthase expression dependent on NF-κB • Activation of A_{1A}R displayed protection against Tat-induced apoptosis in PC12 cells and cerebellar granule cells
By et al [43]	In vitro; CEM cells, a $CD4^+$ human T-lymphoma cell line expressing A_{2A}R, CXCR4, and CCR5	Analysis of the influence of an agonist-like monoclonal antibody to A_{2A}R, Adonis, on $CD4^+$CEM T cells	• Adonis assisted activation of A2AR and inhibited CEM cell growth • A_{2A}R upregulation reduced CXCR4 and CCR5 expression without altering CD4 expression on CEM cells
Moreno-Fernandez et al [44]	Ex vivo; PBMCs from HIV^- participants	Functional inspection of the suppressive abilities of Tregs including extracellular adenosine formation	• Treg suppression occurred through gap junctions via a cAMP-dependent mechanism that activated protein kinase A in conventional T cells • CD39 expression on Treg also played an important role during suppression
Martinez-Navio et al [45]	Ex vivo; 36 HIV^+ and 10 HIV^- participants	Exploration of the influence of HIV on ADA costimulation in T cells	• ADA increased T-cell proliferation and positively correlated with $CD4^+$ percentage and count while negatively correlated with viral load • HIV reduced ADA-induced cytokine production (IFN-γ, IL-6, and IL-10) in T cells • gp120 impaired ADA-CD26 interaction in HIV
Climent et al [46]	Ex vivo; 8 HIV+; HIV^- participants	Measurement of the immunologic usefulness of ADA as an adjuvant in HIV dendritic cell-based therapeutic vaccines	• ADA-induced enhancement of $CD4^+$ and $CD8^+$ T-cell proliferation • ADA increased cytokine production (IFN-γ, TNF-α, and IL-6) to promote a Th1 response to improve T helper and CTL responses
Parish et al [47]	Ex vivo; PBMCs from HIV^- participants	Investigation of the role of ADA on replicative senescence in human $CD8^+$ T cells	• ADA expression on $CD8^+$ T lymphocytes was lost after successive cultures • $CD8^+CD28^+$ T lymphocytes that are ADA^+ had greater telomerase activity than ADA^- • Lack of ADA expression subjected $CD8^+$ T lymphocytes to prolonged adenosine exposure, which accelerated senescence and loss of CD28 expression
Nikolova et al [48]	Ex vivo; 39 HIV^+, c-ART naive; 39 HIV^+, c-ART stable; 25 HIV^- participants	Examination of the CD39/adenosine axis involvement in the pathogenesis and progression of HIV	• Downregulation of CD39 expression on Treg caused a higher $CD8^+$ T-cell proliferation and cytokine production in HIV^+ vs HIV^- individuals • Higher level of A_{2A}R expression on untreated HIV^+ individuals, which caused a higher susceptibility to CD39/adenosine-mediated inhibition • In HIV^+ participants, $CD39^+$ Treg positively correlated with immune activation and inversely with $CD4^+$ T-cell absolute counts • A CD39 gene polymorphism, causing lower levels of expression, associated with LTNPs and may indicate slower progression of HIV-1 disease
Schuler et al [49]	Ex vivo; 36 HIV^+ and 10 HIV^- participants	Investigation of adenosine-induced immunomodulation and $CD4^+CD73^+$ T-cell involvement in HIV-associated immune activation	• Absolute numbers of $CD4^+CD73^+$ T cells are lower in HIV^+ individuals compared to HIV^- regardless of viral suppression • Absolute numbers of $CD4^+CD73^+$ T cells inversely correlate with activated $CD4^+$ T cells, activated $CD8^+$ T cells, and plasma CRP in HIV^+ individuals • Circulating $CD4^+CD39^+$ T cells frequency did not correlate with the frequency of activated $CD4^+$ or $CD8^+$ T cells or with the plasma CRP levels • $CD4^+$ T cells require the presence of both $CD4^+CD39^+$ and $CD4^+CD73^+$ T cells to hydrolyze exogenous ATP to adenosine • Exogenous adenosine decreases in the percentage of cytokine-expressing $CD4^+$ T cells
Tóth et al [50]	Ex vivo; 95 HIV^+ and 27 HIV^- participants	Immunophenotypic analysis of T-cell populations were compared across 5 groups: health controls, ECs, LTNPs, ART patients, and viremic patients	• In HIV^+ individuals, $\%CD73^+$ cells was significantly lower in $CD8^+$ T cells and $CD4^+$ non-Tregs compared with HIV- individuals • Among the HIV^+ participants, ECs and ART-treated participants showed the highest percentages of $CD73^+$ cells • Viremic participants displayed the lowest expression levels of $CD73^+$, followed by LTNPs

Table 1. Continued

Reference	Study Population	Endpoint	Major Findings
Menkova-Garnier et al [51]	Ex vivo; 16 HIV$^+$ IRs; 16 HIV$^+$ INRs; 18 HIV$^-$ participants	Limiting dilution assays of circulating CD34$^+$ hematopoietic progenitor cells; frequency of recent thymic emigrants (RTEs), defined as CD31^{hi}CD27$^+$CCR7$^+$CD45RA$^+$CD4$^+$ cells; RT-qPCR analysis of FAS, P2X7, and CD73, mRNA levels in purified CD34 cells; transcriptomic analysis of CD3$^+$ cells in HIV IRs versus INRs.	• There was no difference in the frequency of CD34$^+$ cells between the 3 groups • The T-cell potential of CD34$^+$ cells was significantly lower in INRs compared to HIV- participants and IRs • $P2X_7$ was more strongly expressed in INRs than in IRs and HIV$^-$ participants • CD73 expression was undetectable in all the INRs studied • $P2X_7$ inhibition with PPAD significantly improved the potential of CD34$^+$ cells from INRs to differentiate into T cells
He et al [5]	In vivo; 14 PTM (pathogenic SIV host) and 15 AGM (nonpathogenic SIV host)	Changes of markers related to ADO production (CD39 and CD73) and breakdown (CD26 and ADO deaminase) on T cells from blood, lymph nodes, and intestine after SIV acute infection	• Coexpression of CD38 and CD73 was low in circulating CD4$^+$ Tregs and CD8$^+$Tregs in both AGMs and PTMs before infection • Coexpression of CD39 and CD73 was highest in intestinal Treg cells and significantly higher in AGMs compared with PTMs • Intestinal levels of adenosine increased during acute SIV infection in AGMs but not in PTMs

Abbreviations: ADA, adenosine deaminase; ADO, adenosine; AGM, African green monkeys; ART, antiretroviral therapy; ATP, adenosine triphosphate; cAMP, 3′5′-cyclic monophosphate; c-ART; combination ART; CRP, C-reactive protein; CTL, cytotoxic T lymphocyte; EC, elite controllers; HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; INR, immunological nonresponder; IR, immunological responder; LTNP, long-term nonprogressor; mRNA, messenger ribonucleic acid; PBMC, peripheral blood mononuclear cell; PTM, pigtail macaques; SIV, simian immunodeficiency virus; RT-qPCR, quantitative reverse-transcription polymerase chain reaction; TNF, tumor necrosis factor; Treg, regulatory T cells.

disruption of the intestinal epithelial integrity and mucosal immune dysfunction [68–71]. Multiple markers of microbial translocation and innate immune activation have been associated with HIV comorbidities, including CVD [72–74]. Extracellular adenosine signaling has been implicated in promoting restoration of the epithelial barrier in inflammatory bowel disease (IBD) [75, 76], and enhanced migration of antigen-presenting cells (APCs) to the gut mucosa can be induced via preferential activation of A_1 receptors [77]. Given these findings, decreased adenosine levels in the gut epithelium could be an important contributing factor to the disrupted epithelial barrier caused by HIV.

NLRP3 inflammasome activation and IL-1β production are factors that contribute to HIV-associated inflammation, and these are strongly implicated in the development of atherosclerosis and hypertension [78, 79]. Activation of the NLRP3 inflammasome and release of IL-1β are strongly tied to purinergic signaling, particularly $P2X_7R$ stimulation [78, 80]. In addition, the inflammasome system may contribute to the loss of CD4$^+$ T-cell populations and overall lack of immune recovery seen in PWH [81]. For example, INRs upregulate NLRP3 inflammasome and caspase-1 expression compared with those who experience immune recovery on ART [82]. Antagonism of $P2X_7R$ may therefore help to suppress inflammasome activity and IL-1β release [78]. The exact role of how other components of the adenosine pathway can influence inflammasome activity is still unclear. In non-HIV studies, adenosine has been shown to be necessary for sustained inflammasome activation via the A_{2A} receptor [83]. In animal models of hypertension, proinflammatory cytokines, including IL-1β, downregulate CD39 expression [84]. However, CD39 has recently been implicated to serve a protective role by limiting NLRP3 activation and IL-1β release [85]. Upregulation of CD39$^+$/CD73$^+$ Tregs are thought to be protective against CVD and unstable plaque rupture [86–89]. Antiretroviral therapy-treated PWH with coronary artery disease have depressed levels of CD39$^+$/CD73$^+$ Tregs, which may predispose them to atherosclerotic development [90]. How the adenosine pathway affects inflammasome activation in treated HIV will require additional studies to fully understand its role in HIV pathogenesis.

It has recently been shown that purinergic signaling is linked to metabolic disease and dyslipidemia, which contributes to CVD and liver disease among PWH [91, 92]. $P2X_7R$ expression is upregulated in the peripheral blood mononuclear cells from patients with type 2 diabetes and in adipocytes of patients with metabolic syndrome [93, 94]. In addition, $P2X_7R$ expression correlates with low-density lipoprotein cholesterol, which is a major component of metabolic syndrome and elevated cardiovascular risk [93]. Furthermore, extracellular nucleotides have been associated with insulin resistance and dysregulated lipoprotein synthesis [95]. There is also emerging evidence that $P2Y_6R$ activation may contribute to obesity [96].

From our current knowledge of the adenosine pathway, focusing on the role of adenosine as an anti-inflammatory agent could prove beneficial in developing safe and effective interventions in clinical settings. However, although chronic immunoactivation has an integral role in HIV-associated chronic conditions, local immunosuppression plays a key role in cancer risk and viral persistence. Although ART has reduced the mortality of AIDS-related cancers [97], PWH still experience accelerated aging and other risk factors for cancer diagnoses [98]. Long-term immunosuppression is likely the main contributor to non-AIDS-defining cancers [99, 100]. Enhanced adenosine activation in tumor microenvironments generates an immunosuppressant environment that supports tumor growth and evasion from T-cell immune defense [101]. Therefore, modulating adenosine activation in PWH, who are at risk for both solid and liquid malignancies, should be closely monitored.

Viruses that are commonly comorbid with HIV can also upregulate the expression and activity of CD39 and CD73 to facilitate infection [102, 103]. Endothelial cells infected with cytomegalovirus demonstrate an increase in local adenosine production due to the upregulation of both ectonucleotidases [102]. This is thought to facilitate viral entry into target cells by creating a locally immunosuppressive environment. In addition, compared with people with resolved hepatitis B virus (HBV) infection, HBV carriers have higher proportions of Tregs, and increase proportions of circulating $CD39^+$ Tregs correlated with serum viral load, thus, suggesting that $CD39^+$ Tregs contribute to chronic viral persistence [103].

Finally, modifying the adenosine pathway can have important implications on quality of life among PWH. Adenosine is a well established sleep regulatory substance, and enhanced extracellular concentrations in the brain are associated with sleep deprivation and promotion of sleep, particularly via stimulation of A_1 and A_{2A} receptors [104]. Indeed, caffeine, an adenosine antagonist, is commonly used to thwart sleepiness in the general population [105]. Sleep disturbances are highly prevalent among PWH and, therefore, they may be especially vulnerable to worsened fatigue [106]. Enhancing adenosine activation may facilitate insomnia and nocturnal sleep quality among PWH. However, adenosine activation may promote daytime sleepiness and fatigue as well. Alternatively, changes in sleep patterns have been demonstrated to augment $P2X_7$ and A_{2A} receptor expression on circulating leukocytes [107]; this suggests that modifying sleep behavior may be a novel nonpharmacologic mechanism to alter purinergic signaling in PWH.

POTENTIAL OF PHARMACOTHERAPIES

There is an understandably heightened interest in developing effective pharmacotherapies to reduce the incidence of non-AIDS-related comorbidities that lead to early mortality in PWH. Targeting the purinergic signaling pathway to shift the balance away from proinflammatory P2 activation towards anti-inflammatory activation of adenosine receptors is an attractive model to test pharmacotherapeutics. For example, A_{2A} receptors are a critical part in the negative feedback loop of limiting and inhibiting inflammatory responses, providing a rationale to develop A_{2A} receptor-targeted therapeutics to either inhibit or enhance immune responses [38, 108, 109]. There has been emerging literature on pharmacological approaches that target purinergic signaling in various ways to reconstitute the subsequent immune damage of HIV-1 infection [110, 111]. This review focuses on potential therapies to reduce inflammation and promote viral clearance (Figure 2).

Investigation into several potential therapies to curb chronic inflammation in ART-treated PWH and show promising preliminary results [112]. Given that T-cell expression of CD73 is reduced among PWH, attempts have been made to assess whether modulating the adenosine signaling pathway may decrease the persistent chronic inflammatory profile experienced in PWH. In a double-blind, placebo-controlled study, we randomized 40 ART-controlled PWH to 12 weeks of dipyridamole versus placebo, followed by 12 weeks of open-label dipyridamole [113]. Dipyridamole is a nucleoside transport inhibitor and phosphodiesterase 3 inhibitor used clinically in patients with a history of peripheral vascular disease and stroke patients to prevent future thrombotic events. It increases extracellular adenosine by blocking ENTs and preventing transport of adenosine intracellularly down its concentration gradient [114, 115]. Initial data showed that dipyridamole decreased $CD8^+$ T-cell activation in the treatment arm versus placebo arm. In pooled analyses, after 12 weeks of dipyridamole, there was a significant decrease in $CD4^+$ T-cell activation and a trend toward decreased $CD8^+$ T-cell activation in blood [113]. In a substudy, we collected rectosigmoid biopsies from 18 participants to further assess the effect of dipyridamole on mucosal immune cells. Those receiving dipyridamole had (1) a median 70.2% decrease from baseline in the Treg population and (2) an 11.3% increase in $CD8^+$ T cells. There were also trends towards decreased $CD4^+$ T-cell activation and $CD8^+$ T-cell activation [116]. Because the population of Tregs increased in response to heightened inflammation, these data suggest that there is a decrease in gut inflammation that obviates a compensatory Treg response.

Modulating ectonucleotidase activity, particularly CD39 and CD73 activity, is an attractive therapeutic target to reduce proinflammatory extracellular ATP concentrations in favor of anti-inflammatory adenosine. Methotrexate and sulfasalazine, immunosuppressants commonly used in IBD, may be partially effective in treating IBD by enhanced CD73 production of adenosine [117]. Among PWH, although low doses of methotrexate had no effect on systemic inflammatory endothelial markers, there were improvements in brachial artery ultrasound measurements, which may indicate favorable vasculature changes

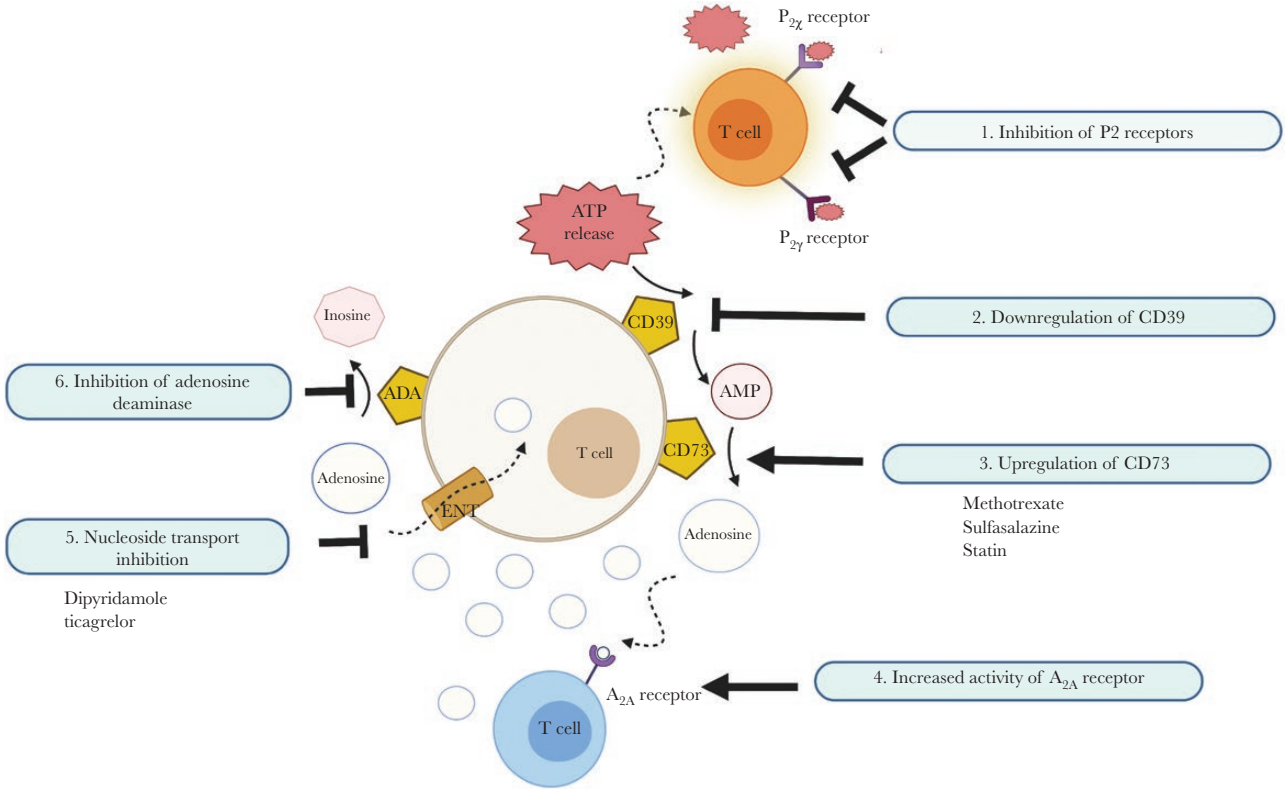

Figure 2. Pharmacologic targets to target adenosine metabolism in human immunodeficiency virus infection. Sites and available therapeutics that decrease purinergic 2 (P2) receptor activity and promote extracellular adenosine and adenosine 2A (A_{2A}) receptor activation are shown. A2A receptor, adenosine 2A receptor; ADA, adenosine deaminase; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; ENT, equilibrative nucleoside transporter; P2Y receptor, purinergic 2Y receptor.

[118, 119]. Rosuvastatin, typically used to treat high cholesterol and triglyceride levels, can also increase extracellular adenosine formation via upregulation of CD73, and it has shown in vivo protection against inflammation [120–122]. The Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE) is an ongoing, prospective, randomized placebo-controlled clinical trial of a pitavastatin strategy for the primary CVD prevention among PWH [123]. In addition to informing the field on the efficacy of statin strategy among PWH, it includes multiple measurements of plaque stability, immune activation, and inflammation [124]. This trial may provide valuable insights on the role of statins in modifying purinergic metabolism.

Selectively inhibiting $P2Y_2$ receptor expression with small interfering ribonucleic acids reduces the HIV-induced inflammatory response and cell death [125, 126]. Inhibiting $P2X_7$ receptors to restore T-cell differentiation from $CD34^+$ hematopoietic progenitor cells could be a potential strategy in PWH, who experience reduced immune recovery while on ART, to regenerate new T-cell populations [51]. Due to the ubiquitous nature of purinergic receptors, there should be a narrowed focus on refining the characterization of cellular patterns and molecular control of expression of crucial enzymes in the purinergic signaling pathway to minimize unwanted side effects. It is important to understand receptor regulation to design and improve purinergic receptor strategies to effectively prevent accelerated aging and control systemic inflammation in PWH.

CONCLUSIONS

Although ART is effective in viral suppression and prolonging the development of AIDS, the concern lies in patient susceptibilities to morbidity and early mortality of age-associated diseases due to immunological dysfunction caused by HIV. Adenosine agonists provide immune advantage by inhibiting T-cell effector function, in conjunction with Tregs, to reduce the chronic immune activation and dysfunction seen in PWH. However, although enhancing CD39 and CD73 activity may improve inflammation and immunoactivation-related comorbidities in HIV, modulating ectonucleotidase activity is a double-edged sword. The adenosine/Treg axis can be detrimental by suppressing HIV-specific immune responses. Additional studies are necessary to determine the proper balance between controlling inflammation but still allowing the generation of an effective immune response against the virus. Novel and innovative strategies targeting these 2 contrasting functions of the adenosine pathway can lead to a decreased risk

for non-AIDS-associated chronic disease and, at the same time, target viral persistence.

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