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Skewed CD39/CD73/adenosine pathway in B cells is associated with innate immune hyperactivation in chronic HIV-1 infection

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Abstract

Background: The CD39/CD73/adenosine suppression pathway correlates with disease progression in patients with chronic human immunodeficiency virus 1 (HIV-1) infection; however, the relationships between this pathway in B cells and innate immune hyperactivation remain largely undefined.

Methods: In this study, we examined CD39 and CD73 expression, and adenosine production by B cells from 136 patients with chronic HIV-1 infection.

Results: The CD39 and CD73 expression levels on total B cells (and their subsets) decreased continuously in typical progressors (TPs), but were gradually recovered in complete responders. In TPs, the frequencies of CD39⁺, CD73⁺, and CD39⁺CD73⁺ B cells inversely correlated with HIV-1 viral load and positively correlated with CD4 T cell counts, but also positively associated with CD4/CD8 ratios and inversely correlated with markers for intestinal epithelial injury and microbial translocation. Furthermore, B cells from TPs showed a markedly reduced capacity to generate CD39/CD73-dependent extracellular adenosine; the loss of this capacity was more serious in patients with acquired immunodeficiency syndrome. In vitro, increased adenosine could inhibit the activation of monocytes and suppress the ability of monocytes and T cells to produce TNF-alpha, regardless of their infection status. Moreover, adenosine could inhibit activation-induced HIV-1 virion production and p24 expression.

Conclusions: These findings provided a new role of B cell pathology in HIV-1 infection, in which the skewed CD39/CD73/adenosine pathway in B cells linked to microbial translocation-induced innate immune hyperactivation, supporting the notion that regulating the adenosine pathway in B cells might be an attractive approach to treat patients with HIV-1-infection.

Keywords: Human immunodeficiency virus 1, B cell, CD39, CD73, Immune hyperactivation

Background

Under physiological or pathological conditions, ATP released from damaged tissues serves as an important signal, eliciting a variety of inflammatory responses. Following its release, ATP is hydrolyzed in a stepwise manner to ADP, AMP, and adenosine (ADO), a process that is controlled by four families of ectonucleotidases, in which the ATP-CD39-CD73-adenosine pathway contributes the

major source of extracellular ADO [1]. ADO exerts its function via four receptors: A1R, A2AR, A2BR, and A3R, to inhibit leukocyte extravasation and decrease immune reactions [2]. ADO also promotes endothelial cell barrier function by phosphorylating tight junction-associated proteins, such as the vasodilator-stimulated phosphoprotein [3, 4]. Thus, the CD39/CD73/adenosine pathway can serve as an “immunological switch” that shifts ATP-driven pro-inflammatory immune cell activity toward an anti-inflammatory state, mediated by ADO [5].

Chronic HIV-1 infection is characterized by gradual CD4⁺ T-cell depletion, associated with abnormal immune manifestations, including immunosenescence, immune

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hyperactivation, and systemic inflammation [6, 7]. Recently, the CD39/CD73/adenosine pathway in T cells has been extensively studied in HIV-1 infection. Briefly, a genetic study showed that patients with HIV-1 infection with a CD39 gene polymorphism that is associated with reduced levels of CD39 expression had a slower progression to AIDS [8]. Numbers of CD39⁺ regulatory T cells increase during chronic HIV-1 infection and strongly correlate with high levels of immune activation and low CD4⁺ T cell counts [8, 9]. Previous studies suggested that patients with HIV-1/AIDS have a reduced CD73 activity in their lymphocytes [10, 11]. Downregulation of CD73 expression on CD4⁺ and CD8⁺ T cells correlated strongly with high levels of T cell activation and C reactive protein levels in patients with HIV-1 infection [12–14]. Furthermore, the CD39/CD73/adenosine pathway has been linked to regulatory T cell-mediated immune suppression, as well as inhibition of HIV-1 replication [15]. In animal models, significant degradation of ADO occurred in pigtailed macaques but not in African green monkeys in response to SIV infection [16]. These studies collectively point to an important role of this pathway in driving disease progression by attenuating HIV-1-associated T-cell responses.

Human B cells have long been recognized to release ADO [17]; however, only limited information about the CD39/CD73/adenosine pathway in B cells has been reported. In healthy individuals, recent studies indicated that activated B cells acquired regulatory activity to suppress the T-cell response by downregulating CD73 expression and upregulating CD39 expression [18]. Moreover, blockade of CD73 activity led to impaired IgG class switching for B cells [19]. In patients with HIV-1 infection, significant downregulation of CD73 on B cells was reported to correlate with CD4 counts and B-cell activation [20]. These studies focused largely on CD39/CD73/adenosine pathway-mediated dysfunction of virus-specific CD8⁺ T cells or B cells; however, little is known regarding the role of the CD39/CD73/adenosine pathway in regulating innate immune hyperactivation.

Considering that B cells, rather than other types of cells, express higher levels of CD39 and CD73, we assessed the characteristics of the CD39/CD73/adenosine pathway in B

cells from a cohort of patients with HIV-1 infection and found that CD39 and CD73 expression on B cells, and their production of ADO, decreased during disease progression. We further analyzed the relationships between the CD39/CD73/adenosine pathway in B cells and innate immune hyperactivation.

Materials and methods

Subjects

Blood samples were collected from 136 HIV-1-infected individuals, including a cohort of 100 typical progressors (TPs), who exhibited typical progressive disease without receiving antiviral treatment; and 36 complete responders (CRs), who underwent antiretroviral therapy (ART) for more than two years and had peripheral CD4⁺ T cells above 200 cells/ μ l and plasma HIV-1 RNA < 80 copies/ml. The diagnosis criteria were defined according to previous reports and the exclusion criteria included co-infection with hepatitis B virus or hepatitis C virus, tuberculosis, pregnancy, and moribund status [21, 22]. The TPs were further divided into patients with AIDS and CD4⁺ T cells below 200 cells/ μ l, and patients without AIDS with CD4⁺ T cells of more than 200 cells/ μ l. Thirty-six age-matched healthy individuals were enrolled as healthy controls (HCs), who tested serologically negative for HIV, hepatitis B virus, and hepatitis C virus. The detailed information for these subjects is listed in Table 1.

Isolation of PBMCs and cell separation

Peripheral blood mononuclear cells (PBMCs) were isolated from freshly heparinized blood and were maintained in RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 20 mM HEPES, 100 U/ml of penicillin, 100 μ g/ml of streptomycin, and 5×10^{-5} M 2-mercaptoethanol. CD4⁺ T cells, CD8⁺ T cells, CD14⁺ monocytes, and CD19⁺ B cells from PBMCs were obtained by positive selection using anti-CD4, anti-CD8, anti-CD14, and anti-CD19 antibody-coated magnetic beads (Miltenyi), respectively. All assays were performed according to the manufacturers' instructions. The purity of the separated cells exceeded 95%, as shown by flow cytometry.

Table 1 Baseline data of the enrolled individuals

Characteristic	HCs	TPs		CRs
		Non-AIDS (CD4 \geq 200)	AIDS (CD4 < 200)	
Cases (n)	36	74	26	36
Age, Median (Range), y	31 (24–52)	29 (17–53)	39 (23–63)	35 (17–55)
CD4, Median (Range) cells/ μ l	ND	385 (208–1144)	117 (10–185)	493 (358–816)
HIV Ag/Ab	Negative	Positive	Positive	Positive
HIV-1, Median (Range), copies/ mL	ND	10,108.50 (568.09–235,400.16)	66,248.40 (1865.27–3,673,035.29)	< 80

Abbreviations: HC healthy control, TP typical progressor, CR complete responder

Antibodies and reagents

All fluorochrome-conjugated anti-human mAbs and their relevant isotype controls were purchased from BD Biosciences, except mouse anti-HIV-p24Gag-FITC (clone KC-57, Beckman Coulter). Rabbit anti-human A2AR mAb (catalog no. PA5-23969) was purchased from Thermo Fisher Scientific. Alexa Fluor 488 goat anti-rabbit IgG (catalog no. A11034) was purchased from life Technologies. ATP (catalog no. 12067-99-1), LPS (catalog no. L2630), and 2-Chloroadenosine (CADO) (catalog no. A9251) were purchased from Sigma-Aldrich. Purified anti-human CD3 and anti-human CD28 Abs used for T cell activation were purchased from Biolegend.

Real-time RT-PCR assay

Relative quantifications of the expression levels of target mRNAs encoding CD39, CD73, A1R, A2AR, A3R, and β -actin (control) were performed on a CFX96 Real-Time System (Bio Rad Laboratories). These gene-specific PCR primers and experimental assays were as previously described [18].

Mass spectrometry

Purified CD19⁺B cells were incubated in 200 μ l of PBS in 96-well plates in the presence of 20 μ M ATP for 5, 30, 45, and 60 min. Cells and supernatants were collected, centrifuged, and boiled for 2 min to inactivate ADO-degrading enzymes, and then stored at -80 °C for subsequent analysis.

Purine levels in the supernatants were analyzed using an LTQ XL Linear Ion Trap Mass Spectrometer (Thermo Scientific), equipped with a heated electrospray ionization source, and selected reaction monitoring with ¹³C₁₀-adenosine (Sigma-Aldrich) as the internal standard. The mass spectrometer was operated in the positive-ion mode and the following mass-to-charge transitions were monitored: 348 \rightarrow 136 for 5'-AMP, 268 \rightarrow 136 for ADO, and 291 \rightarrow 159 for inosine [18, 23].

Monocyte activation assay

Freshly purified monocytes were activated using LPS (500 ng/ml) or an equal volume of medium for 16 h in the presence of CADO (0–2.5 μ M). CADO was used in place of ADO, because of its greater stability. After stimulation, the supernatant from the culture was used for soluble CD14 (sCD14) detection, and adherent monocytes were collected for surface activation marker detection.

For intracellular TNF- α staining, fresh PBMCs were incubated with LPS (500 ng/ml) and brefeldin A for 6 h in the presence of CADO (0–5 μ M) in RPMI medium. Unstimulated PBMCs served as negative controls.

Cytokines and virus production assay

Fresh PBMCs from typical progressors were incubated with anti-CD3 and anti-CD28 antibodies (1 μ g/ml), with or without brefeldin A, in the presence of CADO (0–5 μ M) in RPMI medium for 6 or 24 h at 37 °C. TNF- α (clone Mab11) levels in CD4⁺ and CD8⁺ T cells were determined following 6 h of culture. Virus titers in culture supernatant and p24 expression on CD4⁺ T cells were determined following 24 h of culture.

Flow cytometry

To characterize the CD39 and CD73 expression on B cells, PBMCs were stained with a cocktail of mAbs: mouse anti-human CD45-PerCP (clone 2D1), mouse anti-human CD19-PE-Cyanine 7 (Cy7) (clone SJ25C1), mouse anti-human CD39-FITC (clone TU66), mouse anti-human CD73-Brilliant Violet 421 (clone AD2), mouse anti-human CD10-PE (clone HI10a), mouse anti-human CD27-Brilliant Violet 510 (clone L128), and mouse anti-human CD21-allophycocyanin (clone B-ly4).

To detect monocyte-activation phenotypes, PBMCs were stained with the following surface mAbs: mouse anti-human CD45-PerCP, mouse anti-human CD14-PE-Cy7 (clone M5E2), mouse anti-human CD80-allophycocyanin (clone 2D10, Biolegend), mouse anti-human CD86-Brilliant Violet 421 (clone BU63), and mouse anti-human HLA-DR-Brilliant Violet 500 (clone G46-6).

Cells were stained with above-mentioned mAbs at room temperature for 30 min in the dark. After washing, cells were fixed in 1% paraformaldehyde. Intracellular staining of TNF- α or p24 was performed on previously labeled cells using Fix/Perm solution (BD Bioscience) according to manufacturer's instructions. A minimum of 10,000 CD45⁺CD19⁺ or CD45⁺CD14⁺ cells or CD4⁺T cells or CD8⁺T cells from lymphocyte-gated events were collected for each sample on a FACSVerser and analyzed with FlowJo Software (Treestar).

Plasma HIV-1 RNA monitoring

The HIV-1 reverse transcription PCR Assay V2 (Qiagen, Hilden, Germany) and CFX96 Real-Time System (Bio Rad Laboratories) were used to quantify the HIV-1 RNA levels in plasma, as previously described(21). The cut-off value was 80 copies/ml.

Elisa

Commercial ELISA kits were used to measure sCD14 (R&D Systems, Minneapolis, MN, USA) and intestinal fatty acid-binding protein (I-FABP; R&D Systems) in plasma and culture supernatant, according to the manufacturers' protocols.

Statistical analysis

All data were analyzed using SPSS version 22. For multiple comparisons, the Kruskal-Wallis H nonparametric test was first used between multiple groups, the Mann-Whitney U test was used for comparisons between two groups, and a paired t-test was used for comparisons within the same individual. Correlation analysis was evaluated using the Spearman rank correlation test. For all tests, differences were considered statistically significant at $P < 0.05$ (two-sided).

Results

Frequencies of CD39⁺, CD73⁺, and CD39⁺CD73⁺B cells correlated negatively with disease progression in patients with HIV-1 infection

We detected the expression profile of CD39 and CD73 on various cell populations in peripheral blood. B cells displayed more CD39 and CD73 double positive cells than did other cell types (Fig. 1a). However, B cells from patients with HIV-1 infection showed decreased CD39 and CD73 expression, not only on the cell membrane, but also in terms of mRNA levels (Fig. 1a, b). Compared with HCs, the percentages of CD39⁺, CD73⁺, and CD39⁺CD73⁺ B cells were significantly decreased in patients with chronic HIV-1 infection. Particularly in patients with AIDS, more profound decreases in the percentages of CD39⁺, CD73⁺, and CD39⁺CD73⁺ B cells were observed (Fig. 1c). Analysis showed that the percentages of CD39⁺, CD73⁺, and CD39⁺CD73⁺ B cells in TPs correlated positively with the CD4/CD8 ratios and correlated inversely with the viral load (Fig. 1d, e). Importantly, the percentages of CD39⁺, CD73⁺, and CD39⁺CD73⁺ B cells increased gradually after effective antiretroviral therapy (Fig. 1c), and one patient with AIDS who had received ART for 88 weeks showed recovered levels of CD39⁺, CD73⁺, and CD39⁺CD73⁺ B cells (Fig. 1f). These data revealed that the frequencies of CD39⁺, CD73⁺, and CD39⁺CD73⁺ B cells were closely associated with disease progression in patients with chronic HIV-1 infection.

The expression profiles of CD39 and CD73 on B cell subsets

A previous study showed that the levels of CD39 and CD73 on B cell subsets were generally downregulated [20], but did not address the expression profiles of CD39 and CD73 on B cell subsets. We further compared the expression profiles among different groups. The gating strategy is shown in Fig. 2a, and the observed frequencies of B cell subsets were in line with those reported in previous studies [24, 25]. The frequencies of immature, activated memory (AM) and tissue like memory (TLM) B cells were increased in TPs, especially in patients with AIDS. In contrast, naïve and resting memory (RM) B cell numbers were decreased in TPs; and further decreased

in patients with AIDS (Fig. 2b). CD39 and CD73 levels decreased significantly on immature CD10⁺ B cells and further decreased in these cells from patients with AIDS. In the patients with AIDS, CD39 expression on naïve B cells; CD73 expression on naïve and AM B cells; and CD39 and CD73 expression on naïve and AM B cells were more profoundly decreased (Fig. 2c). These data further indicated that CD39 and CD73 expression on B cell subsets is regulated differentially during chronic HIV-1 infection.

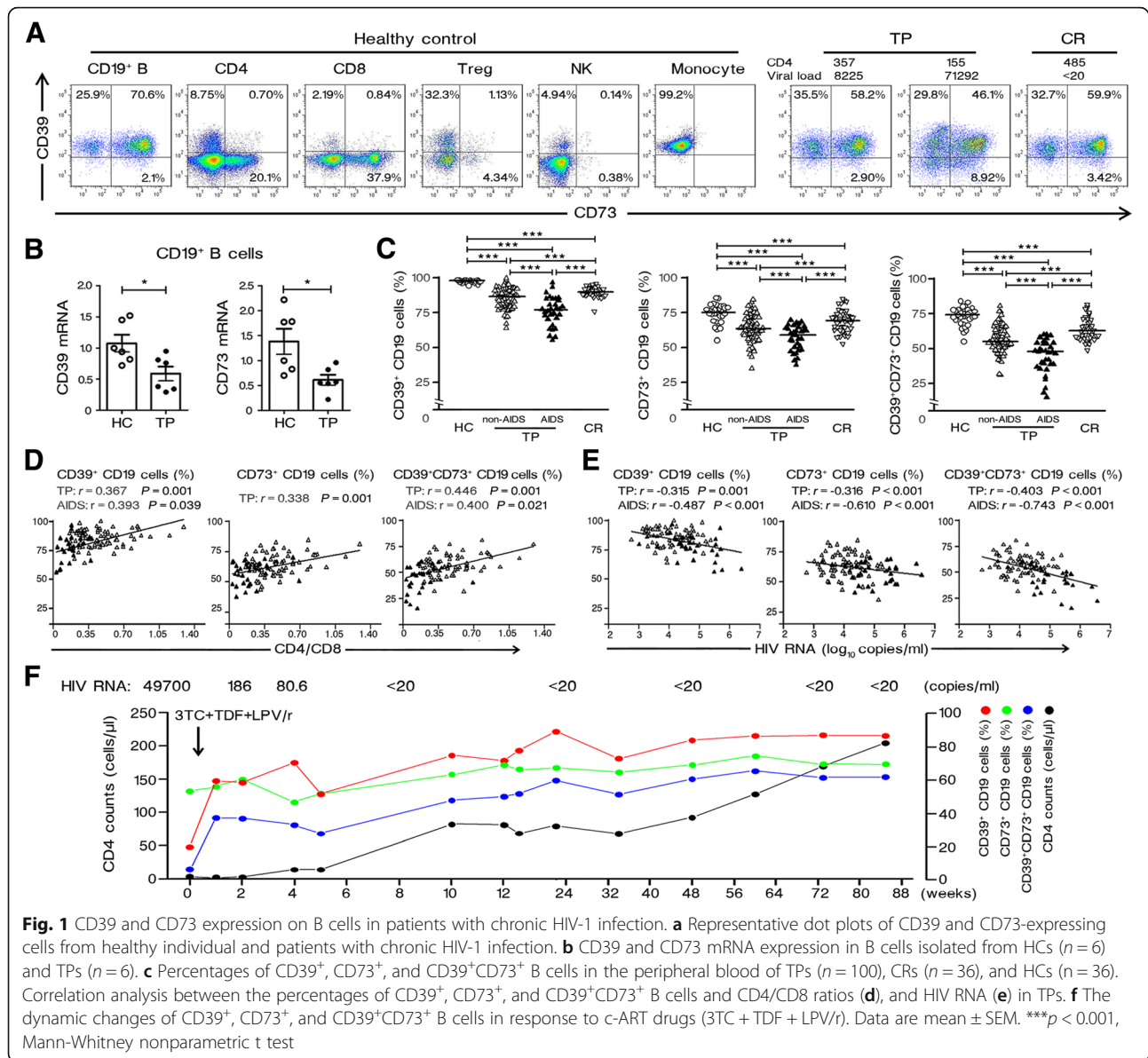
Decreased production of ADO by B cells in patients with HIV-1 infection

Extracellular ATP promotes immune cell activation and elicits pro-inflammatory responses during chronic infection [1]. To address whether the decrease in CD39 and CD73 expression on B cells is associated with reduced ATP consumption, purified B cells from HCs and TPs were incubated in the presence of 20 μM ATP for various time periods. Levels of 5'AMP, ADO, and inosine were measured using mass spectrometry. B cells from patients with AIDS degraded less ATP to 5'AMP (Fig. 3a) and produced less ADO compared with those from HCs (Fig. 3b). By contrast, B cells from patients with higher CD4 numbers produced similar levels of 5'AMP (Fig. 3a), but produced less ADO, compared with those from HCs (Fig. 3b). In contrast, there was no significant difference in inosine production among non-AIDS, AIDS and HC groups (Fig. 3c). These data suggested that B cells from patients with AIDS had an impaired capacity to hydrolyze ATP.

Frequencies of CD39⁺, CD73⁺, and CD39⁺CD73⁺B cells correlated negatively with plasma I-FABP and sCD14 levels

Intestinal barrier dysfunction can be assessed by measuring the levels of I-FABP, which is expressed in epithelial cells and is released when intestinal mucosal damage occurs [26, 27]. We observed that I-FABP levels were obviously increased in patients with HIV-1 infection compared with those in HCs, and were further increased in patients with AIDS (Fig. 4a). Analysis also showed that the percentages of CD39⁺, CD73⁺, and CD39⁺CD73⁺ B cells in TPs correlated negatively with I-FABP levels (Fig. 4b).

Microbial translocation is a cause of systemic immune activation in chronic HIV infection [28]. Plasma levels of the monocyte activation/microbial translocation marker, sCD14, were observed to correlate with the degree of microbial translocation in patients with chronic HIV infection [29]. The sCD14 levels were markedly increased in patients with HIV-1 infection compared with those in the HCs. Particularly in patients with AIDS, the sCD14 levels were further increased over those in CRs (Fig. 4c). Subsequent analysis showed that



the percentages of CD39⁺, CD73⁺, and CD39⁺CD73⁺ B cells in TPs correlated negatively with the sCD14 levels. Interestingly, more significant correlation coefficients were observed in patients with AIDS (Fig. 4d).

Taken together, these data suggested that CD39 and CD73 expression on B cells is associated with an impaired intestinal barrier and subsequent microbial translocation.

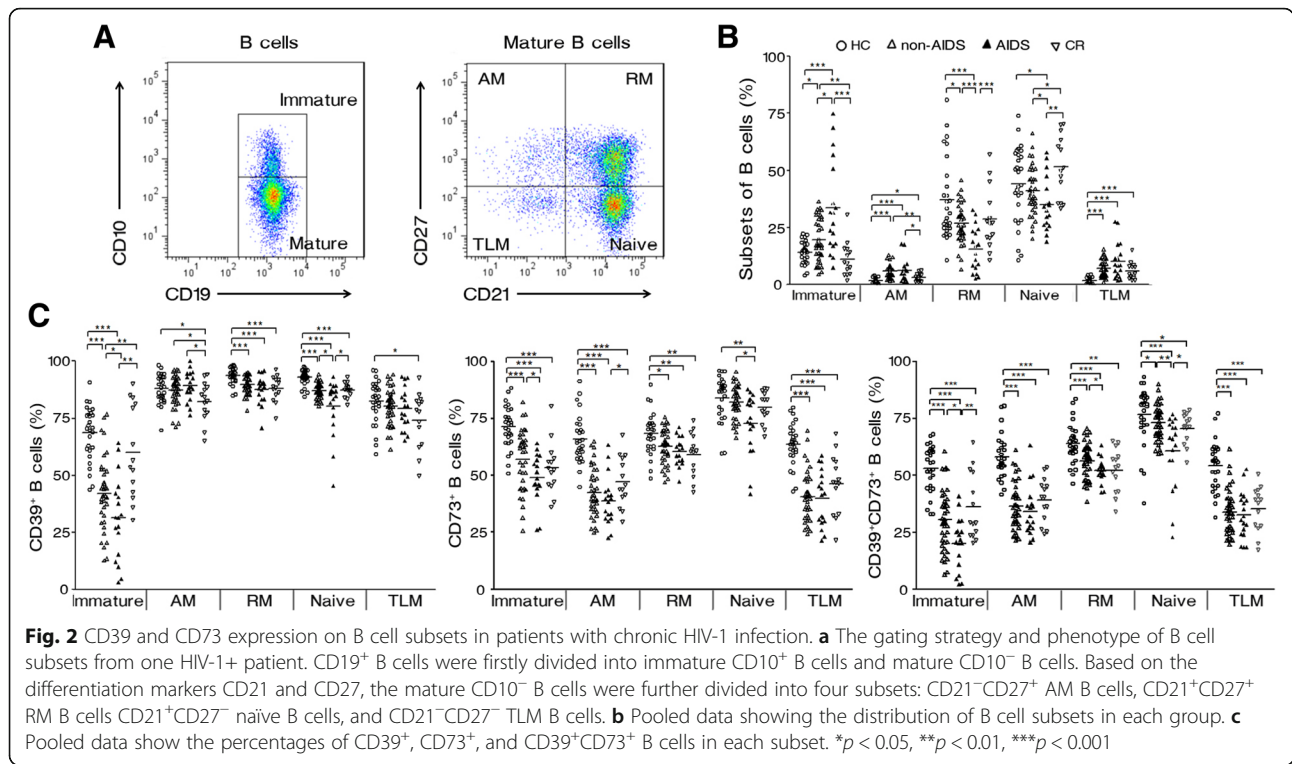
The expression of adenosine receptors in monocytes and inhibition of monocyte activation by adenosine

Monocyte activation contributes to systemic inflammation; therefore, we further investigate the profiles of adenosine receptor subtypes present on monocytes in patients with chronic HIV-1 infection. Monocytes from TPs expressed higher levels of mRNA encoding A1R,

A2AR, and A3R than did monocytes from HCs (Fig. 5a). We further confirmed that monocytes from patients with AIDS expressed higher levels of A2AR (Fig. 5b), as indicated by the mean fluorescence intensity (MFI) scores.

Next, we detected the responsiveness of monocytes to adenosine in vitro. ADO could significantly downregulate CD80, CD86, and HLA-DR expression on monocytes from TPs and HCs, as well as decreased sCD14 production in response to LPS (Fig. 5c). These data suggested ADO inhibits the activation of monocytes in vitro.

TNF- α has been reported to induce mucosal epithelial cell death resulting from tight-junction changes [30]. We found that ADO could significantly downregulate TNF- α production of monocytes in response to LPS from both TPs and HCs, and that increasing ADO doses significantly



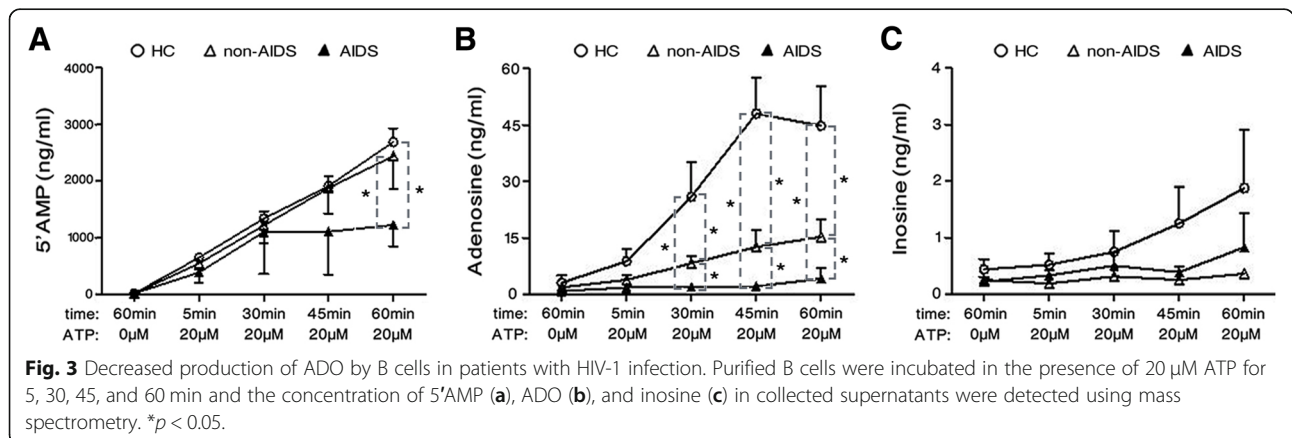
inhibited TNF- α production (Fig. 5d), indicating that the effect of ADO was dose-dependent.

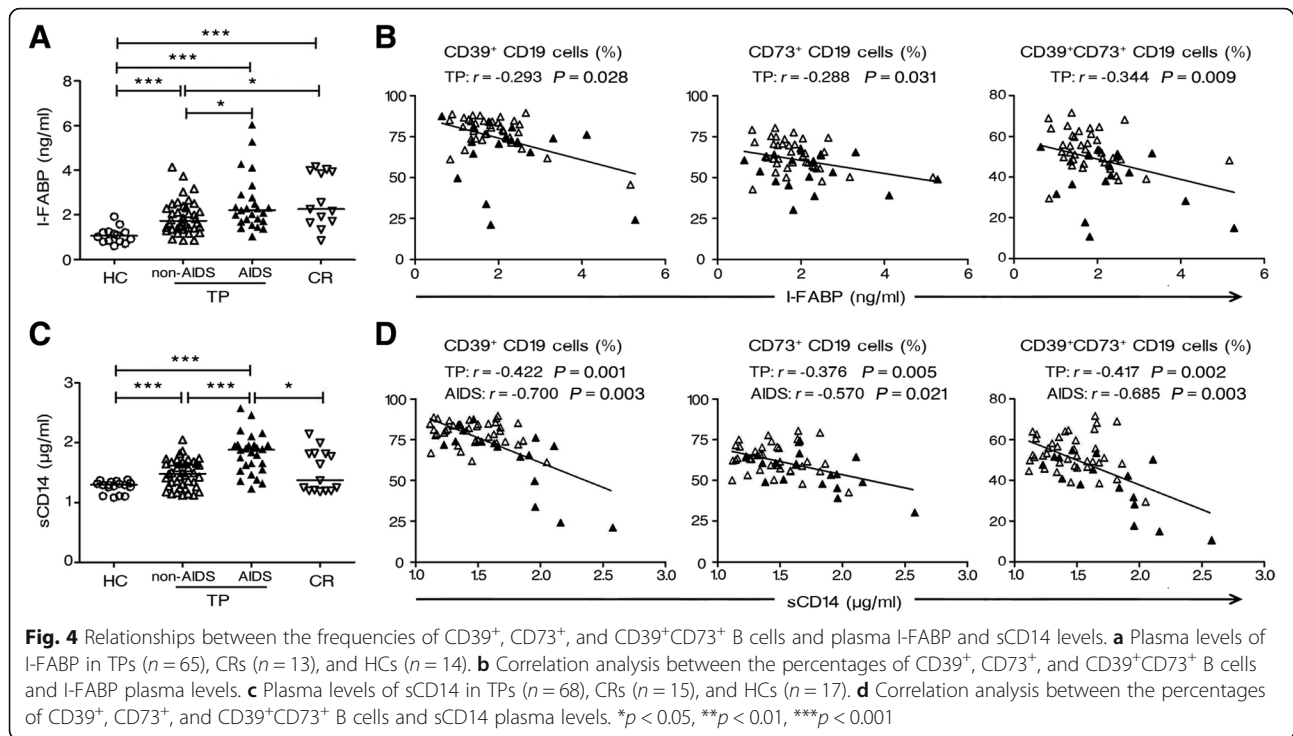
The expression of adenosine receptors in T cells and the inhibition of viral production and proinflammatory cytokine release by adenosine

We also investigated the profiles of adenosine receptors present on T cells in patients with chronic HIV-1 infection. CD4⁺ and CD8⁺ T cells from TPs expressed higher levels of mRNA encoding A1R, A2AR, and A3R than did the same cells from HCs (Fig. 6a, b). We found that A2AR was constitutively expressed by CD4⁺ and CD8⁺ T cells (Fig. 6c) and that the MFI levels of A2AR on

CD4⁺ and CD8⁺ T cells increased gradually with disease progression (Fig. 6d).

We further examined the effect of ADO on HIV virus replication. ADO could significantly downregulate the production of HIV virus in response to anti-CD3 and anti-CD28 stimulation, and increasing ADO doses significantly inhibited the HIV virus production (Fig. 6e), indicating that the effect of ADO was dose-dependent. Interestingly, ADO also affected the levels of p24 per CD4 cell, because the p24 MFI was significantly decreased in response to higher levels of ADO (Fig. 6f). ADO could significantly downregulate the TNF- α production of T cells in response to anti-CD3 and anti-CD28 stimulation, and





increasing ADO doses significantly inhibited TNF- α production in both CD4 and CD8 T cells; the dose-dependent effect of ADO on TNF- α production of T cells was also observed (Fig. 6g).

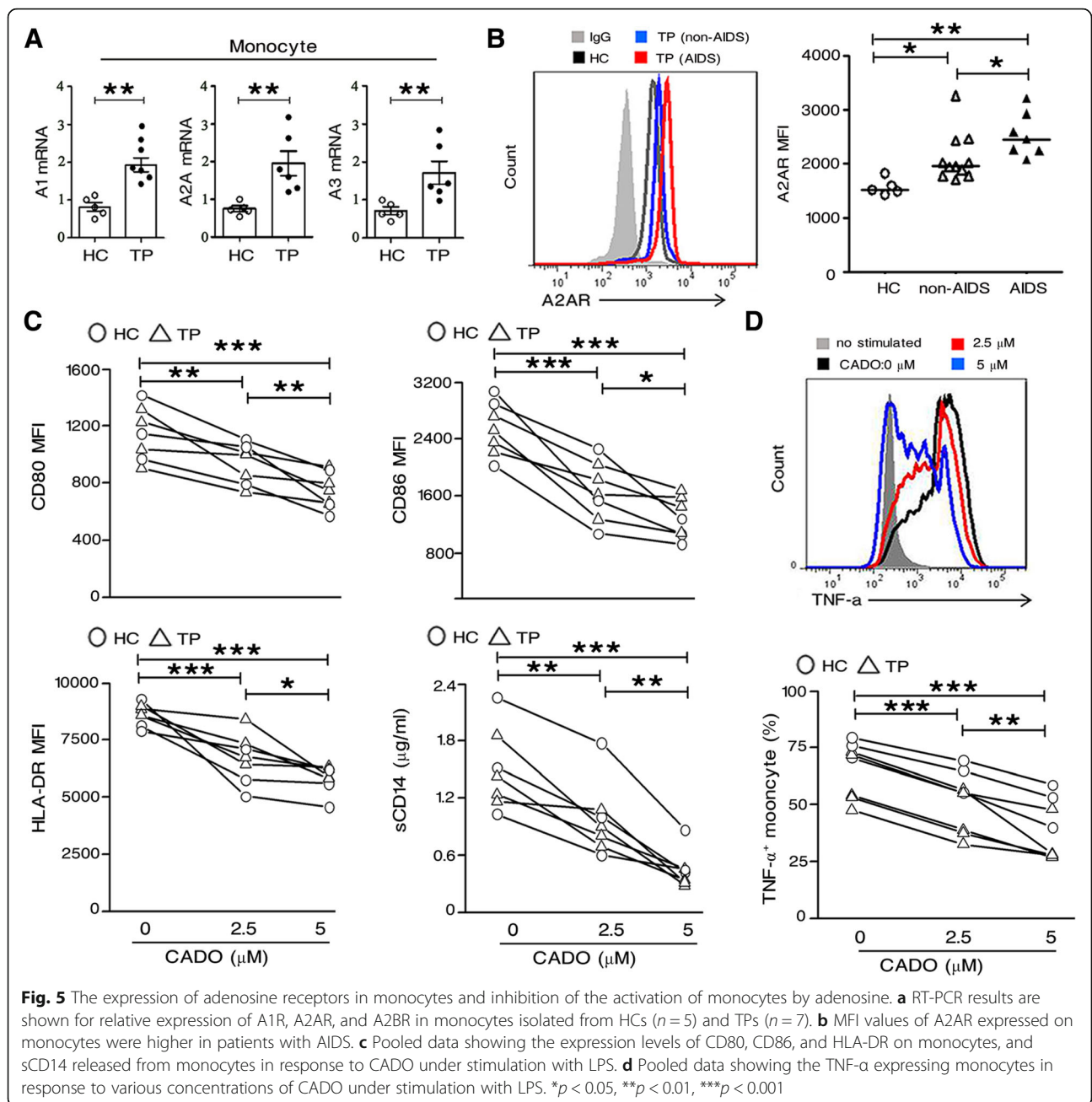
Discussion

Immune activation is a critical component of HIV disease pathogenesis; however, the causes of HIV-associated immune activation remain unclear, but are likely to be multifactorial. In the present study, we demonstrated that the skewed CD39/CD73/adenosine pathway in B cells is not only closely associated with disease progression, but also with immune activation. We showed that the impaired production of B cell-derived ADO might lose its inhibition of immune activation, especially innate immune activation, and decrease its inhibition of HIV-1 replication. Collectively, these findings provided evidence linking this pathway with innate immune hyperactivation and provided a new role for B cell pathology in HIV-1 infection.

The relationship between the expression profiles of CD39 and CD73 on B cells and HIV/AIDS disease progression has not been thoroughly analyzed. In the present study, we observed that CD39 and CD73 expression levels on B cells were downregulated in patients with HIV-1 infection. The levels of CD39 and CD73 decreased progressively in TPs; however, they were maintained at relatively higher levels, but slightly below the level of HCs, in CRs. This finding confirmed and expanded upon the previous observation in patients with

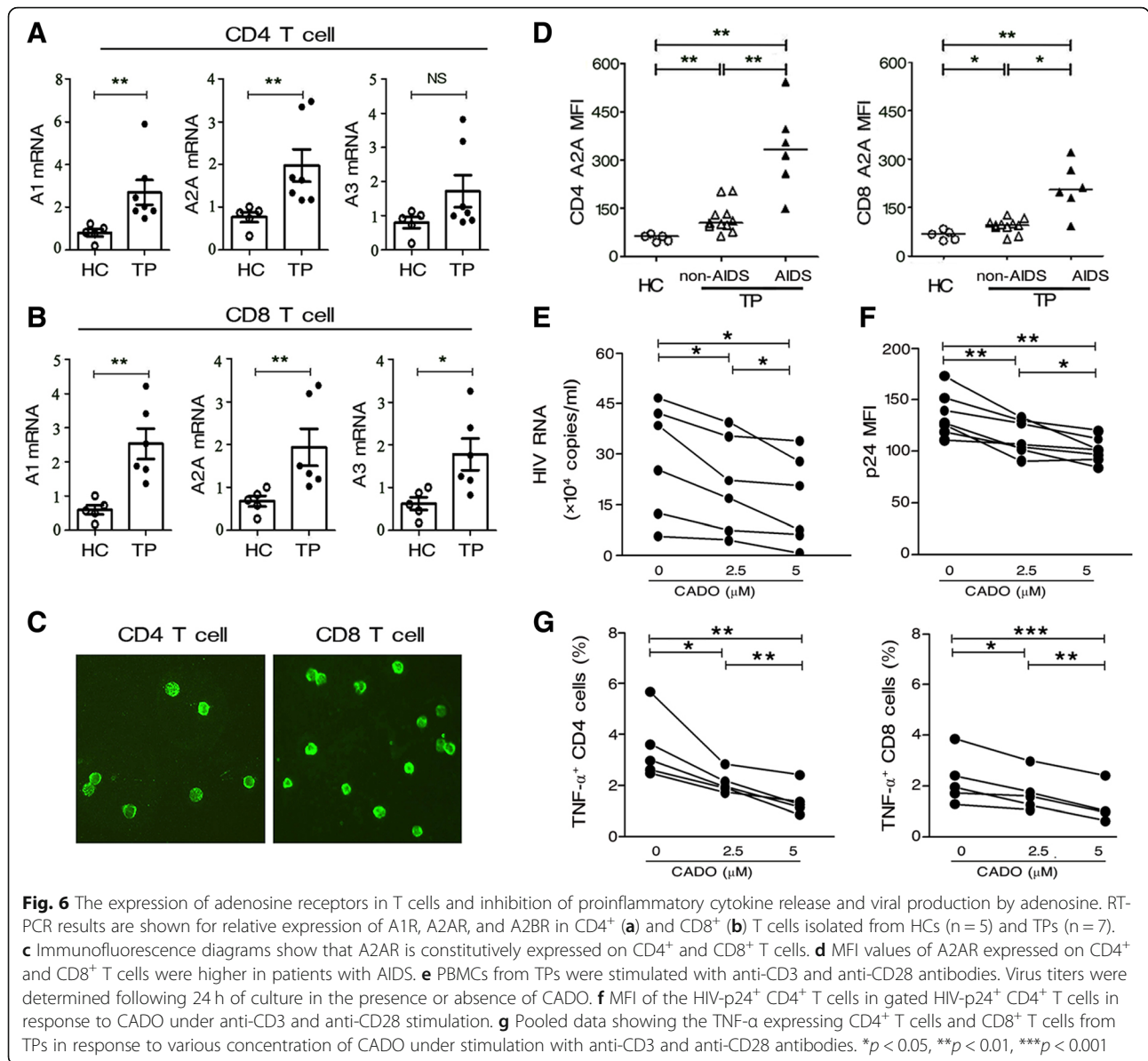
viremic HIV in whom the decreased frequencies of CD39⁺CD73⁺ B cells correlated with low CD4⁺ T-cell counts [20]. The data from our cohort of patients strongly suggested that the frequencies of CD39⁺ and CD73⁺, as well as CD39⁺CD73⁺ B cells, were positively associated with CD4⁺ T-cell counts and negatively correlated with viral load. Importantly, the CD39 and CD73 expression on B cells in TPs was positively associated with impaired immune balance (the CD4/CD8 ratio), and negatively correlated markers for monocyte activation//microbial translocation (sCD14) and for intestinal mucosal damage (I-FABP). The latter three markers are also recognized as independent predictors of morbidity and mortality for HIV/AIDS. For example, higher sCD14 levels could lead to increased CD38 expression on CD4⁺ or CD8⁺ T cells, which is a predictor of CD4⁺ T cell loss and death [31]. Similarly, the elevated I-FABP levels detected in these patients might reflect ongoing structural damage to the gastrointestinal tract, because only dying enterocytes release I-FABP [26, 27]. These associations support the view that decreased CD39 and CD73 expression on B cells in patients with HIV-1 infection is intimately linked with disease progression. Further investigation should be done to evaluate the association of CD39 and CD73 expression with clinical outcome in patients undergoing long-term antiretroviral therapy.

Microbial translocation is a cause of systemic immune activation in chronic HIV infection [28]. Our data increased our knowledge about the role of the CD39/CD73/adenosine pathway under conditions of microbial



translocation. Although the cause of CD39 and CD73 downregulation on these B cells is not yet clear, previous studies have demonstrated that several microbial translocation associated factors, including inflammatory cytokines, oxidative stress, and hypoxia, can dysregulate CD39 and CD73 expression [32]. In our study, it was rational to propose that the decreased CD39 and CD73 expression on B cells might reflect a deficiency in ADO production. This hypothesis was supported by our findings that B cells from patients with HIV-1-infection displayed a reduced ability to convert ATP to ADO. In detail, the procedure was controlled in two successive

steps. First, lower CD39 expression in patients with AIDS led to lower ATP consumption and lower AMP production. This result demonstrated that the decreased CD39 expression is responsible, at least in part, for the lower AMP (substrate for CD73) production. Second, the reduced AMP and decreased CD73 ultimately resulted in lower levels of ADO production in patients with AIDS. In addition, B cell subsets displayed different patterns of CD39 and CD73 expression, which reflected their distinct roles in producing ADO. Thus, the combined effects of CD39 and CD73 modulate ADO production and might tip the balance toward lower



ADO concentrations. Conversely, the impaired production of B cell-derived ADO might exacerbate innate immune activation. There are some observations to support this notion. First, TNF-α could induce mucosal epithelial cell death resulting from tight-junction changes; ADO in vitro could suppress TNF-α production in both T cells and monocytes in a dose-dependent pattern. In addition, ADO could suppress monocyte activation. Third, sCD14 secreted from monocytes in response to LPS could increase monocyte responses to LPS by 50–1000 folds [33]. We found that in vitro, ADO also suppressed sCD14 production in monocytes, which then attenuated the proinflammatory responses. These results indicated that the skewed CD39/CD73/adenosine pathway potentially boosts microbial translocation-induced innate immune hyperactivation.

In non-progressive SIV infection, higher concentrations of ADO control immune activation/inflammation, whereas the effect subsides because of degradation of ADO in progressive SIV infection. Administration of ADO ex vivo strongly suppressed proinflammatory cytokine production by T cells in both species [8]. These data suggested that an increased concentration of ADO could be beneficial by controlling immune activation/inflammation, which would delay disease progress. We found ADO significantly inhibited proinflammatory cytokine production by T cells, and more importantly, inhibits HIV replication. This finding is in accordance with previous studies in which HIV replication control could be achieved through a cAMP-dependent mechanism [9]. This beneficial effect is further supported by above-mentioned

evidence that ADO has a significant role in suppressing monocyte activation and proinflammatory cytokine production.

Conclusions

Taken together, this study found that the reduced expression of CD39 and CD73 on B cells and the loss of B cell-derived ADO might be linked to HIV disease pathogenesis.

These findings extend our understanding of B cell pathology in HIV infection and further support the notion that modifying the ADO pathway might be an attractive approach to treat patients with HIV-infection.

Abbreviations

ADO: Adenosine; AM: Activated memory; ART: Antiretroviral therapy; CR: Complete responders; HC: Healthy control; I-FABP: Intestinal fatty acid binding protein; MFI: Mean fluorescence intensity; RM: Resting memory; sCD14: Soluble CD14; TLM: Tissue like memory; TP: Typical progressors

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

W-XC performed the experiments, analyzed the data. H-HH recruited participants and collected blood samples. LH, J-JS, Y-MJ, CZ, LJ, TY, MS, BT, ZX and T-JJ provided help. F-SW supervised the study. J-YZ designed and performed experiments, analyzed the data and wrote the manuscript. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by the ethics committee of the fifth medical center of PLA general hospital, and written informed consent was obtained from each subject in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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