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Original Article

Specific Patterns of CD39 Expression on T-cells of HIV/HCV Coinfected Patients Receiving Antiretroviral Therapy: A Case-control Study

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Abstract

Background and objectives: Human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfection leads to severe systemic inflammation, increasing non-AIDS morbidity and mortality risk. CD39 ectoenzyme on T-cells, which catalyzes the conversion of pro-inflammatory purines to immunosuppressive adenosine, plays an important role in inflammation control. The role of $CD39^+$ T-cells in systemic inflammation during HIV/HCV coinfection under antiretroviral therapy (ART) remains unexplored. This study aimed to identify specific patterns of CD39 expression on T-cells in ART-treated HIV/HCV coinfected patients and assess their relationship with systemic inflammation.

Methods: We conducted a case-control study that enrolled 41 HIV/HCV coinfected patients on stable ART (cases) and 23 healthy controls. CD39 expression on blood $CD4^+$ and $CD8^+$ T-cells, including $CD45RA^+$ and $CD45RA^-$ subsets, was quantified using flow cytometry. Cytokines were assessed using multiplex and enzyme-linked immunosorbent assays.

Results: A significant proportion of $CD4^+$ T-cells expressed CD39 in both groups (cases – 24.0%; controls – 16.1%). That was not true for $CD8^+$ T-cells (cases – 3.2%; controls – 2.8%). CD39 expression was higher on $CD45RA^+$ than $CD45RA^-$ $CD4^+$ T-cells (cases – 39.4% vs. 19.0%; controls – 24.6% vs. 9.2%). HIV/HCV coinfected patients exhibited a significantly increased proportion of $CD39^+$ $CD4^+$ T-cells compared to uninfected controls ($P < 0.01$). A negative correlation was observed between the percentage of $CD39^+$ $CD4^+$ $CD45RA^-$ T-cells and levels of pro-inflammatory chemokines monocyte chemoattractant protein 1 ($R = -0.392$; $P < 0.01$) and eotaxin ($R = -0.325$; $P < 0.05$).

Conclusions: The data suggest a compensatory expansion of cells with regulatory properties that is ultimately insufficient to control systemic immune activation.

Keywords: HIV infection; Hepatitis C; Chronic; Inflammation mediator; T-Cell; Leukocyte common antigen; Memory T cell; EctoATPase; Cytokine.

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Introduction

The problem of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) coinfection remains highly relevant worldwide. This is supported by extensive recent scientific literature demonstrating its significant global burden, continued transmission, and substantial impact on morbidity and mortality.[1–5]

Although HCV infection may be associated with a decreased viral load suppression rate during antiretroviral therapy (ART), in treated HIV/HCV coinfected patients, HIV replication is typically suppressed.[6] However, in Russia, low hepatitis C treatment access often leads to uncontrolled progression of HCV infection.[7] In HIV/HCV coinfection, HCV replication drives persistent he-

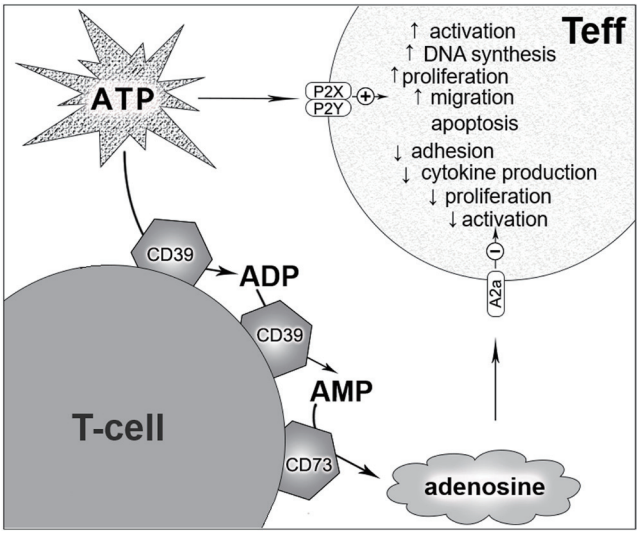

Fig. 1. Conversion of pro-inflammatory ATP to immunosuppressive adenosine by the ectonucleotidases CD39 and CD73. ADP, adenosine diphosphate; ATP, adenosine triphosphate; AMP, adenosine monophosphate; P2X/P2Y/A2a, purinergic receptors; Teff, effector T cell.

patic inflammation and injury, frequently leading to accelerated liver fibrosis progression, cirrhosis, and hepatocellular carcinoma compared to HCV monoinfection or HIV monoinfection.[8–10] The resultant destruction of the liver barrier contributes significantly to systemic inflammation, which is markedly elevated in HIV/HCV coinfected individuals relative to both HIV monoinfected patients and healthy controls.[11] Critically, sustained systemic inflammation in this population is also closely associated with an increased risk of non-AIDS-defining conditions (including liver-related morbidity) and mortality.[12,13] Therefore, HCV coinfection poses a substantial, independent threat to the long-term health outcomes of HIV-positive individuals, with liver disease being a primary contributor to this excess risk.

Various suppressor cells physiologically control excessive inflammatory responses.[14] Among these, $CD4^+$ and $CD8^+$ T-cells expressing immunosuppressive molecules play a significant role. One such key molecule is the ectoenzyme CD39, which is expressed on both regulatory (Treg) and conventional T-cells and functions as a critical regulator of purinergic signaling within the inflammatory microenvironment, including the liver.[15]

The mechanism underlying the suppressive action of CD39 is well established (Fig. 1).[16,17] Under conditions of cellular stress, damage, or activation—common features in chronic viral hepatitis and systemic inflammation—high-energy nucleotides like adenosine triphosphate (ATP) are released into the extracellular space. Extracellular ATP (eATP) acts as a potent danger signal (DAMP), activating purinergic receptors (e.g., P2X and P2Y) on immune cells (including effector T-cells, macrophages, and dendritic cells) and parenchymal cells, thereby promoting pro-inflammatory responses and cell death. Within this inflammatory milieu, particularly at sites of tissue damage like the inflamed liver, the CD39 ectoenzyme, expressed on specific T-cell subsets and other immune cells, catalyzes the sequential hydrolysis of eATP. It first converts ATP to adenosine diphosphate and then to adenosine monophosphate. The resulting adenosine monophosphate is subsequently hydrolyzed by the ectoenzyme CD73 to adenosine. Adenosine, in stark contrast to eATP, signals through adenosine receptors (predominantly A2a) on immune cells, exerting potent anti-inflammatory and immunosuppressive effects, including the dampening of T-cell activation, cytokine production, and cytotoxicity.[18,19] Thus, while the coordinated action of CD39 and CD73 is essential for the generation of immunosuppressive adenosine, CD39 serves as the pivotal, rate-limiting enzyme initiating this purinergic pathway for inflammation resolution.

Understanding CD39 expression dynamics on key immune cells like T-cells in HIV/HCV coinfection is of significant importance, given the critical role of this CD39/CD73/adenosine axis in controlling tissue inflammation and immune-mediated damage,[20] and the central role of unresolved inflammation in driving poor outcomes in this patient group. However, data on CD39 ectoenzyme expression patterns on $CD4^+$ and $CD8^+$ T-cells in HIV/HCV coinfected individuals, particularly those receiving stable ART, remain scarce. Moreover, a critical gap exists in the current literature: there is a complete lack of information correlating established indices of systemic inflammation (which reflect disease activity and predict clinical outcomes like liver fibrosis progression[21]) with CD39 expression levels on immunocompetent cells in this clinically relevant population. Elucidating these relationships is crucial for understanding the immunoregulatory failures contributing to persistent inflammation and liver disease progression in coinfected patients on ART, potentially identifying novel biomarkers or therapeutic targets aimed at modulating purinergic signaling to improve hepatic outcomes.

The aim of the present work was to study the specific patterns of CD39 ectoenzyme expression on T-cells of HIV/HCV coinfected patients receiving ART.

Materials and methods

The following tasks were formulated:

1. To determine the CD39 expression level on $CD4^+$ and $CD8^+$ T-cells of HIV/HCV coinfected and healthy subjects.
2. To assess CD39 expression on $CD45RA^+$ and $CD45RA^-$ $CD4^+$ and $CD8^+$ T-cells of HIV/HCV coinfected and healthy subjects.
3. To determine the concentrations of pro-inflammatory, anti-inflammatory, and immunoregulatory cytokines in the blood plasma of HIV/HCV coinfected and healthy individuals, and to analyze the relationship between systemic inflammation indices and CD39-positive T-cells in the blood of HIV/HCV coinfected patients.

Study participants and selection criteria

This case-control study comprised two groups: a case group (HIV/HCV-coinfected patients; n = 41) and a control group (healthy donors; n = 23). The inclusion criteria for the case group were as follows:

- Confirmed diagnosis of HIV and HCV coinfection;
- Age ≥ 18 years;
- Receipt of ART for ≥2 years;
- HIV viral load < 50 copies/mL;
- Nadir $CD4^+$ T-cell count < 200 cells/μL.

Exclusion criteria for the case group included:

- A history of interferon-based or direct-acting antiviral therapy for HCV;
- Presence of other chronic viral hepatitis (e.g., hepatitis B virus) or tuberculosis coinfection;
- Pregnancy;
- An acute illness or acute exacerbation of a chronic condition at the time of participant enrollment.

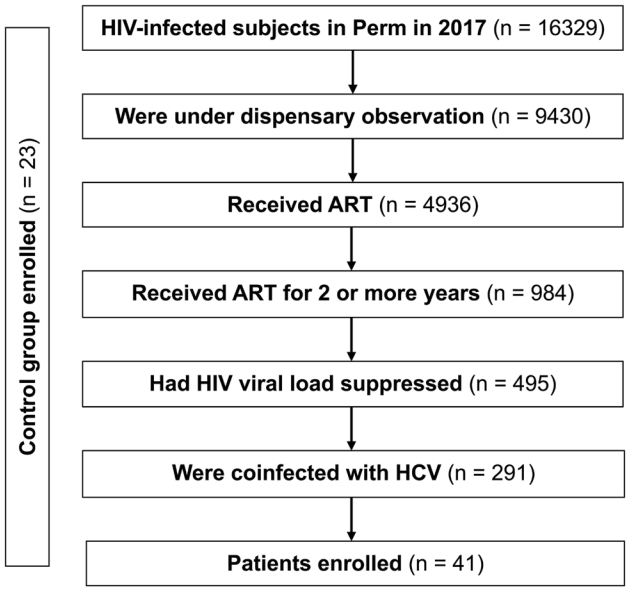

Fig. 2. Flowchart of case cohort selection. ART, antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

All case group participants were selected from the Perm Regional Center for Protection against AIDS and Infectious Diseases electronic database covering the period from 2017 to 2019 (Fig. 2).The control group consisted of voluntary blood donors with serologically confirmed absence of HIV and HCV infection. To enhance comparability, control subjects were matched to cases by sex and age.

The institutional review board of the Perm Regional Center for Protection against AIDS and Infectious Diseases (IRB00008964) reviewed and approved the study plan and the form of the patients' informed consent. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2024). Each participant (both cases and controls) was informed about the study purpose and objectives and provided informed consent.

HIV and HCV levels in plasma

Blood samples (up to 30 mL) were collected into EDTA-coated tubes from each participant. The blood plasma was separated by centrifugation. The HIV and HCV viral loads were determined, respectively, by the branched DNA hybridization method with the Versant HIV-1 RNA 3.0 assay b kits on the Versant 440 analyzer (Siemens, Germany) and by real-time polymerase chain reaction with the HEPATOGEN C quantitative kits (DNA-Tekhnologiya, Russia) on an iCycler IQ5 thermal cycler (BioRad, CA, USA) according to the manufacturer's instruction.

Multiplex and enzyme-linked immunosorbent assay (ELISA) assays

The levels of cytokines – eotaxin, interleukin (IL)-1b, IL-6, IL-10, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein (MIP)-1a, interferon gamma-induced protein (IP)-10, tumor necrosis factor a, vascular endothelial growth factor, transforming growth factor beta 1—were determined by multiplex (Bio-Plex Pro Human Cytokine Standard 27-Plex, Group I, BIO-RAD, CA, USA) and ELISA (R&D Systems, MA, USA) assays.

Blood samples for T-cell phenotyping

The peripheral blood $CD4^+$ and $CD8^+$ T-cell counts were assessed using the commercial Immunocytometry Systems (BDIS) Simultest™ kit (Becton Dickinson, NJ, USA) with a CytoFLEX S flow cytometer (Beckman Coulter, CA, USA).

Mononuclear cells were isolated by centrifugation of peripheral blood, which was diluted twice with Dulbecco's phosphate-buffered saline (Gibco, MA, USA), in a Diacoll density gradient (1.077 g/mL, Diaem, Russia). The isolated cells were collected, washed twice with Dulbecco's phosphate-buffered saline, counted in a Goryaev's chamber, and then frozen in liquid nitrogen in a medium containing 90% heat-inactivated fetal calf serum (Gibco, South America) and 10% dimethyl sulfoxide (AppliChem, Germany). Cells were thawed prior to immunophenotyping. Viable lymphocytes were counted by staining the cells with trypan blue.

A Fortessa multicolor flow cytometer (Becton Dickinson, NJ, USA) was used to identify T-cell subsets using monoclonal antibodies (anti-CD3-AF700, anti-CD8-BUV737, anti-CD25-BUV395, anti-CD127-BV786 (Becton Dickinson, NJ, USA), anti-CD45RA-BV650 (Biolegend, CA, USA), anti-CD4-Qdot605 (eBioscience™, MA, USA), anti-FOXP3-PE (Thermo Fisher Scientific, MA, USA)) and the LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit (Invitrogen, CA, USA). Among $CD4^+$ and $CD8^+$ T-cells, lymphocytes related ($CD45RA^-$) and not related ($CD45RA^+$) to the memory subset were identified. Tregs were identified as $CD4^+CD25^+CD127^{lo/-}FOXP3^+$ cells. The expression of the CD39 ectoenzyme was assessed by staining T-cells with anti-CD39-BV711 antibodies (Becton Dickinson, NJ, USA).

Statistical analysis

Since the data did not follow a normal distribution, statistical analysis was performed using nonparametric methods. The median and interquartile range (25–75 percentile) were calculated. Differences between the groups were established using the Mann–Whitney U-test. Correlation analysis was performed by calculating the Spearman rank correlation coefficient. The sample size for this study was determined *a priori* using preliminary data from pilot studies to ensure adequate statistical power for detecting significant differences in CD39 expression levels on T-cells between the HIV/HCV coinfected and healthy control groups. The minimum sample size required per group was calculated to be 40 for HIV/HCV subjects and 20 for healthy controls (allocation ratio N2/N1 = 0.5). This was determined by setting a significance level (alpha) of 0.05 and aiming for a statistical power (1 – beta) of 80%. Power calculations were performed using G*Power software, version 3.1.9.7.

Results

Clinical characteristics

The two groups of patients had no differences in age ($P > 0.05$) or gender ($P > 0.05$). Their median age was: cases – 37 years (34–39 years); controls – 32 years (29–39 years). Women comprised: cases – 49%; controls – 65%. HIV/HCV coinfected subjects were in an immunodeficient state: their absolute $CD4^+$ T-cell counts in blood (358/μL) were significantly lower than those of healthy subjects (885/μL; $P < 0.05$). In contrast, the $CD8^+$ T-cell counts were higher in cases (681/μL) compared to controls (550/μL; $P < 0.05$). While cases were not treated with anti-HCV therapy, their HCV viral load was detectable and equaled 41,900 copies/mL (835–114,500 cop-

Table 1. Frequency of CD4$^+$ and CD8$^+$ T-cells expressing the CD39 ectoenzyme in HIV/HCV coinfected and healthy individuals

Parameters	Clinical groups	
	HIV$^+$HCV$^+$	HC
CD4$^+$CD39$^+$, %	24.0 (14.6–31.9)*	16.1 (7.8–20.1)
CD8$^+$CD39$^+$, %	3.2 (2.7–4.2)*	2.8 (2.2–3.5)

Medians and interquartile ranges are shown. Statistics were performed using the Mann-Whitney method. *P < 0.05 (when comparing groups). HIV$^+$HCV$^+$, hepatitis C virus/human immunodeficiency virus coinfected patients; HC, healthy controls.

ies/mL). Epidemiological tracing showed that both infections were acquired simultaneously through injection drug use.

Differential CD39 expression in T-cell subsets

In both HIV/HCV coinfected and healthy subjects, CD39-positive cells were more abundant among CD4$^+$ T-cells (Table 1). At the same time, the frequencies of CD4$^+$CD39$^+$ and CD8$^+$CD39$^+$ T-cells were increased in cases compared to controls. Correlation analysis revealed a relationship between the relative number of CD39$^+$CD4$^+$ T-cells and the proportion of Tregs, which held true only in the group of healthy subjects (R = 0.413; P < 0.05).

Among CD45RA$^+$CD4$^+$ T-cells, the frequency of CD39$^+$ elements was twice as high as that among CD45RA$^-$CD4$^+$ T-cells, which was not typical for the corresponding cytotoxic T-cell subsets (Table 2). Regardless of the CD45 isoform expressed by the cells, the frequency of CD4$^+$CD39$^+$ T-cells was increased in cases compared to controls. No differences between the two groups were found in the pool of CD8$^+$ T-cells.

Systemic pro-inflammatory milieu in HIV/HCV coinfection is linked to altered CD39$^+$ T-cell pools

The results of the cytokine study were as follows (Table 3). In HIV/HCV coinfected subjects compared with healthy subjects, higher levels of pro-inflammatory cytokines, including IL-1b, IL-6, tumor necrosis factor a, eotaxin, IP-10, MCP-1, and MIP-1a, were observed. The amounts of anti-inflammatory and immunoregulatory cytokines—IL-10, vascular endothelial growth factor, and transforming growth factor beta 1—in the blood of cases and controls were comparable.

We found a negative relationship between the concentration of pro-inflammatory cytokines and the proportion of CD39-positive blood T-cells. The presence of high concentrations of chemoattractants, such as MCP-1 and eotaxin, in the peripheral blood of the examined individuals was associated with a decrease in the frequencies of CD4$^+$ and CD8$^+$ memory T-cells carrying CD39 (Fig. 3).

Table 2. Frequency of CD45RA$^+$ and CD45RA$^-$ CD4$^+$ and CD8$^+$ T-cells expressing the CD39 ectoenzyme in HIV/HCV coinfected and healthy individuals

Parameters	Clinical groups	
	HIV$^+$HCV$^+$	HC
CD4$^+$CD45RA$^+$CD39$^+$, %	39.4 (16.7–48.3)**	24.6 (9.3–30.3)
CD8$^+$CD45RA$^+$CD39$^+$, %	3.0 (2.6–5.0)	2.9 (2.2–3.8)
CD4$^+$CD45RA$^-$CD39$^+$, %	19.0 (9.5–24.4)***	9.2 (4.9–10.6)
CD8$^+$CD45RA$^-$CD39$^+$, %	3.0 (1.5–4.8)	2.6 (1.8–4.3)

Medians and interquartile ranges are shown. Statistics were performed using the Mann-Whitney method. **P < 0.01; ***P < 0.001 (when comparing groups). HIV$^+$HCV$^+$, hepatitis C virus/human immunodeficiency virus coinfected patients; HC, healthy controls.

Table 3. Plasma concentrations of pro-inflammatory, anti-inflammatory, and immunoregulatory cytokines in HIV/HCV coinfected and healthy individuals

Parameters	Clinical groups	
	HIV$^+$HCV$^+$	HC
IL-1b, pg/mL	0.07 (0.04–0.15)***	0.03 (0–0.04)
IL-6, pg/mL	0.28 (0.21–0.33)*	0.21 (0–0.31)
TNFa, pg/mL	1.68 (0.77–2.37)*	0.67 (0–1.5)
Eotaxin, pg/mL	5.8 (4.0–8.1)*	4.0 (2.3–5.1)
IP-10, pg/mL	218.8 (149.3–292.4)***	64.0 (45.7–107.2)
MCP-1, pg/mL	2.0 (1.6–3.1)**	0.97 (0.64–2.33)
MIP-1a, pg/mL	0.16 (0.09–0.46)**	0 (0–0.16)
IL-10, pg/mL	0.73 (0.29–1.25)	0.54 (0–0.91)
VEGF, pg/mL	4.8 (2.6–6.5)	3.1 (0–5.7)
TGF-b1, pg/mL	9,262.7 (7,636.3–13,797.8)	9,002.2 (6,951.2–12,198.0)

Medians and interquartile ranges are shown. Statistics were performed using the Mann-Whitney method. *P < 0.05; **P < 0.01; ***P < 0.001 (when comparing groups). HC, healthy controls; HIV$^+$HCV$^+$, hepatitis C virus/human immunodeficiency virus coinfected patients; IL, interleukin; IP-10, interferon gamma-induced protein 10; MCP-1, monocyte chemoattractant protein 1; MIP-1a, macrophage inflammatory protein 1a; TGF-b1, transforming growth factor beta 1; TNFa, tumor necrosis factor a; VEGF, vascular endothelial growth factor.

Discussion

In this work, we studied the specific patterns of CD39 ectoenzyme expression on CD4$^+$ and CD8$^+$ T-cells of HIV/HCV coinfected patients receiving ART. The absence of HCV treatment in our HIV/HCV-coinfected cohort, recruited in Russia in 2017, reflected the specific healthcare access barriers prevalent at that time. In accordance with the national clinical guidelines and reimbursement policies, direct-acting antiviral therapy was primarily restricted to patients with advanced liver fibrosis (stages F3/F4).[7] Furthermore, treatment was centralized, requiring prescription from specialized infectious disease or hepatology centers, creating significant bottlenecks. This systemic limitation, rather than a study design choice, resulted in a treatment-naive cohort, providing a unique opportunity to assess unmodified T-cell phenotypes in the context of untreated HCV coinfection.

We found that the CD39 molecule was expressed on both cytotoxic and helper T-cells in HIV/HCV coinfected and healthy individuals. At the same time, CD39 was found approximately six times more often on the surface of CD4$^+$ T-cells. The wide representation of CD39 on CD4$^+$ T-cells can be partly explained by the presence of regulatory T-cells that use the CD39 ectoenzyme as one of the important suppressor mechanisms.[22] However, correlation analysis showed that the relationship between Tregs and CD39$^+$CD4$^+$ T-cell frequencies was present only in the group of healthy controls and explained only 17% of the variation in a regression model. Apparently, during HIV/HCV coinfection, not only regulatory but also conventional CD4$^+$ T-cells actively expressed the CD39 suppressor molecule for more effective control of systemic inflammation.

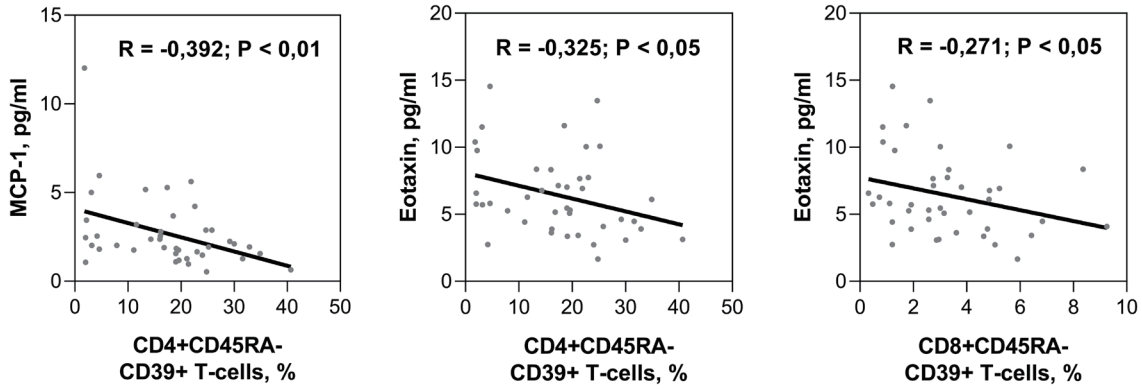

Fig. 3. Correlation between the frequency of CD39-positive CD4$^+$ and CD8$^+$ T-cells and the pro-inflammatory chemokine concentrations in the blood of HIV/HCV coinfected and healthy individuals. Individual values (points) and regression lines are presented. HCV, hepatitis C virus; HIV, human immunodeficiency virus; MCP-1, monocyte chemoattractant protein 1; R, Spearman's rank correlation coefficient.

In HIV/HCV coinfected patients compared with healthy subjects, the frequency of CD39-positive CD4$^+$ T-cells was increased in both naive and memory subsets. It was noteworthy that among naive (CD45RA$^+$) T-helper cells, the percentage of CD39$^+$ cells was twice as high as that among the corresponding memory cells (CD45RA$^-$) in both clinical groups. At present, there is no explanation for this phenomenon. At the same time, it is known that naive CD39$^+$ T-cells can be precursors for Tregs that acquire higher proliferative and suppressive capacity than those induced from naive CD39$^-$ T-cells.[23] But it is worth noting that only half of all CD4$^+$CD39$^+$ memory T-cells were CD25$^+$FOXP3$^+$ and could perform a suppressor function.[24]

In the present work, we showed that the frequency of CD39-positive cytotoxic T-cells was slightly increased ($P < 0.05$) in the blood of HIV/HCV coinfected patients compared with healthy individuals. According to the literature, in chronic inflammatory diseases, the CD39^{hi}CD8$^+$ T-cell population is enriched for cells with the phenotypic and functional profile of terminal exhaustion.[25,26] The authors found that although such "exhausted" CD39$^+$CD8$^+$ T-cells had reduced cytotoxic activity, they effectively performed their suppressor function. It appeared that in HIV/HCV coinfected individuals, CD39$^+$CD8$^+$ T-cells might also be involved in the control of inflammatory responses, though no functional tests were performed to support this notion.

In our previous studies and in the present work,[9] we showed that HIV/HCV coinfected patients developed severe systemic inflammation, which was not compensated by the production of anti-inflammatory and/or immunoregulatory cytokines. At the same time, it seemed that an increase in the content of pro-inflammatory cytokines and chemokines stimulated CD4$^+$ and CD8$^+$ memory T-cells, which have a suppressive ability, to migrate to the sites of inflammation. This notion was supported by the negative correlations we found between the frequencies of CD4$^+$ and CD8$^+$ memory T-cells, on the one hand, and pro-inflammatory cytokines, on the other hand. This interpretation, however, should be approached with caution, as peripheral blood may not fully reflect immune cell dynamics within tissues.

This study has several limitations. The absence of functional assays and monoinfection control groups warrants careful consideration when interpreting our findings. While our phenotypic data clearly demonstrate an expansion of CD39$^+$ T-cells in HIV/HCV coinfection, we cannot definitively conclude whether these cells exhibit fully competent suppressive function *in vivo*. It is plausible that the chronic inflammatory milieu could induce CD39 expression as a compensatory mechanism, yet also lead to functional exhaustion, ultimately resulting in insufficient control of inflammation. This potential disconnect between phenotype and function could explain the observed coexistence of elevated CD39$^+$ T-cell frequencies and persistent systemic inflammation.

Similarly, the lack of HIV-monoinfected and HCV-monoinfected cohorts limits our ability to observe the unique contribution of each virus to the CD39 signature found. The expanded CD39$^+$ CD4$^+$ T-cell pool could be a hallmark of the intensified inflammatory burden specific to coinfection, a consequence of persistent HCV replication in an immunologically altered HIV-infected host, or a combination of both. Future studies incorporating functional analyses and well-matched monoinfection groups will be crucial to determine if the CD39 pathway is differentially regulated and functionally compromised in coinfection compared to single infections, and whether it represents a viable target for immunomodulatory interventions aimed at reducing inflammation-driven pathology in this population.

Notwithstanding these limitations, our data contribute to understanding the immunoregulatory landscape mediated by CD39$^+$ T-cells in the context of HIV/HCV coinfection. The findings also promote consideration of their potential clinical implications. The observed inverse correlation between the frequency of memory CD39$^+$CD4$^+$ T-cells and pro-inflammatory chemokines suggests that this T-cell subset could be further investigated as a potential peripheral biomarker of the systemic inflammatory state in ART-treated HIV/HCV coinfection. However, establishing its actual utility would require longitudinal studies to determine if CD39$^+$ T-cell frequencies correlate with clinical outcomes, such as the progression of liver fibrosis or the development of non-AIDS comorbidities.

The CD39/CD73/adenosine axis represents a fundamental immunoregulatory pathway from a therapeutic perspective. Our data demonstrate its alteration in HIV/HCV coinfection, aligning with the broader hypothesis that this pathway might be a target for immunomodulation in chronic inflammatory conditions. However, any potential therapeutic strategy aimed at modulating this pathway in the context of persistent viral infections would require extreme caution. Therefore, while our phenotypic data highlight the relevance of the CD39 pathway in HIV/HCV coinfection, its suitability as a therapeutic target remains an open question that might be resolved upon future functional validation and a deeper under-

standing of its role in maintaining overall immune homeostasis in this patient population.

Conclusions

Thus, in this work, we have demonstrated that $CD4^+$ T-cells, namely their $CD45RA^+$ subset, act as the main regulators of purinergic signals in HIV/HCV coinfected and healthy individuals. At the same time, the proportion of $CD4^+$ T-cells involved in the control of purinergic signaling was increased in HIV/HCV coinfected patients compared with healthy subjects. These CD39-positive T-cells circulated between lymphoid organs and migrated to inflamed sites in response to increased concentrations of pro-inflammatory cytokines and chemokines.

At present, it remains unclear how effectively CD39-positive T-cells neutralize purinergic molecules. Moreover, our results raise a new question: how might a shift from effector to suppressor $CD4^+$ T-cells affect the ability of the immune system to withstand newly emerging threats in HIV/HCV coinfected patients? Solving this and other questions requires further research.

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Study concept and design (ES, LK), acquisition of data (LK, VV), analysis and interpretation of data (ES, VV), drafting of the manuscript (ES), critical revision of the manuscript for important intellectual content (ES, LK, VV), and technical and material support (VV). All authors made significant contributions to this study and approved the final manuscript.

Ethical statement

The institutional review board of the Perm Regional Center for Protection against AIDS and Infectious Diseases (IRB00008964) reviewed and approved the study plan and the form of the patients' informed consent. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2024). Each participant (both cases and controls) was informed about the study purpose and objectives and provided informed consent.

Data sharing statement

The flow cytometry and ELISA data used to support the findings of this study are available from the corresponding author upon request.

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