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Correlation between virological indexes and immune inflammation-related factor levels in acute HIV-1-infected patients

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Abstract

Purpose: This study aimed to compare the different expression levels of 12 immune inflammation-related factor in plasma of untreated acute HIV-1-infected patients with different HIV-1 RNA or HIV-1 DNA levels.

Methods: 70 acute HIV-1-infected patients without any anti-retrovirus therapy (ART) were selected. The levels of patients' HIV-1 RNA, HIV-1 DNA and 12 different immune inflammation-related factors in plasma were detected and compared.

Results: The expression levels of CXCL10, CXCL11, CXCL13, IL-6, IL-16 and IL-18 were different between the different HIV-1 RNA level groups and the different HIV-1 DNA level groups. There was no significant difference in the expression levels of the chemokines IL-8, CCL2, CCL20 and CCL22 between the groups. The stratified analysis using 200 cells/μL as the CD4+ T cell count cut-off value showed similar intergroup differences.

Conclusion: CXCL10, CXCL11, CXCL13, IL-6, IL-16 and IL-18 may be involved in the regulation of viral replication or viral reservoirs, and may also be used as important indicators for the evaluation of HIV-1 replication.

Keywords: HIV-1; chemokines; interleukins; HIV-1 RNA; HIV-1 DNA

Introduction

After human immunodeficiency virus-1 (HIV-1) infects humans, it often leads to local or systemic inflammatory reactions, which affect the prognosis of patients. The occurrences of the inflammatory response are generally correlated with the chemokines and interleukins (ILs) (1-3). Chemokines are a class of low molecular weight proteins secreted by cells. To date, more than 40 chemokines have been identified in humans, most of which have molecular weights of 8-10 kDa (4). The chemokine family have similar structural characteristics and are mainly divided into four subtribes, CXC, CC, C and CX3C, according to the arrangement of the amino terminus of cysteine. Among the subtribes of CXC chemokines, CXCL8, CXCL10, CXCL11 and CXCL13 are mainly associated with HIV-1 infection and the post-infection inflammatory response (5). ILs are involved in various kinds of immune regulation, immune response, immune activation, and information transmission (6). According to the main properties of ILs, they are divided into IL-1, IL-2, chemokines, IL-10, IL-12 and IL-17 subtribes. ILs are strongly related to the immune inflammatory activation of HIV-1-infected, including IL-1 family, which are mainly proinflammatory cytokines (7-9), and IL-10 family, which mainly play the immunoregulatory roles (10,11). Although it has been shown that the levels of these immune inflammation-related factors change in patients after HIV-1 infection, it is unclear whether the expression levels in plasma are correlated with the virology levels of untreated HIV-1-infected persons, especially the levels of HIV-1 DNA. In this study, acute HIV-1-infected patients were divided into different groups according to the levels of HIV-1 RNA or HIV-1 DNA, and the different expression levels of 6 chemokines and 6 ILs in plasma among groups were observed.

Methods

Clinical data

70 HIV-1-infected patients admitted to the Second Affiliated Hospital of Air Force Medical University were selected. All HIV-1-infected patients underwent primary screening and HIV-1 antibody confirmation tests; none had opportunistic infection or tumours or coinfection with HBV/HCV, and none had received any ART before blood sample collection. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Air Force Medical University, and blood samples from patients were used for scientific research purposes only. The general data and partial test results of 70 patients are shown in Table 1. The sample size of this study was determined by combining the following sample size calculation formula and the actual sample size that met the inclusion criteria:

$$N = Z^2 \times （P \times （1 - P））\div E^2$$

where N is the sample size, Z is the statistic, E is the error value, and P is the probability value. When the confidence level was 90%, Z=1.64, P=0.5, and E=10%, the sample size calculated by the above formula was at least N=67, and the number of samples that met the inclusion criteria after screening was 70.

DNA extraction from PBMCs

Peripheral blood was extracted from the patients for PBMC isolation, flow cytometry and quantitative viral nucleic acid detection. PBMCs were isolated by density gradient centrifugation (lymphocyte separation solution) for subsequent detection. A QIAamp DNA Blood Mini Kit (QIAGEN®) was used to extract total DNA from PBMC samples. The extracted DNA was detected by NanoDrop for nucleic acid concentration and then directly quantitatively detected for HIV-1 DNA.

Quantitative detection of HIV-1 RNA

HIV-1 nucleic acid detection kit (SUPBIO®) was used for the quantitative detection of HIV-1 RNA in plasma samples on ABI 7500 fluorescent PCR instrument, and the operation was strictly followed by the instructions of the kit. The result unit was expressed as IU/ml, and the minimum detection limit was 20 IU/ml.

Quantitative detection of HIV-1 DNA

HIV-1 DNA quantitative detection kit (SUPBIO®) was used for the quantitative detection of

HIV-1 DNA extracted from PBMC samples on ABI 7500 fluorescent PCR instrument. The operation was strictly followed by the instructions of the kit. The result unit was expressed as copies/10^6 cells. The minimum detection limit was 20 copies/10^6 cells.

CD4+ T cell count and CD8+ T cell count in peripheral blood

CD4 FITC/CD8 PE/CD3 PerCP (BD Tritest™), BD Trucount™ Tubes and BD Facsaria II flow cytometry were used for detection. 50μl whole blood was taken and incubated for 20 min with a mixture of 20μl monoclonal antibodies. The mix was lysed by erythrocyte lysate and then tested on the machine. The results were analysed with Flowjo software.

Detection of immune inflammation-related factors

CCL2, CCL20, CCL22, CXCL10, CXCL11, CXCL13, IL-1β, IL-6, IL-8, IL-10, IL-16 and IL-18 were selected to customize the Luminex Assay Human Premixed Multi-Analyte Kit (R&D Systems). 50μl plasma samples were taken and analysed in strict accordance with the manufacturer's instructions. The Luminex 200 instrument was used for detection.

Statistical Methods

The data obtained were statistically processed, analysed and plotted by SPSS 23.0 statistical software and GraphPad Prism 8 software. Descriptive analysis was used for general data, and measurement data are expressed as $\bar{x} \pm s$. Independent sample *t* test was used to compare the measurement data between the different groups. $P<0.05$ was considered statistically significant.

Results

Baseline characteristics of patients

The baseline characteristics of 70 untreated acute HIV-1-infected patients is shown in Table 1.

Table 1. Basic characteristic of untreated patients

Baseline characteristics		$\bar{x} \pm s$ / *n(%)*
Number of untreated patients		70
Gender (male/female)		62/8
Age (years)		29.62±7.48
Infection way	Sexual transmission	70(100)
	Blood transmission	0
Infection time (days)		23.43±3.81
$CD4^+$ T cell count（cells/μl）		374.76±222.57
$CD8^+$ T cell count（cells/μl）		1199.23±553.54

$CD4^+/CD8^+$	0.32±0.15

Comparison of the baseline characteristics between different groups

Patients were divided into four groups according to whether HIV-1 DNA was less than 10^3copies/10^6 cells or HIV-1 RNA was less than 10^6 IU/ml: DNA high level group (DHG), DNA low level group (DLG), RNA high level group (RHG) and RNA low level group (RLG).There were no significant differences in baseline characteristics between each group (P>0.05), as shown in Table 2 and Table 3.

Table 2. Differences between RHG and RLG

Baseline characteristics	RLG	RHG	t value χ^2value	P value
Number of untreated patients	36	34	/	/
Gender (male/female)	32/4	30/4	0.572	0.682
Age (years)	29.42±7.54	30.32±7.13	0.332	0.741
Infection time (days)	23.29±2.86	24.54±3.12	1.203	0.233
$CD4^+$ T cell count（cells/μl）	393.76±241.12	356.31±205.44	0.671	0.505
$CD8^+$ T cell count（cells/μl）	1213.43±489.88	1186.76±615.54	0.192	0.849
$CD4^+/CD8^+$	0.32±0.16	0.31±0.14	0.147	0.883

Table 3. Differences between DHG and DLG

Baseline characteristics	DLG	DHG	t value χ^2value	P value
Number of untreated patients	26	44	/	/
Gender (male/female)	23/3	29/5	0.892	0.591
Age (years)	28.61±7.32	30.22±7.19	1.426	0.159
Infection time (days)	23.78±2.91	23.54±3.25	0.484	0.630
$CD4^+$ T cell count（cells/μl）	406.42±247.76	357.13±210.92	0.839	0.404
$CD8^+$ T cell count（cells/μl）	1276.62±464.80	1160.22±594.76	0.801	0.426
$CD4^+/CD8^+$	0.32±0.18	0.32±0.13	0.068	0.946

Comparison of plasma immune inflammation-related factor expression levels between different groups

The results showed that the expression levels of CXCL10, CXCL11, CXCL13, IL-1β, IL-6, IL-10, IL-16 and IL-18 were significantly different between the RLG and RHG (P<0.05), as shown in Figure 1. The expression levels of CXCL10, CXCL11, CXCL13, IL-6, IL-16 and IL-18 were statistically significant between the DLG and DHG (P<0.05), as shown in Figure 2.

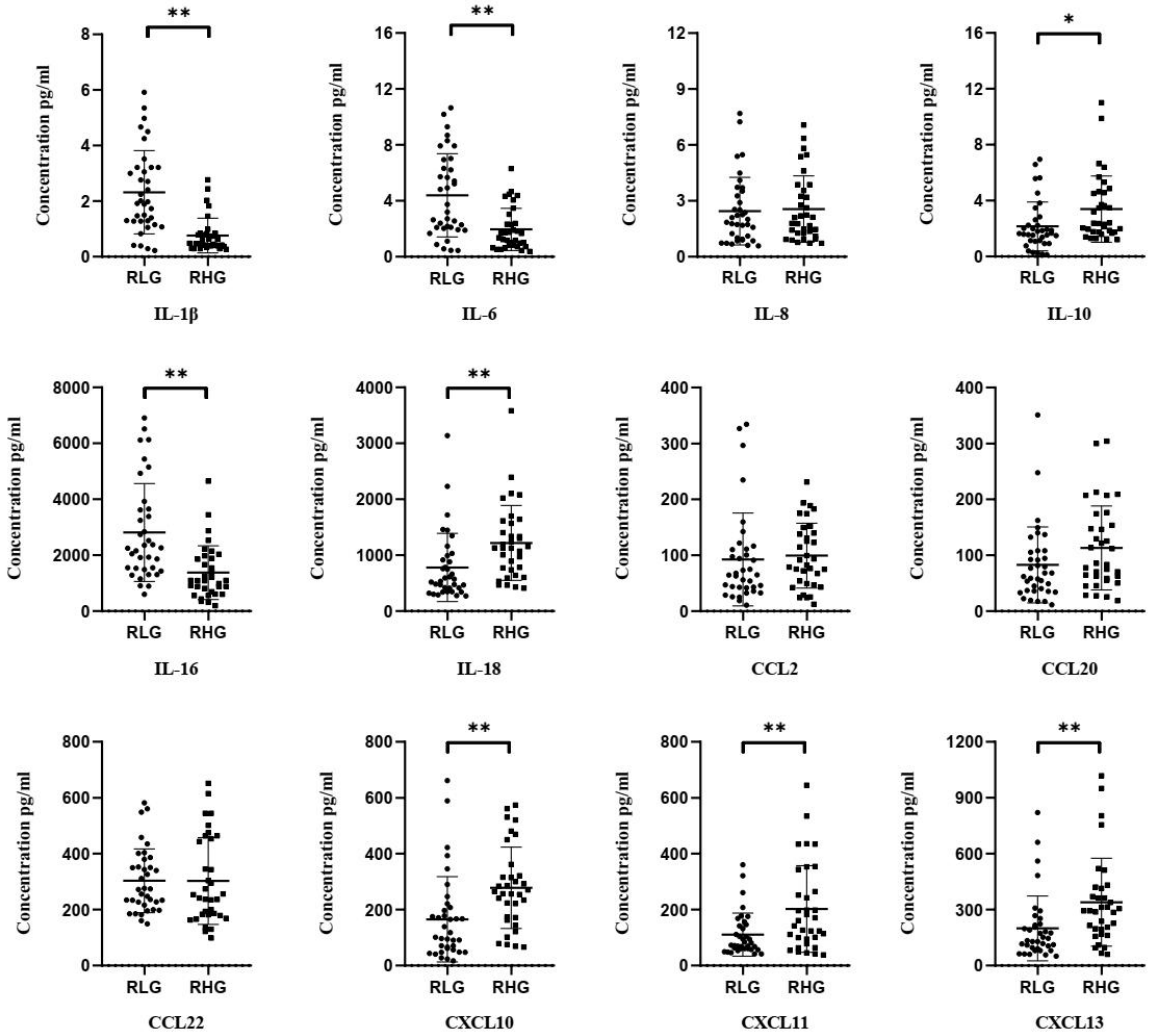

Figure 1 Comparison of the 12 immune inflammation-related factors expression levels between the RLG and the RHG.

Note: *: $P<0.05$, ** $P<0.01$.

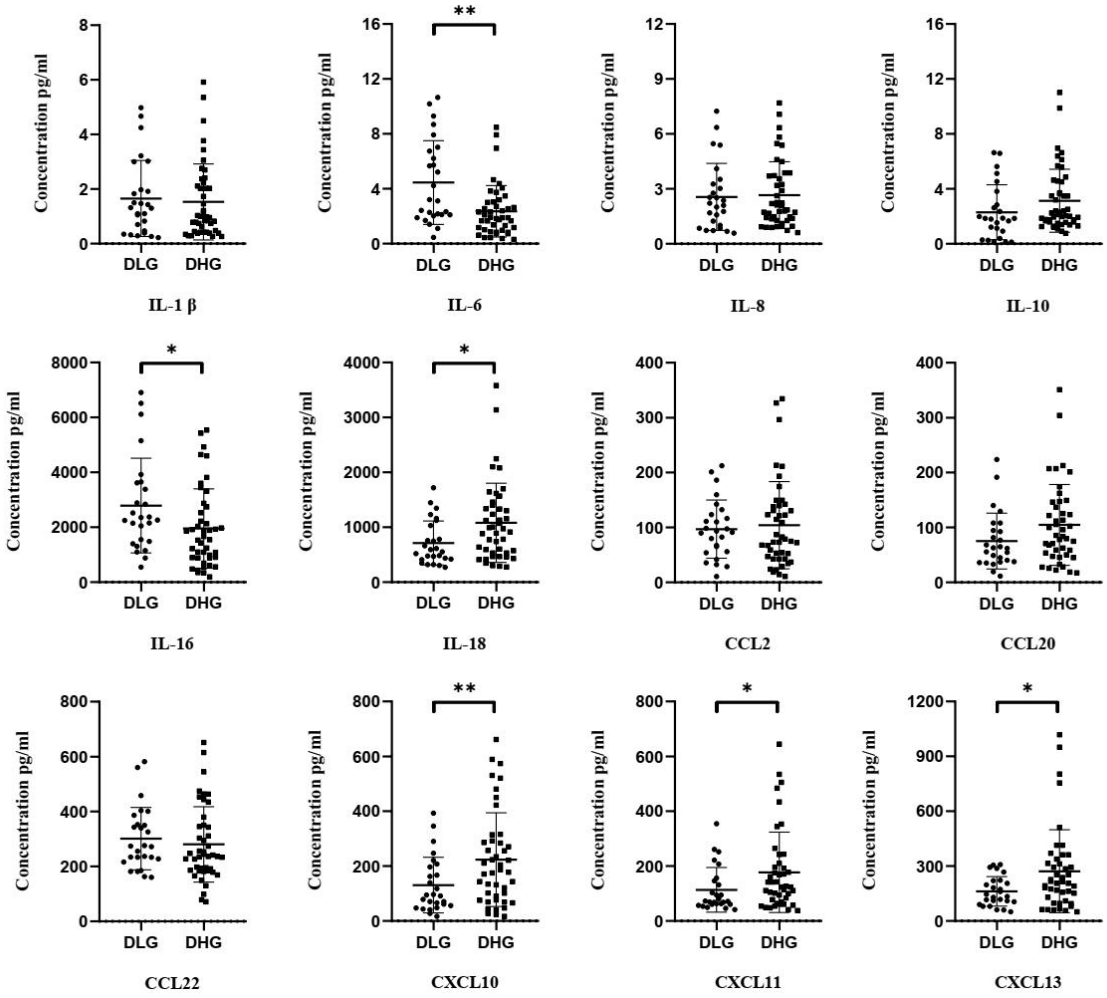

Figure 2 Comparison of the 12 immune inflammation-related factors expression levels between the DLG and the DHG.

Note: *: $P<0.05$, ** $P<0.01$.

Stratified comparison by CD4+ T cell count between different groups

The cut-off value of CD4+ T cell count was 200 cells/μL. The results showed that regardless of whether the CD4+ T cell count >200 cells/μL or ≤ 200 cells/μL, there were significant differences in the CXCL10, CXCL11, CXCL13, IL-1β, IL-6, IL-10, IL-16 and IL-18 expression levels between the groups ($P<0.05$), as shown in Table 4 and Table 5.

Table 4. Differences between RHG and RLG with stratification of CD4+T cell count

Chemokines	$CD4^+$T cell count ≤ 200（cells/μl）				$CD4^+$T cell count > 200（cells/μl）			
	RLG (N=17)	RHG (N=14)	*t*	*P* value	RLG (N=19)	RHG (N=20)	*t*	*P* value
IL-1β	2.76±0.35	0.54±0.08	5.54	0.01	1.92±0.33	0.92±0.17	2.75	0.01
IL-6	4.12±0.73	1.83±0.44	2.54	0.02	4.63±0.69	2.04±0.32	3.46	0.01

IL-8	2.62±0.42	2.95±0.45	0.52	0.61	2.29±0.43	2.29±0.41	0.01	0.99
IL-10	1.81±0.38	3.45±0.62	2.33	0.03	2.04±0.28	3.36±0.55	2.09	0.04
IL-16	2212.31±287.13	1393.43±207.91	2.22	0.03	3352.21±462.60	1164.32±144.63	4.61	0.01
IL-18	732.21±90.96	1151.33±123.31	2.79	0.01	770.62±160.73	1266.41±176.94	2.07	0.04
CCL2	113.72±25.44	98.27±10.85	0.52	0.61	73.47±12.12	100.23±15.33	1.36	0.18
CCL20	82.95±21.97	94.09±17.14	0.39	0.70	82.57±9.37	106.62±12.11	1.56	0.13
CCL22	316.12±30.50	277.13±45.04	0.74	0.47	291.81±23.92	320.24±32.83	0.69	0.49
CXCL10	164.42±26.57	296.43±28.81	3.36	0.01	145.02±33.27	265.04±37.71	2.37	0.02
CXCL11	100.64±15.22	211.71±42.61	2.64	0.01	109.2±15.59	195.6±34.73	2.23	0.03
CXCL13	214.43±44.19	400.73±66.08	2.41	0.02	154.52±17.42	296.71±50.06	2.63	0.01

Table 5. Differences between DHG and DLG with stratification of CD4+T cell count

Chemokines	$CD4^+$T cell count ≤ 200（cells/μl）				$CD4^+$T cell count > 200（cells/μl）			
	DLG (N=12)	DHG (N=19)	*t*	*P* value	DLG (N=14)	DHG (N=25)	*t*	*P* value
IL-1β	1.05±0.26	1.24±0.30	0.44	0.66	2.17±0.42	1.75±0.29	0.85	0.40
IL-6	3.42±0.55	2.05±0.26	2.52	0.01	5.34±0.96	2.20±0.35	3.69	0.01
IL-8	2.54±0.54	2.42±0.39	0.18	0.85	2.58±0.50	2.83±0.39	0.39	0.70
IL-10	1.59±0.31	2.36±0.32	1.54	0.13	3.21±0.57	3.05±0.37	0.23	0.81
IL-16	2393.34±377.12	1457.44±164.23	2.58	0.01	3273.23±507.61	1852.23±282.01	2.66	0.01
IL-18	625.62±93.86	1119.25±144.62	2.49	0.01	738.82±100.41	1223.22±147.31	2.29	0.03
CCL2	72.71±7.54	94.37±10.79	1.45	0.16	124.12±14.38	111.73±19.49	0.44	0.66
CCL20	68.28±9.91	104.01±17.31	1.54	0.13	95.74±15.07	105.13±14.70	0.41	0.68
CCL22	275.81±30.87	291.12±36.02	0.29	0.77	323.31±31.67	272.53±24.52	1.25	0.21
CXCL10	78.61±10.92	179.23±32.45	2.39	0.02	175.52±31.43	285.42±35.03	2.09	0.04
CXCL11	89.96±24.43	184.92±30.43	2.21	0.03	133.72±20.27	223.62±30.36	2.07	0.05
CXCL13	156.32±22.91	307.62±54.40	2.12	0.04	147.42±20.82	268.53±42.06	2.07	0.04

Discussion

CXCL10 is secreted by a variety of cells, including monocytes, white blood cells(WBC), endothelial cells, and epithelial cells (12). The expression of CXCL10 are upregulated in many diseases, such as hepatitis B infection, tuberculosis, cancer, diabetes and autoimmune diseases (13). It's found that the CXCL10 increases in the early stage of HIV-1 infection and may lead to immune dysfunction in infected patients by inhibiting the secretion of IFN-γ and inducing the cytotoxicity of T cells and NK cells (14). CXCR3+ Th cells are decreased in response to CXCL10-mediated migration after HIV-1 infection, which may lead to impaired cellular immune function (15). CXCL11 is expressed highly in the WBC, the pancreas and the liver (16, 17), which has a high affinity for CXCR3 as well. The expression of CXCL11 was upregulated after HIV-1 infection, which was positively correlated with CXCL9 and CXCL10 expression levels, and negatively correlated with the CD4+ T cell counts (18). The expression of CXCL11 mRNA was increased in monocyte-derived macrophages and dendritic cells

infected by HIV-1 in vitro, which suggest that CXCL11 may affect the HIV-1 infective inflammatory response and immune function (19). CXCL13 is mainly synthesized and secreted by stromal cells, which will mediate the homing process of B cells to inflammatory lesions when an inflammatory response occurs (20). Among them, the CXCL13-CXCR5 axis plays an important role in physiological and pathological immunity, and this axis is related to the pathogenesis of many infectious diseases (21). In HIV-1 infection, the expression of CXCR5 and CXCL13 changes, the number of CXCR5+ B cells decreases with the progression of HIV-1 infection, and the plasma level of CXCL13 increases with the duration of infection (22). Subsequent studies have confirmed a slow elevation of CXCL13 in serum during chronic HIV-1 infection and have shown that CXCL13 levels are associated with disease progression and the duration of HIV-1 infection (23).

IL-1β is an important inflammatory lymphokine which is mainly secreted by peripheral blood monocytes and macrophages. Previous studies have shown that it is closely related to apoptosis, and often increased by the stimulation of inflammation and infection (24). For example, the expression of the IL-1β gene in the gut-associated lymphoid tissue of HIV-1-infected people is significantly higher than healthy people (1). However, IL-1β can also inhibit HIV-1 replication in lymphocytes, Jurkat cells and PBMCs in vitro, and it may inhibit viral replication by blocking the expression and activation of caspase-3 (12,25). IL-6 is a proinflammatory lymphokine which participates in the regulation of various physiological processes, especially in acute inflammation and the transformation from acute-inflammation to chronic-inflammation (26). For a long time, HIV-1 infection has been proven to induce the expression and secretion of IL-6 by monocytes and macrophages. Even in the patients under virological inhibition, the plasma IL-6 level is significantly higher than the healthy people (27,28). Recent studies have shown that the increase of plasma IL-6 in HIV-1 infected patients is correlated with various adverse clinical results, such as anaemia, cardiovascular disease and cancer (29–31). The continuous increase in IL-6 proves that active inflammation may have far-reaching clinical significance for the prognosis of HIV-1-infected people, but whether it affects the viral replication process remains unclear (8). IL-10 is considered to be a Th2 lymphokine which can indirectly inhibit the Th1 response. IL-10 can continuously inhibit T cells in chronic infection caused by HIV-1, HBV, HCV and other viruses, in which promoting viral infection activity (32). It can also directly act on antigen-presenting cells, reduce the expression of stimulating molecules, prevent the maturation of initial T cells, or limit T cells' proliferation and functional differentiation (10). It has been observed that IL-10 produced by

PBMCs from HIV-infected patients can inhibit the proliferation of CD4+ and CD8+ T cells and the production of cytokines, and blocking IL-10 can effectively restore the function of these cells in vitro (33). IL-16 is a natural soluble ligand of CD4 and has the characteristics of promoting inflammation and immune regulation. It also has chemotaxis effects on CD4+ T cells, monocytes and eosinophils (11). The antiviral activity of IL-16 was first found in isolated and cultured CD8+ T cells, which can inhibit the replication of HIV-1 and SIV (34). Although both IL-16 and HIV-1 use CD4 as receptors, they do not share common binding sites (35). Therefore, IL-16, as an endogenous antiviral lymphokine, plays an important role in inhibiting HIV-1 replication. IL-18 is a proinflammatory, proapoptotic and atherosclerotic lymphokine belonging to the IL-1 lymphokine family, and its impact on HIV-1 replication has been controversial. IL-18 promotes the development and differentiation of initial CD4+ T cells into Th2 effector cells, which inhibiting the immune effect of anti-HIV-1 and indirectly promoting HIV-1 replication (36,37).

In this study, 6 chemokines and 6 ILs in plasma of acute HIV-1 infected patients without ART were detected, and the patients were grouped according to HIV-1 RNA or HIV-1 DNA levels to compare the above Whether other chemokines and ILs are also correlated with the size of the HIV-1 reservoirs, alymphokines. The results showed that CXCL10, CXCL11, CXCL13, IL-10 and IL-18 in patients with higher HIV-1 RNA were higher, and the expression levels of IL-1β, IL-6 and IL-16 in patients with higher HIV-1 RNA were lower. This result is consistent with previous studies suggesting that these chemokines and ILs are related to the degree of HIV-1 infection (14, 18, 22). In addition, CXCL10, CXCL11, CXCL13, IL-16 and IL-18 in patients with higher HIV-1 DNA were higher, while the expression levels of IL-6 in patients with higher HIV-1 DNA were lower. To avoid the effects of the CD4+ T cell count on the conclusion, we further performed stratified analysis with a CD4+ T cell count cut-off value of 200 cells/μL and obtained similar intergroup differences. Since HIV-1 DNA is considered an important indicator of the size of the viral reservoir in patients, we believe that the CXCL10, CXCL11, CXCL13, IL-6, IL-16 and IL-18 may be both related to the viral replication and the size of the viral reservoirs (38, 39). In conclusion, the CXC subfamily of chemokines and ILs play important roles in HIV-1 infection. Whether other chemokines and ILs are also correlated with the size of the HIV-1 reservoirs, and the specific mechanism by which these lymphokines may affect the viral reservoir remain to be further explored.

Data Availability Statements

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest statement

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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