Effects of HIV on metabolic and biological pathways of CD4 T lymphocytes

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Abstract. The effects of human immunodeficiency virus (HIV) on the metabolic and biological pathways of cluster of differentiation (CD)4 T lymphocytes were investigated. A total of 150 patients with acquired immune deficiency syndrome (AIDS) and 50 healthy individuals who were admitted to hospital for physical examination during the period of June 2016 to January 2017, were selected as subjects in the present study. According to the virus load, 150 AIDS patients were divided into three groups: i) Viral load >10^6 copies/ml (group A, n=39), ii) 10^4 copies/ml < viral load <10^5 copies/ml (group B, n=76), and iii) viral load <10^4 copies/ml (group C, n=35). The relationship between viral loads in the three groups and CD4 T lymphocyte counts was assessed. Active lymphocytes were isolated from T lymphocytes in the subjects, and the ratio of Th1 to Th2 was measured by flow cytometry. Effects of HIV on human T-lymphocyte differentiation were observed. Differences in T-lymphocyte metabolites were detected by proton nuclear magnetic resonance and their biological pathways analyzed. The results showed that CD4 T-cell counts were decreased with the increase of the viral loads of patients. The viral loads of AIDS patients differentiated T lymphocytes. In other words, high viral loads accelerated the differentiation of T lymphocytes into Th1 cells. In the high HIV viral load group, the levels of glycerol phosphodiesterase, 7-dehydrocholesterol, p-hydroxyphenylacetic acid, cholesterol and deoxyxuridine were increased, but the levels of 3-methoxytyramine, cytidine deaminase, deoxyxorticosterone and 3-hydroxybutyric acid were decreased. The viral loads of AIDS patients are associated with CD4 T-cell counts and the ratio of CD4+ T to CD8+ T cells. At the same time, HIV viral loads can affect the lipid biosynthesis of T-lymphocyte membranes, thus affecting the differentiation and proliferation of T lymphocytes and finally intervening its mediated immune responses.

Introduction

Acquired immune deficiency syndrome (AIDS) is a type of acquired immune deficiency syndrome caused by human immunodeficiency virus (HIV) infection. The number of AIDS patients has been on the increase annually (1). The main transmission modes of the disease include sexual, blood and mother-to-fetus transmission.

At present, the main diagnosis of AIDS is based on laboratory tests. The number of peripheral blood cluster of differentiation (CD)4 T lymphocytes and viral load can be used as important test indicators to assess the severity of AIDS patients, determine the progression of the disease and evaluate the prognosis of AIDS patients following antiviral therapy (2). The CD4 T lymphocyte is one of the central cells involved in immune responses in vivo. HIV mainly infects CD4 T lymphocytes. T lymphocytes in healthy individuals contain approximately 65% of CD4 T lymphocytes and 35% of CD8 T lymphocytes (3,4). Once HIV infection occurs, the body’s immune system is damaged resulting in decreased CD4 T lymphocyte and dysfunction as well as increased CD8 T lymphocyte levels, eventually leading to CD4+/CD8 imbalance. Therefore, the main assessment indicator is immune system damage condition in AIDS patients. In other words, the CD4 T lymphocyte expression level is used to determine whether the immune cell function is disordered (5-7). The detection of viral load in the peripheral blood of patients is the most important factor affecting the progress of AIDS (8).

Therefore, in the present study, we analyzed the relationship of viral loads with CD4 T-cell counts, T lymphocyte differentiation and metabolites in AIDS subjects. The aim of the present study was to provide a theoretical basis for conducting antiviral therapies as early as possible to reduce the incidence and death rate of AIDS.

Materials and methods

General materials. A total of 150 AIDS patients admitted to Qingdao Sixth People’s Hospital (Shandong, China) from June...
Detection of HIV viral loads. Ethylenediaminetetraacetic acid dipotassium salt dihydrate (EDTA-K2) anticoagulants were taken and centrifuged for 10 min at 1,300 x g to separate the plasma. The separated plasma was stored in the refrigerator at -70°C for standby application. Plasma (1,100 µl) was taken from each sample and the HIV RNA content in the plasma samples was detected by reverse transcriptase-polymerase chain reaction in an automatic virus loader (Roche Diagnostics, Basel, Switzerland). During the operation, an internal standard was added to the specimens and incubated in the dark at room temperature for 15 min, and the levels of CD4 T lymphocytes were detected in accordance with the Guidelines for Quality Assurance of CD4 T lymphocyte Detection and the National AIDS Test Specification as follows: i) Viral load >10⁶ copies/ml (group A, n=39), ii) 10⁵ copies/ml < viral load <10⁶ copies/ml (group B, n=76), and iii) viral load <10⁵ copies/ml (group C, n=35).

Detection of the number of CD4⁺ T lymphocytes. The levels of CD4⁺ T lymphocytes were detected in accordance with the Guidelines for Quality Assurance of CD4⁺ T lymphocytes Detection and the National AIDS Test Specification as follows: The test specimens and Immuno-Trol™ controlled blood were operated simultaneously; the CD4 fluorescent-marked antibodies (20 µl) and mixed anticoagulants (50 µl) were successively added to the tubes for detection using flow cytometry (FCM), and then incubated for 15 min at room temperature. The CaliBRITE™ beads and FACSComp™ software were used to calibrate the FCM before the operation. Hemolysin was added to the specimens and incubated in the dark at room temperature for 15 min, and the levels of CD4⁺ T lymphocytes were detected using the aforementioned software. The results were retrieved and automatically analyzed by MultiSET™ software.

Detection of T-lymphocyte differentiation. Active lymphocytes were isolated from T lymphocytes in subjects, and T lymphocytes and CD3⁺CD8 IFN-γ+(Th1)/CD3⁺CD8 IL-4+(Th2) cells were isolated and analyzed by FCM. At the same time, the ratio of Th1 to Th2 was measured by FCM, and the effects of HIV on human T-lymphocyte differentiation were observed.

| HIV viral load (copies/ml) | Percentage (n, %) |
|---------------------------|-------------------|
| >10⁶                      | 39/150 (26.00)    |
| 10⁵-10⁶                  | 76/150 (50.67)    |
| <10⁵                     | 35/150 (23.33)    |

Detection of metabolites of T lymphocytes cultured in vitro and analysis of their biological pathways. The metabolites of T lymphocyte lysates were analyzed using proton nuclear magnetic resonance. The differences in T-lymphocyte metabolites among groups were analyzed, and the biological pathways were then analyzed.

Statistical analysis. The data in this study were all analyzed using SPSS 20.0 (IBM Corp., Armonk, NY, USA) software. The measurement data were analyzed by analysis of variance. The data results were expressed as mean ± standard deviation, and verified using a t-test and Chi-square test. The Mann-Whitney U non-parametric test was performed for variables. The grouping variable was ‘group’ and test variable was ‘chemical shift value (shift)’. P<0.05 was considered to indicate a statistically significant difference.

Results

Detection of HIV viral load. Results of the detection on the HIV viral load of AIDS patients showed that the highest HIV viral load was 3.8x10⁶ copies/ml and the lowest was 6.2x10⁵ copies/ml in 150 patients. Of these, 39 subjects had >10⁶ copies/ml HIV viral load, 76 patients had 10⁵-10⁶ copies/ml HIV viral load and 35 subjects had <10⁵ copies/ml HIV viral load, accounting for 26.00, 50.67 and 23.33%, respectively (Table I).

Association between HIV viral load of AIDS patients and CD4⁺ T lymphocyte counts. CD4⁺ T lymphocyte counts of subjects in group A were <50 cells/µl, accounting for 71.79% of the total number of group A; CD4⁺ T lymphocyte counts of subjects in group B were mainly ≤400 cells/µl. In group C, 23 subjects had 200-399 cells/µl CD4⁺ T lymphocyte counts, accounting for 65.71% of the total subjects in group C (Table II).

Association between HIV viral load and T lymphocyte differentiation. HIV viral loads of AIDS patients differentiated T lymphocytes. In other words, high HIV viral loads accelerated the differentiation of T lymphocytes to Th1 cells (Table III).

Detection of metabolites of human T lymphocytes cultured in vitro. The Mann-Whitney U non-parametric test was performed for the variables. The test results showed that different HIV viral loads exerted significant effects on the metabolites of T lymphocytes. There were a total of 11 chemical shift values representing significant changes in metabolites (Table IV).

We searched the Human Metabolome Database and selected endogenous metabolites with high matching degree.
Table II. Association between the HIV viral load of AIDS patients and CD4⁺T lymphocyte counts.

| Groups | CD4⁺T lymphocyte counts (cells/µl) |
|--------|----------------------------------|
|        | <50  | 50-199 | 200-399 | ≥400 | χ² value | P-value |
| A (n, %) | 28/39 (71.79) | 4/39 (10.26) | 5/39 (12.82) | 2/39 (5.13) | 10.764 | 0.001 |
| B (n, %) | 20/76 (26.32) | 28/76 (36.84) | 20/76 (26.32) | 8/76 (10.53) | 6.979 | 0.004 |
| C (n, %) | 0/35 (0.00) | 12/35 (34.29) | 23/35 (65.71) | 0/35 (0.00) | 12.582 | <0.01 |
| χ² value | 11.524 | 8.221 | 9.275 | 13.124 | - | - |
| P-value | <0.01 | 0.001 | 0.007 | 0.001 | - | - |

Table III. Association between HIV viral load and T lymphocyte differentiation (mean ± SD).

| Groups | T-lymphocyte differentiation (Th1/Th2) |
|--------|----------------------------------|
| A (n=39) | 35.28±4.37 |
| B (n=76) | 26.45±2.91 |
| C (n=35) | 18.75±2.37 |
| F-value | 15.97 |
| P-value | 0.003 |

in the database. After checking the displacement variables, we found that most metabolites were intermediates metabolized by lipids, end proteins, which were closely related to organic acid, amino acid, purine pyrimidine, lipid metabolism and other metabolic pathways. The detection results showed that glycerol phosphocholine diesterase, 7-dehydrocholesterol, p-hydroxyphenylacetic acid, cholesterol and deoxyuridine levels were increased in the high HIV viral load group, whereas 3-methoxytyramine, cytidine deaminase, deoxycorticosterone and 3-hydroxybutyric acid were reduced (Table V).

Discussion

HIV-1 AIDS is a kind of cellular immunodeficiency disease. Its main feature is that after T lymphocytes are damaged and HIV-1 enters the body, the disease does not immediately develop into AIDS, but goes through an asymptomatic period (9,10). The current international assessment determines the progression of AIDS mainly based on two indicators, that is, HIV-1 viral loads in the peripheral blood and CD4⁺ T lymphocyte counts (11). Foreign scholars have found that the expression levels of CD4⁺ T lymphocytes in AIDS patients are closely related to their HIV-1 virus loads. HIV-1 can directly attack CD4⁺ T lymphocytes, causing the body's immune dysfunction and different degrees of damage to other immune cells. Another study has suggested that, CD4⁺ T lymphocyte count levels can accurately reflect the immune status of the body, and the exacerbation of HIV-1 AIDS patients can be reflected by largely reduced expression levels of CD4⁺ T lymphocytes in the short term (12,13).

In the present study, the detection results of the viral load of AIDS patients showed that the highest viral load in the 150 patients was 3.8x10⁷ copies/ml while the lowest was 6.2x10⁵ copies/ml, in which 39 subjects had more than 10⁶ copies/ml HIV viral load, 76 patients had 10³-10⁶ copies/ml HIV viral load and 35 subjects had less than 10⁵ copies/ml HIV viral load, accounting for 26.00, 50.67 and 23.33%, respectively. Results of the analysis of the relationship between viral loads and CD4⁺ T lymphocyte counts in AIDS patients showed that CD4⁺ T lymphocyte counts of subjects in group A were less than 50 cells/µl, accounting for 71.79% of the total number of group A; while CD4⁺ T lymphocyte counts of subjects in group B were mainly no more than 400 cells/µl. In group C, 23 subjects had 200-399 cells/µl CD4⁺ T lymphocyte counts, accounting for 65.71% of the total subjects in group C. It could be inferred that the higher the viral load was, the lower was the CD4⁺ T lymphocyte count. The occurrence mechanism may include the following six aspects: i) HIV infection may directly kill CD4⁺ T lymphocytes; ii) HIV DNAs not integrated in the cytoplasm could infect CD4⁺ T lymphocytes; iii) intracellularly replicated HIV may cause interference with the synthesis of proteins within the cells; iv) CD4⁺ T lymphocytes were damaged by specific cytotoxicity of cytotoxic T lymphocytes; v) Gp41 and gp120-mediated cell fusion cause accelerated infection of CD4⁺ T lymphocytes and cause death; and vi) immune dysfunction existed in patients prior to a sharp decrease in the expression levels of CD4⁺ T lymphocytes (14-16). The HIV viral load reflects the number of replicated HIVs within the body, and the number of CD4⁺ T lymphocytes represents the number of HIV-infected target cells, thus both can be used independently for the prediction of disease development and prognosis of HIV-1 AIDS patients (17,18). CD4⁺ T lymphocyte counts indicate different immune levels of the human body. A low or reduced CD4⁺ T lymphocyte count level has a parallel relationship with disease progression, which is a useful indicator for following disease development and is closely related to opportunistic infection and patient survival time. Thus, the more the number of replicated HIVs is, the more the number of CD4⁺ T lymphocytes are and the less the number of residual CD4⁺ T lymphocytes.

Changes in metabolic quantities reflect metabolic trends in major biochemicals, suggesting metabolic status and metabolic cell regulation, including energy, nucleotide and amino acid metabolism (19,20). From the present study, it was found that the differences between the different HIV viral load groups were significant, including differences in cholesterol, adenosine and deoxyinosine (dl). Adenosine and dl are the compounds of adenine metabolism. dl is the metabolite of deoxyadenosine (dA) after it has been hydrolyzed by adenosine deaminase for
Adenosine belongs to the intracellular endogenous nucleoside, which can participate in the synthesis of adenylate, adenine and adenosine triphosphate, which are involved in cell energy metabolism. Adenosine and dA can lead to the accumulation of dA triphosphate (dATP), thus inhibiting the nucleotide reductase and eventually leading to lymphopenia and causing immune deficiency. The over-accumulation of dATP can inactivate adenosine homocysteine hydrolase, which affects involvement of the enzyme in the decomposition of adenosine homocysteine, and ultimately inhibits the physiological action of adenosylmethionine-dependent trans methylation and interferes with methylation-dependent processes. dA can inhibit adenosine homocysteine hydrolase, which induces the accumulation of adenosine homocysteine deaminase (21,22). dA is closely associated with lymphocyte toxicity and dATP accumulation as well as adenosine homocysteine hydrolase inactivation. Adenosine deaminase has a detoxification effect on adenosine and dA. Adenosine and dA can stimulate the adenylate cyclase system, upregulate adenylate deaminase activity, and ultimately accelerate the metabolism of T-lymphocyte nucleotides (25,26). The present study indicated that HIV viral load affected adenosine deaminase to catalyze the deamination of adenosine and dA, reduced their toxicity to lymphocytes, and then affected T lymphocyte-mediated immune responses (25,26).

**Table IV. Displacement of metabolites of T lymphocyte cell lysates.**

| Shift (ppm) | z   | P-value | Correlation coefficient | Coefficient |
|------------|-----|---------|-------------------------|-------------|
| 0.03       | -2.265 | 0.009   | -                       | -2.87214    |
| 0.67       | -2.887 | 0.002   | +                       | 8.10393     |
| 1.35       | -2.887 | 0.002   | -                       | -29.7471    |
| 1.39       | 2.727  | 0.004   | -                       | -2.02653    |
| 3.39       | 2.887  | 0.002   | -                       | -3.03678    |
| 3.63$^a$   | -2.727 | 0.004   | +                       | 18.4743     |
| 3.67       | -2.727 | 0.004   | +                       | 15.6276     |
| 3.72       | -2.082 | 0.041   | +                       | 8.52371     |
| 3.35       | -2.882 | 0.002   | -                       | -1.08921    |
| 3.43       | -2.722 | 0.004   | +                       | 0.434467    |
| 3.47       | -2.882 | 0.002   | +                       | 0.50965     |
| 3.51       | -2.882 | 0.002   | +                       | 0.476585    |
| 3.59       | -2.882 | 0.002   | +                       | 1.40816     |
| 4.12       | -2.882 | 0.002   | -                       | -1.493351   |
| 4.16       | -2.882 | 0.002   | -                       | -1.35287    |

$^a$+, upregulation; - -, downregulation.

**Table V. Metabolites of T lymphocyte cell lysates and relevant metabolic pathways.**

| Shift (ppm) | Endogenous | Possible endogenous metabolites | Pathway                                      |
|------------|------------|--------------------------------|----------------------------------------------|
| 0.03       | Adenine    | Adenine                        | Purine metabolic pathway                     |
| 0.67       | Cholesterol| Cholesterol                    | Bile acid metabolic pathway                  |
| 1.35/1.39  | Deoxycorticosterone| Deoxycorticosterone              | Steroid metabolic pathway                    |
| 3.39       | L-carnitine| L-carnitine                    | Long-chain fatty acid β-oxidation metabolic pathway |
| 3.63/3.67  | 7-Dehydrocholesterol| 7-Dehydrocholesterol           | Steroid metabolic pathway                    |
| 3.72       | Deoxyuridine| Deoxyuridine                   | Pyrimidine metabolic pathway                 |
| 3.35       | 3-Methoxytyramine| 3-Methoxytyramine              | Tyrosine metabolic pathway                   |
| 3.43/3.47/3.51 | P-hydroxyphenylacetic acid | P-hydroxyphenylacetic acid | Tyrosine metabolic pathway                   |
| 3.59       | Glycerophosphocholine| Glycerophosphocholine          | Glycerophosphoric acid metabolic pathway     |
| 4.12       | Cytidine   | Cytidine                       | Pyrimidine metabolic pathway                 |
| 4.16       | (R)-3-hydroxybutyric acid| (R)-3-hydroxybutyric acid    | Ketone body metabolic pathway                |
physiological function of T lymphocytes (27,28). The results suggested that HIV viral loads may regulate the lipid biosynthesis of T lymphocyte membranes and affect the proliferation of T lymphocytes, thus interfering with its immune processes. In summary, the viral loads of AIDS patients are associated with CD4+ T lymphocyte count and the ratio of CD4+/CD8+. At the same time, HIV viral loads can affect the lipid biosynthesis of T lymphocyte membranes, thus affecting the differentiation and proliferation of T lymphocytes and finally interfering with its mediated immune responses. Therefore, the levels of CD4+ T lymphocytes and viral loads of AIDS patients should be regularly measured in order to begin antiviral therapies as early as possible, thus reducing the incidence and death rates of AIDS.

Competing interests

The authors declare that they have no competing interests.

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