Expansion of circulating T follicular helper cells is associated with disease progression in HIV-infected individuals

Jianhua Lu [a,1], Ying Lv [c,1], Zhuo Lv [b], Yi Xu [a], Yan Huang [c], Meilan Cui [c], Huimin Yan [c,*]

[a] Department of Laboratory Medicine, Shijiazhuang Fifth Hospital, Shijiazhuang, Hebei 050021, China
[b] Graduate College of Hebei Medical University, Hebei Medical University, Shijiazhuang, Hebei 050017, China
[c] Clinical Research Center, Shijiazhuang Fifth Hospital, Shijiazhuang, Hebei 050021, China

ABSTRACT

Background: T follicular helper (Tfh) cells within germinal centers (GC) of lymphoid tissue play an important role in HIV infection. Recently, circulating Tfh cells have been described, which share phenotypic and functional characteristics with GC Tfh cells. This study aimed to investigate the effect of HIV infection on four circulating Tfh subsets, including CD4+CXCR5+, CD4+CXCR5+ICOS+, CD4+CXCR5+PD-1+, and CD4+CXCR5+ICOS+PD-1+ cells.
Patients and methods: Peripheral blood samples were collected from 33 HIV-infected individuals and 21 healthy controls. The frequency and absolute number of CD3, CD4 and CD8 cells were detected by flow cytometry. The frequency of circulating Tfh cell subsets was also determined by flow cytometry. The correlation between the frequency of Tfh subsets and CD4 T cells counts was assessed by Pearson correlation analysis.
Results: There was no significant difference in the frequency of peripheral CD4+CXCR5+ Tfh cells between HIV-infected individuals and healthy controls. However, the percentages of circulating CD4+CXCR5+ICOS+, CD4+CXCR5+PD-1+, and CD4+CXCR5+ICOS+PD-1+ Tfh cells were significantly higher in individuals with HIV infection than those of healthy controls. Furthermore, the percentage of CD4+CXCR5+PD-1+ Tfh cells showed negative correlation with CD4 T cell counts in HIV-infected individuals.
Conclusion: Our results suggested the potential involvement of circulating CD4+CXCR5+PD-1+ Tfh cells during the development of HIV infection.

Introduction

Infection with human immunodeficiency virus (HIV) remains one of the major public health problems, affecting about 40 million people worldwide. During HIV infection, a progressive depletion of CD4 T lymphocytes is the major immunological manifestation, which eventually results in severe deficiency of immune competence and consequent development of acquired immunodeficiency syndrome (AIDS) [1]. The predilection sites of HIV replication and latency are lymphoid tissue compartments [2], and CD4 T cells in germinal centers (GC) of lymphoid tissues are highly susceptible to HIV [3].

Among the cells that reside within the GC, T follicular helper (Tfh) cells have been described to be the key cell type required for the formation of GC [4]. Tfh cells have a central memory phenotype and express CCR5, suggesting their potential involvement in the pathological processes of HIV infection [5]. Recent studies have revealed that infected GC Tfh cells within lymph nodes constitute a major HIV reservoir [6,7]. GC Tfh cells have also been shown to be increased in HIV-1-infected subjects and the frequency correlates with plasma viremia [8,9].

Although several studies have confirmed the abnormal frequency of GC Tfh cells, it is relative difficult to better understand Tfh cell function because of the relative inaccessibility and paucity of lymph node samples. Recently, a memory Tfh-like population of CD4+CXCR5+ T cells has been identified in human peripheral blood. These cells share functional properties with GC Tfh cells [10,11]. However, different from GC Tfh cells, circulating Tfh cells highly express CCR7 and CD62L, but lowly express activation molecule,

such as ICOS and CD69, and meanwhile Bcl-6 protein expression is absent [12]. These findings suggest that there are phenotypical and functional distinctions between circulating Tfh and GC Tfh cells. Up to the present, studies regarding the role of circulation Tfh cells in HIV infection are limited. In the present study, we explored the frequency of Tfh subsets, including CD4+CXCR5+ (CXCR5+ Tfh), CD4+CXCR5+ICOS+ (ICOS+ Tfh), CD4+CXCR5+PD-1+ (PD-1+ Tfh) and CD4+CXCR5+ICOS+PD-1+ (ICOS+PD-1+ Tfh) cells in peripheral blood from naive-treated HIV-infected individuals, and evaluated the potential involvement of these cells during the development of HIV infection.

Patients and methods

Patients

A total of 33 HIV-infected individuals were recruited at the Shijiazhuang Fifth Hospital from March 2015 to February 2016. HIV infection status was defined as testing positive for an HIV-1 on ELISA and Western blot analysis. Individuals on highly active antiretroviral therapy, pregnant women, and pediatric age group were excluded. The healthy controls were recruited from participants of regular physical check-ups, who were negative for HIV, had normal liver function and without any history of chronic disease. The study protocol was approved by the ethics committee of Shijiazhuang Fifth Hospital. The methods used in relation with humans were carried out in accordance with the approved guidelines.

Sample collection

Blood samples from peripheral vein were collected into vacutainer blood collection tubes coated with ethylenediamine tetraacetic acid dipotassium salt. All samples were processed within 6 h after collection.

Detection of frequency and absolute number of CD3, CD4 and CD8 cells by flow cytometry

A single-platform lyse/no-wash procedure was performed using Trucount tubes and TriTEST CD4-FITC/CD8-PE/CD3-PerCP reagents (BD Biosciences). Briefly, 50 μL of whole blood samples was incubated with 10 μL of monoclonal antibodies conjugated with fluorochromes at room temperature. After 15 min, FACS lysing solution was added, and all samples were analyzed using a FACS Canto^{II} flow cytometer (Beckton Dickinson) with FACS Diva software.

Detection of frequency of Tfh cells by flow cytometry

Samples were treated with red blood cell lysing buffer and washed twice with 5% fetal bovine serum in phosphate-buffered saline. Cells were incubated with the following antibodies: CD4-FITC, PD-1-APC, ICOS-PE (all from BioLegend), and CXCR5-PE/Cy7 (eBioscience) for 15 min. Appropriate isotype controls were used for nonspecific staining. Cells were washed twice and analyzed using FACS Canto^{II} flow cytometer with FACS Diva software.

Statistical analysis

All statistical analyses were performed by SPSS 19.0 software (SPSS Inc, Chicago, USA). Data were expressed as the mean ± SD. Comparison of differences was carried out using Student's t test, Mann–Whitney U test or Kruskal Wallis H test. Pearson correlation tests were performed for correlation analysis. A two-sided P value of <0.05 was considered to be significant.

Table 1
Demographic and clinical characteristics of study subjects.

		HIV+ (n = 33)	HIV− (n = 21)
Gender (male/female; n)		32/1	15/6
Age (years; mean ± SD)		32.36 ± 11.84	37.90 ± 10.94
WBC counts (10⁹ cells/L)		5.51 ± 1.29	6.08 ± 1.61
CD4 T cell	counts (cells/μL)	289.84 ± 118.25	686.58 ± 174.64
	percentage (%)	14.96 ± 5.60	32.44 ± 5.42
CD8 T cell	counts (cells/μL)	1010.53 ± 371.53	602.52 ± 185.78
	percentage (%)	51.07 ± 9.72	28.55 ± 6.74

Results

Clinical characteristics of study populations

The demographic and clinical characteristics of the 33 HIV-infected subjects and 21 healthy controls were summarized in Table 1. Of the 33 enrolled infected individuals, 32 (97.0%) were males and 1 (3.0%) were females, with mean ages of (32.36 ± 11.84) years. Among healthy controls, 15 (71.4%) were males and 6 (28.6%) were females, with mean ages of (37.90 ± 10.94) years.

The change of CD3, CD4 and CD8 T cells in the peripheral blood of HIV-infected individuals

As expected, neither the percentage nor absolute number of CD3+ T cells was different between HIV-infected individuals and healthy controls (Fig. 1A). However, when compared to healthy controls, the percentage and absolute number of CD4+ cells were significantly decreased, whereas those of CD8+ T cells were significantly increased in the HIV-infected individuals (Fig. 1B and 1C). HIV-infected individuals had significantly lower CD4/CD8 ratios than healthy controls (Fig. 1D).

The change of Tfh subsets in the peripheral blood of HIV-infected individuals

Since CD4+CXCR5+ T cells are initially identified as Tfh cells, the frequency of CXCR5+ Tfh cells among CD4+ T cells was firstly analyzed. Furthermore, three subpopulations were differentiated by ICOS and PD-1 expression, including ICOS+ Tfh, PD-1+ Tfh, and ICOS+PD-1+ Tfh cells, and the frequencies were investigated. Representative flow cytometric results were shown in Fig. 2. The result showed that there was no significant difference in the percentage of CD4+CXCR5+ cells between HIV-infected individuals and healthy controls. However, the percentages of other three subsets in HIV-infected individuals were all significantly increased than those in healthy controls (Fig. 3).

Association between the percentage of Tfh cells and CD4 T cell counts in HIV-infected individuals

Since Tfh subsets were dysregulated in HIV-infected individuals, this further drove us to study whether these Tfh subsets are associated with the progression of HIV infection. The HIV-infected subjects were classified into three groups according to the absolute number of CD4 T cells. The results showed that the percentages of all phenotypically defined Tfh subsets tended to be highest in subjects with CD4 T cell counts less than 200 cells/μL and lowest in subjects with CD4 T cell counts greater than 350 cells/μL, although there was no significant statistically difference among three groups. Pearson correlation analysis revealed that only the percentage of PD-1+ Tfh subsets was negatively correlated with CD4 T cell counts, suggesting a close association between the expression of PD-1 on Tfh cells and HIV-infected progression (Fig. 4).