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Research paper

Alteration of CCR6$^+$CD95$^+$CD4$^+$ naïve T cells in HIV-1 infected patients: Implication for clinical practice

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ABSTRACT

The profound deficiency of Th17 cells contributes to HIV disease progression. The mechanisms of their perturbation remain unclear. Recently, CCR6$^+$CD95$^+$CD4$^+$ naïve T cells (CCR6$^+$CD95$^+$CD4$^+$ T_{NA}), identified as pre-committed Th17 precursors, were recognized as a subpopulation of CD4$^+$ T cells with stem cell properties. Following phenotypical identification, we evaluated their level in patients during chronic HIV infection and following antiretroviral therapy (ART) using flow cytometry. The levels of CCR6$^+$CD95$^+$CD4$^+$ T_{NA} were decreased during chronic HIV infection and correlated with CD4$^+$ T cell counts. Immunological responders harbored higher frequency of CCR6$^+$CD95$^+$CD4$^+$ T_{NA}, which was associated with CD4/CD8 T cell ratio. Immunological non-responders with lower frequency of CCR6$^+$CD95$^+$CD4$^+$ T_{NA} failed to exhibit a correlation between CCR6$^+$CD95$^+$CD4$^+$ T_{NA} and CCR6$^+$CD95$^+$CD4$^+$ T_{CM}, and displayed elevated ratio of CCR6$^+$CD95$^+$CD4$^+$ T_{CM}/T_{NA}. The number of CCR6$^+$CD95$^+$CD4$^+$ T_{NA} was increased following early ART. These findings shed light on the importance of targeting pre-committed Th17 precursors that enhance immune reconstitution.

1. Introduction

HIV infection is accompanied by depletion of CD4$^+$ T cells with destruction of mucosal integrity. The deficiencies in Th17 cells are potential causes of mucosal dysfunction, microbial translocation, and persistent immune activation, contributing to HIV disease progression [1,2]. Th17 cells, expressing chemokine receptor 6 (CCR6), can migrate to mucosa tissue and enhance mucosal defense by secreting interleukin 17 (IL-17) [3]. During HIV/SIV infection, Th17-polarized CCR6$^+$CD4$^+$ T cells are preferentially infected and disturbed, either in blood or tissues, including gastrointestine and reproductive tract [4–7]. The transcriptional characteristics of Th17 cells may facilitate the susceptibility to HIV [8]. Moreover, Th17-polarized CCR6$^+$CD4$^+$ T cells containing heterogenous subpopulations contribute to HIV persistence despite suppressive ART as evidenced by our study [9] as well as several others [10–12]. The mechanisms underlying Th17 defects in HIV infection are needed to be investigated.

Stem cell memory T cells (T_{SCM}), recognized as a novel T cell subset with multipotential and self-renewal properties [13], display the phenotype of naïve T cells and express markers of memory cells such as CD95 and IL-2RB. Preserved levels of CD4$^+$ T_{SCM} with decreased HIV infection in these cells are related to maintained CD4$^+$ T cell counts in HIV-infected patients despite persistently high viral loads, implying a critical role of T_{SCM} in lack of HIV disease progression [14–16]. Recent studies have reported that CCR6$^+$CD95$^+$CD4$^+$ naïve T cells (CCR6$^+$CD95$^+$CD4$^+$ T_{NA}), as part of the CD4$^+$ T_{SCM} compartment, exclusively give rise to IL-17-producing CD4$^+$ T cells *ex vivo* in the presence or absence of Th17-inducible cytokines [17–19]. The findings highlight the role of CCR6$^+$CD95$^+$CD4$^+$ T_{NA} as Th17 precursors in functionating in human immunity. However, whether and how CCR6$^+$CD95$^+$CD4$^+$ T_{NA} change during HIV infection has not been fully elucidated.

In this study, we assessed CCR6$^+$CD95$^+$CD4$^+$ T_{NA} in HIV infection and the impact of ART on these cells. Resembling the markers of T_{SCM}, CCR6$^+$CD95$^+$CD4$^+$ T_{NA} were diminished and correlated with the loss of CD4$^+$ T cells in antiretroviral therapy (ART)-naïve chronic patients. The level of CCR6$^+$CD95$^+$CD4$^+$ T_{NA} was not restored following ART and the sustained low level of CCR6$^+$CD95$^+$CD4$^+$ T_{NA} was associated

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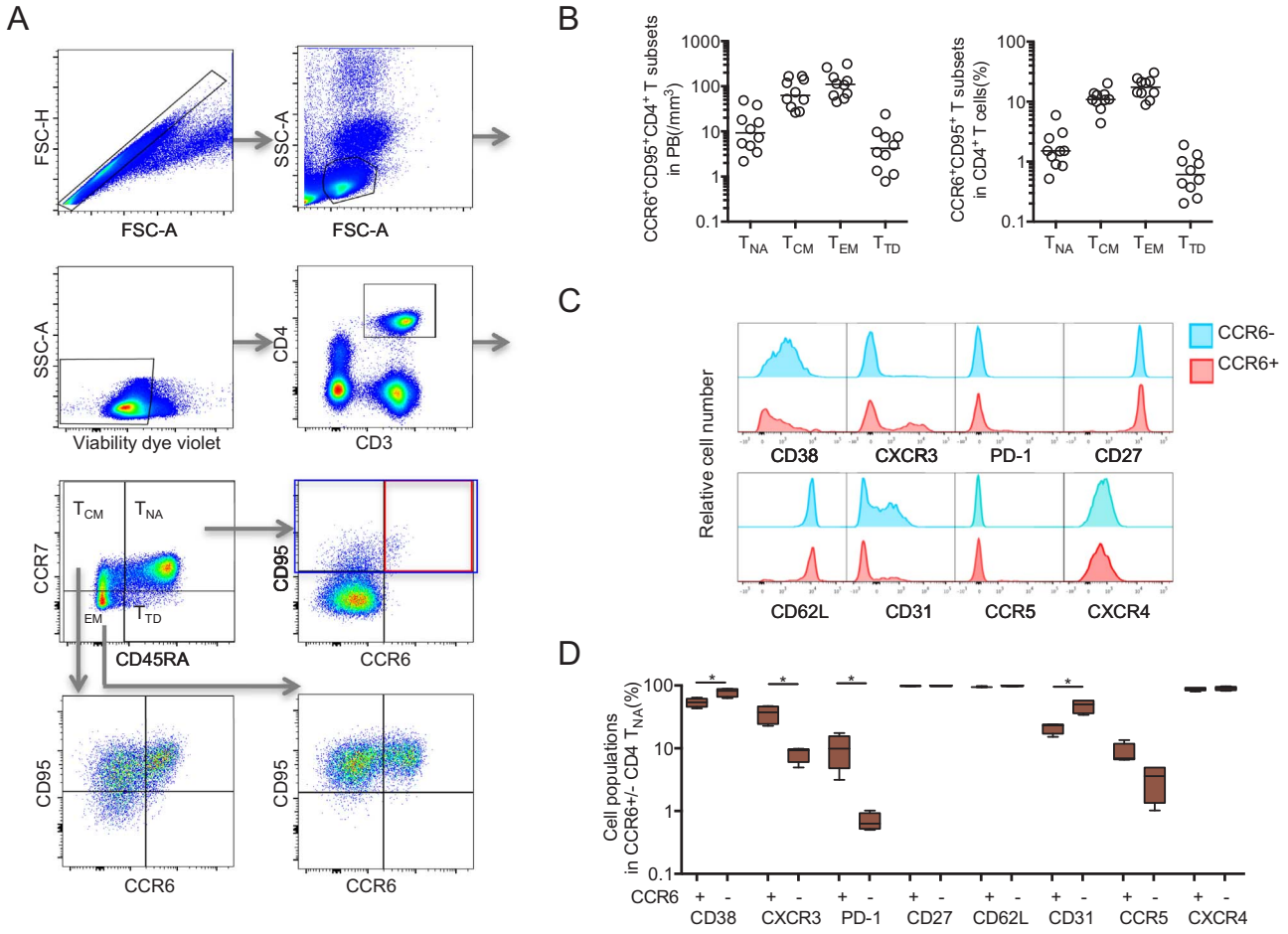

Fig. 1. Flowcytometric gating and phenotypic characterization of $CCR6^+CD95^+CD4^+$ T_{NA} in PB. (A) Representative flow cytometric gating of $CCR6^+CD95^+CD4^+$ T_{NA} from a cohort donor. Red square indicates $CCR6^+CD95^+CD4^+$ T_{NA}. Blue square indicates broad-sense $CD4^+$ T_{SCM}. (B) Comparison of absolute number and percentage of $CCR6^+CD95^+$ cells among different $CD4^+$ T subsets. (C) Flow cytometric analysis of phenotypic markers including CD27, CD62L, CD31, CD38, CXCR3, PD-1, CCR5, and CXCR4 (n = 3–5) expression of $CCR6^+CD4^+$ T_{NA} compared with $CCR6^-CD4^+$ T_{NA} from healthy donors. Data were presented as the median with range using box-and-whisker plot graphs (*, $p < 0.05$).

with poor $CD4^+$ T cell recovery in chronic ART-treated patients. Longitudinal observation demonstrated that the level of $CCR6^+CD95^+CD4^+$ T_{NA} was elevated obviously along with the decrease of $CCR6^+$ T cell activation in early HIV-1 infected patients since initiation of ART.

2. Materials and methods

2.1. Study subjects

Nineteen ART-naïve HIV-infected patients (HIV + ART−), 26 chronic ART-treated (HIV + ART+) HIV-infected patients, and 10 HIV-negative donors (HIV−) were recruited in the cross-sectional cohort. In the longitudinal cohort of early treatment, ten HIV-1 patients treated during early infection stage (infection time < 1 year) were enrolled and followed up for around 2 years, and serial samples were collected and frozen at baseline, 48 weeks and 96 weeks since initiation of treatment. All participants were from First Hospital of China Medical University. Ethical approval was obtained from the Institutional Review Board and patients provided written informed consent. The chronic ART-treated patients had received at least 2 years of successful treatment (HIV RNA < 20 copies/ml). As previously described [20–22], HIV immunological responders (IRs, n = 14) were defined as patients under ART with $CD4^+$ T cell counts above 350 cells/mm^3, while $CD4^+$ T cell counts lower than 350 cells/mm^3 defined HIV immunologic non-responders (INRs, n = 12).

2.2. Measurement of $CD4^+$ T cell counts and plasma HIV RNA

$CD4^+$ T cell counts were measured with whole blood and performed using a FACSCalibur Flow Cytometer (BD Biosciences, NJ). The standard lyse/no-wash procedure was applied using anti-CD4-FITC/CD8-PE/CD3-PerCP reagents and TruCOUNT tubes (BD Biosciences, NJ). TruCOUNT Control Beads were used for the quality control.

A Roche Amplicor Monitor Standard Assay (COBAS AmpliPrep/COBAS Taqman HIV Test, Roche, Switzerland) was used to detect plasma HIV RNA. The value was calculated according to the manufacturer's reference standards. The lowest detection limit was 20 copies per milliliter.

2.3. Flow cytometric analysis

Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation for immunofluorescence staining. The following mouse antihuman antibodies (Biolegend) were used in different combinations: PerCP/Cy5.5 anti-CD3 (clone HIT3a), APC/Cy7 anti-CD4 (RPA-T4), FITC anti-CD45RA (HI100), APC anti-CCR7 (G043H7), PE/Cy7 anti-CCR6 (G034E3), and PE anti-CD95 (DX2). PE anti-CD38 (HB7), APC anti-HLA-DR (L243), PE anti-CD27 (O323), FITC anti-CD62L (DREG-56), PE anti-CXCR3 (G025H7), PE anti-PD-1 (EH12.2H7), PE anti-CCR5 (J418F1) and PE anti-CXCR4 (12G5) were also employed where applicable. A Live/Dead Violet Stain kit (L34964, Life technologies) was used to gate live cells. After staining with antibodies for 30 min at 4 °C, cells were washed, and more than 3×10^5