A Human Immunodeficiency Virus Controller With a Large Population of $CD4^+CD8^+$ Double-Positive T Cells

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Human immunodeficiency virus (HIV) controllers are patients who control viral replication without antiretroviral therapy. We present the case of an HIV controller who had CD4 and CD8 coexpressed on 40% of his T cells. Although a recent study found that double-positive T cells had superior antiviral capacity in HIV-1 controllers, in this case, the $CD4^+CD8^+$ T cells did not have strong antiviral activity.

Keywords. AIDS; double-positive cells; elite controllers; HIV; HIV controllers; immune activation; long-term nonprogressors; viremic controllers.

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CASE PRESENTATION

The patient is a 62-year-old African American male with human immunodeficiency virus (HIV) infection diagnosed 12 years ago. His CD4 count was 711 cells/μL, and his viral load was 141 copies/mL plasma without antiretroviral therapy (ART) at diagnosis. His viral load remained undetectable to low (<400 copies/mL), and although his absolute CD4 count was very high, his $CD4^+$ T cell percentage was low and his $CD8^+$ T cell percentage was elevated (Figure 1A). At year 3, it was noted that 26% of lymphocytes coexpressed CD4 and CD8 and the proportion eventually peaked at 40%. These cells were not reported before year 3, but the aggregate percentage of $CD4^+$ and $CD8^+$ T cells exceeded 100%, suggesting that $CD4^+CD8^+$ cells had always been present. A malignancy workup included a polymerase chain reaction (PCR) for clonal T cell rearrangement (negative), fluorescent in situ hybridization for B-cell chronic lymphocytic leukemia (negative), and cytogenetics (normal chromosomes). Human T-lymphotropic virus type-1 serology was negative.

Flow cytometry showed that the density of CD4 was lower on $CD4^+CD8^+$ T cells than on $CD4^+$ T cells (Figure 1B). Because HIV infection results in down-regulation of CD4, we measured the frequency of HIV infection with semiquantitative PCR. Infection was detected in 0.01% of $CD4^+$ T cells but <0.001% of $CD4^+CD8^+$ cells (Table 1); therefore, double-positive cells were not a major infection target. Consistent with this finding, expression of CCR5 and CXCR4 was lower in $CD4^+CD8^+$ T cells than in $CD4^+$ T cells, and there was less viral entry of $CD4^+CD8^+$ T cells than $CD4^+$ T cells after spinoculation with CCR5-tropic and CXCR4-tropic viruses (Table 1).

A much larger fraction of $CD8^+$ and $CD4^+CD8^+$ T cells was activated compared with $CD4^+$ T cells as measured by HLA-$DR/CD38^+$ expression (Table 1). The percentage of activated $CD8^+$ T cells was comparable to that seen in untreated individuals and higher than that in treated patients, HIV controllers, and uninfected individuals (Figure 1C).

$CD4^+CD8^+$ and $CD8^+$ T cell fractions contained high levels of effector memory cells (Table 1) that exceeded the percentage seen in healthy donor and HIV-infected subjects (Figure 1D). The $CD4^+CD8^+$ T cells, $CD4^+$ T cells and $CD8^+$ T cell produced comparable levels of cytokines in response to polyclonal stimulation (Table 1), but the pattern of the cytokine production in $CD4^+CD8^+$ T cells was more similar to $CD8^+$ T cells than $CD4^+$ T cells (Figure 1E). To determine whether the cells were HIV-1 specific, they were stimulated with HIV-1 antigens. A similar

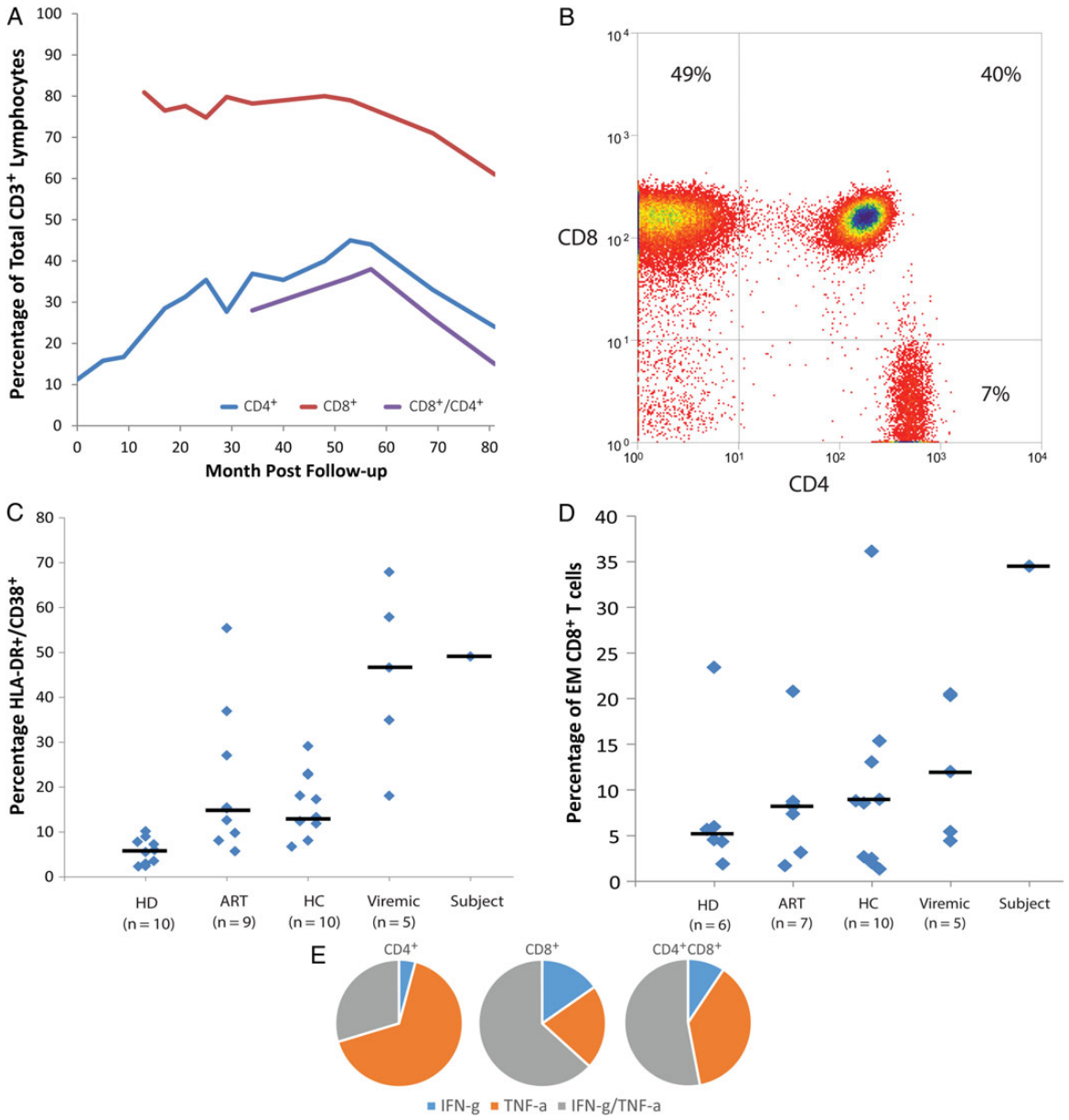

Figure 1. Percentage of different T cell populations over time in the subject (A). Flow cytometry showing the large population of $CD4^+CD8^+$ T cells (B). Comparison of activated (C) and effector memory (EM; D) $CD8^+$ T cells in the subject compared with healthy donors (HD), untreated viremic patients, patients on antiretroviral therapy (ART), and human immunodeficiency virus controllers (HC). The percentage of $CD4^+CD8^+$ T cells was too low in the other patients for a meaningful comparison to be performed. Percentage of cells that expressed interferon-γ or tumor necrosis factor-α alone or in combination after stimulation with anti-CD3 and anti-CD28 monoclonal antibodies.

proportion of all 3 cell populations expressed TNF-α in response to stimulation with Gag peptides (Table 1). In contrast to a prior study in which $CD4^+CD8^+$ T cells were often multifunctional [2], <0.1% of $CD4^+$, $CD8^+$, and $CD4^+CD8^+$ simultaneously expressed TNF-α, IFN-γ, and IL-2 (Table 1). Furthermore, although $CD8^+$ T cells had a modest inhibitory effect on viral replication, $CD4^+CD8^+$ T had no detectable effect (Table 1).

CONCLUSIONS

We present the case of an HIV controller with a very large, persistent population of $CD4^+CD8^+$ T cells (15%–40%). There is a prior report of an HIV-infected individual with progressive disease who had elevated $CD4^+CD8^+$ T cells (approximately 7.5%) over 8 years [3]. In that case, $CD4^+CD8^+$ T cells had a low CD8 density and were phenotypically similar to $CD4^+$ cells, whereas in

Table 1. Features of T Cell Subsets

	$CD4^+$ T Cells	$CD8^+$ T Cells	$CD4^+CD8^+$ T Cells
T cell phenotype			
Activated ($CD38^+$/HLA-DR^+)	8%	49%	53%
Naive ($CCR7^+$/$CD45RA^+$)	9%	2%	1%
Terminal Effector ($CCR7^-$/$CD45RA^+$)	0%	18%	2%
Central memory ($CCR7^+$/$CD45RA^-$)	31%	2%	1%
Effector memory ($CCR7^-$/$CD45RA^-$)	18%	35%	55%
HIV-1 susceptibility			
% infected cells (PCR analysis)	0.01%	NA	<0.001%
CCR5 positive	35%	25%	15%
CXCR4 positive	88%	59%	76%
% CCR5-tropic virus entry	27%	0.3%	6%
% CXCR4-tropic virus entry	20%	0.4%	7%
Polyclonal T cell responses			
IFN-γ and/or TNF-α expression: Unstimulated cells	0%	0.5%	0.4%
IFN-γ and/or TNF-α expression: PHA-stimulated cells	11.9%	30.4%	44.8%
IFN-γ and/or TNF-α expression: CD3/CD28-stimulated cells	25.9%	40.9%	39.1%
IFN-γ and/or TNF-α expression: PMA/ionomycin-stimulated cells	72.5%	78.3%	69.4%
HIV-1-specific responses			
TNF-α expression: Unstimulated cells	0.4%	0.1%	0.1%
TNF-α expression: Gag-stimulated cells	5.2%	7%	6.1%
TNF-α, IFN-γ, IL-2 expression: Gag-stimulated cells	<0.1%	<0.1%	<0.1%
% Inhibition of HIV-1 replication: Day 5	NA	29%	0%

Abbreviations: HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; NA, not applicable; PCR, polymerase chain reaction; PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate; TNF, tumor necrosis factor.

our case, the $CD4^+CD8^+$ T cells are similar to $CD8^+$ T cells based on the cytokine expression pattern and activation and memory markers. Although some studies suggest that $CD4^+CD8^+$ T cells have enhanced antiviral activity [2, 4], this was not observed here.

Although we were not able to determine the etiology of the mutual expression of CD4 and CD8 on T cells, flow cytometry revealed that the majority of these $CD4^+CD8^+$ T cells were effector memory cells with an activated phenotype. Human immunodeficiency virus controllers generally have higher levels of activated $CD8^+$ T cells [5], elevated inflammatory biomarkers [6, 7], and higher rates of atherosclerosis [8, 9] compared with treated and uninfected individuals. The significance of this inflammation is an active area of investigation. A recent study reported HIV controllers have higher rates of hospitalization than treated patients [10]. Preliminary studies suggest that ART decreases immune activation in some HIV controllers [11–13]. Randomized controlled trials are needed to determine the impact of ART and/or anti-inflammatory treatment in HIV controllers.

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References

1. O'Connell KA, Bailey JR, Blankson JN. Elucidating the elite: mechanisms of control in HIV-1 infection. Trends Pharmacol Sci **2009**; 30:631–7.
2. Frahm MA, Picking RA, Kuruc JD, et al. $CD4^+CD8^+$ T cells represent a significant portion of the anti-HIV T cell response to acute HIV infection. J Immunol **2012**; 188:4289–96.
3. Weiss L, Roux A, Garcia S, et al. Persistent expansion, in a human immunodeficiency virus-infected person, of V beta-restricted $CD4^+CD8^+$ T lymphocytes that express cytotoxicity-associated molecules and are committed to produce interferon-gamma and tumor necrosis factor-alpha. J Infect Dis **1998**; 178:1158–62.
4. Nascimbeni M, Shin EC, Chiriboga L, et al. Peripheral CD4(+)CD8(+) T cells are differentiated effector memory cells with antiviral functions. Blood **2004**; 104:478–86.
5. Hunt PW, Brenchley J, Sinclair E, et al. Relationship between T cell activation and $CD4^+$ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. J Infect Dis **2008**; 197:126–33.
6. Krishnan S, Wilson EM, Sheikh V, et al. Evidence for innate immune system activation in HIV type 1-infected elite controllers. J Infect Dis **2014**; 209:931–9.
7. Noel N, Boufassa F, Lecuroux C, et al. Elevated IP10 levels are associated with immune activation and low CD4(+) T-cell counts in HIV controller patients. AIDS **2014**; 28:467–76.
8. Hsue PY, Hunt PW, Schnell A, et al. Role of viral replication, antiretroviral therapy, and immunodeficiency in HIV-associated atherosclerosis. AIDS **2009**; 23:1059–67.
9. Pereyra F, Lo J, Triant VA, et al. Increased coronary atherosclerosis and immune activation in HIV-1 elite controllers. AIDS **2012**; 26:2409–12.

10. Crowell TA, Gebo KA, Blankson JN, et al. Elite controllers are hospitalized more often than persons with medically controlled HIV [Epub ahead of print]. J Infect Dis **2015**; doi:10.1093/infdis/jiu809.
11. Sedaghat AR, Rastegar DA, O'Connell KA, et al. T cell dynamics and the response to HAART in a cohort of HIV-1-infected elite suppressors. Clin Infect Dis **2009**; 49:1763–6.
12. Chun TW, Shawn Justement J, Murray D, et al. Effect of antiretroviral therapy on HIV reservoirs in elite controllers. J Infect Dis **2013**; 208:1443–7.
13. Hatano H, Yukl SA, Ferre AL, et al. Prospective antiretroviral treatment of asymptomatic, HIV-1 infected controllers. PLoS Pathog **2013**; 9: e1003691.