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BRIEF REPORT

Preferential depletion of CD2^{low} plasmacytoid dendritic cells in HIV-infected subjects

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Plasmacytoid dendritic cells (pDCs) are decreased in number and are functionally impaired in HIV act reasons for pDCs depletion are still unknown. It was recently reported that pDCs can be divided into two functionally distinct populations based on their CD2 expression level. To determine how the CD2^{high} and CD2^{low} populations are affected by HIV infection, we analyzed their frequencies in the peripheral blood of HIV-infected subjects and healthy controls. We found that the CD2^{low} pDC subset was preferentially depleted in infected individuals. The frequency of CD2^{low} pDCs correlated with the CD4$^+$ T-cell count but not with the plasma viral load. This finding furthers our understanding of the causes and consequences of pDC depletion during HIV infection.
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INTRODUCTION

Plasmacytoid dendritic cells (pDCs) play important roles in controlling viral infection.[1] However, the exact roles of pDCs in HIV infection are poorly understood. pDCs are decreased in number and are functionally impaired[2] in HIV-infected individuals. The decline of type I interferon production correlates with opportunistic infection independently of CD4$^+$ T-cell count,[3] suggesting an important role for pDCs in controlling viral replication. However, pDCs may play detrimental roles in HIV infection. They can be activated by HIV virions[4] or infected cells,[5] and sustained pDC activation and type I interferon production may contribute to immune activation and disease progression. This hypothesis is supported by data from both HIV-infected subjects[6–8] and simian immunodeficiency virus-infected monkey models.[9,10] Moreover, HIV infection stimulates pDCs to express tumor-necrosis factor-related apoptosis-inducing ligand and to participate in the killing of CD4$^+$ T cells.[11] However, the role of tumor-necrosis factor-related apoptosis-inducing ligand in inducing CD4$^+$ T cell death remains controversial.[12]

The results of a recent study suggest that pDCs can be divided into two populations based on their CD2 expression level.[13] In this report, we analyzed the frequencies of these two pDC subpopulations in HIV-negative (HIV$^-$) and -positive (HIV$^+$) subjects.

MATERIALS AND METHODS

Human subjects

Peripheral blood was collected from 30 (HIV$^-$) and 102 HIV-infected individuals who were not receiving antiretroviral therapy. The median age of the controls was 29 years (23–45 years) and that of the infected group was 35 years (9–75 years). The CD4$^+$ T-cell count of the HIV$^+$ subjects ranged from 9 to 781 cells/μl (median: 267 cell/μl), and the RNA viral load ranged from 75 to 3 100 000 copies/ml (median: 15 300 copies/ml). All samples tested were negative for hepatitis B, hepatitis C and herpes simplex virus infection. This study was approved by the Institutional Review Boards of the You-An Hospital, and informed consent was given by the participants before blood donation.

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were stained with anti-BDCA2-FITC (Miltenyi, Bergisch Gladbach, Germany), anti-CD2-PE (Biolegend, San Diego, CA, USA) and 7-AAD. Analysis was performed on the gate that included viable PBMCs, according to forward scatter and side-scatter characteristics. pDCs were defined as 7-AAD$^-$BDCA2$^+$ cells and were further divided into CD2^{high} and CD2^{low} populations as shown in Figure 1a and c. For apoptosis analysis, PBMCs were labeled with anti-BDCA2-FITC, anti-CD2-PE, 7-AAD and Annexin V-Alexa 647 (Biolegend). Otherwise, PBMCs were stained with anti-BDCA2-FITC, anti-CD2-PE, anti-CCR7-PerCP/Cy5.5

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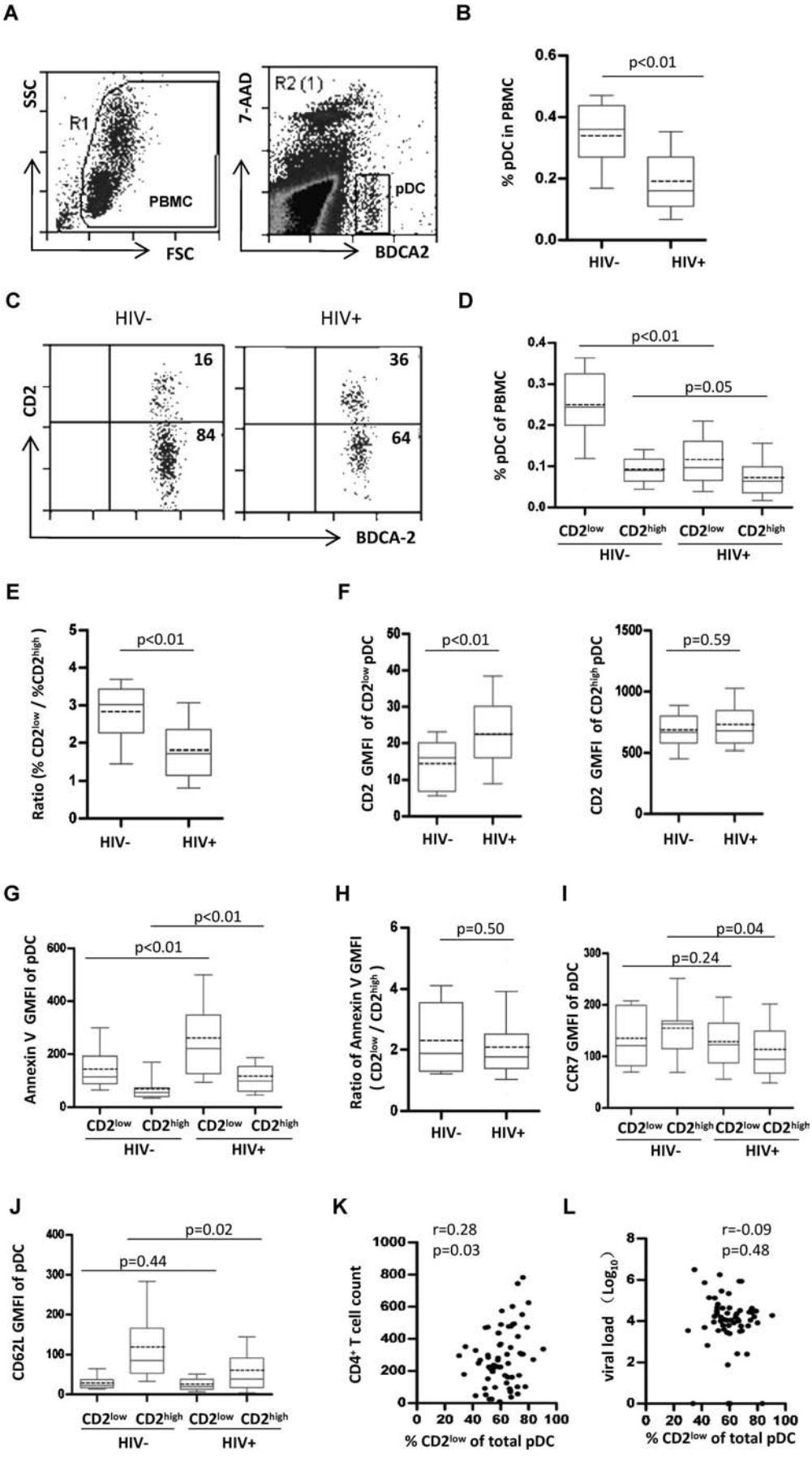
A
SSC
R1
PBMC
FSC
7-AAD
R2 (1)
pDC
BDCA2
B
% pDC in PBMC
p<0.01
HIV-
HIV+
C
HIV-
HIV+
CD2
16
84
36
64
BDCA-2
D
% pDC of PBMC
p<0.01
p=0.05
CD2low CD2high CD2low CD2high
HIV-
HIV+
E
Ratio (% CD2low / %CD2high)
p<0.01
HIV-
HIV+
F
CD2 GMFI of CD2low pDC
p<0.01
CD2 GMFI of CD2high pDC
p=0.59
HIV-
HIV+
G
Annexin V GMFI of pDC
p<0.01
p<0.01
CD2low CD2high CD2low CD2high
HIV-
HIV+
H
Ratio of Annexin V GMFI
(CD2low / CD2high)
p=0.50
HIV-
HIV+
I
CCR7 GMFI of pDC
p=0.04
p=0.24
CD2low CD2high CD2low CD2high
HIV-
HIV+
J
CD62L GMFI of pDC
p=0.02
p=0.44
CD2low CD2high CD2low CD2high
HIV-
HIV+
K
CD4+ T cell count
r=0.28
p=0.03
% CD2low of total pDC
L
viral load (Log10)
r=-0.09
p=0.48
% CD2low of total pDC

Figure 1 Characterization of CD2^{low} and CD2^{high} pDC subpopulations in HIV-infected subjects and healthy controls. (**a**) Total PBMCs were gated on forward and side scatter characteristics (left panel), and pDCs were identified as BDCA2^{+}7-AAD^{-}cells (right panel). (**b**) The frequencies of pDCs among the PBMCs of healthy controls (n=18; median=0.36) and HIV^{+} subjects (n=66; median=0.16) are shown. (**c**) pDCs were divided into CD2^{low} and CD2^{high} populations, and the relative proportions of these two populations from one representative healthy control (left panel) and one representative HIV^{+} patient (right panel) are shown. The numbers in the plots represent the percentage of the indicated population among pDCs. (**d**) The frequencies of the CD2^{low} and CD2^{high} subsets among PBMCs from healthy controls (n=18) and HIV^{+} subjects (n=66) are shown. (**e**) The ratio of the CD2^{low} pDC frequency in PBMCs relative to those of CD2^{high} pDCs in healthy controls (n=18; median=3.0) and HIV^{+} subjects (n=66; median=1.7). (**f**) The GMFIs of CD2 on CD2^{low} (left panel) and CD2^{high} pDCs (right panel) from healthy controls (n=18) and HIV^{+} subjects (n=66) are shown. (**g**, **i**, **j**) The GMFIs of Annexin V, CCR7 and CD62L on CD2^{low} and CD2^{high} pDCs in healthy controls (n=12) and HIV^{+} subjects (n=36) are shown. (**h**) The ratio of Annexin V GMFI on CD2^{low} pDCs relative to CD2^{high} pDCs in healthy controls (n=12; median=1.88) and HIV^{+} subjects (n=36; median=1.78) are shown. (**k**, **l**) The correlation between the percentage of CD2^{low} cells among total pDCs and both the CD4^{+} T-cell count (**k**) and plasma viral load (**l**) in HIV-infected subjects (n=66); the correlation coefficient (r) and significance (P) were calculated by the Spearman test. (**b**, **d**–**j**) Boxes showing the 25th to 75th percentile values (bottom and top of boxes) with the median (solid line) and mean value (dotted line); the bottom and top of the bars represent the 10th and 90th percentiles. The P values shown on each plot were calculated with the Mann–Whitney U test. GMFI, geometric mean fluorescence intensity; PBMC, peripheral blood mononuclear cell; pDC, plasmacytoid dendritic cell.

(Biolegend) and anti-CD62L-APC (Biolegend). Samples were analyzed using a FACSCalibur (BD Biosciences, Franklin Lakes, NJ, USA) and Summit (Dako, Glostrup, Denmark).

CD4^{+} T-cell count and viral load

After red blood cells were lysed, T-lymphocyte counts were determined by three-color flow cytometry using CD3, CD4 and CD8 antibodies (BD Biosciences). HIV-1 viral loads were determined by using the Roche Amplicor assay, and the cutoff value for positive detection was 50 copies/ml.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.00. For evaluation of the statistical significance of the differences between the subject groups, the Mann–Whitney U test was used. The correlations between the CD4^{+} T-cell count, the viral load and the percentage of CD2^{low} pDCs were assessed by Spearman test. $P<0.05$ was considered statistically significant.

RESULTS

A recent report suggests that pDCs can be divided into two functionally distinct populations based on their CD2 expression level.[13] In an attempt to elucidate how these two populations are affected by HIV infection, we compared pDCs in HIV-infected (n=66) and healthy subjects (n=18). As shown in Figure 1a and b, the median percentage of pDCs in PBMC dropped from 0.36% in healthy controls to 0.16% in HIV-infected subjects ($P<0.01$). Then, pDCs were divided into two populations, CD2^{low} and CD2 ^{high} (Figure 1c and Supplementary Figure 1). The frequency of CD2^{low} pDCs was significantly lower in HIV-infected subjects than in healthy controls (0.24% versus 0.10%, $P<0.01$; Figure 1d and Table 1). The frequency of CD2^{high} pDCs also dropped but to a much lesser extent (0.09% versus 0.06%, P=0.05; Figure 1d and Table 1). The ratio of CD2^{low} to CD2^{high} cells was 3.0 in (HIV^{-}) subjects and 1.7 in HIV^{+} subjects ($P<0.01$; Figure 1e). The geometric mean fluorescence intensity (GMFI) of CD2 expression on CD2^{low} cells was 16 in healthy controls, and this increased to 22 in HIV-infected subjects ($p<0.01$). But the GMFI of the CD2^{high} pDCs was not significantly different between the two groups (GMFI, 666 versus 678, P=0.59; Figure 1f and Table 1).

We analyzed the contribution of apoptosis to the selective loss of CD2^{low} pDCs by Annexin V staining. Both pDC populations appeared to be more apoptotic in HIV-infected subjects. The median GMFI of Annexin V on CD2^{high} pDC increased from 55 in healthy controls to 98 in HIV^{+} subjects, and that of CD2 ^{low} pDCs increased from 114 in (HIV^{-}) subjects to 221 in HIV^{+} subjects (Figure 1g and Table 1). However, the ratio of the GMFIs of Annexin V for the CD2^{low} and CD2^{high} populations was not significantly different between HIV-infected and healthy subjects (median value: 1.88 versus 1.78, P=0.50; Figure 1h).

To determine the role of migration to the lymph nodes in loss of CD2^{low} pDCs, we also stained cells for CCR7 and CD62L. On these cells, the expression levels of both markers were not significantly different between HIV^{+} and (HIV^{-}) subjects (Figure 1i and j and Table 1). However, the levels of both molecules decreased on CD2^{high} pDCs as a result of HIV infection; the median CCR7 GMFI dropped from 163 in healthy subjects to 95 in HIV-infected subjects (P=0.04; Figure 1i and Table 1), and that of CD62L decreased from 85 to 39 (P=0.02; Figure 1j and Table 1).

We also analyzed the correlation of the CD2^{low} pDC frequency with the CD4^{+} T-cell count and the plasma viral load. The frequency of the CD2^{low} subset among total pDCs was correlated with CD4^{+} T-cell loss (r=0.28, P=0.03; Figure 1k), but was not correlated with the plasma viral load (r=0.09, P=0.48, Figure 1l).

DISCUSSION

pDCs were depleted from the peripheral blood of HIV-infected individuals,[2] including those with a primary infection.[14] In this study, we demonstrated that CD2^{low} pDCs were preferentially depleted during HIV infection (Figure 1d and e). Apoptosis and lymphoid organ migration did not contribute to the selective loss of CD2^{low} pDCs

Table 1 Characterization of CD2^{low} and CD2^{high} pDC subpopulations in HIV-infected subjects and healthy controls

	HIV^{-}		*HIV^{+}*	
	CD2^{low} pDCs	*CD2^{high} pDCs*	*CD2^{low} pDCs*	*CD2^{high} pDCs*
% pDCs among PBMCs	0.24 (0.20–0.32)	0.09 (0.06–0.12)	0.10 (0.07–0.16)	0.06 (0.04–0.10)
CD2 GMFI	16 (7–20)	666 (577–798)	22 (16–30)	678 (577–843)
Annexin V GMFI	114 (88–192)	55 (39–73)	221 (127–348)	98 (60–154)
CCR7 GMFI	120 (82–199)	163 (115–169)	122 (87–164)	95 (68–1489)
CD62L GMFI	22 (17–37)	85 (53–166)	20 (13–38)	39 (17–91)

Abbreviations: GMFI, geometric mean fluorescence intensity; pDC, plasmacytoid dendritic cell.
Data are shown as the median value with the 25th–75th percentiles in parenthesis.

(Figure 1i and j). Even though the lower level of lymph node migration of CD2^{high} pDCs could result in a lower frequency of CD2^{low} pDCs, this migration cannot explain the increase in the CD2 GMFI of CD2^{low} cells. Several possible reasons could contribute to the decrease in CD2^{low} pDCs from blood. First, HIV infection inhibits hematopoiesis,[15,16] and the differentiation or migration from bone marrow of CD2^{low} pDCs may be preferentially inhibited by HIV. Second, because HIV may kill pDCs through direct infection[17,18] or fusion with infected cells,[19] CD2^{low} pDCs may be selectively killed. In addition, even though the previous report demonstrated that CD2^{low} and CD2^{high} pDCs cannot differentiate into each other upon stimulation,[13] we cannot exclude the possibility that, in the case of HIV infection, CD2^{low} pDCs may upregulate their CD2 expression and convert to CD2^{high} pDCs. We also observed a correlation between the frequency of CD2^{low} pDCs among total pDCs and the CD4$^+$ T-cell count. Thus, the preferential loss of peripheral CD2^{low} pDCs may be another indicator in HIV infection, in addition to the loss of total pDCs.

In summary, we report here the selective loss of the CD2^{low} pDC subset from the peripheral blood in HIV-infected subjects. Unlike the CD2^{low} subset, CD2^{high} pDCs express lysozyme and possess cytotoxic activity. Additionally, these cells express higher levels of costimulatory molecules and are more efficient at triggering allogeneic naive T-cell proliferation.[13] The accumulation of CD2^{high} pDCs may contribute to immune activation and pathogenesis in HIV infection. The mechanisms underlying preferential loss of CD2^{low} pDCs and its consequences on disease progression need to be further investigated.

Note: Supplementary information is available on the Cellular & Molecular Immunology website (http://www.nature.com/cmi/).

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