研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
高橋秀実	免疫応答不全	神谷　茂、河野　茂	微生物学	メディカル・サイエンス・インターナショナル社	東京	2012.4.20	343-364 (総ページ664)

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Imbalanced Production of Cytokines by T Cells Associates with the Activation/Exhaustion Status of Memory T Cells in Chronic HIV Type 1 Infection

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Abstract

Chronic HIV-1 infection is characterized by immune cell dysfunctions driven by chronic immune activation. Plasma HIV-1 viral load (VL) is closely correlated with disease progression and the level of immune activation. However, the mechanism by which the persistent presence of HIV-1 damages immune cells is still not fully understood. To evaluate how HIV-1 affects disruption of T cell-mediated immune responses during chronic HIV-1 infection we determined the functional profiles of T cells from subjects with chronic HIV-1 infection. We measured the capacity of peripheral blood mononuclear cells (PBMCs) to produce 25 specific cytokines in response to nonspecific T cell stimulation, and found that the capacity to produce Th-1-related cytokines (MIP-1α, MIP-1β, RANTES, IFN-γ, and MIG), sIL-2R, and IL-17, but not Th-2-related cytokines, was inversely correlated with plasma VL. The capacities to produce these cytokines were interrelated; notably, IL-17 production had a strong direct correlation with production of MIP-1α, MIP-1β, RANTES, and IFN-γ. In both $CD4^+$ and $CD8^+$ T cells, dysfunctional production of cytokines was associated with T cell activation (CD38 expression) and exhaustion (PD-1 and/or CTLA-4 expression) status of memory subsets. Although the capacity to produce these cytokines was recovered soon after multiple $\log_{10}$ reduction of plasma viral levels by antiretroviral therapy, memory $CD8^+$ T cells remained activated and exhausted after prolonged virus suppression. Our data suggest that HIV-1 levels directly affect the ability of memory T cells to produce specifically Th1- and Th17-related cytokines during chronic HIV-1 infection.

Introduction

Plasma viral load (VL) and CD4-positive T cell count are two surrogate markers of HIV-1 disease progression.[1] Throughout the course of infection both innate and adaptive immune systems are highly activated, and disease progression is strongly correlated with immune activation status.[2,3] Notably, immune activation is observed in both HIV-1 and pathogenic SIV infection, but not in nonpathogenic SIV infection in a natural host.[4,5] Moreover, studies have shown that T cells in patients with high VL and progressive disease are less functional, have less proliferative capacity, and are more exhausted than T cells in patients with low VL and slow disease progression.[6–12] In those patients, exhaustion is seen not only in HIV-1-specific T cells, but also in nonspecific T cells.[11,13] These data suggest that immune cells have lost their original function due to persistent hyperactivation, which depends on VL, during chronic HIV-1 infection.

The immune system is highly coordinated: the cytokine network regulates interactions between cells, and cytokine balance dictates how the immune system responds. Cytokine production determines the specific helper functions of $CD4^+$ T cells and allows balance in immune responses *in vivo*.[14–16] A possible explanation of the impaired immune response in chronic HIV-1 infection is that the ability of T cells to balance cytokine production has been altered, just as alteration of balance between Th1- and Th2-type immune response affects the clinical course of certain infectious diseases and autoimmune syndromes.[17,18]

To evaluate T cell impairment resulting from persistent immune activation during chronic HIV-1 infection, we compared the cytokine expression spectra of peripheral blood mononuclear cells (PBMCs) in response to nonspecific T cell stimulation in treatment-naive HIV-1-infected subjects with low or high VL. We also examined the differentiation states and activation levels of $CD4^+$ and $CD8^+$ T cells from

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HIV-1-infected subjects to elucidate relationships between cytokine expression capacity and T cell phenotypic status.

Materials and Methods

Study design

HIV-1-infected individuals who were under medical supervision at our clinic were asked to provide blood samples for this study. Blood samples were taken from selected patients in the chronic phase of HIV-1 infection, with CD4 counts >200 cells/ml. We requested blood samples from antiretroviral therapy (ART)-naive patients with either low plasma viral load (VL) values (<5000 copies/ml; LVL group) or high VL values (>25,000 copies/ml; HVL group), and from treatment-experienced patients who had received ART >2 years (Tx group). Blood samples were also obtained from a small number of HIV-1-infected patients who had first initiated ART within the previous 1–2 months. As controls, blood samples were obtained from HIV-1-seronegative individuals (healthy controls; HC).

All participants gave written informed consent, and the study was approved by the institutional review boards of the Institute of the Medical Science of the University of Tokyo (No. 11-2-0329 and 20-47-210521).

PBMC cultures and PHA stimulation

PBMCs were isolated from heparinized whole blood by Ficoll-Paque PLUS density gradients (GE Healthcare, Piscataway, NJ) and cryopreserved in liquid nitrogen until use. The frozen cells were thawed 1 day before stimulation and cultured in R10 medium [RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 10% heat-inactivated fetal calf serum (FCS; Sigma), 100 U penicillin/ml, 100 μg/ml streptomycin (Sigma), 2 mmol/liter L-glutamine (Sigma), and 10 mmol/liter 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; Sigma)] at 37°C, 5% CO_2. The following day 5×10^5 cells/well were cultured in 250 μl/well of R10 medium with or without 2 μg/ml phytohemagglutinin L (PHA; Roche Applied Science, Mannheim, Germany). Culture supernatants were harvested after 48 h and stored at −80°C until use for multiple cytokine assays.

Quantification of cytokines

The human cytokine 25-plex antibody kit (Invitrogen Corporation, Carlsbad, CA) was used to measure the levels of 25 cytokines in culture supernatants: interleukin (IL)-1 receptor antagonist protein (IL-1RA), IL-1β, IL-2, soluble IL-2R (sIL-2R), IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40/70, IL-13, IL-15, IL-17, eotaxin, interferon gamma (IFN-γ)-induced protein 10 kDa (IP-10), monocyte chemoattractant protein-1 (MCP-1), monokine induced by IFN-γ (MIG), macrophage inflammatory protein 1alpha (MIP-1α), MIP-1β, regulated on activation normal T cell expressed and secreted (RANTES), tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-α, and IFN-γ. The detection limits for the cytokines measured by the kit were as follows: IL-5, IL-6, IL-8, 3 pg/ml; MIG, 4 pg/ml; IL-4, IL-10, IFN-γ, IP-10, eotaxin, 5 pg/ml; IL-2, 6 pg/ml; IL-7, IL-13, IL-15, IL-17, TNF-α, MIP-1α, MIP-1β, MCP-1, 10 pg/ml; IL-1β, IL-12p40/70, IFN-α, GM-CSF, RANTES, 15 pg/ml; IL-1RA, sIL-2R, 30 pg/ml. As the amounts of IL-6, IL-8, TNF-α, MIP-1α, MIP-1β, IP-10, MIG, and MCP-1 produced from PHA-stimulated PBMCs were beyond the range of the assay, we diluted the samples 10-fold prior to measurement of these cytokines. However, IL-8 levels were out of range in most patient samples and could not be measured accurately.

Samples were loaded onto the Luminex100 system (Luminex Corporation, Austin, YX), and samples were quantified by analysis of the median fluorescence intensity of the beads using MasterPlex QT version 2.5 (Luminex Corporation). The assays were performed according to the manufacturer's instructions, and all samples were run in duplicate.

Identification of cytokine-producing cells in PBMCs

$CD14^+$ cells (monocytes), $CD8^+$ T cells, $CD4^+$ T cells, and $CD56^+CD16^+$ (NK) cells were isolated sequentially from PBMCs of each healthy subject. Magnetic cell separation (MACS) selection was performed using anti-CD14, anti-CD8, and anti-CD4 antibody-conjugated microbeads or using the $CD56^+CD16^+$ NK cell isolation kit (Miltenyi Biotic, Bergisch Gladbach, Germany). The purity of each cell fraction was >95% as determined by flow cytometry.

Fractionated cells were cultured separately or were co-cultured in the presence of 2 μg/ml PHA at 37°C, 5% CO_2, for 48 h. Levels of MIP-1α, MIP-1β, RANTES, IL-2R, IFN-γ, and IL-17 in culture supernatants were measured with DuoSet ELISA Development Systems (R&D Systems). The absolute numbers of each cell fraction used in the experiments were calculated from the average proportion of each subset in PBMCs.

Antibodies

The fluorochrome-conjugated monoclonal antibodies (mAb) used in the study were as follows: fluorescein isothiocyanate (FITC)-labeled anti-MIP-1α, anti-MIP-1β, and anti-RANTES (R&D Systems, Minneapolis, MN); FITC-labeled anti-PD-1 and anti-Ki67, phycoerythrin (PE)-labeled anti-Bcl-2, peridinin cholorophyll protein/cyanin5.5 (PerCP Cy5.5)-labeled anti-CD38 and anti-CD3, PE Cy7-labeled anti-CCR7, allophycocyanin (APC)-labeled anti-CD45RA, and pacific blue-labeled anti-CD4 (BD Biosciences, San Jose, CA); APC AlexaFluor 750-labeled anti-CD4, pacific blue-labeled anti-IFN-γ, and AlexaFluor 647-labeled anti-IL-17A (eBioscience, San Diego, CA); PE-labeled anti-IL-4 (Becton Dickinson, Franklin Lakes, NJ); APC Cy7-labeled anti-CD3 (BioLegend, San Diego, CA); and Pacific Orange-labeled anti-CD8 (Invitrogen).

Surface phenotypic and intracellular cytokine staining

For intracellular cytokine staining, cryopreserved PBMCs were thawed and cultured in R10 overnight. The following day cells were stimulated with phorbol ester (PMA)/calcium ionophore (ionomycin) in the presence of Golgi inhibitor (brefeldin A) for 5 h. Cells were stained with a panel of fluorescently labeled antibodies against cell-surface markers. For detection of dead cells, the cells were also stained with 5 μg/ml ethidium monoazide bromide (EMA; Sigma). Cells were washed twice and exposed to fluorescent light for 10 min on ice to allow the EMA to bind to DNA in dead cells. Cells were then fixed in 2% paraformaldehyde and permeabilized in BD FACS Permeabilizing Solution 2 (BD Biosciences) prior to antibody staining for intracellular molecules.

Dead or dying cells were detected by surface phenotypic staining with propidium iodide (PI; Sigma).

Flow cytometric analysis

Samples were analyzed on a FACSAria multilaser cytometer (Beckton Dickinson) running FACSDiva software, with collections of 60,000–100,000 lymphocyte-gated events. Data were analyzed with FlowJo software (Tree Star, Ashland, OR).

Statistical analysis

GraphPad Prism5 software (San Diego, CA) was used for all statistical analysis. Differences between groups were tested for statistical significance using the nonparametric Mann–Whitney *U* test. Since previous studies revealed that production of multiple cytokines by HIV-specific T cells was limited in progressors compared to nonprogressors,[6,19] the production levels of cytokines were expected to differ among LVL, HVL, and healthy control subjects. For this reason, we did not consider multiple comparison correction for Mann–Whitney *U* tests to avoid false-negative results. Correlation analysis was performed using Spearman's rank correlation. The level of significance for all analyses was set at $p < 0.05$.

Results

Study population

Most analyses were performed using blood samples collected from 35 HIV-1-infected, ART-naïve patients, 15 HIV-1-infected, treatment-experienced patients, and 16 HIV-1-seronegative individuals. Demographic characteristics of these 50 HIV-1-infected patients are presented in Table 1. The 35 HIV-1-infected, ART-naive patients included 19 patients with low VL (LVL group; median VL: 1200, range: 53 to 3600) and 16 patients with high VL (HVL group; median VL: 62,000; range: 25,000 to 500,000). The median CD4 counts in the LVL and HVL groups were 449 (range: 316 to 749) and 407 (range: 228 to 520), respectively; the difference was not statistically significant. The groups also showed no significant difference in age, another factor that influences immune status.

The 15 HIV-1-infected individuals recruited into the study to represent treatment-experienced patients had received ART and successfully controlled their disease over a long period of time (median: 66 months; range: 22 to 149 months). To examine the impact of actively decreasing VL on the functional profile of PBMCs, blood samples were also collected from six HIV-1-infected patients who had initiated treatment only in the previous 1–2 months.

Cytokine production in PHA-stimulated PBMCs

Cytokine measurements from cells cultured for 48 h in an unstimulated state were at the limit of detection (data not shown). We initially compared anti-CD3-antibody and PHA as a nonspecific stimulus of PBMCs to induce cytokine production, and found that the production levels of most cytokines were much higher in PHA-stimulated PBMCs than in anti-CD3-antibody-stimulated PBMCs (data not shown). When cells were stimulated with PHA and cultured for 48 h, production of most cytokines increased dramatically (Fig. 1A). There were no significant differences between any groups in IL-2, IL-13, IL-15, IL-1β, IFN-α, TNF-α, eotaxin, or IP-10 production (data not shown).

Cytokine production in PBMCs from treatment-naive HIV-1 subjects was compared to cytokine production in PBMCs from healthy control subjects. Median levels of many cytokines in the HVL group were significantly different from those in the healthy control group: MIP-1α [6.33 (range 0.99–21.01) vs. 16.92 (10.36–23.87) ng/ml; $p = 0.0005$], MIP-1β [8.51 (1.37–26.42) vs. 21.44 (10.26–34.11) ng/ml; $p = 0.0036$], IFN-γ [1.50 (0.30–5.75) vs. 2.64 (0.79–5.78) ng/ml; $p = 0.0402$], IL-7 [<0.01 (<0.01–0.67) vs. 0.04 (<0.01–0.07) ng/ml; $p = 0.0077$], IL-1Ra [18.93 (0.59–27.61) vs. 1.50 (0.55–14.95) ng/ml; $p = 0.0184$], IL-6 [0.63 (0.11–12.23) vs. 1.77 (0.68–4.93) ng/ml; $p = 0.0254$], and IL-10 [0.08 (<0.005–0.40) vs. 0.55 (0.13–0.83) ng/ml; $p = 0.0031$]. In contrast, significant differences between the LVL group and the healthy control group were seen only in levels of IL-10 [0.12 (<0.005–0.72) vs. 0.55 (0.13–0.83) ng/ml; $p = 0.0050$] and IL-1Ra [17.05 (0.49–28.31) vs. 1.50 (0.55–14.95) ng/ml; $p = 0.0282$] (Fig. 1A). These data suggest that although PBMCs from HVL subjects are abnormal in some way, PBMCs from LVL subjects are almost normal in terms of cytokine production.

As shown in Fig. 1A, mean cytokine levels were significantly lower in HVL subjects compared to LVL subjects, as follows: MIP-1α [6.33 (0.99-21.01) vs. 14.36 (2.29–29.16) ng/ml; $p = 0.0077$], MIP-1β [8.51 (1.37–26.42) vs. 20.14 (4.31–48.75) ng/ml; $p = 0.0034$], RANTES [2.01 (<0.015–4.57) vs. 3.40 (1.33–6.90) ng/ml; $p = 0.0014$], sIL-2R [2.30 (0.02–4.96) vs. 3.72 (1.72–7.38) ng/ml; $p = 0.0136$], IL-17 [0.04 (<0.01–0.12) vs. 0.08 (<0.01–0.17) pg/ml; $p = 0.0256$], and IL-7 [<0.01 (<0.01–0.67) vs. 0.05 (<0.01–1.05) pg/ml; $p = 0.0029$]. Notably, there was an inverse correlation between VL and production of these cytokines, and of IFN-γ (Fig. 1B). No relationship was observed between cytokine levels and CD4 cell count (data not shown). These data suggest that VL directly affects the capacity of PBMCs to produce certain cytokines during chronic infection.

Th1- and Th17-type T cells have impaired cytokine production in HVL subjects

Although PHA is considered a T cell mitogen, other cell populations also produce cytokines in response to PHA stimulation.[20–22] The next step was to determine which cells were responsible for the alterations in cytokine production observed under our experimental conditions. The cytokines whose production was inversely correlated with VL can be produced by several cell populations in PBMCs. To identify the major cell population producing these cytokines, we fractionated PBMCs in healthy donors by positive selection and determined the cell population producing these cytokines. $CD4^+$ T cells, $CD8^+$ T cells, monocytes ($CD14^+$), and NK cells ($CD56^+CD16^+$) were isolated from PBMCs, cultured separately or cocultured, and stimulated with PHA. We then measured levels of MIP-1α, MIP-1β, RANTES, IFN-γ, sIL-2R, and IL-17. Little or no production of these cytokines was detected in any of the single cell fractions (Fig. 2A). Production of cytokines MIP-1α, MIP-1β, RANTES, IFN-γ, and sIL-2R was observed in cocultures of $CD4^+$ and $CD14^+$ cells and in cocultures of $CD8^+$ and $CD14^+$ cells (Fig. 2A, and data not shown). IL-17 production was detected only in cocultures of $CD4^+$ and $CD14^+$ cells (Fig. 2A). As T cell stimulation by

TABLE 1. PATIENT CHARACTERISTICS

	Diagnosis (month)	Sex	Age	VL	CD4	CD8	Treatment period (month)
19 LVL							
S70	*9*	*M*	*47*	*53*	*481*	*746*	
T16	*60*	*F*	*41*	*240*	*492*	*804*	
O12	*61*	*M*	*32*	*450*	*559*	*1,187*	
S33	*125*	*M*	*52*	*470*	*400*	*888*	
K2	*60*	*M*	*30*	*510*	*316*	*871*	
M3	*160*	*F*	*34*	*730*	*444*	*859*	
S81	*6*	*M*	*32*	*1,700*	*381*	*1,475*	
Y1	*131*	*M*	*36*	*1,700*	*358*	*753*	
F4	*101*	*M*	*32*	*2,000*	*404*	*699*	
E6	*13*	*M*	*29*	*2,100*	*348*	*649*	
T24	30	M	46	400	455	469	
O16	23	M	36	1,100	362	812	
F9	65	M	35	1,100	517	1,066	
K11	33	M	30	1,200	521	1,137	
F1	41	M	35	1,600	424	1,151	
H25	6	M	43	1,700	749	821	
K16	61	F	23	2,000	560	1,049	
A10	32	M	25	3,400	586	1,603	
T26	28	M	36	3,600	449	864	
median	41		35	1,200	449	868	
16 HVL							
S60	*22*	*M*	*36*	*35,000*	*321*	*486*	
H24	*23*	*M*	*35*	*42,000*	*462*	*907*	
F13	*22*	*M*	*34*	*51,000*	*520*	*831*	
O29	*9*	*M*	*21*	*56,000*	*314*	*880*	
Y24	*14*	*M*	*25*	*58,000*	*381*	*2,023*	
K54	*10*	*M*	*24*	*82,000*	*386*	*1,056*	
K43	*11*	*M*	*29*	*110,000*	*361*	*507*	
S78	*5*	*M*	*48*	*260,000*	*492*	*1,441*	
K46	*15*	*M*	*47*	*280,000*	*454*	*1,579*	
S55	*26*	*M*	*56*	*500,000*	*427*	*510*	
T37	3	M	30	25,000	254	626	
K33	24	M	39	27,000	314	832	
M11	58	M	38	33,000	228	908	
O17	24	M	33	66,000	494	632	
T35	4	M	20	78,000	434	1,133	
S5	49	M	62	85,000	516	1,029	
median	18.5		35	62,000	407	894	
15 Tx							
U5	*22*	*M*	*42*	*30*	*308*	*581*	*22*
S19	*82*	*M*	*35*	*30*	*382*	*533*	*22*
T18	*70*	*M*	*40*	*40*	*508*	*747*	*66*
T8	*39*	*M*	*42*	*50*	*423*	*491*	*51*
K4	*73*	*M*	*31*	*50*	*480*	*718*	*71*
Y17	*33*	*M*	*36*	*50*	*365*	*508*	*31*
I9	*90*	*M*	*50*	*50*	*406*	*643*	*80*
I5	*59*	*M*	*44*	*50*	*466*	*668*	*56*
N11	*119*	*M*	*39*	*67*	*440*	*886*	*117*
N17	*60*	*M*	*29*	*67*	*382*	*571*	*46*
K24	150	M	54	40	610	805	149
N5	72	M	37	45	633	706	41
O9	61	M	41	50	335	629	72
Y5	81	M	36	110	753	605	79
S15	113	M	38	130	814	1,048	111
median	72		39	50	440	643	66

Italics indicates the patients used for phenotype and activation/exhaustion status of T cells.
VL, viral load; LVL, low viral load; HVL, high viral load; Tx, treatment experienced.

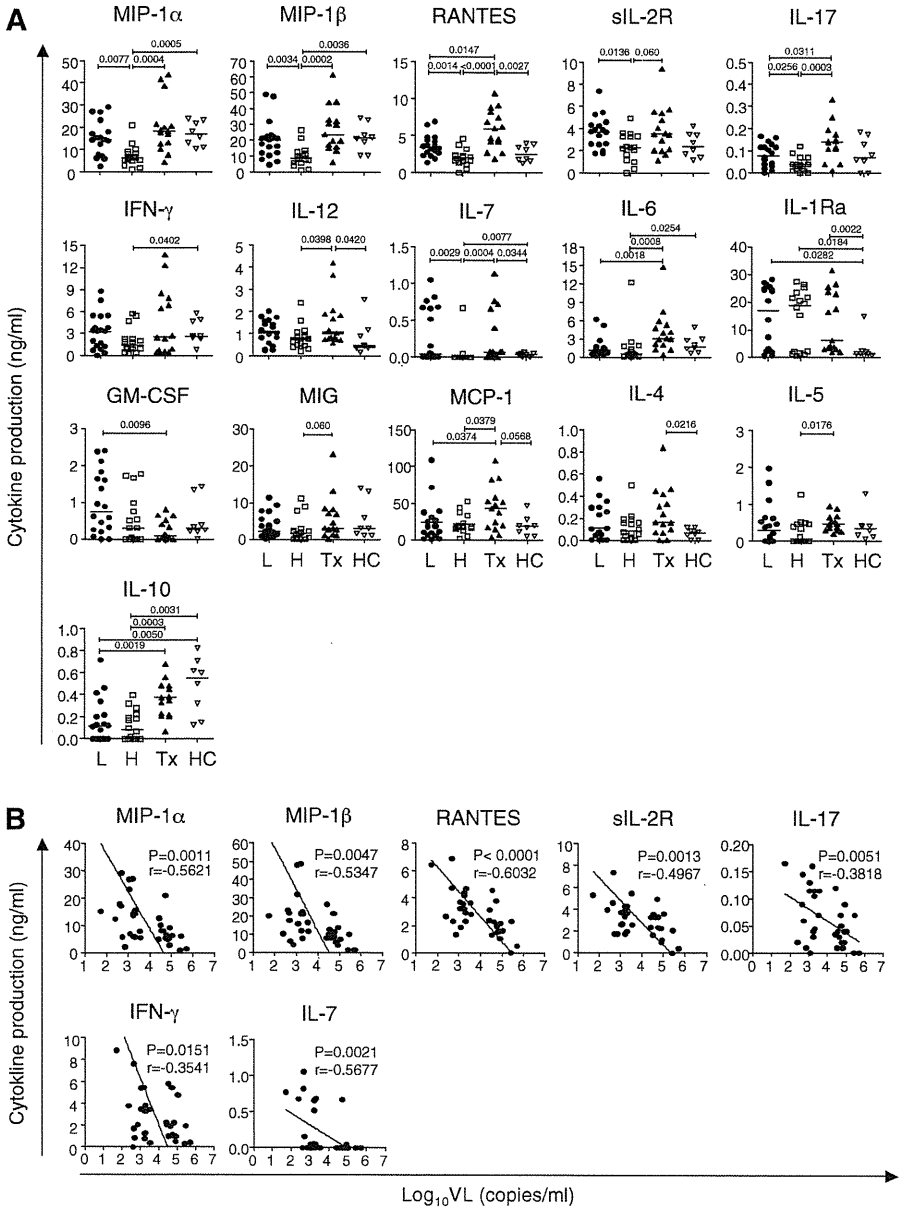

FIG. 1. Multiple cytokine production of phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) in chronic HIV-1-infected subjects and healthy individuals. **(A)** Comparison of cytokine production from PHA-stimulated PBMCs. L, LVL subjects (●), H, HVL subjects (□), Tx, HIV-1-infected subjects with prolonged antiretroviral therapy (ART) (▲), HC, healthy control (□). The horizontal bars indicate the median value. Differences between groups were tested for statistical significance by the Mann–Whitney *U* test. **(B)** Correlation between cytokine production and viral load. Correlation analysis was performed with Spearman's rank correlation to determine correlations between variables.

PHA requires accessory cells such as monocytes and macrophages,[23,24] these data indicate that $CD4^+$ and $CD8^+$ T cells are the sources of MIP-1α, MIP-1β, RANTES, IFN-γ, and sIL-2R production, and that only $CD4^+$ T cells are the source of IL-17.

$CD4^+$ T cells were classified into subsets based on cytokine secretion.[14,16] We found VL-associated reductions in levels of IFN-γ and IL-17 levels, which are typical cytokines secreted by Th1 and Th17 cells, respectively. In contrast, as shown in Fig. 1A, the LVL and HVL groups had comparable levels of IL-4, IL-5, and IL-13, which are associated with a Th2-type response, and IL-10, which is produced by regulatory T cells (Treg) (Fig. 1A). These results suggest that $CD4^+$ T cell dysfunction in HVL may occur in a type-specific manner, especially in Th1 and Th17 cells.

To determine which types of T cells could secrete MIP-1α, MIP-1β, and RANTES under our experimental conditions, we examined the expression pattern of MIP-1α/MIP-1β/RANTES, IFN-γ, and IL-17 by intracellular cytokine staining (ICS) after nonspecific T cell stimulation. Production of MIP-1α/MIP-1β/RANTES occurred in IFN-γ-expressing $CD4^+$ T cells, particularly in the subset of cells that expressed high levels of IFN-γ (Fig. 2B left). IL-17 was also produced in $CD4^+$ T cells, but was secreted by a different $CD4^+$ T cell subset. In $CD8^+$ T cells, most IFN-γ-expressing cells produced MIP-1α/MIP-1β/RANTES, and IL-17 was not produced at all (Fig. 2B right). Thus, our assays showed that MIP-1α, MIP-1β, and RANTES are secreted from Th1-type $CD4^+$ T cells and $CD8^+$ T cells, and that IL-17-secreting cells (Th17 cells) are clearly distinct. These data suggest that cytokine production by T

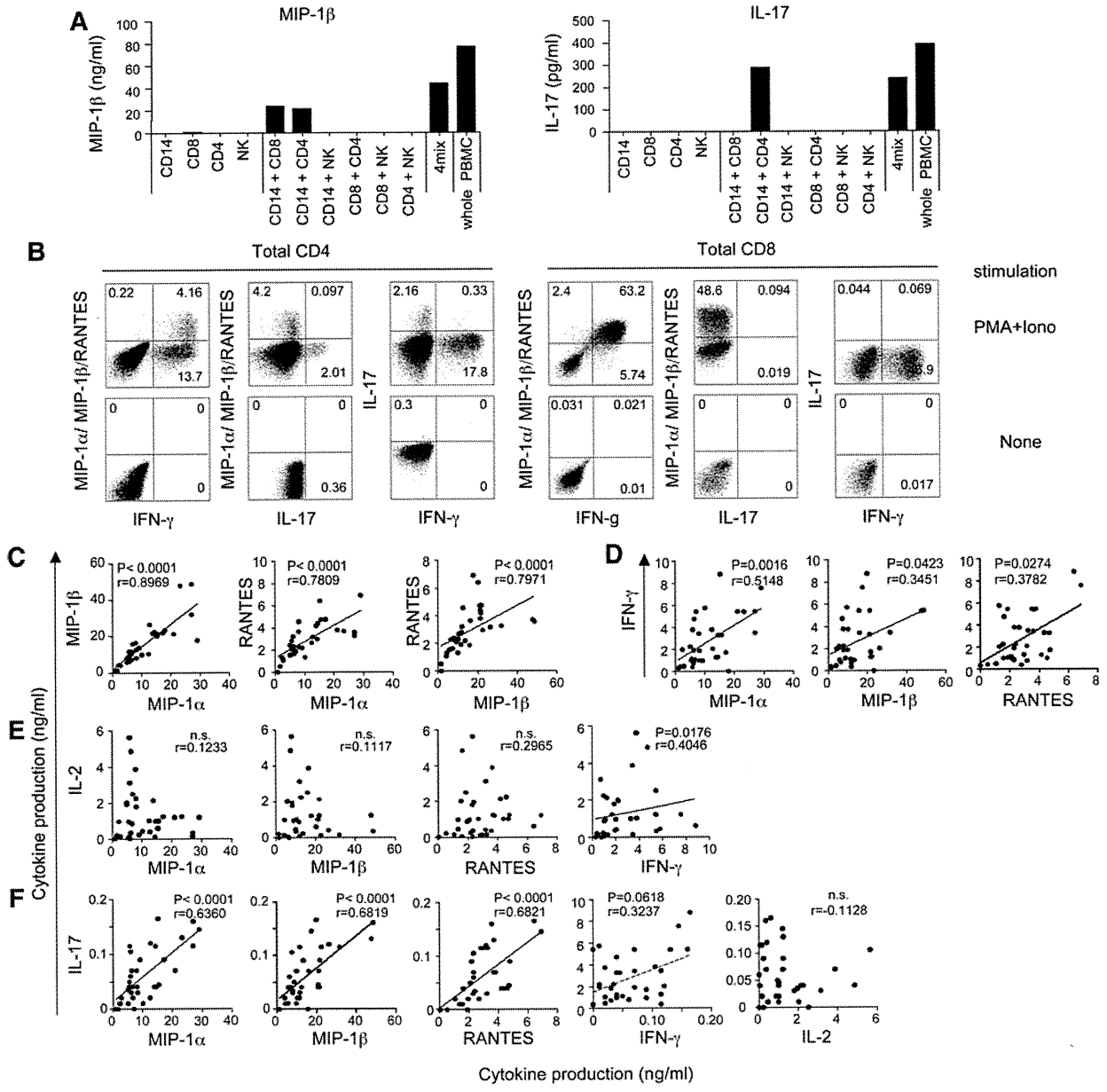

FIG. 2. Identification of cytokine-producing cells and relationships between cytokines. **(A)** Cytokine production in cell fractions from PBMCs. The results of MIP-1β and IL-17 production are shown. Fractionated $CD4^+$, $CD8^+$, $CD14^+$, and NK ($CD56^+CD16^+$) cells from PBMCs in healthy individuals were cultured separately or cocultured for 48 h after PHA stimulation. The experiment was repeated twice with PBMCs from different donors. **(B)** Representative flow cytometric analysis of intracellular cytokine staining for MIP-1α, MIP-1β, RANTES, IFN-γ (Th1 cytokine), and IL-17 after PMA/ionomycin stimulation in PBMCs of healthy individual. **(C–F)** Correlation between each cytokine production in treatment-naive HIV-1-infected subjects. MIP-1α, MIP-1β, and RANTES production **(C)**, IFN-γ production and MIP-1α, MIP-1β, or RANTES production **(D)**, IL-2 production and MIP-1α, MIP-1β, RANTES, or IFN-γ production **(E)**, IL-17 production and MIP-1α, MIP-1β, RANTES, IFN-γ, or IL-2 production **(F)** are shown. Correlation analysis was performed with Spearman's rank correlation to determine correlations between variables.

cells from the HVL group is dysfunctional, specifically in some of the Th1-related cytokines and in IL-17.

We next analyzed the correlation between production of Th1 cytokines (IFN-γ and IL-2), MIP-1α/MIP-1β/RANTES, and IL-17 in treatment-naive HIV-1 subjects. The levels of MIP-1α, MIP-1β, and RANTES showed strong positive correlations to each another (Fig. 2C), and correlations between IFN-γ and each of them were also significant (Fig. 2D). However, IL-2, another typical Th1 cytokine, did not show any significant correlation with MIP-1α, MIP-1β, and RANTES (Fig. 2E). Surprisingly, we found strong correlations between IL-17 production and MIP-1α/MIP-1β/RANTES

levels or IFN-γ levels, despite the fact that these cytokines are produced by different cells (Fig. 2F). These data suggest interrelated production of IFN-γ, MIP-1α, MIP-1β, RANTES, and IL-17 in T cells, but not of IL-2. Moreover, the capacity of T cells to produce these cytokines appears to be affected by HIV-1 VL *in vivo*.

Both central and effector memory CD4$^+$ and CD8$^+$ T cells are highly activated and exhausted in HVL subjects

The mechanism underlying the reduction in levels of specific cytokines in the HVL group could result either from decreased numbers of the cytokine-producing cells or from decreased productive capacity in those cells. We quantitated CD4$^+$ and CD8$^+$ T cells in HIV-1-infected patients (Table 1) and healthy control subjects (data not shown) by FACS. Although the number of CD4$^+$ T cells was significantly higher and the number of CD8$^+$ T cells significantly lower in HC than in HIV-positive patients, the differences in these T cell subsets were not significant between HVL and LVL.

As the number of monocytes seemed to affect T cell stimulation by PHA, we also analyzed monocytes (CD14$^+$ cells) and found there was no quantitative difference between any of the groups (data not shown).

As the cytokine productive capacity of T cells differs according to their differentiation status,[25] we explored the differentiation status of CD4$^+$ and CD8$^+$ T cells. We divided CD4$^+$ and CD8$^+$ T cells into four subsets depending on the expression pattern of CD45RA and CCR7: naive (CD45RA$^+$/CCR7$^+$), central memory (CM; CD45RA$^-$/CCR7$^+$), effector memory (EM; CD45RA$^-$/CCR7$^-$), and effector (CD45RA$^+$/CCR7$^-$) subsets. The proportion of each subset was highly heterogenic between subjects. The HVL and LVL subjects showed no significant differences in distribution of T cell subsets except in the proportion of naive CD8$^+$ T cells (data not shown), which cannot secrete cytokines even following PHA stimuli (Fig. 3A).[26]

To investigate whether there are qualitative differences in T cells between HVL and LVL subjects, we analyzed the expression of CD38, Ki67, Bcl2, PD-1, and CTLA-4 as markers of the activation and exhaustion status of T cells, which seems to affect their capacity to produce cytokines (Fig. 3B). In both CD4$^+$ and CD8$^+$ T cells, CM and EM subsets that mainly secrete these cytokines were highly activated (CD38$^+$, Ki67$^+$, and/or Bcl-2$^-$) in HVL subjects compared to LVL subjects (Fig. 3C). Especially in CM subsets of CD4$^+$ T cells, the frequency of exhausted cells (PD-1$^+$ and CTLA-4$^+$) was also significantly higher in HVL subjects compared to LVL subjects ($p < 0.05$ for both comparisons). EM subsets in CD4$^+$ T cells and CM and EM subsets in CD8$^+$ T cells also tended to be highly exhausted, although these differences were statistically insignificant in HVL subjects. These data indicate that memory CD4$^+$ and CD8$^+$ T cells, but not naive and effector subsets, are highly activated and exhausted in HVL subjects.

Poor cytokine production is directly correlated with activation/exhaustion status in memory T cells

As exhausted memory CD8$^+$ T cells fail to produce effector cytokines, such as IL-2, IFN-γ, and TNF-α, upon antigen stimulation,[27,28] we analyzed the relationship between the expression level of activation/exhaustion markers (CD38, Ki67, Bcl2, PD-1, and CTLA-4) on memory CD4$^+$ and CD8$^+$ T cells and the reduced production of cytokines seen in HVL subjects in response to PHA stimulation. The proportions of PD-1$^+$ and CD38$^+$ cells in CM subsets were inversely correlated with the capacity to produce MIP-1α, MIP-1β, RANTES, IFN-γ, and IL-17 (Fig. 4, and data not shown). In the EM subsets, proportions of PD-1$^+$ and CD38$^+$ cells, but not of CTLA4$^+$ cells, were inversely correlated with cytokine production. These data suggest that the compromised productive capacity of Th1-related and IL-17 cytokines is directly associated with persistent activation and exhaustion in memory T cells.

Cytokine production capacity is recovered soon after ART initiation, but memory CD8$^+$ T cells remain activated and exhausted even after prolonged viral suppression by ART

To explore whether the low cytokine production in HVL subjects is a cause or a consequence of high viral load, we compared cytokine production in subjects whose VL had been suppressed by ART for a prolonged period (>22 months) and whose CD4 count was at a similar level to that of HVL and LVL subjects (Tx subjects). In these subjects, production of the cytokines that were decreased in HVL subjects (MIP-1α, MIP-1β, RANTES, IFN-γ, sIL-2R, IL-7, and IL-17) was significantly higher than in HVL subjects, and production of MIP-1α, MIP-1β, sIL-2R, IL-7, and IFN-γ was at a similar level to that seen in LVL and HC subjects (Fig. 1A). Production of RANTES and IL-17 was higher in subjects with long-term viral suppression than in the other groups.

To clarify the relationship between VL and cytokine production capacity, we performed a similar analysis in subjects with dramatic reductions in VL due to recent ART initiation. We measured cytokine production from PBMCs isolated from blood drawn from six HIV-1-infected subjects within 1–2 months after starting ART, when VL had undergone dramatic reduction (mean VL = 440 copies/ml, range 63 to 1100) (Fig. 5A). The levels of cytokines MIP-1α, MIP-1β, RANTES, and IL-7 produced after PHA stimulation were comparable to those seen in subjects with long-term suppression from ART (Fig. 5B). These data indicate that dysfunction of these cytokine production in individuals with high VL is reversible and is recovered soon after the VL reduction.

We also analyzed the activation and exhaustion status of CD4$^+$ and CD8$^+$ T cells in treatment-experienced (Tx) subjects (Fig. 3C). With the sole exception of CTLA-4 expression on the EM subset in CD8$^+$ T cells, proportions of activated (CD38$^+$, Ki67$^+$, and Bcl-2$^-$) cells and exhausted (PD-1$^+$ and CTLA-4$^+$) cells within both memory CD4$^+$ and CD8$^+$ T cell populations were significantly lower in Tx subjects compared to HVL subjects (Fig. 3C).

We next examined the activation/exhaustion status of memory CD4$^+$ and CD8$^+$ cells in Tx subjects compared to uninfected control subjects to determine whether the T cell status can revert to normal status after prolonged viral suppression by ART. The Tx and HC groups did not differ significantly in expression levels of markers in memory CD4$^+$ T cells (Fig. 3C). In contrast, the two groups differed significant in the activation/exhaustion status of memory CD8$^+$ T cells, with higher levels of Ki67, PD-1, and/or CTLA-4 expression and lower levels of Bcl-2 expression, in Tx subjects compared

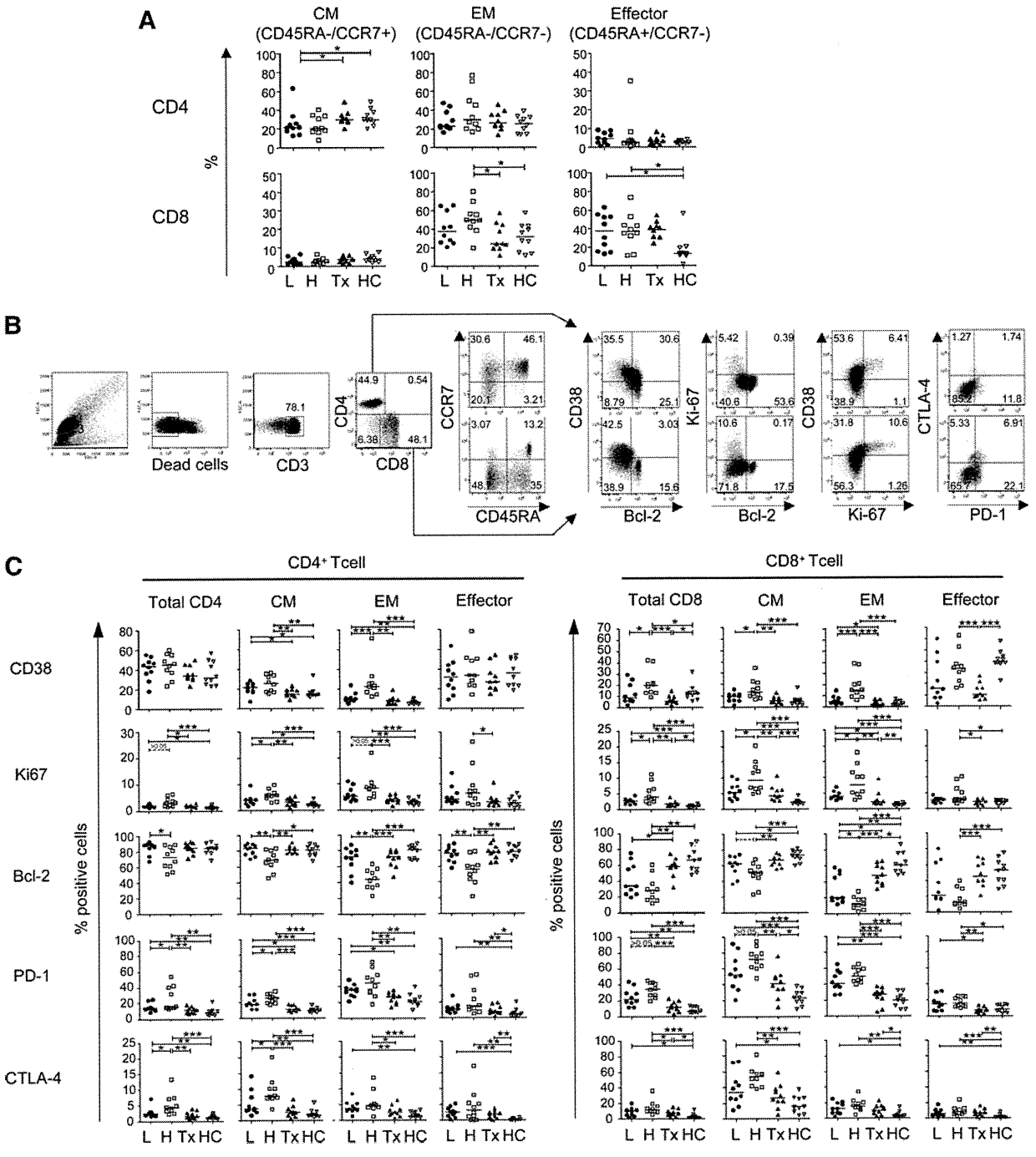

FIG. 3. Differentiation and activation/exhaustion status of $CD4^+$ and $CD8^+$ T cells in HIV-1-infected subjects. **(A)** Comparison of the frequency of $CD4^+$ and $CD8^+$ T cell subsets. The percentages of central memory (CM; $CD45RA^-/CCR7^+$), effector memory (EM; $CD45RA^-/CCR7^-$), and effector ($CD45RA^+/CCR7^-$) subsets in $CD4^+$ and $CD8^+$ T cells are shown. **(B)** Representative flow cytometric analysis of activation (CD38, Ki67, and Bcl2 expression) and exhaustion (PD-1 and CTLA-4 expression) status in $CD4^+$ and $CD8^+$ T cells. **(C)** Comparison of the activation/exhaustion status in $CD4^+$ and $CD8^+$ T cell subsets. L, LVL subjects (●), H, HVL subjects (□), Tx, HIV-1-infected subjects with prolonged ART (▲), HC, healthy control (□). The horizontal bars indicate the median value. Differences between groups were tested for statistical significance by the Mann–Whitney *U* test. $*p = 0.01$ to 0.05, $**p = 0.001$ to 0.01, $***p < 0.001$ (Mann–Whitney test).

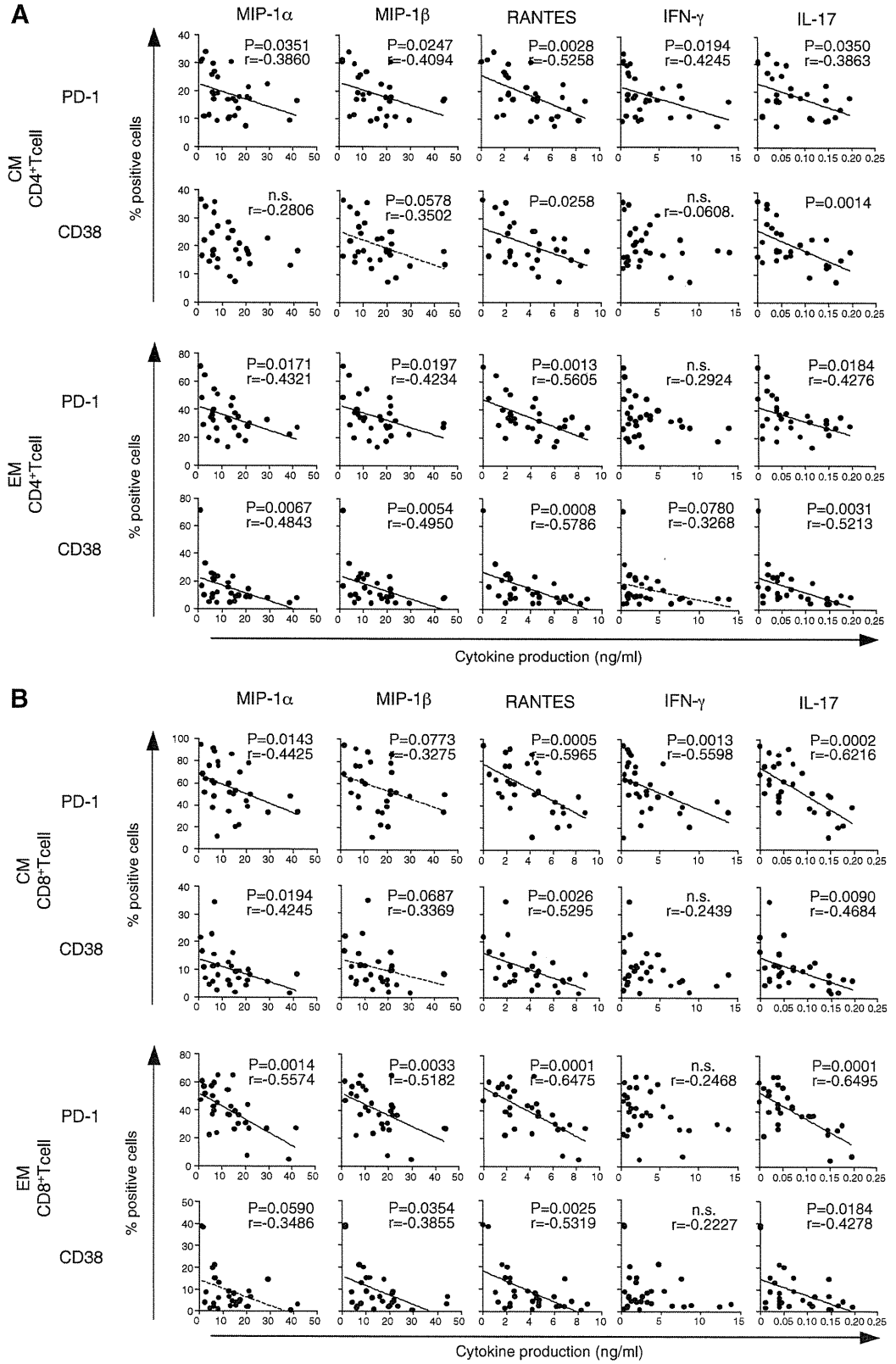

FIG. 4. Correlation between activation/exhaustion status in memory T cell subsets and cytokine production. Each panel indicates the relationship between the frequency of PD-1 or CD38 expressing cells in central memory (CM) and effector memory (EM) T cells and each cytokine production. The results of $CD4^+$ T cells and $CD8^+$ T cells are shown in **(A)** and **(B)**, respectively. Correlation analysis was performed with Spearman's rank correlation to determine correlations between variables.

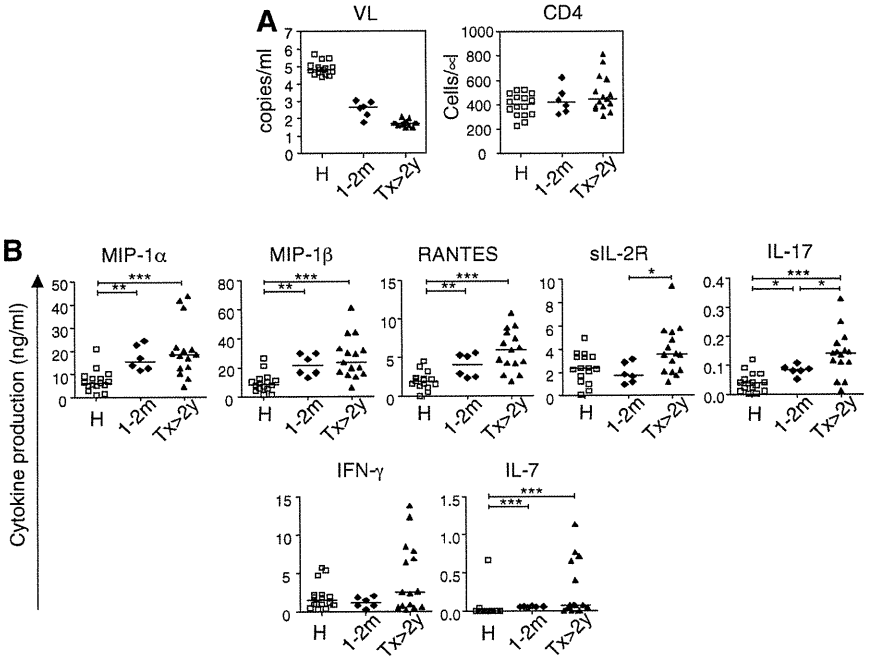

FIG. 5. Rapid recovery of cytokine production after initiation of antiretroviral therapy. **(A)** Viral load and CD4 count of each group. **(B)** Comparison of cytokine production by PHA-stimulated PBMCs. H, HVL subjects (□), 1–2 months, subjects 1–2 months after starting ART (□), Tx>2 years, subjects with prolonged ART (▲). $^{*}p=0.01$ to 0.05, $^{**}p=0.001$ to 0.01, $^{***}p<0.001$ (Mann–Whitney test).

to HC subjects. These data suggest that although suppression of HIV-1 replication by ART dramatically improves the cytokine production capacity of T cells to a normal level, memory $CD8^+$ T cells, but not memory $CD4^+$ T cells, remain somewhat activated even after prolonged viral suppression.

Discussion

Despite intensive research, it remains unclear how HIV-1 can cause the collapse of the host immune system and development of AIDS after chronic infection. In this study, we demonstrate that high HIV-1 viral load associates with skewed T cell dysfunction in cytokine production, independently of CD4 T cell count. Diminished cytokine production in subjects with high VL is specific for some Th1-related cytokines (MIP1-α, MIP-1β, RANTES, and IFN-γ), IL-17, IL-7, and sIL-2R, and is associated with activation and exhaustion status in both $CD4^+$ and $CD8^+$ T cells, especially in memory subsets. The dysfunctional production of these cytokines in HVL subjects appears to be reversible, with recovery occurring after VL reduction by ART.

In this study, we tried to find as many cytokines as possible that differ between LVL and HVL subjects. For this reason, we used a strong stimulus and long incubation times to show the results clearly. The 48-h culture period is long enough to allow expression both of late-response genes and of secondary response genes that may be induced following the primary response.

Production of MIP-1α, MIP-1β, and RANTES was dramatically reduced in HVL subjects and showed a close inverse correlation with plasma VL (Fig. 1). As the natural ligands of HIV-1 coreceptor CCR5, MIP-1α/MIP-1β/RANTES are potent inhibitors of CCR5-tropic HIV-1 (R5-HIV-1) infection.[29] Physiologically, these chemokines also play a key role in induction of cellular immune responses by recruiting $CCR5^+$ Th1 lymphocytes to the infectious site *in vivo*.[30–33] In the case of HIV-1 infection, decreased production of these chemokines seems to favor both viral expansion and reduced migration of effector T cells *in vivo*. In recent studies, a high copy number of CCL3L1 (one of the genes encoding MIP-1α) combined with a low CCR5 expression genotype was associated with low VL in HIV-1-infected subjects,[34,35] suggesting that CCL3L1-CCR5 genotypes may be able to modify the clinical course of HIV-1 infection.

In our study plasma VL affected the ability of T cells to produce IFN-γ, one of the cytokines that defines Th1 cells, and IL-17, which is a Th17-type cytokine. However, no effect was seen on Th2-type cytokines (IL-4, IL-5, and IL-13) or IL-10. Interferon-γ, MIP-1α, MIP-1β,and RANTES are produced by Th1 cells (Fig. 2B), which preferentially express CCR5,[30] and Th17 cells are known to express CCR5 in peripheral blood.[36,37] However, Th2 cells do not express CCR5.[30] Transcription of these cytokines in T cells may be influenced by CCR5 signaling. Large amounts of R5-HIV-1 or the Env protein might persistently trigger the signaling pathway by binding to CCR5, thereby causing reductions in levels of specific cytokines in chronically HIV-1 subjects.

In our experiments, MIP-1α, MIP-1β, and RANTES were produced by IFN-γ-expressing cells in subsets of $CD4^+$ and $CD8^+$ T cells, and IL-17 was produced by a different subset of $CD4^+$ T cells. Surprisingly, IL-17 production was strongly correlated with MIP-1α, MIP-1β, RANTES, and IFN-γ production even though the producer cells are different (Fig. 2F). This correlation might reflect a general ability of Th1 and Th17 cells to produce cytokines. However, IL-2 production was not correlated with MIP-1α, MIP-1β, RANTES, and IFN-γ production, despite the fact that IL-2 should be produced by the same IFN-γ-producing cells (Fig. 2C–F). Critical regions of IFN-γ promoter (i.e., consensus GATA motif and essential functional motif) are not found in the IL-2 promoter region, but are found in the MIP-1α and MIP-1β promoters.[38] In addition, the same sequence in the promoter region of IFN-γ,

MIP-1α, and MIP-1β was found in the IL-17 promoter.[39] Interferon-γ, MIP-1α, MIP-1β, RANTES, and IL-17 production in T cells may be coordinately regulated, and the productive capacity of these cytokines appears to be affected by HIV-1 VL in a similar fashion. Alternatively, we measured cytokine production 48 h after PHA stimulation in this study. The period is long enough to develop sequential reactions occurring in response to primary reaction. As IFN-γ is known as an early-response gene and has the potential to affect multiple immune responses,[40] the production of MIP-1α, MIP-1β, RANTES, and IL-17, but not IL-2 may depend on the amount of IFN-γ as the primary response. Further studies are required to elucidate the mechanism by which IL-17 production is correlated with MIP-1α, MIP-1β, RANTES, or IFN-γ production. The IFN-γ pathway protects against intracellular pathogens through cellular immunity, and IL-17 provides protection against extracellular pathogens and fungal infections.[41,42] Although their target pathogens differ, IL-17 regulates the Th1 immune response through IL-17 receptor-expressing dendritic cells (DC) and macrophages.[43] These data suggest that Th1-type and Th17-type immune responses are closely related, and that their interaction is crucial for immune protection.

In a pathogenic SIV infection model, the loss of Th17 cells in the gastrointestinal tract dampens the intestinal mucosal barrier, resulting in microbial translocation, which in turn induces systemic immune activation.[44–47] In SIV infection the loss of Th17 cells in intestinal mucosa and in PBMCs is inversely correlated with plasma VL.[48] In this study, we observed a strong inverse correlation between IL-17 production and the proportion of activated and exhausted memory T cells. Our results suggest that not only the number of IL-17-producing cells but also the quality of those cells may account for the dysfunction of the Th17-type immune response in HVL subjects.

During chronic HIV-1 infection expression of the inhibitory coreceptors PD-1 and CTLA-4 on total T cells (not only HIV-1-specific T cells) is associated with plasma VL and CD4 count.[11,12] In this study, we found that the proportions of PD-1^+, $CTLA4^+$, and $CD38^+$ cells in total memory subsets of $CD4^+$ and $CD8^+$ T cells were inversely correlated with the ability of T cells to produce MIP-1α, MIP-1β, RANTES, IFN-γ, sIL-2R, and IL-17 in response to PHA stimulation (Fig. 4). It has been reported that PD-1 expression depends on the status of activation markers such as CD38 and on the differentiation stage of T cells.[49,50] Other studies have shown that blocking the pathway of the PD-1/PD-L1 interaction augments the cytokine production capacity of HIV-1-specific $CD4^+$ and $CD8^+$ T cells *in vitro*.[11,51] In our study, prolonged virus suppression by ART resulted in cytokine production capacities returning to normal (Fig. 1A). Memory subsets of $CD4^+$ T cells were no longer activated and exhausted (Fig. 3C), although memory subsets of $CD8^+$ T cells remained slightly activated/exhausted. These data suggest that activation and/or exhaustion of T cells is directly associated with the ability to produce these specific cytokines and that the impairment in T cell function is reversible.

Our study is the first to show that the T cell impairment in high VL subjects is specific for production of some of Th1-type and Th17-type cytokines, and that production of these cytokines is strongly correlated with one another. In subjects with high VL, a vicious cycle occurs, as T cells increasingly lose the capacity to produce these important cytokines. Notably, we also found that subjects who maintain a low VL, yet who are not "elite controllers," are capable of producing normal levels of these cytokines. These findings could be useful in guiding the development of new therapies focusing on immune control to reduce T cell activation in chronic HIV-1 infection.

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Author Disclosure Statement

No competing financial interests exist.

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CASE REPORT

Favourable outcome of progressive multifocal leukoencephalopathy with mefloquine treatment in combination with antiretroviral therapy in an HIV-infected patient

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Summary: A 33-year-old man who developed progressive multifocal leukoencephalopathy (PML) with HIV infection is reported. The patient exhibited rapid decline in neurological status after initiation of antiretroviral therapy (ART), which was attributed to the PML-immune re-constitution inflammatory syndrome. Following the administration of mefloquine in combination with ART, the patient's neurological status improved substantially. This case suggests that further investigation of the use of mefloquine might be warranted for treatment of PML in HIV-infected patients.

Keywords: HIV, AIDS, progressive multifocal leukoencephalopathy, PML, IRIS, mefloquine

CASE REPORT

A 33-year-old HIV-infected man started to develop slurred speech, according to his family, in mid-August 2009. He had never received antiretroviral therapy (ART) and never had opportunistic infections. Upon initial visit to our institution, neurological examination revealed dysarthria and sensory defect of left angle of the mouth. Brain magnetic resonance imaging (MRI) showed multiple hyperintense white matter lesions of the occipital lobes bilaterally on fluid-attenuated inversion-recovery imaging (Figures 1a and b). He was admitted the following week for further work-up. His CD4 T-cell count and plasma HIV-RNA were 269 cells/μL and 8700 copies/mL, respectively. The results of cerebrospinal fluid (CSF) examination were as follows: nucleated cells 12/μL, glucose 3 mmol/L, protein 27 g/L; polymerase chain reaction (PCR) for John Cunningham virus (JCV) was positive. We diagnosed his status as progressive multifocal leukoencephalopathy (PML) from neurological and radiological findings. ART consisting of abacavir/lamivudine and lopinavir/ritonavir was initiated. Upon initiation of ART, he exhibited deterioration of dysarthria and hemiparesis with left upper dominance. In addition, he presented dysphagia, right central pattern facial paresis and sensory aphasia. Repeat brain MRI performed two weeks later showed the progression of the white matter lesion (Figures 1c and d) and mild contrast enhancement, suggesting PML-immune re-constitution inflammatory syndrome (PML-IRIS) (Figures1e and f). He received prednisolone 30 mg/day and a 5-hydroxytryptamine receptor 2a (5HT2a) antagonist, risperidone, was initiated to inhibit JCV propagation.[1] Since his status remained unchanged following these therapies, we tapered the dose of prednisolone and discontinued over the next two weeks. The lack of expected clinical improvement led us to search for unconventional therapy for salvage. We found a report that mefloquine, an antimalarial drug, inhibited JCV replication *in vitro*[2] and decided to add mefloquine after the permission from Institutional Review Board (accession number: 21-32). After initiating mefloquine, no further clinical deterioration was noted. Furthermore, within 30 days, his neurological symptoms started to improve gradually, with the reduction of abnormal lesion volume on MRI (Figures 1g and h). PCR for JCV in the CSF two months later was negative. The patient did not need any rehabilitation and was discharged from our hospital soon after. Risperidone was discontinued after six months of administration. The administration schedule of mefloquine was 250 mg/day for the first three days, and then 250 mg weekly for total of six months. No adverse events were identified during this time and there has been no evidence of clinical PML recurrence.

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DISCUSSION

At present, the main approach to treatment of PML is to restore the host adaptive immune response to JCV. For HIV-infected

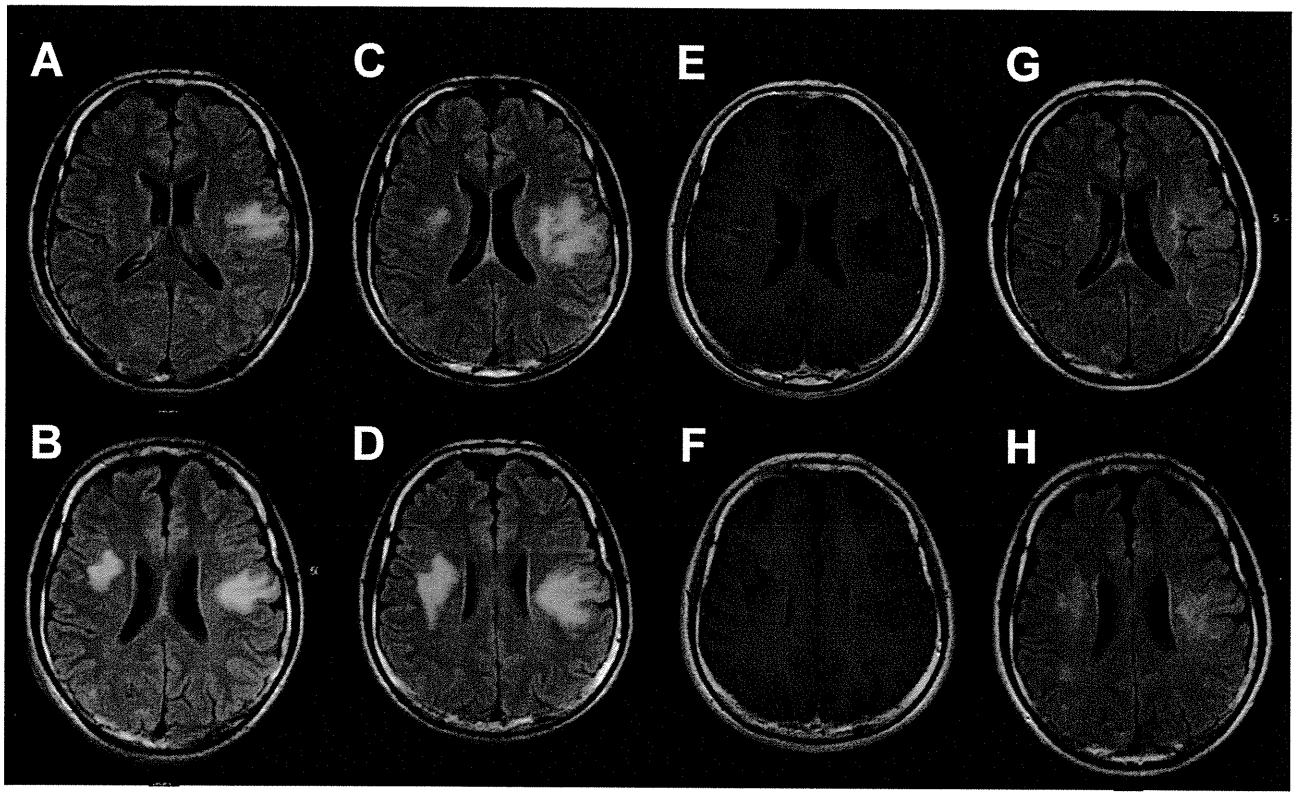

Figure 1 Magnetic resonance imaging (MRI) findings. (a, b) On 28 August 2009, fluid-attenuated inversion-recovery (FLAIR) imaging demonstrates areas of increased signals in the right and left bilateral occipital white matter. (c–f) Follow-up MRI on 14 September 2009. FLAIR imaging shows progression of white matter abnormalities (c, d). T1-weighted image after gadolinium injection shows mild contrast enhancement suggesting progressive multifocal leukoencephalopathy-immune re-constitution inflammatory syndrome (e, f). (g, h) FLAIR imaging shows regression of the white matter lesions in both hemispheres six months after mefloquine administration

PML patients, initiation or optimization of ART is the only therapy that has proven to be effective. However, PML still carries a significant mortality rate. Berenguer *et al.*[3] reported that one-half of the survivors exhibited some partial neurological improvement, but generally neurological deficits persist or progress due to irreversible brain damage. Hence, the search for specific treatment targeting JCV is imperative. Brickelmaier *et al.*[2] recently reported that mefloquine could inhibit replication of JCV in a cell culture system. Although the exact mechanisms by which mefloquine acts against JCV remain unclear, Phase I and II clinical trials have been in progress since September 2008 (ClinicalTrials.gov number, NCT00746941). One case report of non-AIDS-associated PML describes successful treatment with combination of mirtazapine (another 5-hydroxytryptamine receptor a [5HT2a] antagonist, like risperidone) and mefloquine.[4] Another case report of PML after umbilical cord blood transplant described partial clinical improvement after treatment with mefloquine.[5] This case exhibited significant recovery of neurological deficit and improvement of radiological findings after initiation of mefloquine. As both ART and risperidone had also been administered to our patient, it is difficult to determine whether any improvement can be attributed to mefloquine use. In addition, the administration of corticosteroids also complicates the evaluation of mefloquine's efficacy. However, we would like to highlight the substantial improvement of neurological symptoms after initiating mefloquine in spite of rapid deterioration after introduction of ART. In some studies, a higher CD4 T-cell count was associated with long-term survival for PML.[6] The good outcome observed in this case may have been affected by the patient's higher CD4 T-cell count at the time of PML onset.

In this case we were confronted by rapid progression of PML disease after initiation of ART. The neurological symptoms of PML may worsen in the setting of recovery of immune system, termed PML-IRIS. The pathophysiology of PML IRIS is poorly understood, it is speculated that activated CD4 and CD8 T-cells trafficking into the central nervous system are the main mediators of this phenomenon. Although the diagnosis of PML-IRIS can be challenging, contrast-enhancing lesions present in the brain can support this diagnosis. We regarded this case's initial neurological exacerbation as PML-IRIS based on clinical and radiological findings. Although a couple of studies showed a partial benefit of corticosteroids in PML-IRIS,[7] treatment with corticosteroids remains highly controversial.[8] However, in a patient with severe inflammation, we believe the anti-inflammatory effects of corticosteroids are warranted.

To our knowledge, this is the first case report describing potential benefit of mefloquine in an HIV-infected PML patient with IRIS. Although the efficacy and safety of mefloquine therapy awaits validation by ongoing clinical trials, our case demonstrates the potential benefit of mefloquine to improve neurological deficits in PML-IRIS patients.

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Cell Stem Cell
Short Article

Generation of Rejuvenated Antigen-Specific T Cells by Reprogramming to Pluripotency and Redifferentiation

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SUMMARY

Adoptive immunotherapy with functional T cells is potentially an effective therapeutic strategy for combating many types of cancer and viral infection. However, exhaustion of antigen-specific T cells represents a major challenge to this type of approach. In an effort to overcome this problem, we reprogrammed clonally expanded antigen-specific CD8$^+$ T cells from an HIV-1-infected patient to pluripotency. The T cell-derived induced pluripotent stem cells were then redifferentiated into CD8$^+$ T cells that had a high proliferative capacity and elongated telomeres. These "rejuvenated" cells possessed antigen-specific killing activity and exhibited T cell receptor gene-rearrangement patterns identical to those of the original T cell clone from the patient. We also found that this method can be effective for generating specific T cells for other pathology-associated antigens. Thus, this type of approach may have broad applications in the field of adoptive immunotherapy.

INTRODUCTION

T cells play a central role in acquired immunity and the configuration of systemic immunity against pathogens. In particular, cytotoxic T lymphocytes (CTLs) are major components of this systemic response to microorganisms, viral infections, and neoplasms (Greenberg, 1991; Zhang and Bevan, 2011). T cells initiate their proliferative and effector functions upon human leukocyte antigen (HLA)-restricted recognition of specific antigen peptides via T cell receptors (TCRs). This is greatly beneficial in enabling the selective recognition and eradication of target cells, and also in long-term immunological surveillance by long-lived memory T cells (Butler et al., 2011; Jameson and Masopust, 2009; MacLeod et al., 2010). However, viruses in chronic infection or cancers often hamper or escape the T cell immunity by decreasing the expression of molecules required for T cell recognition or by inhibiting antigen presentation (Virgin et al., 2009). In addition, continuous exposure to chronically expressed viral antigens or cancer/self-antigens can drive T cells into an "exhausted" state. This is characterized by loss of effector functions and the potential for long-term survival and proliferation, ultimately leading to the depletion of antigen-responding T cell pools (Klebanoff et al., 2006; Wherry, 2011).

The infusion of ex vivo-expanded autologous antigen-specific T cells is being developed clinically for T cell immunotherapy. However, up to now, highly expanded T cells have not proven to be particularly effective (June, 2007). This is in part explained by losses of function that occur during the ex vivo manipulation of patient autologous T cells. In another instance, genetic modification of antigen receptors is an ambitious but only partially successful way to add desired antigen specificity to nonspecific T cells (Morgan et al., 2006; Porter et al., 2011). The therapeutic effect also strongly depends on the extent of functional loss that occurs during the ex vivo manipulation of T cells and on the stability of exogenous antigen receptor expression specific to target molecules in the presence of the endogenous TCR genes (Bendle et al., 2010; Brenner and Okur, 2009).

For the purpose of overcoming these obstacles, the therapeutic potential of induced pluripotent stem cells (iPSCs) is being