RNA interference assay. siRNA was synthesized chemically at Hokkaido System Science. The sequences of siRNA oligonucleotides were as follows: Tax, 5′-GGCCUUAUUUGGACAUUUATT-3′ and 5′-UAAAUGUCCAAAUAAGGCCTT-3′ (31); Luc, 5′-CGUACGCG-GAAUACUUCGATT-3′ and 5′-UCGAAGUAUUCCGCGUACGTT3′. Next, 100 pmol annealed RNA duplex was transfected using Human T cell Amaxa Nucleofector Kit according to the manufacturer's recommendations (Lonza). 100 pmol Luc siRNA was used as a negative control. Cells were incubated for 48 hours and then harvested and subjected to real-time RT-PCR analysis.

Measurement of IFN-γ. IFN-γ concentration in the culture supernatant was measured with a cytometric bead array kit (BD Biosciences) using a FACSCalibur flow cytometer (BD Biosciences) according to the manufacturer's instructions.

IP. Approximately 1 mg of MT-2 nuclear extracts were incubated with 5 μg anti-Tax, anti-Sp1, or normal IgG coupled with protein G–agarose (Roche Applied Science) in IP buffer (10 mM HEPES [pH 7.9], 100 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 0.1% NP-40, 1 mM Na_3VO_4, 5 mM NaF, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 2 μg/ml pepstatin) for 2 hours. The precipitated proteins were washed with the IP buffer, separated by 10% SDS-PAGE, and immunoblotted with anti-Tax or anti-Sp1 antibodies.

ChIP assay. ChIP assay was performed using a ChIP assay kit (Upstate Biotechnology) with some modifications. Briefly, 5×10^6 MT-2 cells were fixed with 1% formaldehyde at 37°C for 25 minutes and washed twice with PBS. Cells were subsequently harvested and sonicated in lysis buffer. Precleared chromatin samples were immunoprecipitated with 5 μg anti-Tax antibody, anti-Sp1 antibody, or normal IgG for 16 hours at 4°C. Immune complexes were collected with salmon sperm DNA/protein G–sepharose for 90 minutes with rotation, washed, and then incubated at 65°C for 6 hours for reverse cross-linking. Chromatin DNA was extracted and analyzed using PCR with primers for the *TBX21* promoter region (–179 to –59; forward, 5′-GCCAAGAGCGTAGAATTTGC-3′; reverse, 5′-CGCTTT-GCTGTGGCTTTATG-3′) (25, 61). Amplification was performed using ExTaq (Takara Bio) with 1 cycle at 95°C for 5 minutes followed by 30 cycles of 95°C for 30 seconds, 54°C for 30 seconds, and 72°C for 30 seconds. Amplified products were analyzed using 8% polyacrylamide gel electrophoresis.

Luciferase assay. For transient transfection, HEK293 cells were seeded at 5×10^4 cells/well into 24-well plates. After 12 hours, medium was changed to MEM supplemented with 10% FBS and 1% P/S, and each plasmid was transfected with CellPhect Transfection Kit according to the manufacturer's recommendations (GE Healthcare). 50 ng pRSV-βgal plasmid was included in each transfection experiment to control for the efficiency of transfection. The total amount of transfected DNA was kept constant with pcDNA3 in all samples. After 48 hours, cells were lysed with Passive Lysis Buffer (Promega), and luciferase activity was measured using the Promega luciferase assay system and MicroLumat Plus LB96V (Berthold Technologies). Values were normalized to β-galactosidase activity as an internal control.

Tissue staining. Formalin-fixed thoracic spinal cord tissue sections were deparaffinized in xylene and rehydrated in a series of graded alcohols and distilled water. The antigenicity of the tissue sections was recovered using a standard microwave heating technique. For immunofluorescence, the slides were incubated in PBS with 10% goat serum for 1 hour at room temperature, then in anti-CCR4 antibody, anti-T-bet antibody, anti-IFN-γ antibody, and anti-CXCR3 antibody overnight at 4°C, labeled with Alexa Fluor 488– or Alexa Fluor 594–conjugated secondary antibody, and examined under a fluorescence microscope (Nikon eclipse E600 with fluorescence filter Nikon F-FL; Nikon Instech) with rabbit or goat IgG as the negative control. Tissue sections were also stained with H&E.

Immunofluorescence staining and immunofluorescence-FISH. Jurkat cells, MT-2 cells, and cells from the CSF of 3 HAM/TSP patients were attached to slides using a cytospin centrifuge (Thermo Fisher Scientific) and fixed in 4% paraformaldehyde (Wako Pure Chemical Industries) for 30 minutes. The slides were washed with PBS and then pretreated as follows: slides were immersed in room temperature 0.2M HCl for 20 minutes, followed by 0.2% Triton-X/PBS for 10 minutes, and finally 0.005% pepsin/0.1M HCl heated to 37°C for 5 minutes. After pretreatment, the slides were stained using the immunofluorescence Can Get Signal kit (TOYOBO) according the manufacturer's instructions with anti-CCR4 as the primary antibody and Alexa Fluor 488–conjugated anti-goat IgG as the secondary antibody. After again being fixed with 4% paraformaldehyde, cells were incubated with a nick-translated (Spectrum Red) pUC/HTLV-1 DNA probe, first for 5 minutes at 70°C and then overnight at 37°C. Images were obtained under an automated research microscope (Leica DMRA2) and analyzed with CW4000 FISH software (Leica Microsystems).

Proliferation assay. PBMCs from HAM/TSP patients were plated into 96-well round-bottomed plates (1×10^5 cells/well) and cultured without any mitogenic stimuli. Cell proliferation was measured using a ^{3}H-thymidine incorporation assay as described previously (19).

Statistics. Paired 2-tailed Student's *t* test and Wilcoxon test were used for within-group comparisons. Unpaired 2-tailed Student's *t* test or Mann-Whitney *U* test was used for between-group comparisons. 1-way ANOVA was used for multiple comparisons, followed by Dunnett or Tukey test. Friedman test was used for paired multiple comparisons, followed by Dunn test. Statistical analyses were performed using Graphpad Prism 5 (GraphPad Software Inc.). A *P* value less than 0.05 was considered significant.

Study approval. Written informed consent was obtained from all patients before the study, which was reviewed and approved by the Institutional Ethics Committee at St. Marianna University and conducted in compliance with the tenets of the Declaration of Helsinki.

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MAJOR ARTICLE

Mogamulizumab, an Anti-CCR4 Antibody, Targets Human T-Lymphotropic Virus Type 1–infected $CD8^+$ and $CD4^+$ T Cells to Treat Associated Myelopathy

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Background. Human T-lymphotropic virus type 1 (HTLV-1) can cause chronic spinal cord inflammation, known as HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). Since $CD4^+CCR4^+$ T cells are the main HTLV-1 reservoir, we evaluated the defucosylated humanized anti-CCR4 antibody mogamulizumab as a treatment for HAM/TSP.

Methods. We assessed the effects of mogamulizumab on peripheral blood mononuclear cells from 11 patients with HAM/TSP. We also studied how $CD8^+$ T cells, namely $CD8^+$ $CCR4^+$ T cells and cytotoxic T lymphocytes, are involved in HTLV-1 infection and HAM/TSP pathogenesis and how they would be affected by mogamulizumab.

Results. Mogamulizumab effectively reduced the HTLV-1 proviral load (56.4% mean reduction at a minimum effective concentration of 0.01 μg/mL), spontaneous proliferation, and production of proinflammatory cytokines, including interferon γ (IFN-γ). Like $CD4^+CCR4^+$ T cells, $CD8^+CCR4^+$ T cells from patients with HAM/TSP exhibited high proviral loads and spontaneous IFN-γ production, unlike their $CCR4^-$ counterparts. $CD8^+CCR4^+$ T cells from patients with HAM/TSP contained more IFN-γ–expressing cells and fewer interleukin 4–expressing cells than those from healthy donors. Notably, Tax-specific cytotoxic T lymphocytes that may help control the HTLV-1 infection were overwhelmingly $CCR4^-$.

Conclusions. We determined that $CD8^+CCR4^+$ T cells and $CD4^+CCR4^+$ T cells are prime therapeutic targets for treating HAM/TSP and propose mogamulizumab as a new treatment.

Keywords. HTLV-1; HAM/TSP; CCR4; mogamulizumab; CD8.

Human T-lymphotropic virus type 1 (HTLV-1) infects 10–20 million people worldwide, causing HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia/lymphoma (ATL) in a small fraction of infected individuals [1–3]. HAM/TSP is an inflammatory disease of the central nervous system (CNS) that is thought to develop via so-called bystander damage, meaning that the host immune responses to HTLV-1–infected cells in the CNS damage the spinal cord [4]. Currently established treatments for HAM/TSP, such as corticosteroids [5] and interferon alfa [6], do not effectively reduce the HTLV-1 proviral load, which is well correlated with disease severity [7]. Reverse transcriptase inhibitors, which are used against human immunodeficiency virus type 1, were not effective against HTLV-1 in

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clinical trials [8, 9]. These and other existing antiviral drugs usually block the viral replication process [10], but HTLV-1 may escape these drugs by replicating using host cell division [11, 12]. The ideal treatment strategy for HAM/TSP would be selectively targeting and eliminating the HTLV-1–infected cells, but no such treatments currently exist.

Mogamulizumab, a defucosylated humanized anti-CCR4 immunoglobulin G1 (IgG1) monoclonal antibody, was recently approved in Japan as a novel therapy for ATL [13]. Importantly, ATL cells usually express chemokine receptor CCR4 [14]. Mogamulizumab strongly binds to Fcγ receptor IIIa (FcγRIIIa) on natural killer (NK) cells and elicits powerful antibody-dependent cellular cytotoxicity (ADCC) against the $CCR4^+$ ATL cells [15, 16].

Recently, we found that $CD4^+CD25^+CCR4^+$ T cells are the main HTLV-1 reservoir in HAM/TSP [17]. These cells abnormally produce interferon γ (IFN-γ) and are thought to play an important role in producing the chronic inflammation in HAM/TSP [17]. Thus, we began investigating the possibility of treating HAM/TSP and ATL by targeting $CCR4^+$ cells. We have already established that the defucosylated human/mouse chimeric anti-CCR4 antibody KM2760 effectively reduces the HTLV-1 proviral load in cultured peripheral blood mononuclear cells (PBMCs) from patients with HAM/TSP [18]. Here, we evaluate for the first time the effects of the humanized antibody mogamulizumab on cells from patients with HAM/TSP.

There is a population of $CD8^+$ T cells that express CCR4, but these cells have so far received much less attention than $CD4^+$ $CCR4^+$ T cells from HTLV-1 researchers. Although it has been shown that HTLV-1 infects $CD8^+$ T cells [19], it is as of yet unknown which among $CD8^+$ T cells are predominantly infected, as well as whether and how the infection influences the functions of those cells. $CD8^+CCR4^+$ T cells are reported to suppress inflammation and play a beneficial role in controlling chronic inflammatory diseases [20, 21]. It is important to determine whether $CD8^+CCR4^+$ T cells are protective or harmful during HAM/TSP pathogenesis, as well as how these cells would be affected by mogamulizumab.

We hypothesized that $CCR4^+$ cells among $CD8^+$ and $CD4^+$ T cells are highly infected and liable to develop proinflammatory traits. It has been reported that HTLV-1 preferentially transmits to $CCR4^+$ T cells through CCL22 (a CCR4 ligand) production induced by the HTLV-1 protein product Tax [22]. Tax has also been reported to induce IFN-γ production via transcriptional alterations within the infected cells themselves [18].

In the present study, we determined that mogamulizumab is effective at reducing the proviral load and proinflammatory character in PBMCs from patients with HAM/TSP. Next, we revealed that $CD8^+CCR4^+$ T cells are indeed highly infected by HTLV-1 and become proinflammatory. Finally, we determined that the majority of Tax-specific cytotoxic T lymphocytes (CTLs) were $CCR4^-$, indicating that they would not be inadvertently targeted by mogamulizumab. Our results indicate that $CD8^+CCR4^+$ T cells should be considered a key therapeutic target when developing treatments for HAM/TSP and that mogamulizumab represents a viable candidate for such a treatment.

METHODS

Subjects

This study was approved by the Institutional Ethics Committee at St. Marianna University and conducted in compliance with the Declaration of Helsinki. All participants gave written informed consent. Blood samples were obtained from 11 patients with HAM/TSP (8 females and 3 males; median age, 57 years [range, 47–72 years]; proviral load, 4.7 copies/100 cells [range, 1.26–9.71 copies/100 cells]), 8 HTLV-1–positive asymptomatic carriers (6 females and 2 males; median age, 57 years [range, 28–76 years]; proviral load, 4.7 copies/100 cells [range, 2.43–13.19 copies/100 cells]), and 8 HTLV-1–negative healthy volunteers (6 females and 2 males; median age, 59 years [range, 51–72 years]). HTLV-1 seropositivity was determined by a particle agglutination assay (Fujirebio, Tokyo, Japan) and confirmed by Western blot (SRL Inc., Tokyo, Japan). HAM/TSP was diagnosed according to the World Health Organization guidelines [23]. PBMCs were separated by Ficoll-Hypaque density gradient centrifugation (Pancoll; PAN-Biotech, Aidenbach, Germany) and viably cryopreserved in liquid nitrogen with freezing medium (Cell Banker 1; Mitsubishi Chemical Medience Corporation, Tokyo, Japan).

Cell Culture

PBMCs were seeded at 1×10^5 cells/200 μL/well in 96-well round-bottomed plates in the presence or absence of mogamulizumab or KM2760 (gifts from Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan) or human control IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) or prednisolone (LKT Laboratories, Inc., St. Paul, MN) and incubated at 37°C in 5% CO_2. Roswell Park Memorial Institute 1640 medium was supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin and streptomycin antibiotic solution (Wako Pure Chemical Industries Ltd., Osaka, Japan). The supernatants were collected and stored at −80°C. The cells were harvested for DNA extraction or fluorescence-activated cell-sorter (FACS) analysis. The HTLV-1 proviral load was measured using ABI Prism 7500 SDS (Applied Biosystems, Carlsbad, CA), as described previously [24].

Cell Proliferation Assay

PBMCs from patients with HAM/TSP were cultured for 7 days as described above. PBMCs from healthy donors were stimulated with 4 μg/mL of phytohemagglutinin-P (PHA; Sigma-Aldrich, St. Louis, MO) and cultured in the presence or absence of mogamulizumab or prednisolone for 3 days. During the last 16 hours, 1 μCi of ^{3}H-thymidine was added to each well, and then cells were harvested and counted with a β-plate counter (Wallac-Perkin Elmer, Waltham, MA). The assay was performed in triplicate, and average values were used for analysis.

Measurement of Cytokines

The concentrations of 6 cytokines (IFN-γ, interleukin 2 [IL-2], interleukin 4 [IL-4], interleukin 6 [IL-6], interleukin 10 [IL-10], and tumor necrosis factor α [TNF-α]) in culture supernatants were measured with a cytometric bead array kit (BD Biosciences, San Diego, CA), using a FACSCalibur flow cytometer (BD Biosciences).

Flow Cytometric Analysis

Cells were immunostained with various combinations of the following fluorescence-conjugated antibodies to surface antigens: anti-CD3 (UCHT1), anti-CD4 (RPA-T4), anti-CD8 (RPA-T8), anti-CD14 (61D3), and anti-CD19 (HIB19), from eBiosciences (San Diego, CA); and anti-CD56 (B159) anti-CCR4 (1G1), from BD Biosciences. The epitope of the anti-CCR4 antibody (1G1) is different from that of mogamulizumab and KM2760, and thus these treatments do not affect the binding of 1G1 to CCR4 [16]. In some experiments, allophycocyanin-conjugated HLA-A*2402/HTLV-1 Tax301-309 tetramer (Medical & Biological Laboratories, Nagoya, Japan) was used. To identify HTLV-1–infected cells, cells were fixed and permeabilized using a staining buffer set (eBiosciences) and then intracellularly stained with anti-Tax antibody (Lt-4) [25]. To analyze intracellular effector molecules, cells were fixed and stained with the antibodies to perforin (δG9) and granzyme B (GB11; BD Biosciences). For intracellular cytokine staining, PBMCs were stimulated for 6 hours with phorbol 12-myristate 13-acetate (50 ng/mL) and ionomycin (1 μg/mL, Sigma-Aldrich Japan, Tokyo, Japan) in the presence of monensin (GolgiStop, BD Biosciences). After being harvested, the cells were fixed and stained with the antibodies to IFN-γ (B27) and IL-4 (MP4-25D2; BD Biosciences). The stained cells were analyzed using FACSCalibur, and the data were processed using FlowJo software (TreeStar, San Diego, CA). For cell sorting, $CD8^+$ T cells were negatively selected from PBMCs, using MACS beads (Miltenyi Biotec, Bergisch Gladbach, Germany). The purified $CD8^+$ T cells were stained with anti-CD3, anti-CD8, and anti-CCR4 antibodies, and then $CD3^+CD8^+$, $CD3^+CD8^+CCR4^+$, and $CD3^+CD8^+CCR4^-$ T cells were separated using a cell sorter (JSAN, Bay Bioscience Co., Ltd., Hyogo, Japan). The purity exceeded 95%.

Statistical Analysis

Values are expressed as means ± standard deviations. The paired *t* test or the Wilcoxon signed-rank test was used for within-group comparisons. The Mann–Whitney *U* test was used for comparisons between groups. Repeated-measures analysis of variance (ANOVA) followed by the Dunnett test or the Friedman test followed by the Dunn test were used for paired multiple comparisons. Statistical analyses were performed using GraphPad Prism 5 and Prism statistics (GraphPad Software, Inc., La Jolla, CA), and *P* values of <.05 were considered statistically significant.

RESULTS

Mogamulizumab and KM2760 Reduce the HTLV-1 Proviral Load and Inhibit Spontaneous Proliferation of PBMCs From Patients With HAM/TSP

The effects of mogamulizumab and KM2760 were assessed by measuring proviral loads in treated PBMCs from patients with HAM/TSP. ^{3}H-thymidine incorporation was used to assess the inhibitory effects of the antibodies on spontaneous cell proliferation, a distinctive phenomenon associated with PBMCs from HTLV-1–infected individuals by which the cells proliferate without mitogens or stimuli in vitro [26]. Mogamulizumab and KM2760 both reduced proviral load in a dose-dependent manner at concentrations of ≥0.01 μg/mL (mean reduction [±SD], 56.4% ± 21.1% and 61.1% ± 17.0%, respectively; $P < .01$ and $P < .001$, respectively; Figure 1*A*). Notably, there was a mean reduction (±SD) of 66.4% ± 20.2% in the proviral load with 10 μg/mL mogamulizumab ($P < .001$), which is the blood concentration of the antibody in patients with ATL treated with 1 mg/kg mogamulizumab [13]. Mogamulizumab and KM2760 similarly inhibited spontaneous proliferation in a dose-dependent manner at concentrations of ≥0.01 μg/mL (mean inhibition [±SD], 25.6% ± 31.9% and 22.1% ± 35.9%, respectively; $P < .01$ and $P < .05$, respectively; Figure 1*B*). Because mogamulizumab and KM2760 showed such similar results, only mogamulizumab was used in the next experiments as representative of the 2. Mogamulizumab was also tested against human IgG to control for nonspecific antibody effects and more effectively reduced the proviral load and spontaneous proliferation (Supplementary Figure 1*A* and 1*B*). Mogamulizumab reduced the HTLV-1 proviral load in cells from asymptomatic carriers, as well, in a dose-dependent manner (Figure 1*C*). Finally, mogamulizumab inhibited PHA-stimulated proliferation of PBMCs from healthy donors at concentrations of ≥0.1 μg/mL ($P < .01$), but the effects of prednisolone were much more pronounced than those of mogamulizumab (prednisolone vs mogamulizumab, $P < .001$; Figure 1*D*).

Prednisolone suppressed spontaneous proliferation (mean inhibition [±SD], 37.4% ± 35.2%; $P < .001$; Figure 1*B*) but did not decrease proviral load (Figure 1*A*). The combination of mogamulizumab and 0.1 μg/mL of prednisolone, which corresponds to the serum concentration achieved when 5 mg of prednisolone is administered orally [27], reduced proviral load as much as but no more than did mogamulizumab alone (Supplementary Figure 2*A*). On the other hand, ^{3}H-thymidine incorporation was substantially more inhibited by the combination treatment than with mogamulizumab alone (mean inhibition [±SD], 81.3% ± 18.3% vs 71.3% ± 19.3%; $P = .01$; Supplementary Figure 2*B*).

Mogamulizumab and KM2760 Inhibit Proinflammatory Cytokine Production in PBMCs From HAM/TSP Patients

Here we examined the effects of mogamulizumab and KM2760 on cytokine production in PBMCs from patients with HAM/

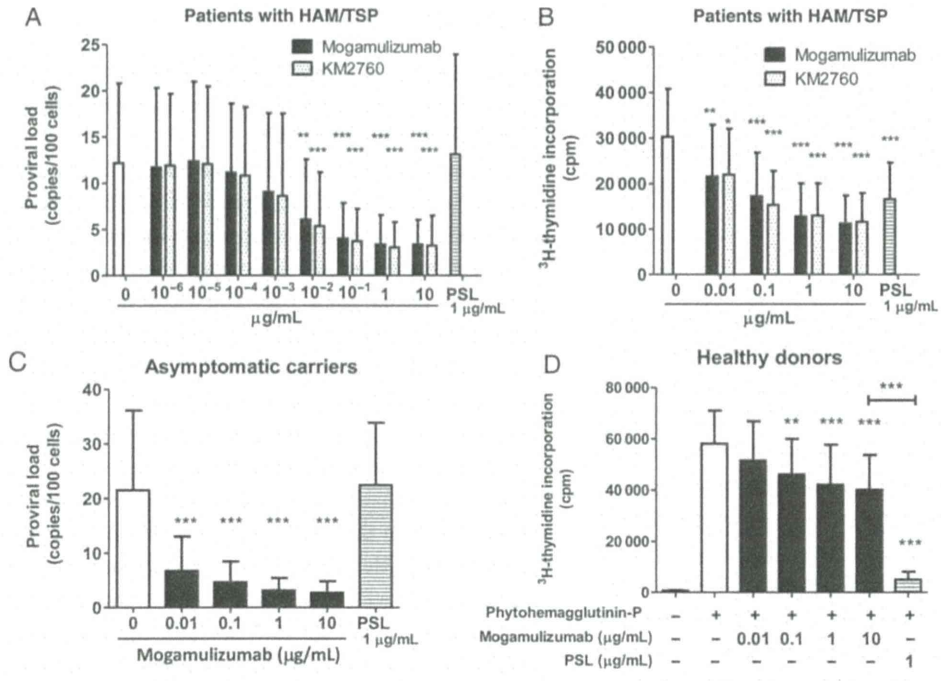

Figure 1. Mogamulizumab and KM2760 reduce the human T-lymphotropic virus type 1 (HTLV-1) proviral load and inhibit spontaneous proliferation of peripheral blood mononuclear cells (PBMCs) from patients with HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). *A* and *B*, PBMCs from 11 patients with HAM/TSP were cultured for 7 days without stimuli and without treatment or in the presence of mogamulizumab, KM2760, or prednisolone (PSL). Cells were harvested, and the proviral load was measured using real-time polymerase chain reaction (*A*). ^{3}H-thymidine was added during the last 16 hours of culturing. Cells were then harvested and analyzed for ^{3}H-thymidine incorporation (*B*). Because mogamulizumab and KM2760 were equally effective, only mogamulizumab was used thereafter as representative of the 2. *C*, PBMCs from 8 asymptomatic carriers were cultured for 7 days without treatment or in the presence of mogamulizumab or PSL, and the proviral load was measured as described above. *D*, PBMCs from 8 healthy donors were stimulated with 4 μg/mL of phytohemagglutinin-P and cultured for 3 days without treatment or in the presence of mogamulizumab or PSL. ^{3}H-thymidine incorporation was analyzed as described above. Data are presented as the mean ± SD. Statistical analyses were performed using repeated-measures analysis of variance, followed by the Dunnett test, for comparison with PBMCs alone (*A–C*) or with PBMCs stimulated with PHA (*D*). The paired *t* test was used to compare 10 μg/mL of mogamulizumab and PSL (*D*). **P* < .05, ***P* < .01, and ****P* < .001. Abbreviation: SD, standard deviation.

TSP. In line with previous reports [28], PBMCs produced various cytokines, most notably IFN-γ, in 7-day cultures without stimuli (Figure 2*A*). Mogamulizumab and KM2760 both reduced the production of the proinflammatory cytokines IFN-γ, IL-6, IL-2, and TNF-α, as well as the immunosuppressive cytokine IL-10 (Figure 2*B–F*). Mogamulizumab reduced IFN-γ production more than did human IgG (Supplementary Figure 1*C*). Prednisolone at a concentration of 1 μg/mL effectively reduced IFN-γ and TNF-α but not IL-2, IL-6, or IL-10 production.

Mogamulizumab Eliminates CCR4^{+} Cells Among Both CD4^{+} and CD8^{+} T cells

Mogamulizumab effectively eliminated the CD4^{+}CCR4^{+} T cells in cultured PBMCs from patients with HAM/TSP (Figure 3*A* and 3*B*). FACS analysis also revealed a population of CD4^{-} CCR4^{+} cells similarly affected by the antibody, and these cells were found to be CD8^{+} T cells (Figure 3*C*). Detailed investigation confirmed that CCR4^{+} T cells among the CD8^{+} subset were eliminated by mogamulizumab just as they were from the CD4^{+} subset (Figure 3*D–E*).

The ADCC Activity of Mogamulizumab Is Fast Acting and Specific

FACS analysis showed that mogamulizumab reduced the frequency of CCR4^{+} T cells among both CD4^{+} and CD8^{+} subsets within 6 hours ($P = .0003$ and $P = .004$, respectively), with a similar reduction in Tax^{+} T cells observed within 24 hours ($P = .01$ and $P = .03$, respectively; Figure 4*A–C* and Supplementary Figure 3). By contrast, mogamulizumab did not reduce the frequency of B cells, NK cells, or monocytes after 24 hours (Figure 4*D*).

CD8^{+}CCR4^{+} T Cells From Patients With HAM/TSP Are Numerous and Highly Infected by HTLV-1

CD8^{+}CCR4^{+} T cells were then further analyzed to assess their role in HAM/TSP and predict the potential benefits and risks of eradicating them with mogamulizumab. Samples from patients with HAM/TSP, compared with those from age-matched healthy donors, contained a higher proportion of CCR4^{+} cells among both the CD4^{+} T-cell subset ($P = .003$) and the CD8^{+} T-cell subset ($P = .02$; Figure 5*A*). In addition, the proviral

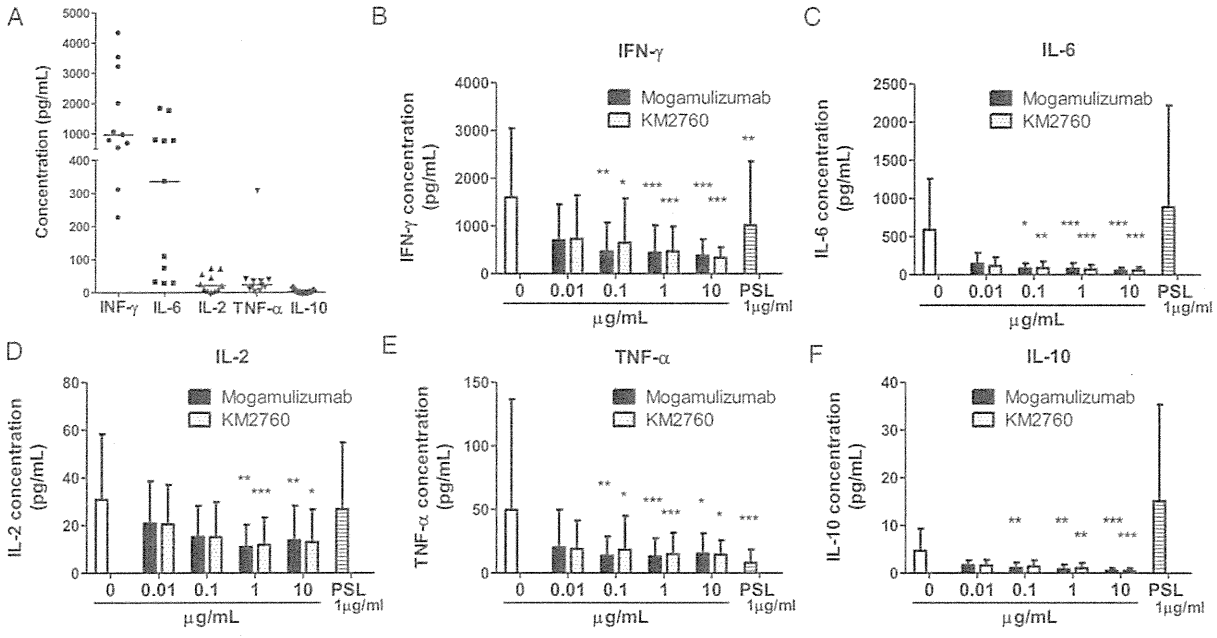

Figure 2. Mogamulizumab and KM2760 inhibit cytokine production in peripheral blood mononuclear cells (PBMCs) from patients with human T-lymphotropic virus type 1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). PBMCs from 11 patients with HAM/TSP were cultured for 7 days without stimuli and without treatment or in the presence of mogamulizumab, KM2760, or prednisolone (PSL). The concentrations of cytokines (interferon γ [IFN-γ], interleukin 6 [IL-6], interleukin 2 [IL-2], tumor necrosis factor α [TNF-α], and interleukin 10 [IL-10]) in the supernatants were then measured. *A*, Direct comparison of the concentrations of these cytokines in the supernatants of untreated PBMC cultures. Horizontal bars represent the median values. *B–F*, The effects of the treatments on the concentrations of these cytokines. Data are presented as the mean ± SD. Statistical analyses were performed using the Friedman test followed by the Dunn test for comparison with PBMCs alone. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. Abbreviation: SD, standard deviation.

load was significantly higher in $CD8^+CCR4^+$ T cells than in $CD8^+CCR4^-$ T cells (mean load [±SD], 13.6 ± 7.9 copies/100 cells and 0.72 ± 0.65 copies/100 cells, respectively; $P = .0002$; Figure 5*B*).

$CD8^+CCR4^+$ T Cells From Patients With HAM/TSP Possess Proinflammatory Properties

Here we investigated the functional differences between $CD8^+CCR4^+$ T cells from patients with HAM/TSP and those from healthy donors. $CD8^+CCR4^+$ cells from both groups expressed minimal perforin and granzyme B (Figure 5*C* and 5*D*). In the $CD8^+CCR4^-$ T-cell subset, the frequency of perforin-expressing cells was unremarkable, but the frequency of granzyme B–expressing cells was higher in patients with HAM/TSP than in healthy donors ($P = .04$; Figure 5*C* and 5*D*). Next, cytokine expression was evaluated in PBMCs stimulated with phorbol 12-myristate 13-acetate and ionomycin in the presence of monensin. Interestingly, samples from patients with HAM/TSP included more IFN-γ–producing cells ($P = .02$; Figure 5*E*) but fewer IL-4–producing cells ($P = .01$; Figure 5*F*) in the $CD8^+CCR4^+$ T-cell subset than did samples from healthy donors. On the other hand, there were no such significant differences among $CD8^+CCR4^-$ T cells (Figure 5*E* and 5*F*). Finally, the concentrations of cytokines in the supernatants of unstimulated cultures of isolated total $CD8^+$, $CD8^+CCR4^-$, and $CD8^+CCR4^+$ T cells were measured to assess spontaneous cytokine production in these cell populations. Spontaneous IFN-γ production, like spontaneous proliferation, is a hallmark of PBMCs from patients with HAM/TSP [29, 30]. Unsurprisingly, IFN-γ was detected in no cell population from healthy donors. Among samples from patients with HAM/TSP, $CD8^+CCR4^+$ T cells produced remarkably more IFN-γ than did $CD8^+CCR4^-$ cells (mean level [±SD], 364.0 ± 445.3 pg/mL vs 1.9 ± 4.5 pg/mL; $P = .001$; Figure 5*G*). IL-4 was not detected in any of the samples (data not shown).

The Majority of HTLV-1 Tax-Specific Cytotoxic T Lymphocytes Are $CCR4^-$

We analyzed CCR4 expression in Tax-specific CTLs to determine whether CTLs against HTLV-1 also become targets of mogamulizumab. Among the 11 patients studied, 7 had HLA-A*2402 and were analyzed using the HLA-A*2402/HTLV-1 Tax301-309 tetramer. The majority of Tax-specific CTLs did not express CCR4, and the percentage of $CCR4^+$ cells was lower in CTLs than in total $CD8^+$ T cells (mean frequency [±SD], 2.3% ± 1.0% and 8.5 ± 4.7%, respectively; $P = .02$; Figure 6).

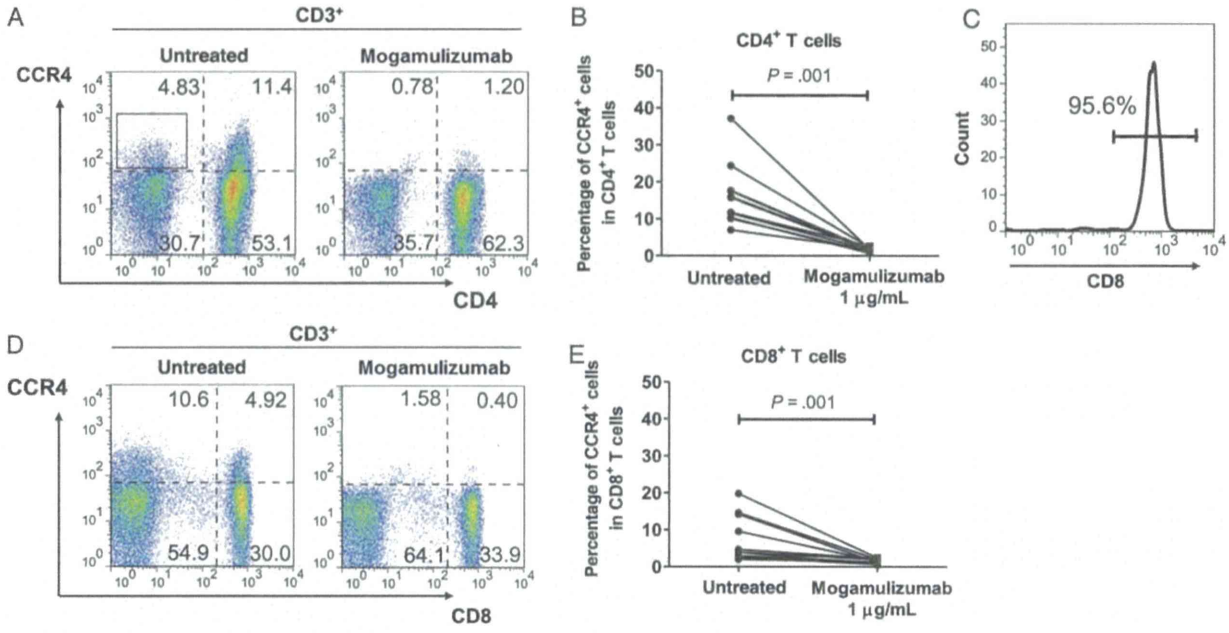

Figure 3. Mogamulizumab eliminates $CCR4^+$ cells in both $CD4^+$ and $CD8^+$ T cells. *A*, Representative dot plots of fluorescence-activated cell-sorter analysis of CCR4 and CD4 expression in $CD3^+$ T cells among peripheral blood mononuclear cells from patients with human T-lymphotropic virus type 1–associated myelopathy/tropical spastic paraparesis after 5-day culture in the presence or absence of 1 μg/mL of mogamulizumab. *B*, Percentages of $CCR4^+$ cells in $CD3^+CD4^+$ T cells were compared between the untreated and mogamulizumab groups (n = 11). Statistical analysis was performed using the Wilcoxon signed-rank test. *C*, The population enclosed in the box in panel *A* (the $CD3^+CD4^-CCR4^+$ subset) was gated and analyzed for the expression of CD8. The percentage of $CD8^+$ cells is shown. *D* and *E*, CCR4 and CD8 expression in $CD3^+$ T cells was analyzed as described above.

DISCUSSION

In this study, we established mogamulizumab as a novel candidate treatment for HAM/TSP that targets infected cells by marking $CCR4^+$ T cells for elimination. Mogamulizumab reduced the number of infected cells, as measured via the proviral load, and thus inhibited the excessive immune responses such as spontaneous proliferation and proinflammatory cytokine production that are attributed to those infected cells (Figures 1 and 2). Effects of mogamulizumab-induced ADCC activity were detectable by FACS after as little as 6 hours of culturing (Figure 4*A*–*C*).

The remaining proviral load after mogamulizumab therapy was higher than expected (mean load [±SD], 3.25 ± 2.58 copies/100 cells; Figure 1*A*). $CD4^+CCR4^-$ T cells [18] and $CD8^+CCR4^-$ T cells (Figure 5*B*) from patients with HAM/TSP were predominantly uninfected, and the antibody therapy should have destroyed the vast majority of the infected $CCR4^+$ T cells (Figure 3), yielding an expected proviral load of <1.0 copy/100 cells. It is possible that some $CCR4^-$ T cells became infected while the samples were being cultured, which is a potential limitation of such in vitro experiments.

The inhibitory effects of mogamulizumab on PHA-stimulated proliferation in PBMCs from healthy donors were statistically significant but still minimal, compared with those of prednisolone (Figure 1*D*), indicating that mogamulizumab, in contrast to immunosuppressive agents, acts via specific reduction of infected cells rather than via nonspecific immune suppression. Interestingly, prednisolone was considerably less effective at suppressing the proliferation of T cells from patients with HAM/TSP (Figure 1*B*) than from healthy donors. Although we cannot be sure of the reasons behind this discrimination, it appears that spontaneous proliferation is less vulnerable to suppression by steroids since it is not a simple T-cell response to antigens [31, 32]. Nevertheless, compared with mogamulizumab alone, the combination with prednisolone enhanced the suppressive effect of mogamulizumab on spontaneous proliferation without hampering proviral load reduction (Supplementary Figure 2).

Mogamulizumab also reduced the proviral load in PBMCs from asymptomatic carriers (Figure 1*C*), which suggests that it can be administered as a preventive treatment to asymptomatic carriers with high proviral loads who are at risk for developing HAM/TSP or ATL. It is well established that high proviral load is associated with the onset and progression of HAM/TSP [7, 33], as well as with the development of ATL [34].

It was well known that although the main reservoir for HTLV-1 is $CD4^+$ T cells, the virus also infects $CD8^+$ T cells in patients

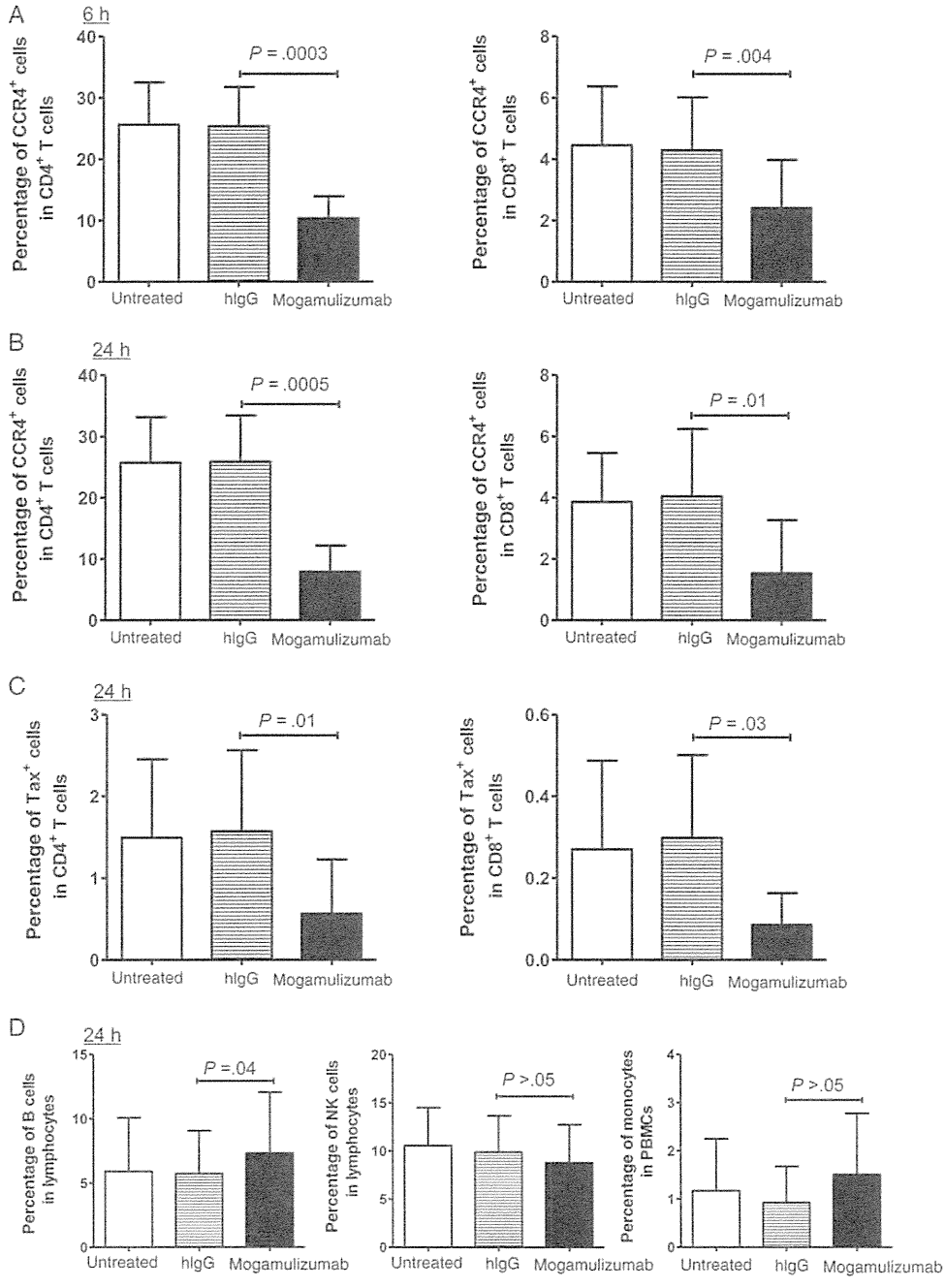

Figure 4. The antibody-dependent cellular cytotoxicity activity of mogamulizumab is fast acting and specific. Peripheral blood mononuclear cells (PBMCs) from 6 patients with human T-lymphotropic virus type–associated myelopathy/tropical spastic paraparesis were cultured in the presence of 1 μg/mL of mogamulizumab or human immunoglobulin G (hIgG) or without treatment. $CD4^+$ and $CD8^+$ T cells were analyzed using fluorescence-activated cell-sorter analysis, and the frequencies of $CCR4^+$ cells after 6 hours (*A*) and 24 hours (*B*), as well as that of Tax^+ cells (*C*) after 24 hours, are shown here. The frequencies of $CD19^+$ B cells and $CD3^-CD56^+$ natural killer (NK) cells among lymphocytes, as well as $CD14^+$ monocytes among PBMCs after 24 hours are also shown (*D*). Data are presented as the mean ± SD. The paired *t* test was used to compare the effects of mogamulizumab and hIgG. Abbreviation: SD, standard deviation.

with HAM/TSP [19]. Here we revealed for the first time that the overwhelming majority of infected $CD8^+$ T cells also expressed CCR4 (Figure 5*B*). Our findings in this study suggest that it is important to eliminate both $CD4^+CCR4^+$ and $CD8^+CCR4^+$ T-cell subsets because both have elevated proviral loads and a tendency to develop proinflammatory traits (Figure 5).

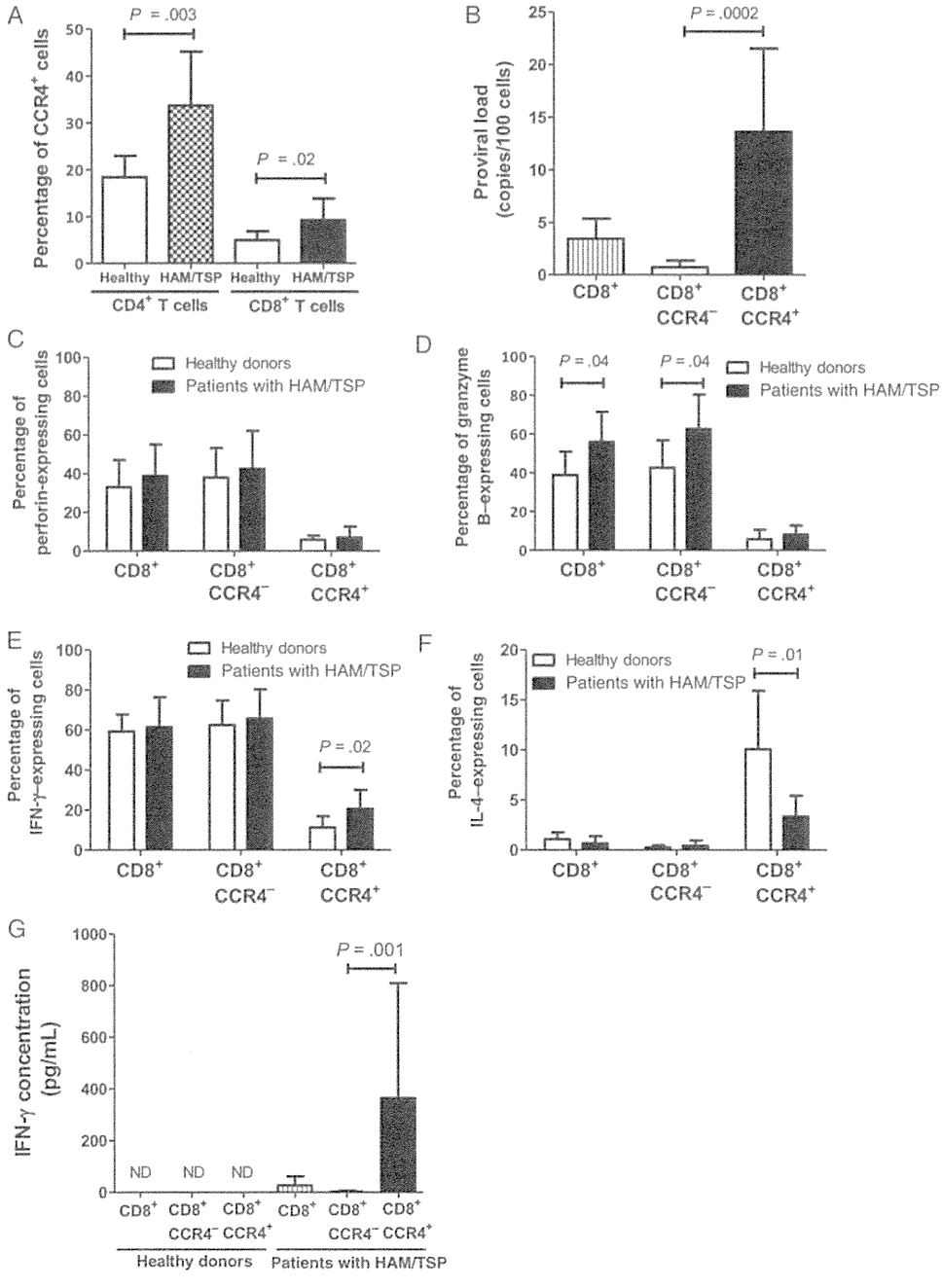

Figure 5. $CD8^+CCR4^+$ T cells from patients with human T-lymphotropic virus type 1 (HTLV-1)–associated myelopathy/tropical spastic paraparesis (HAM/TSP) are numerous, highly HTLV-1 infected, and proinflammatory. *A*, Proportions of $CCR4^+$ cells among $CD4^+$ and $CD8^+$ T cells in 8 healthy donors and 11 patients with HAM/TSP were analyzed by fluorescence-activated cell-sorter (FACS) analysis. *B*, The HTLV-1 proviral load in total $CD3^+CD8^+$, $CD3^+CD8^+CCR4^-$, and $CD3^+CD8^+CCR4^+$ T-cell subsets. $CD8^+$ T cells from 11 patients with HAM/TSP were isolated using negative separation with magnetic beads, and then $CD3^+CD8^+$, $CD3^+CD8^+CCR4^-$ and $CD3^+CD8^+CCR4^+$ T cells were separated with FACS analysis. Proviral loads in each subset were measured using real-time polymerase chain reaction. *C–F*, Peripheral blood mononuclear cells (PBMCs) from 8 healthy donors and 11 patients with HAM/TSP were stained for CD8 and CCR4, as well as intracellular perforin or granzyme B, and analyzed using FACS analysis. PBMCs from the same individuals were stimulated with phorbol 12-myristate 13-acetate (50 ng/mL) and ionomycin (1 μg/mL) in the presence of monensin for 6 hours. The cells were then analyzed for CD8, CCR4, and intracellular interferon γ (IFN-γ) or interleukin 4 (IL-4) expressions. The percentages of perforin-expressing (*C*), granzyme B–expressing (*D*), IFN-γ–expressing (*E*), and IL-4–expressing (*F*) cells in total $CD8^+$, $CD8^+CCR4^-$, and $CD8^+CCR4^+$ T-cell subsets from healthy donors versus patients with HAM/TSP are shown. *P* values are indicated only when <.05. *G*, $CD3^+CD8^+$, $CD3^+CD8^+CCR4^-$, and $CD3^+CD8^+CCR4^+$ T cells were isolated from 6 healthy donors and 11 patients with HAM/TSP as described above. These cells (3×10^4 cells/well) were cultured for 3 days without stimuli, and the concentration of IFN-γ in the supernatants was measured. Statistical analysis was performed using the Mann–Whitney *U*-test (*A* and *C–F*) or the Wilcoxon signed-rank test (*B* and *G*). Data are presented as the mean ± SD. Abbreviation: SD, standard deviation.

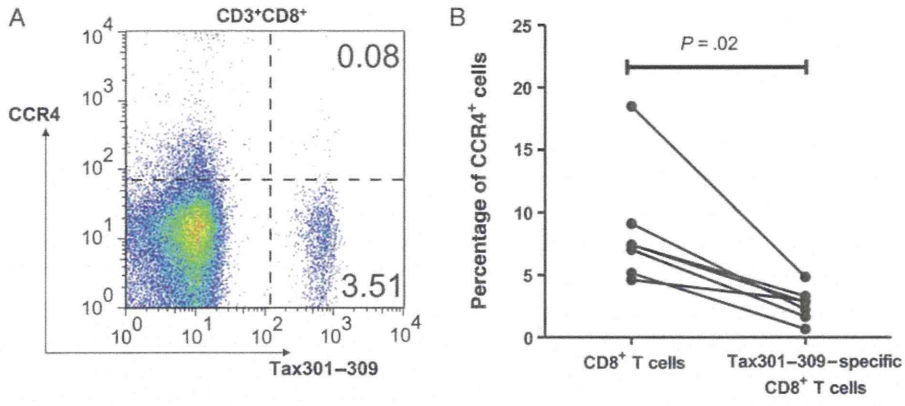

Figure 6. $CD8^+CCR4^+$ T cells from patients with human T-lymphotropic virus type 1 (HTLV-1)–associated myelopathy/tropical spastic paraparesis (HAM/TSP) do not include many Tax-specific cytotoxic T lymphocytes (CTLs). *A*, A representative dot plot of fluorescence-activated cell-sorter (FACS) analysis of the expression of CCR4 in HTLV-1 Tax-specific CTLs. Peripheral blood mononuclear cells from a patient with HAM/TSP and HLA-A*2402 were stained with antibodies for CD3, CD8, CCR4, and HLA-A*2402-restricted Tax301-309–specific tetramer. The $CD3^+CD8^+$ subset was gated. The values in the upper and lower right quadrants indicate the percentages of $CCR4^+$ and $CCR4^-$ Tax-specific CTLs among $CD3^+CD8^+$ T cells, respectively. *B*, Proportions of $CCR4^+$ cells among total $CD8^+$ T cells and Tax301-309–specific $CD8^+$ T cells are compared (n = 7). Statistical analysis was performed using the Wilcoxon signed-rank test.

$CD8^+CCR4^+$ T cells normally produce IL-4 more often than IFN-γ and hardly produce any cytotoxic granules [35, 36]; these cells are thought to be protective against type 1-skewed inflammation [21, 37]. In patients with HAM/TSP, these $CD8^+CCR4^+$ but not $CD8^+CCR4^-$ T cells are altered to produce IFN-γ rather than IL-4 (Figure 5*E* and 5*F*). $CD8^+CCR4^+$ T cells cultured alone exhibited spontaneous IFN-γ production (Figure 5*G*), a hallmark of PBMCs from patients with HAM/TSP [29, 30]. These results suggest that abnormal cells contributing to the pathogenesis of HAM/TSP exist not only among $CD4^+CCR4^+$ T cells but also among $CD8^+CCR4^+$ T cells. It is thought that the functional abnormalities of these cells may arise through transformations occurring within the infected cells themselves, whereby HTLV-1 Tax induces transcriptional alterations via T box transcription factor [18].

In the present study, HTLV-1 infection did not influence cytotoxic granule production in $CD8^+CCR4^+$ T cells (Figure 5*C* and 5*D*). The slightly increased fraction of granzyme B^+ cells in $CD8^+CCR4^-$ T cells from patients with HAM/TSP is presumably attributable to the immune activation resulting from the chronic viral infection [38–40].

Although eliminating the abnormal immune responses of the infected cells should alleviate inflammation and related symptoms of the infection, it is also true that immune responses against HTLV-1 are important for controlling said infection [41]. We evaluated CCR4 expression in HTLV-1 Tax-specific CTLs for fear that use of mogamulizumab might inadvertently destroy CTLs that would have helped to control the infection [42, 43]. Since Tax-specific CTLs have been reported to be preferentially infected by HTLV-1 [44], there was some concern that our finding that infected $CD8^+$ T cells are predominantly $CCR4^+$ meant that these CTLs would also be targeted by mogamulizumab. However, we found that the majority of Tax-specific CTLs do not express CCR4 (Figure 6), meaning that they should essentially be spared during mogamulizumab treatment.

Also concerning is that mogamulizumab is expected to target $CD4^+CCR4^+$ regulatory T (Treg) cells [45], which could elicit autoimmune problems and even exacerbate the chronic inflammation plaguing patients with HAM/TSP. However, there are also $CCR4^-$ Treg cells [45], which would be spared, and there have been no reports of increased incidence of autoimmune disease in patients with ATL treated with mogamulizumab. Furthermore, reducing the number of Treg cells may benefit patients with HAM/TSP by preventing abundant Treg cells from dampening immune control of the HTLV-1 infection [28, 46].

We expect that eliminating HTLV-1–infected cells in the peripheral blood with mogamulizumab would reduce the number of proinflammatory cells and mitigate the inflammation in the CNS. Although HAM/TSP is a disease of the CNS, recent reports suggest that it is indeed effective to target HTLV-1–infected cells in the peripheral blood because continued migration of infected cells from the peripheral blood maintains and even exacerbates the inflammation in the CNS [30].

Based on the results of this study, we have begun conducting a clinical trial to test the efficacy of mogamulizumab on patients with HAM/TSP (UMIN000012655). Our data suggest that as little as one thousandth of the dose administered to patients with ATL (1 mg/kg body weight [13]) may be effective for patients with HAM/TSP. In contrast to patients with an aggressive cancer such as ATL, those with a chronic inflammatory disorder like HAM/TSP would benefit from a more conservative approach that is safer but still effective.

In conclusion, we have demonstrated that mogamulizumab shows promise as a novel treatment for HAM/TSP. Our results indicate that $CD8^+CCR4^+$ T cells and $CD4^+CCR4^+$ T cells are key therapeutic targets and, thus, that the CCR4-targeting therapy mogamulizumab can be expected to effectively ameliorate chronic inflammation in patients with HAM/TSP. The lack of success with classic antiviral therapies [8, 9] suggests that blocking viral replication is ineffective against HTLV-1, which mainly spreads by cell division [11, 12]. Targeting the infected cells themselves on the basis of their characteristic markers may be the key to combating this tricky virus. If successful, mogamulizumab would become the first treatment for a chronic viral infection that effectively targets infected cells.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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J. Y. performed most of the experiments, performed data analysis, created the figures, and wrote the manuscript. A. C. R. performed data interpretation and wrote the manuscript. T. S., N. A., N. Y., H. A., Y. K., and K. T. performed data analysis and interpretation. Y. T., Y. S., K. N., T. N., Y. H., A. U., and K. K. reviewed and edited the manuscript. Y. Y. developed the project, performed data analysis, and wrote the manuscript. All authors approved the final manuscript.

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Potential conflicts of interest. Y. Y. has 1 established patent and another pending for the use of anti-CCR4 antibodies as a treatment for HAM/TSP. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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NEW DEVELOPMENT FROM ASIA

Positive feedback loop through astrocytes causes chronic inflammation in human T-lymphotropic virus type 1-associated myelopathy/tropical spastic paraparesis

Human T-lymphotropic virus type 1 (HTLV-1) is a retrovirus infecting 10–20 million people worldwide, 2–3% of whom develop the chronic spinal cord inflammation that characterizes HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).[1] Evidence suggests that HTLV-1-infected CD4+ T cells migrate across the blood–brain barrier (BBB) and secrete pro-inflammatory cytokines, such as interferon-gamma (IFN-γ), within the central nervous system.[2] The present authors and others have previously shown that the chemokine CXC motif ligand 10 (CXCL10), which binds the CD4+ T helper type 1 (Th1) receptor CXC motif receptor 3 (CXCR3), stands out as particularly elevated in the cerebrospinal fluid (CSF) of HAM/TSP patients and is well-correlated with disease progression.[3] We therefore hypothesized that chemokines, namely CXCL10, play an important role in the pathogenesis of HAM/TSP by continuously recruiting pro-inflammatory cells to the CNS.

We first confirmed that the CSF of HAM/TSP patients contains extraordinarily high levels of CXCL10 and CXCR3+ cells.[4] Importantly, the levels of CXCL10 were much higher in the CSF than the

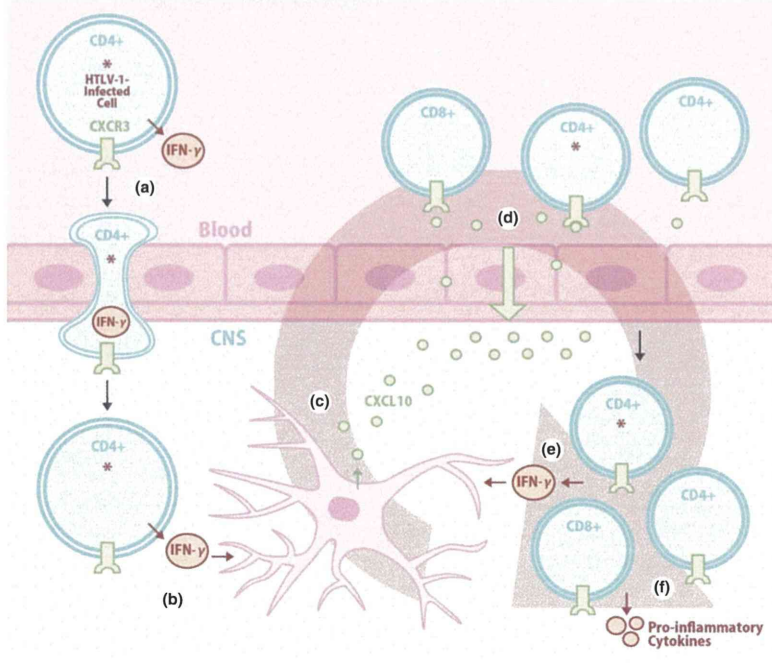

Figure 1 Human T-lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) pathogenesis comprises an inflammatory positive feedback loop. (a) HTLV-1-infected interferon-gamma (IFN-γ)-producing CD4+ Th1 cells migrate across the blood–brain barrier into the central nervous system, where (b) they produce IFN-γ, (c) which stimulates astrocytes to produce CXCL10. (d) The abundant CXCL10 in the central nervous system (CNS) creates a concentration gradient by which CXCR3-expressing CD4+ and CD8+ T cells undergo chemotaxis to the CNS. (e) These Th1 cells attracted by the CXCL10 also produce pro-inflammatory cytokines including IFN-γ, which further stimulates the astrocytes, (f) creating a positive feedback loop that generates abundant pro-inflammatory cytokines in the CNS. The inflammation in the CNS gradually damages the spinal cord.

serum, yielding a concentration gradient towards the CNS. Additionally, levels of CXCL10 were correlated with known features of HAM/TSP, namely increased CSF cell count. Other chemokines, such as CXCL9, CCL5 and CCL4, were considered but did not show similar trends. We then analyzed samples of peripheral blood mononuclear cells (PBMC), CSF cells, and spinal cord tissue to show that CD4+CXCR3+ cells are indeed infected with HTLV-1, do migrate across the BBB into the CNS and do produce IFN-γ in HAM/TSP patients.

Together, these results show that the pathogenesis of HAM/TSP involves CXCR3+ cells crossing the BBB, at least in part as a result of chemotactic attraction to the abundant CXCL10 in the CNS, and secreting pro-inflammatory cytokines that cause spinal cord inflammation. The question remains: from where does this abundant CXCL10 originate?

Immunohistochemical analysis of the spinal cord tissue not only confirmed that CXCL10 is produced in the spinal cords of HAM/TSP patients, but also showed that astrocytes might be the main producers. Co-culture of human astrocytoma cells with CD4+ T cells from HAM/TSP patients confirmed that astrocytes produce CXCL10 in response to IFN-γ secreted by CD4+ T cells.

We concluded that these astrocytes likely represent the missing piece of the puzzle, and we postulated the existence of an inflammatory positive feedback loop: infected CD4+ T cells cross the BBB and produce IFN-γ, which stimulates astrocytes to produce CXCL10, which recruits more CXCR3+ cells of both CD4+ and CD8+ subtypes to the CNS, where they produce more IFN-γ (Fig. 1). As for the initial trigger that starts the vicious cycle, it is thought that HTLV-1-infected cells could be inherently likely to cross the BBB as a result of HTLV-1-induced expression of certain cell surface proteins.[5]

Finally, chemotaxis assays showed that it might be possible to disrupt this loop with anti-CXCL10 neutralizing antibodies. As the current data points to a virtually exclusively Th1-dominant pathogenesis, disruption of the Th1 inflammatory process could effectively cure the disease.

Thus, we described a Th1-centric inflammatory positive feedback loop critical for HAM/TSP pathogenesis and suggested that disrupting this loop might lead to a cure.

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CASE STUDY Open Access

A case of post-transplant adult T-cell leukemia/lymphoma presenting myelopathy similar to but distinct from human T-cell leukemia virus type I (HTLV- I)-associated myelopathy

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Abstract

Introduction: Adult T-cell leukemia/lymphoma (ATL) responds poorly to conventional chemotherapy, but allogeneic stem cell transplantation (allo-SCT) may improve disease prognosis. Herein, we report a female patient with human T-cell leukemia virus type I (HTLV-I)-associated myelopathy (HAM)-like myelopathy following allo-SCT for ATL.

Case report: She developed crural paresis 14 months after allo-SCT. Initially, she was diagnosed with central nervous system (CNS) relapse of ATL and treated with intrathecal injection and whole brain and spine irradiation. Her symptoms recurred 5 months later, when a cerebrospinal fluid (CSF) specimen showed increased CD4 + CXCR3 + CCR4+ cell numbers and levels of neopterin and CXCL10 (IP-10).

Discussion: These results suggest the possible involvement of a certain immunological mechanism such as HAM in her symptoms, irrespective of the lack of anti-HTLV-I antibody in her CSF. Because a definitive diagnosis of CNS manifestation of ATL is sometimes difficult, multi-modal laboratory data are required for differential diagnosis.

Keywords: Adult T-cell leukemia/lymphoma; Post-transplant myelopathy; HTLV-I-associated myelopathy (HAM); Neopterin; CXCL10 (IP-10)

Introduction

Human T-cell leukemia virus type I (HTLV-I) was the first retrovirus identified in humans, isolated from a patient with cutaneous lymphoma (Poiesz et al. 1980). HTLV-I is the cause of not only adult T-cell leukemia/lymphoma (ATL) (Uchiyama et al. 1977; Hinuma et al. 1981) but also HTLV-I-associated myelopathy (HAM)/tropical spastic paraparesis (TSP) (Osame et al. 1986), HTLV-I-associated uveitis (HU) (Ohba et al. 1989; Mochizuki et al. 1992) and infective dermatitis (McGill et al. 2012; de Oliveira et al. 2010).

ATL is one of the most intractable T-cell malignancies, and it responds poorly to conventional chemotherapy, with a median survival time (MST) of approximately 8 months (Shimoyama et al. 1988). Among such treatments, modified LSG-15 (mLSG-15) has shown the best results; in a previous study, the progression free survival (PFS) at 1 year among patients treated with mLSG-15 was 28% and the overall survival (OS) at 3 years was 24% (Tsukasaki et al. 2007). However, the improvement in survival time by mLSG-15 treatment is not satisfactory. Allo-HSCT is a promising treatment option to cure ATL because it may improve disease prognosis (Utsunomiya et al. 2001; Kami et al. 2003).

Herein, we describe a case of HAM-like myelopathy that was difficult to distinguish from central nervous system (CNS) relapse of ATL following allogeneic peripheral blood stem cell transplantation. This case report suggests that there might be immunological myelopathy after HSCT. In the present case, flow cytometric analysis of the cells in cerebrospinal fluid (CSF) was helpful to differentiate it from CNS relapse of ATL.

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Case report

A 63-year-old female patient recognized cervical lymph nodes swelling in October 2010. Lactate dehydrogenase (LDH) and serum corrected calcium levels kept within normal limit, but soluble interleukin-2 receptor (sIL-2R) elevated significantly at the initial visit (Table 1). Diagnostic imaging by computed tomography (CT) revealed systemic lymphadenopathies (cervical, axial, mediastinal, abdominal and mesenteric lymphadenopathy) before the following chemotherapy. Although appetite loss and abdominal distention were added with lymphadenopathy, any other abnormal finding of physical examination could not be detected. Her ECOG performance status was grade 1 before chemotherapy. She received cervical lymph node biopsy and pathological findings of cervical lymph node revealed T cell lymphoma compatible, and HTLV-I provirus DNA analysis (Southern blot) revealed monoclonal integration. Abnormal lymphocytes were not detected in peripheral blood (PB) and HTLV-I provirus DNA analysis of PB did not show monoclonal integration. She was diagnosed as ATL (lymphoma type). She has past histories of glaucoma and pulmonary cryptococcosis. None of ATL patient was in her family.

She was referred to our hospital and received four sessions of mLSG-15 therapy in our hospital. Prophylactic intrathecal injection was performed twice, during chemotherapy and before allogeneic stem cell transplantation. No meningeal involvement of ATL cells was detected at that time. She went into complete remission (Response criteria for adult T cell leukemia-lymphoma from an international consensus meeting (Tsukasaki et al. 2009)) in April 2011. She received following allogeneic peripheral blood stem cell transplantation (allo-PBSCT) in the National Cancer Center Hospital (Tokyo, Japan) (Figure 1). The transplantation conditioning regimen consisted of fludarabine (30 mg/m^2 per day for 5 days) plus busulfan (3.2 mg/kg per day for 2 days) and only cyclosporine A (CyA) was used for GVHD prophylaxis. Transplanted CD34-positive cells were 2.67×10^6/kg and rapid engraftment was achieved. Grade III (gastrointestinal tract and skin) acute graft-versus-host disease (GVHD) was observed 1 month after transplantation, but it improved after treatment with methylpredonisolone (mPSL) (1 mg/kg). No chronic GVHD was observed. CyA was tapered gradually and discontinued 9 months after transplantation, in February 2012. After that point, only 5 mg/day predonisolone (PSL) was continued.

In July 2012 (14 months after allo-PBSCT), the patient developed hemiparesis of the left side. Although left upper-limb paresis improved, lower-extremity paresis progressed to paraplegia. Magnetic resonance imaging (MRI) revealed multiple high-intensity lesions in T2-weighted images of the medulla oblongata, cervical spinal cord, and thoracic spinal cord (Figure 2A), and a CSF specimen showed increased cell counts (Figure 3). Morphologically, typical ATL cells such as flower cells were not detected in CSF, but abnormal small to middle size lymphocytes indistinguishable from ATL cells increased. She was diagnosed as CNS relapse of ATL, and received mPSL pulse, intrathecal injection of MTX 15 mg + Ara-C 40 mg + PSL 20 mg, and irradiation of the whole brain and spine. Following these treatments, the paraplegia improved gradually to such a degree that she could walk with a walker. During the course of these treatments, she was complicated by neurogenic bladder dysfunction, and diabetes insipidus.

In January 2013 (20 months after allo-PBSCT), she again developed left lower-limb weakness, which gradually progressed. She was admitted to our hospital in February 2013. On admission, neurological examination revealed

Table 1 Laboratory data of onset of ATL (lymphoma type) in October 2010

WBC	4100/μl	GOT	67 IU/L	CRP	0.06 mg/dl
Myelo	1.0%	GPT	72 IU/L	sIL-2R	5802 U/ml
St	8.0%	LDH	215 IU/L		
Seg	71.0%	ALP	277 IU/L	HTVL-I Ab	(+)
Ly	11.0%	γ-GTP	46 IU/L	HBs-Ag	(−)
Mo	8.0%	Alb	3.5 mg/dl	HBs-Ab	(−)
Baso	1.0%	BUN	15.6 mg/dl	HBc-Ab	(−)
RBC	423×10^4/μl	Cre	0.58 mg/dl	HCV-Ab	(−)
Hb	13.2 g/dl	Na	142.4 mEq/L	HIV-Ab	(−)
Hct	39.0%	K	4.2 mEq/L	TPHA	(−)
MCV	92.2 fl	Cl	103.8 mEq/L		
MCH	31.2 pg	Corrected Ca	9.9 mg/dl		
MCHC	33.8%				
Plt	21.9×10^4/μl				

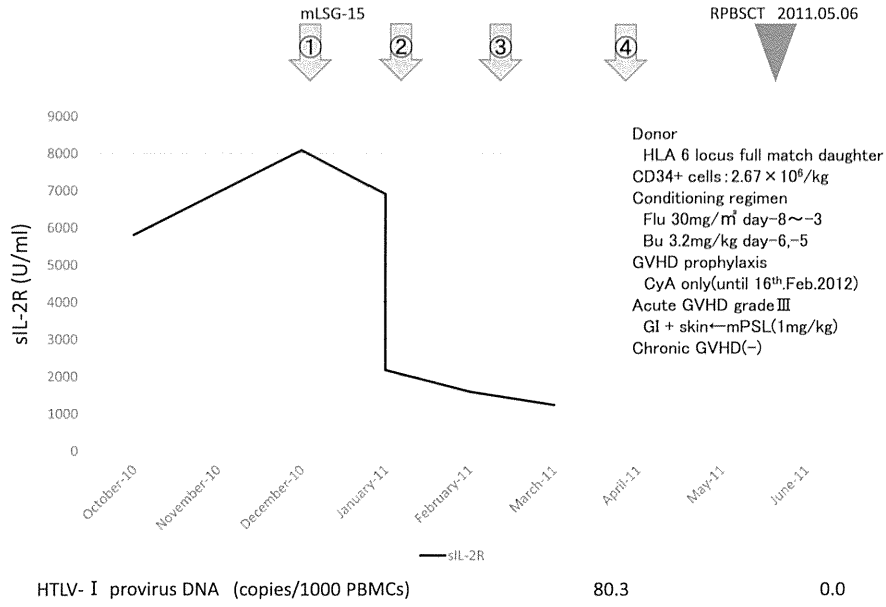

Figure 1 Clinical course from conventional chemotherapy (mLSG-15) to allogeneic peripheral blood stem cell transplantation. The patient received four sessions of mLSG-15 therapy and achieved complete remission (CR) before receiving allo-PBSCT.

no abnormality of cranial nervous system, but abnormal reflex such as Babinski and Chaddock reflex in bilateral lower-limb. Thermal hypoalgesia under right Th4 and left Th6 dermatome was detected, but tactile sense was intact. She was accompanied with bladder dysfunction and severe constipation. Brain and spinal MRI revealed a residual spinal lesion at Th3-7 (Figure 2B). The cell numbers in CSF did not increase, but myelin basic protein (MBP) level was elevated (Figure 4B). Morphologically, ATL cells could not be detected in CSF. Flow cytometric analysis to determine the specific immunophenotype of CD4+ lymphocytes in CSF revealed an expansion of the $CD4^+CXCR3^+CCR4^+$

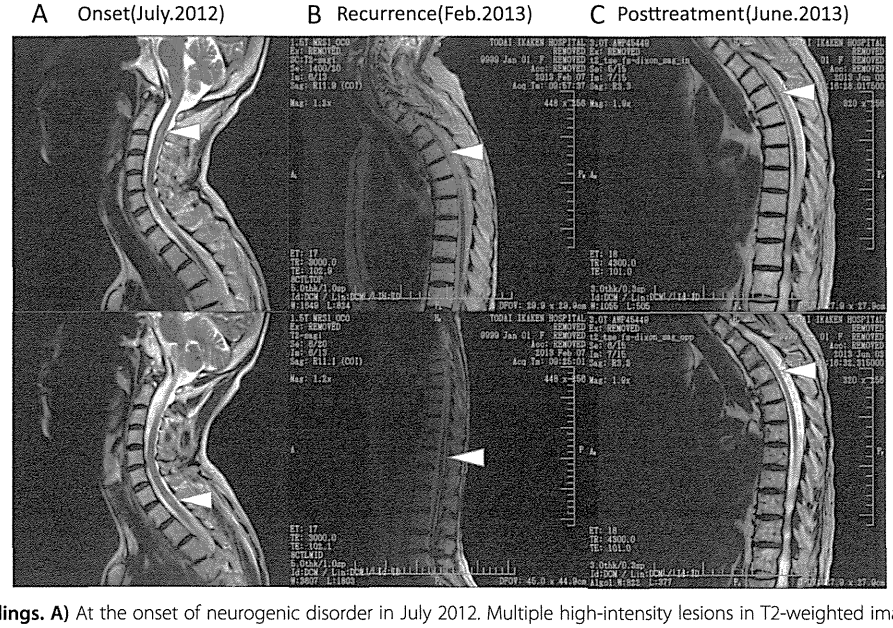

Figure 2 MRI findings. A) At the onset of neurogenic disorder in July 2012. Multiple high-intensity lesions in T2-weighted images (T2WI) of the medulla oblongata, cervical spinal cord, and thoracic spinal cord were revealed. **B)** Residual high-intensity lesion in Th3-7. **C)** Following treatment, the residual lesion in the thoracic spinal cord improved.

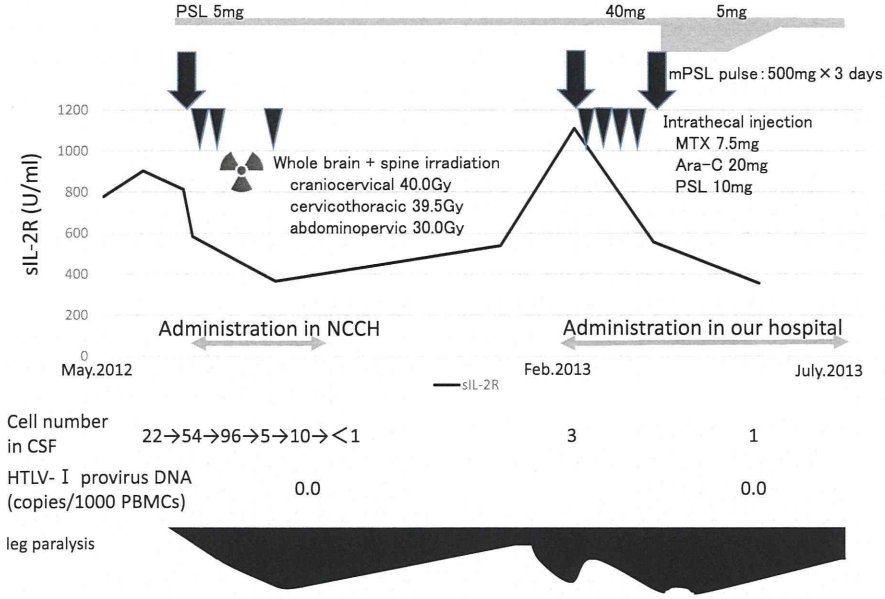

Figure 3 Clinical course after onset of the neurogenic disorder. The patient developed paraplegia 14 months after allo-PBSCT. Neurological findings were partially relieved following treatment with a high dose of mPSL accompanied by intrathecal injection of MTX + Ara-C + PSL and irradiation of the whole brain and spine. Three months later, her neurological deficit worsened again. Ultimately, her neurological disorder improved after treatment with a high dose of steroid.

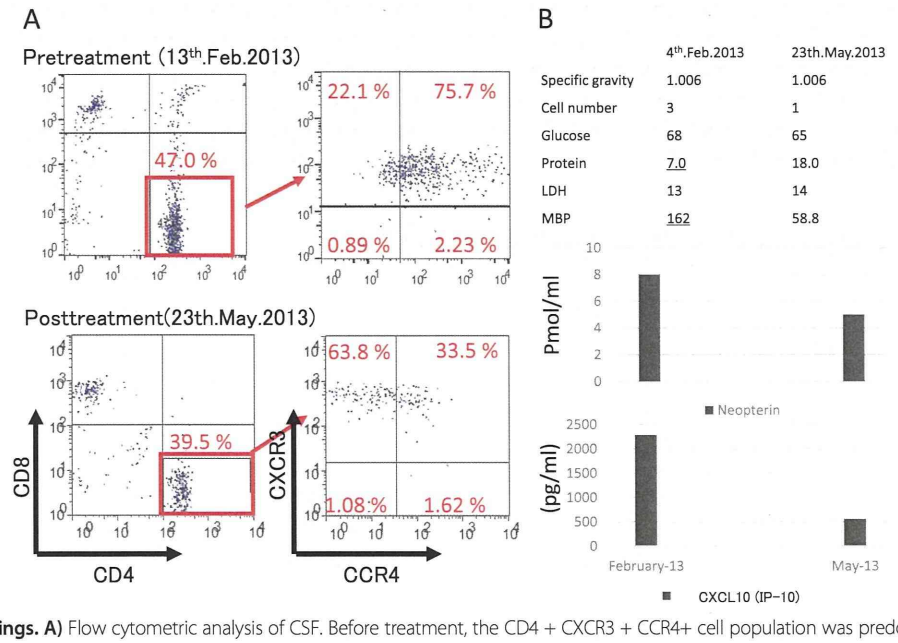

Figure 4 CSF findings. A) Flow cytometric analysis of CSF. Before treatment, the CD4 + CXCR3 + CCR4+ cell population was predominantly elevated. Following treatment, it decreased and the CD4 + CXCR3 + CCR4- cell population increased. **B)** Neopterin and CXCL10 (IP-10) concentrations in CSF. Before treatment, both neopterin and CXCL10 (IP-10) concentrations were significantly elevated. Following treatment, both biomarkers decreased to within the range of the therapeutic goal for HAM patients.