Received: 28 October 2020 | Revised: 1 January 2021 | Accepted: 4 January 2021

DOI: 10.1111/cas.14800

ORIGINAL ARTICLE

Cancer Science WILEY

Short-term cultured autologous peripheral blood mononuclear cells as a potential immunogen to activate Tax-specific CTL response in adult T-cell leukemia patients

Miku Ishizawa[1] | Undrakh Ganbaatar[1] | Atsuhiko Hasegawa[1] | Natsuko Takatsuka[1] | Nobuyo Kondo[1] | Takeru Yoneda[1] | Kuniko Katagiri[1] | Takao Masuda[1] | Atae Utsunomiya[2] | Mari Kannagi[1,3,4]

[1]Deparment of Immunotherapeutics, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

[2]Department of Hematology, Imamura General Hospital, Kagoshima, Japan

[3]Department of Molecular Virology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

[4]Department of Microbiology, Kansai Medical University, Osaka, Japan

Correspondence
Mari Kannagi, Department of Immunotherapeutics, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan.
Email: kann.impt@tmd.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Number: JP20K07512; Japan Agency for Medical Research and Development, Grant/Award Number: 19cm0106365h0001; Japan Cancer Society

Abstract

Activation of $CD8^+$ Tax-specific CTL is a new therapeutic concept for adult T-cell leukemia (ATL) caused by HTLV-1. A recent clinical study of the dendritic cell vaccine pulsed with Tax peptides corresponding to CTL epitopes showed promising outcomes in ATL patients possessing limited human leukocyte antigen (HLA) alleles. In this study, we aimed to develop another immunotherapy to activate Tax-specific CTL without HLA limitation by using patients' own HTLV-1-infected cells as a vaccine. To examine the potential of HTLV-1-infected T-cells to activate CTL via antigen presenting cells, we established a unique co–culture system. We demonstrated that mitomycin C-treated HLA-A2-negative HTLV-1-infected T-cell lines or short-term cultured peripheral blood mononuclear cells (PBMC) derived from ATL patients induced cross–presentation of Tax antigen in co–cultured HLA-A2-positive antigen presenting cells, resulting in activation of HLA-A2-restricted $CD8^+$ Tax-specific CTL. This effect was not inhibited by a reverse transcriptase inhibitor. IL-12 production and CD86 expression were also induced in antigen presenting cells co–cultured with HTLV-1-infected cells at various levels, which were improved by pre–treatment of the infected cells with histone deacetylase inhibitors. Furthermore, monocyte-derived dendritic cells induced from PBMC of a chronic ATL patient produced IL-12 and expressed enhanced levels of CD86 when co–cultured with autologous lymphocytes that had been isolated from the same PBMC and cultured for several days. These findings suggest that short-term cultured autologous PBMC from ATL patients could potentially serve as a vaccine to evoke Tax-specific CTL responses.

KEYWORDS
ATL, CTL, HTLV-1, Tax, tumor vaccine

Miku Ishizawa and Undrakh Ganbaatar contributed equally to this study.

1 | INTRODUCTION

Adult T-cell leukemia/lymphoma (ATL) is an aggressive lymphoproliferative disease caused by HTLV-1.[1-3] HTLV-1 Tax and HTLV-1 bZIP factor (HBZ) have been shown to contribute to viral pathogenesis through multiple functions, including transcriptional regulation of cellular genes related to cell survival and proliferation.[4-6] Despite the strong oncogenic potential of HTLV-1, it takes more than 40 years from infection to ATL development, and the majority of HTLV-1-infected individuals remain asymptomatic for life. A small percentage of HTLV-1-infected individuals develop HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a disease distinct from ATL, manifesting as chronic inflammation with demyelination of the spinal cord.[7,8]

One of the host factors differing between these diseases is HTLV-1 Tax-specific $CD8^+$ CTL, which are detectable in most asymptomatic HTLV-1 carriers (AC) and are often activated in patients with HAM/TSP[9,10] but are either undetectable or dysfunctional in patients with ATL even in the early stages.[11] Tax-specific CTL have been shown to produce anti–tumor effects in animal models of HTLV-1-infected lymphomas,[12,13] implying that impairment of the Tax-specific CTL response could be an underlying risk factor for ATL development. Reasons for the impaired CTL response include immune tolerance,[14] interleukin (IL)-10-dominant immune environment,[15-18] and immune exhaustion.[19,20]

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) following chemotherapy is currently recommended for anti–ATL treatment in Japan because one-third of ATL patients receiving HSCT achieve long-term survival. However, it also has a risk of graft-versus-host disease and treatment-related mortality.[21,22] Interestingly, Tax-specific CTL are often activated in recipient ATL patients following allo-HSCT,[23] suggesting a potential contribution of these CTL to the therapeutic effects.

In these several years, we developed an anti–ATL therapeutic vaccine to activate Tax-specific CTL by using autologous dendritic cells (DC) pulsed with Tax peptides corresponding to the epitopes recognized by Tax-specific CTL.[24] A clinical study of this therapeutic vaccine in patients with acute-type ATL after initial therapies showed that two of three patients lived more than 4 years without serious adverse effects,[24,25] supporting the notion that vaccines to activate Tax-specific CTL could be a promising therapeutic approach to ATL.

The prototype Tax peptide-pulsed DC vaccine is, however, only available for patients with human leukocyte antigen (HLA)-A2, A24, or A11 for which the dominant CTL epitopes have been identified.[23,26,27] Therefore, we intended to develop an alternative anti–ATL immunotherapy without HLA-restriction by using patients' own HTLV-1-infected cells as a vaccine to raise Tax-specific CTL. This is based on our previous findings in a rat model of oral HTLV-1 infection,[28] in which the otherwise impaired HTLV-1-specific T-cell responses were restored by subsequent subcutaneous inoculation of syngeneic HTLV-1-transformed cells, resulting in reduction of proviral load (PVL).[29]

In the present study, we investigate the potential of ATL patients' own HTLV-1-infected cells as a vaccine to generate Tax-specific CTL. Unlike animal models, there are no available syngeneic HTLV-1-transformed cell lines. In addition, HTLV-1 antigens in peripheral blood mononuclear cells (PBMC) of HTLV-1-infected individuals are undetectable. However, HTLV-1 expression is rapidly induced when PBMC are cultured in vitro,[30,31] presumably due to the absence of microenvironmental interferon (IFN)-response and activation of stress-induced MAPK in vitro.[32,33] Although Tax expression in ATL cells is defective in approximately half of ATL patients because of genetic alterations and epigenetic silencing of proviral DNA,[34] the ability to express Tax is retained in the remaining ATL cases[31] and probably more frequently in non–ATL HTLV-1-infected cells or at earlier stages of ATL. Such Tax-expressing HTLV-1-infected cells in cultured PBMC might be used as an immunogen.

Antigen presenting cells (APC), especially DC, play important roles in induction of T-cell responses.[35] Generally, antigens engulfed by DC are processed and presented mostly on MHC-II molecules. However, to induce $CD8^+$ CTL responses, DC should also present the processed antigen fragments on MHC-I molecules via antigen cross–presentation.[36,37] Furthermore, the DC must be matured to express co–stimulatory molecules and produce cytokines such as IL-12 and IFNs so that Th1-type responses occur.[35] Here, we demonstrate that HTLV-1-infected cells, including short-term cultured PBMC derived from ATL patients, potentially activate APC to induce these functions.

2 | MATERIALS AND METHODS

2.1 | Blood samples

Primary PBMC were derived from two HLA-A2-negative patients with chronic ATL in Imamura General Hospital (Kagoshima, Japan) who donated peripheral blood after providing written informed consent. PBMC were isolated on a Ficoll-Paque PLUS (GE Healthcare) density gradient and stored in liquid nitrogen. PBMC were thawed and used immediately or cultured for several days in the presence of 30 U/mL of recombinant human (rh)IL-2 (Shionogi) either with or without depletion of $CD8^+$ cells using Dynabeads coated with anti–CD8 antibodies (Invitrogen). Another aliquot of PBMC was used for induction of monocyte-derived dendritic cells (MoDC), as described below. An HLA-A2-positive HTLV-1-uninfected donor also donated peripheral blood for preparation of MoDC. This study was approved by the Medical Research Ethics Committee of Tokyo Medical and Dental University.

2.2 | Preparation of monocyte-derived dendritic cells

Primary PBMC were cultured in RPMI 1640 medium (Sigma-Aldrich) containing 10% FBS (Sigma-Aldrich), 100 U/mL penicillin,

and 100 μg/mL streptomycin (FUJIFILM Wako Pure Chemical Corporation) for 2 hours in a plastic dish. Non–adherent peripheral blood lymphocytes (PBL) were removed and adherent cells were cultured for the next 5-6 days in the presence of 1000 U/mL granulocyte macrophage-colony stimulating factor (GM-CSF; Miltenyi Biotec) and 500 U/mL IL-4 (Miltenyi Biotec). In ATL samples, 10 μM azidothymidine (AZT) (GlaxoSmithKline, Research Triangle Park) was added to the culture medium throughout the procedure to avoid secondary infection.

2.3 | Cell lines

We also used previously established IL-2 or IL-15-dependent HTLV-1-infected T-cell lines (ILT), ILT-H2[17] and ILT-156,[27] derived from PBMC of patients with chronic ATL and acute ATL, respectively, as described previously. These lines were maintained in RPMI 1640 medium containing 10% FBS and antibiotics, supplemented with 30 U/mL rhIL-2 or 5 ng/mL rhIL-15 (PeproTech), respectively. An HTLV-1-negative monocyte cell line THP-1[38] was maintained in RPMI 1640 medium containing 10% FBS and antibiotics. A $CD8^+$ HLA-A2-restricted Tax-specific CTL line, Tc-M1,[39] derived from a patient with HAM/TSP was maintained in medium containing 100 U/mL rhIL-2.

2.4 | Antibodies

Mouse mAbs used for flow cytometry were APC-Cy7-conjugated anti–human CD4, APC-conjugated anti–human CD25, PE-conjugated anti–human CD30, PerCP/Cy5.5-conjugated anti–human CD83 (BioLegend), Pacific Blue-conjugated anti–human CD3, PE-conjugated anti–human CD11c, FITC-conjugated anti–human CD86 (BD Pharmingen), and PE-conjugated anti–human CCR4 (R&D Systems). Unconjugated anti–Tax mAb Lt-4[40] was kindly provided by Professor Yuetsu Tanaka (University of the Ryukyus, Okinawa, Japan) and used together with control mouse IgG3 (BioLegend) and Alexa 488-conjugated goat anti–mouse IgG (BioLegend).

2.5 | Reagents

Mitomycin C (MMC) (Sigma-Aldrich) was used at 50 μg/mL for 1 h at 37°C, while formalin (FUJIFILM Wako Pure Chemical) was used at 1% for 15 minutes at room temperature to treat cells, followed by extensive washing of the cells. To inhibit HTLV-1 reverse transcription, AZT was added in culture at 10-20 μM. Histone deacetylase (HDAC) inhibitors, suberoylanilide hydroxamic acid (SAHA; Cayman Chemical) and valproic acid (VPA; FUJIFILM Wako Pure Chemical) were used at 1 μM and 1 mM, respectively. DMSO (FUJIFILM Wako Pure Chemical Corporation) was used as a vehicle control.

2.6 | Flow cytometry

For cell surface staining, cells were incubated with antibodies for 30 minutes on ice. For intracellular staining, cells were fixed and permeabilized using a fixation/permeabilization kit (eBioscience) according to the manufacturer's instructions prior to incubation with antibodies. To detect intracellular Tax protein, permeabilized cells were stained with anti–Tax mAb Lt-4[40] or control mouse IgG3 for 1 hour followed by staining with A488-conjugated anti–mouse IgG. The stained cells were analyzed on a MACSQuant® Analyzer (Miltenyi Biotec), and the data were analyzed using FlowJo software (Tree Star Inc).

2.7 | Antigen cross–presentation (CTL assays)

To evaluate cross–presentation of Tax antigen on APC, HLA-A2-negative HTLV-1-infected cells (10^5) treated with various reagents were co–cultured with HLA-A2-positive APC (either 10^5 THP-1 or 5-7 × 10^4 MoDC) overnight, and then fixed with 1% formalin, washed, and further co–cultured overnight with 5 × 10^4 HLA-A2-restricted Tax-specific CTL. CTL activities were evaluated by ELISA measurement of IFN-γ concentrations in the supernatants.

2.8 | ELISA

Concentrations of IFN-γ, IL-12 and IL-10 in culture supernatants were measured using BD OptEIA ELISA sets for human IFN-γ, IL-12, and IL-10 (BD Bioscience) according to the manufacturer's instructions.

2.9 | PCR

DNA was extracted from cells by using DNeasy blood & tissue kits (QIAGEN) and subjected to PCR using One Taq DNA Polymerase (New England BioLabs) with the primer pairs specific for HTLV-1 Tax (pX2: 5′-CGGATACCCAGTCTACGTGTTTGGAGACTGT-3′, and pX3: 5′-GAGC CGATAACGCGTCCATCGATGGGGTCC-3′) and β-globin (5′-ACAC AACTGTGTTCACTAGC-3′ and 5′-CAACTTCATCCACGTTCACC-3′). The PCR products were visualized with Midori Green Advance DNA stain (NIPPON Genetics) after electrophoresis in an agarose gel.

2.10 | Cell counting kit

Viable cell numbers were evaluated by colorimetric assay based on formazan color development using Cell Counting Kit-8 (Dojindo).

2.11 | Statistical analysis

Statistical significance was tested by Student *t* test, and the difference between groups was considered significant at $P < .01$.

3 | RESULTS

3.1 | HTLV-1-infected cells induced Tax cross–presentation in antigen presenting cells following co–culture

To investigate the potential of HTLV-1-infected cells from ATL patients to induce cross–presentation of Tax antigen in APC, we designed an in vitro co–culture assay system (Figure 1A). In this assay, HLA-A2-positive APC were first co–cultured overnight with MMC-treated HLA-A2-negative HTLV-1-infected cells to allow phagocytosis and subsequent antigen processing (first co–culture). The co–cultured cells were then fixed with formalin to avoid further cytokine production and then further co–cultured with HLA-A2-restricted Tax-specific CTL (second co–culture). These CTL were supposed to be unable to recognize these ILT directly because of mismatched HLA but recognize THP-1 cells if they cross–present Tax antigen on their HLA-A2.

Because of the limited availability of clinical materials, we initially used ILT-156 and ILT-H2 cells as HTLV-1-infected cells, which were IL-15 and IL-2-dependent HTLV-1-infected cell lines previously established by long-term culture of PBMC from HLA-A2-negative acute and chronic ATL patients, respectively. These ILT cells expressed Tax but not HLA-A2 (Figure S1). For APC, an HLA-A2-positive uninfected monocyte cell line THP-1 was used, as THP-1 is reportedly capable of antigen presentation to stimulate T-cell responses.[41]

Representative results of the HLA-A2-restricted Tax-specific CTL response in this system are shown in Figure 1B. CTL produced considerable levels of IFN-γ in response to THP-1 cells that had been co–cultured with MMC-treated ILT-156 or ILT-H2 cells but not to ILT or THP-1 alone (Figure 1B).

A time-course study indicated that THP-1 cells began to present Tax antigen to CTL at 8 hours after co–culture with MMC-treated ILT-156 cells, and this became more efficient upon longer culture (Figure 1C).

These findings suggested that MMC-treated HTLV-1-infected cells induced cross–presentation of Tax antigen on MHC-I in the co–cultured APC.

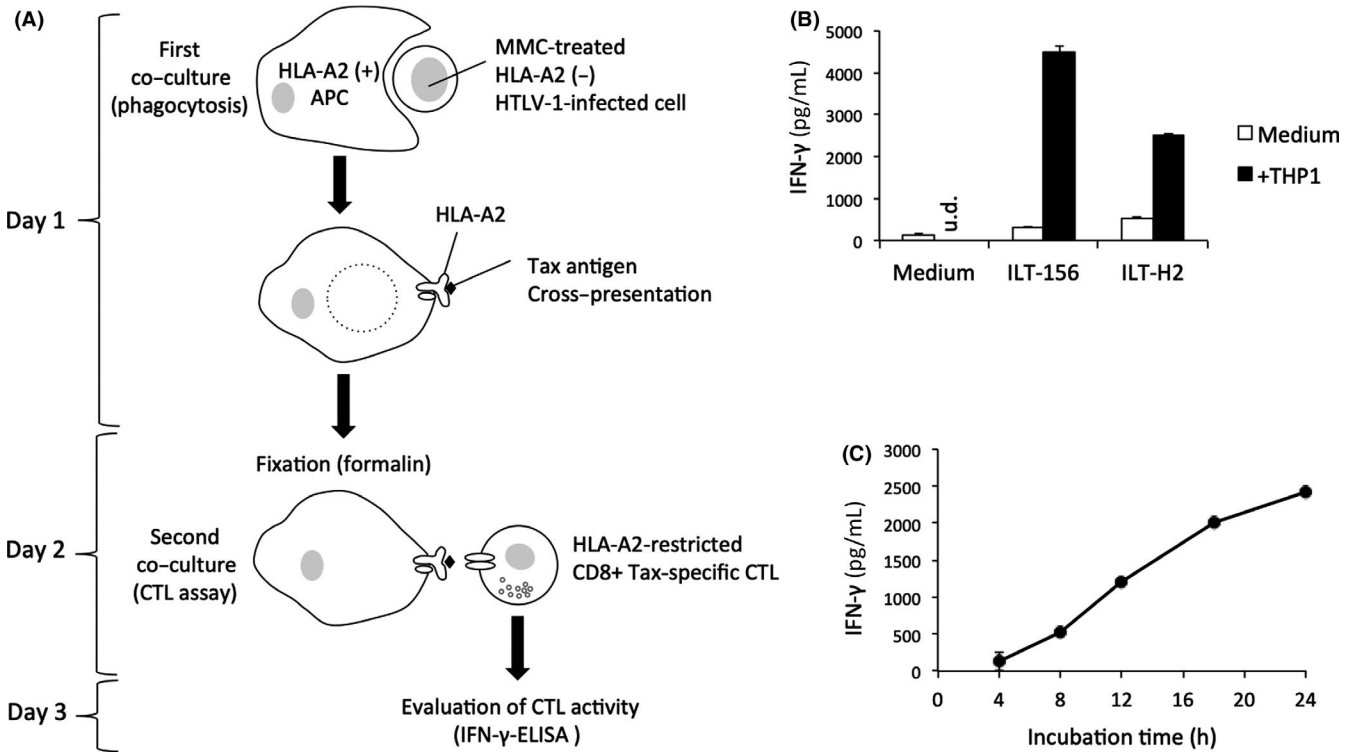


FIGURE 1 HTLV-1 infected cells induce Tax cross–presentation in antigen presenting cells (APC). A, Schematic showing evaluation system for Tax antigen cross–presentation. HLA-A2-negative HTLV-1-infected cells (ILT or peripheral blood mononuclear cells [PBMC] from adult T-cell leukemia [ATL] patients) were pre–treated with mitomycin C (MMC) and co–cultured with HLA-A2-positive APC (THP-1 or monocyte-derived dendritic cells [MoDC]) overnight (first co–culture). Co–cultured cells were then fixed with formalin and further co–cultured with HLA-A2-restricted Tax-specific CTL overnight (second co–culture). Cross–presentation of Tax antigen on HLA-A2 of APC was evaluated by ELISA measurement of supernatant interferon (IFN)-γ released from CTL. B, Representative results of A. HLA-A2-restricted Tax-specific CTL were incubated with fixed THP-1 cells (black bar) that had been co–cultured overnight with MMC-treated HLA-A2-negative HTLV-1-infected ILT-156 and ILT-H2 cells. IFN-γ concentrations in the supernatants were measured by ELISA. CTL activities without THP-1 cells (white bar) were also indicated. Results show mean and SD of duplicate samples. Data are representative of at least three independent experiments. u.d., undetectable. C, THP-1 cells were co–cultured with MMC-treated ILT-156 cells for the times indicated, fixed, and then co–cultured with Tax-specific CTL overnight to evaluate Tax antigen cross–presentation. IFN-γ concentrations in the supernatants at each time point were plotted

3.2 | Azidothymidine did not affect cross–presentation of Tax antigen

Mitomycin C-treated HTLV-1-infected cells still retain infectivity, and, therefore, it is also possible that the APC presented Tax antigen on MHC-I as a de novo antigen produced by infected APC. To exclude this possibility, we next examined the effect of a reverse transcriptase inhibitor AZT on Tax antigen cross–presentation (Figure 2). THP-1 cells were pre–treated with AZT, and co–cultured with MMC-treated ILT-156 cells in the presence of AZT (10 μM). The resulting co–cultured sample was capable of activating Tax-specific CTL to a level comparable with that of the co–culture without AZT (Figure 2A).

The presence of HTLV-1 proviral DNA in the prolonged co–culture of THP-1 cells and MMC-treated ILT was examined by PCR, when all the ILT cells died out in culture. Although HTLV-1 proviral DNA was detected in the culture without AZT treatment, no proviral DNA was detectable in the culture with AZT treatment (Figure 2B), confirming that AZT inhibited HTLV-1 infection.

The results indicate that, although THP-1 cells were susceptible to HTLV-1 infection, reverse transcription or later steps of HTLV-1 replication were dispensable for Tax antigen cross–presentation, proving that THP-1 cells cross–presented the Tax antigen originating from the ILT cells incorporated in the THP-1 cells.

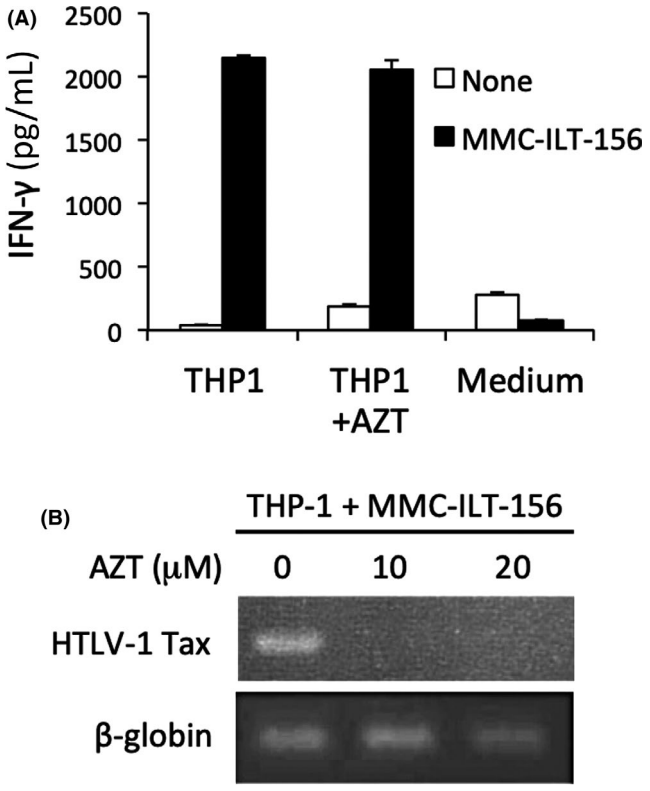


FIGURE 2 Azidothymidine (AZT) did not affect Tax antigen cross–presentation. A, THP-1 cells pre–treated with or without 20 μM AZT 1 day before were co–cultured with (black bar) or without (white bar) mitomycin C (MMC)-treated ILT-156 cells overnight. For AZT-treated THP-1, AZT was also added during co–culture at a concentration of 10 μM. Cells were then fixed and co–cultured with Tax-specific CTL. Supernatant concentrations of interferon (IFN)-γ released from CTL were measured by ELISA and shown as mean and SD of duplicate samples. Data are representative of at least three independent experiments. B, Possible HTLV-1 infection in THP-1 cells that had been co–cultured with MMC-treated ILT-156 in the absence or presence of AZT was examined by PCR analysis using Tax-specific (top) or β-globin-specific (bottom) primers in the DNA samples 3 weeks after the initiation of co–culture. Data are representative of at least two independent experiments

3.3 | Histone deacetylase inhibitors enhanced ILT-induced antigen presentation in antigen presenting cells

Expression of co–stimulatory molecules and production of cytokines, especially IL-12, are important tasks for APC to induce CTL responses. We therefore next assessed whether MMC-treated ILT cells could induce CD86 and IL-12 in APC (Figure 3). In these experiments, monocyte-derived immature dendritic cells (MoDC) from uninfected individuals were used as APC. We also investigated the effect of HDAC inhibitors on the abilities of ILT to activate APC, as HDAC inhibitors are known to enhance Tax expression,[42] and Tax has been reported to activate APC.[43]

Preliminary experiments indicated that treatment of ILT-156 cells with SAHA and VPA at the concentrations of 1 μM and 1 mM, respectively, exhibited similar levels of mild toxicity (Figure S2) in 24 hours. Under these conditions, SAHA and VPA slightly enhanced expression of intracellular Tax in a small proportion of ILT cells (Figure 3A).

We then co–cultured HLA-A2-positive MoDC from an uninfected individual with MMC-treated ILT cells that had been pre–treated with HDAC inhibitors in the presence of AZT. SAHA or VPA-pre–treated ILT-156 cells induced CD86 expression in the co–cultured MoDC, while the control ILT-156 cells that had been pre–treated with DMSO failed to do so (Figure 3B). For ILT-H2, the DMSO-treated control ILT-H2 cells already significantly induced CD86 in the co–cultured MoDC, which was not further enhanced by pre–treatment with SAHA or VPA (Figure 3B).

Interleukin-12 production in the MoDC co–cultured with MMC-treated ILT-156 was undetectable but was slightly enhanced by pre–treatment of ILT cells with HDAC inhibitors. ILT-H2 induced much higher levels of IL-12 production than ILT-156 in the co–cultured MoDC, which was further enhanced by pretreatment with HDAC inhibitors (Figure 3C). Similar HDAC inhibitor-mediated enhancement was observed in IFN-γ production in the co–cultured MoDC. Both ILT-156 and ILT-H2 produced IL-10, with ILT-156 demonstrating much higher levels than ILT-H2, but these cells also induced IL-10 in co–cultured MoDC to some extent, which was not altered by HDAC inhibitors.

Monocyte-derived immature dendritic cells possessed HLA-A2, and, therefore, we also examined the effect of HDAC inhibitors on Tax antigen cross–presentation. The results indicated that the HLA-A2-restricted Tax-specific CTL activities against MoDC

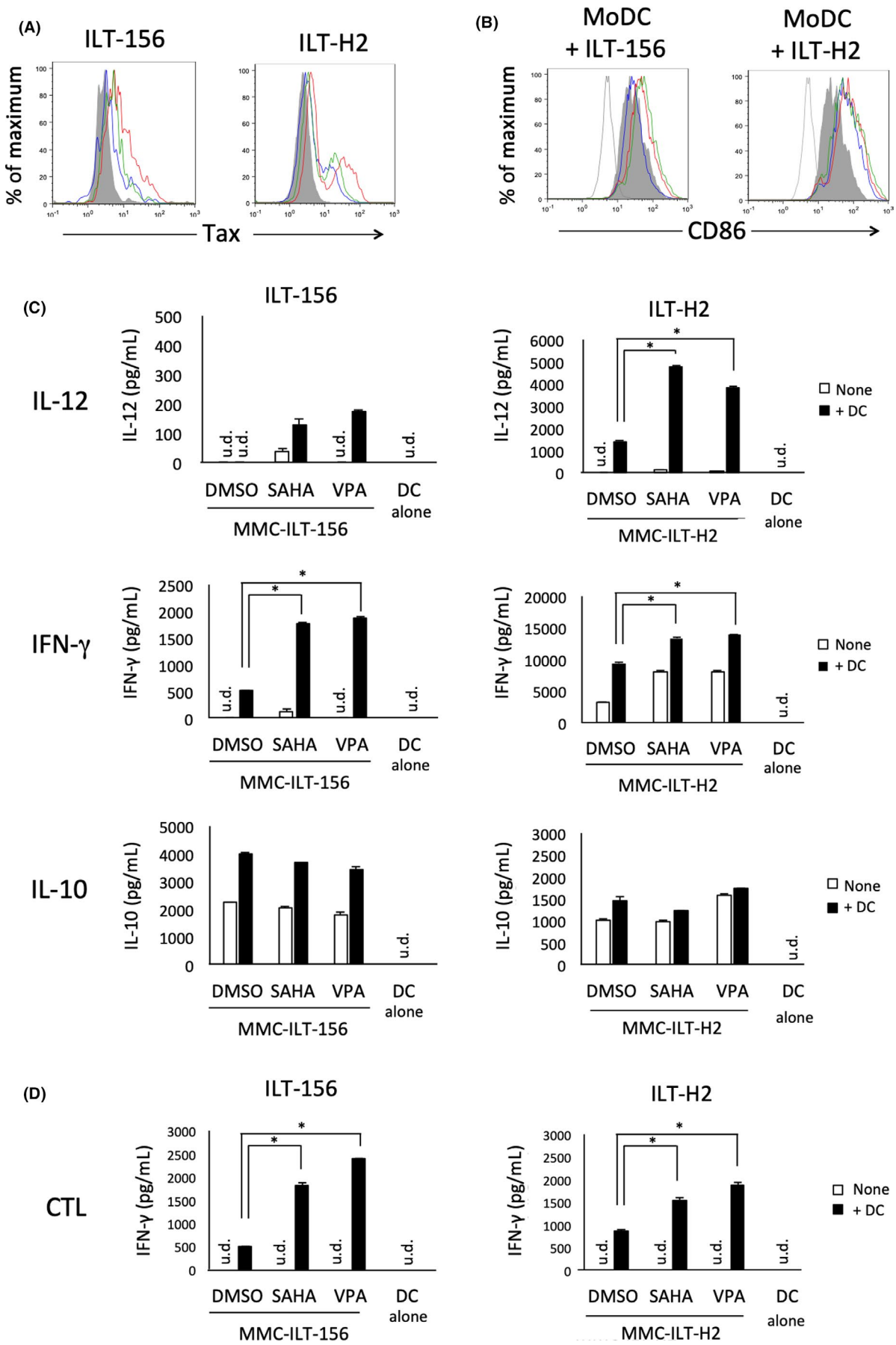

(A)
ILT-156
ILT-H2
% of maximum
Tax
(B)
MoDC + ILT-156
MoDC + ILT-H2
% of maximum
CD86
(C)
IL-12
IFN-γ
IL-10
ILT-156
ILT-H2
IL-12 (pg/mL)
IFN-γ (pg/mL)
IL-10 (pg/mL)
u.d.
DMSO SAHA VPA DC alone
MMC-ILT-156
MMC-ILT-H2
None
+ DC
(D)
CTL
ILT-156
ILT-H2
IFN-γ (pg/mL)
DMSO SAHA VPA DC alone
MMC-ILT-156
MMC-ILT-H2
None
+ DC

FIGURE 3 Histone deacetylase (HDAC) inhibitors enhanced ILT-mediated antigen presentation in antigen presenting cells (APC). A, Intracellular Tax expression in ILT-156 and ILT-H2 cells treated with .08% DMSO (blue), 1 μM suberoylanilide hydroxamic acid (SAHA; red), or 1 mM valproic acid (VPA; green) for 24 h was analyzed by flow cytometry. Shaded histogram indicates staining with control mouse IgG. B, Expression of CD86 in the $CD11c^+$ monocyte-derived dendritic cells (MoDC) co–cultured in the presence of azidothymidine (AZT) with ILT-156 or ILT-H2 cells that had been treated with DMSO (blue), SAHA (red), or VPA (green) as described in (A) was evaluated by flow cytometry. The sample of MoDC alone was similarly stained (shaded histogram) or left unstained (light grey line). C, Interleukin (IL)-12 (top), interferon (IFN)-γ (middle), and IL-10 (bottom) concentrations in the supernatants of HDAC inhibitor-treated ILT-156 or ILT-H2 cells co–cultured with (black bar) or without (white bar) MoDC in (B) were evaluated by ELISA. Results show mean and SD of duplicate samples. u.d., undetectable. $^*P < .01$. D, HDAC inhibitor-treated ILT-156 or ILT-H2 cells co–cultured with (black bar) or without (white bar) MoDC in (B) were formalin-fixed and further co–cultured with HLA-A2-restricted Tax-specific CTL. IFN-γ concentrations in the supernatants were analyzed by ELISA. Results show mean and SD of duplicate samples. u.d., undetectable. $^*P < .01$. Data are representative of at least three independent experiments

co–cultured with MMC-treated ILT-156 and ILT-H2 cells were significantly enhanced by pre–treatment of ILT with SAHA or VPA (Figure 3D).

Treatment of ILT cells with HDAC inhibitors thus augmented Tax expression and induction of CD86 expression, IL-12 production, and Tax cross–presentation in co–cultured MoDC.

3.4 | Short-term cultured primary adult T-cell leukemia cells induced Tax cross–presentation

We next investigated whether the immunogenic potential observed in ILT cells could be reproduced in short-term cultured PBMC from ATL patients.

Peripheral blood mononuclear cells from an HLA-A2-negative patient with chronic ATL (ATL #1) contained 73% $CD3^+CD4^+$ cells, 45% of which were $CD25^+CCR4^+$, compatible with the phenotype of ATL cells (Figure 4A). These PBMC were cultured in vitro for 3 days with or without depletion of $CD8^+$ cells in the presence of IL-2. Although intracellular Tax antigen was undetectable before culture, the levels of Tax expression increased after 3 days of culture (Figure 4B). Depletion of $CD8^+$ cells from the PBMC slightly enhanced Tax induction. These PBMC were MMC-treated and co–cultured with THP-1 cells in the presence of AZT for 24 hours, then fixed with formalin prior to a CTL assay. The HLA-A2-restricted Tax-specific CTL clearly produced IFN-γ against the THP-1 cells co–cultured with ATL cells but not against the ATL cells alone, indicating that the 3 day-cultured ATL cells induced Tax cross–presentation in THP-1 cells (Figure 4C).

We then assessed the effect of HDAC inhibitors on the PBMC-induced Tax cross–presentation. PBMC from another HLA-A2-negative patient with chronic ATL (ATL #2) containing $CD4^+CD25^+CCR4^+$ ATL cells (Figure 4D) were cultured in the presence of IL-2 for 3 days, with HDAC inhibitors for the last 18 hours. Tax expression was induced in approximately 8% of the PBMC cultured with control DMSO, and further enhanced by treatment with SAHA or VPA (Figure 4E). THP-1 cells co–cultured with SAHA or VPA-treated PBMC induced significantly higher levels of IFN-γ production from HLA-A2-restricted Tax-specific CTL than THP-1 co–cultured with DMSO-treated PBMC (Figure 4F).

Thus, short-term cultured PBMC from patients with chronic ATL could induce Tax cross–presentation in the co–cultured APC, which was further augmented by treatment with HDAC inhibitors.

3.5 | Short-term cultured primary adult T-cell leukemia cells can activate autologous monocyte-derived dendritic cells

Finally, we examined whether short-term cultured ATL cells could activate the patients' own MoDC to induce co–stimulatory molecule expression and IL-12 production (Figure 5). The primary PBMC of the patient ATL #1 were first divided into an adherent monocyte-rich fraction and a non–adherent PBL fraction, and then MoDC were induced by culturing adherent cells in the presence of IL-4 and GM-CSF for 5 days, while the non–adherent PBL were simultaneously cultured for 5 days in the presence of IL-2. These PBL containing ATL cells were then treated with MMC and co–cultured with autologous MoDC in the presence of AZT (Figure 5A). The $CD11c^+$ MoDC co–cultured with autologous PBL exhibited enhanced expression of CD86 and CD83, compared with MoDC alone (Figure 5B). The culture supernatants of the ATL patient-derived MoDC co–cultured with autologous PBL contained significantly higher amount of IL-12 and IFN-γ than those of MoDC or PBL alone (Figure 5C).

When MoDC from an uninfected individual were similarly co–cultured with MMC-treated autologous PBL, CD86 expression in the $CD11c^+$ MoDC was also enhanced (Figure 5D). However, the PBL from the uninfected individual hardly induced cytokine production in co–cultured autologous MoDC, whereas MMC-treated ILT-H2 cells, as a positive control, clearly induced IL-12 and IFN-γ production in the MoDC (Figure 5E).

Short-term cultured primary ATL cells were, therefore, capable of activating autologous MoDC to induce expression of co–stimulatory molecules and production of IL-12 and IFN-γ.

4 | DISCUSSION

Although freshly isolated PBMC of HTLV-1-infected individuals do not express detectable levels of Tax, Tax becomes detectable

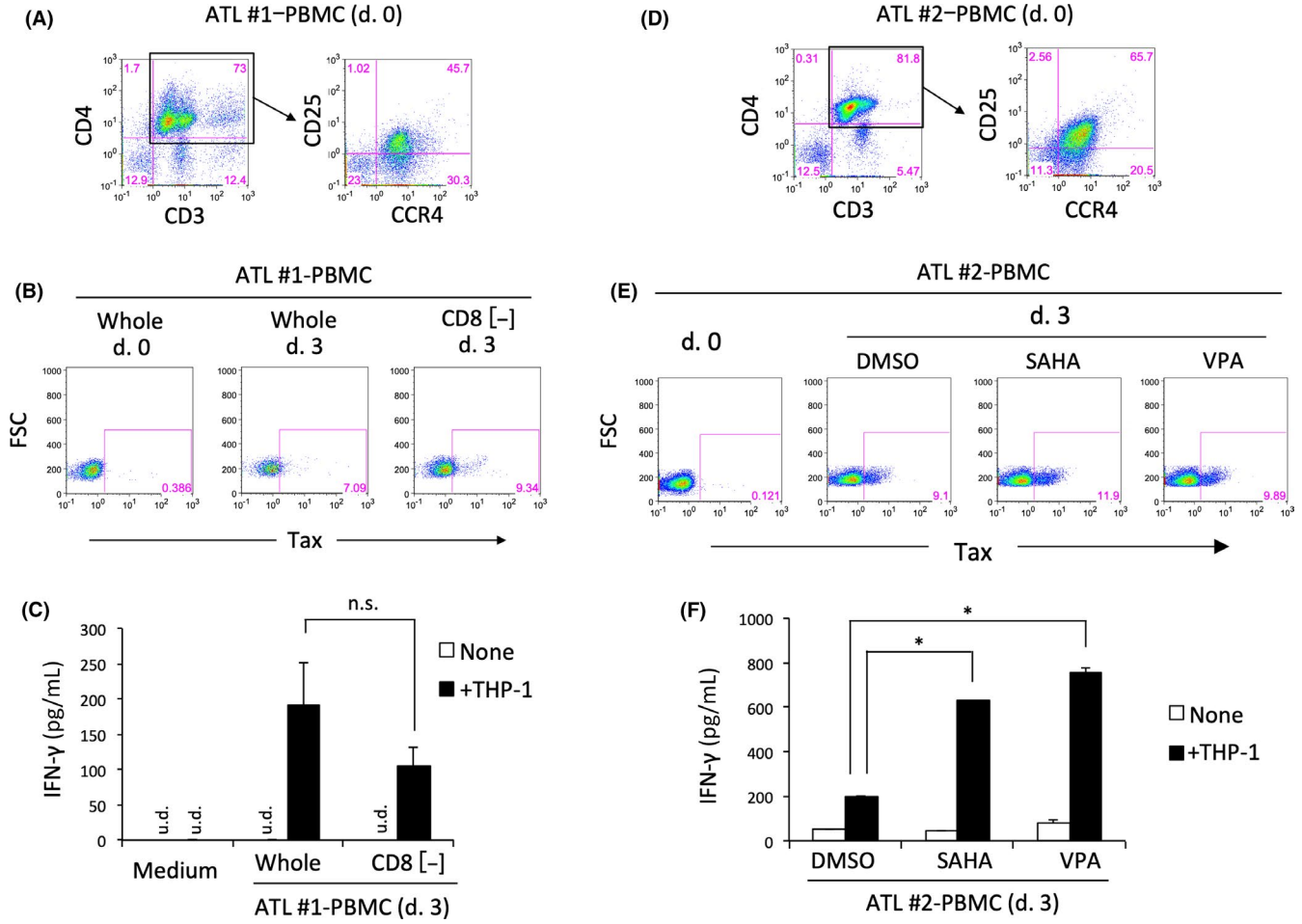
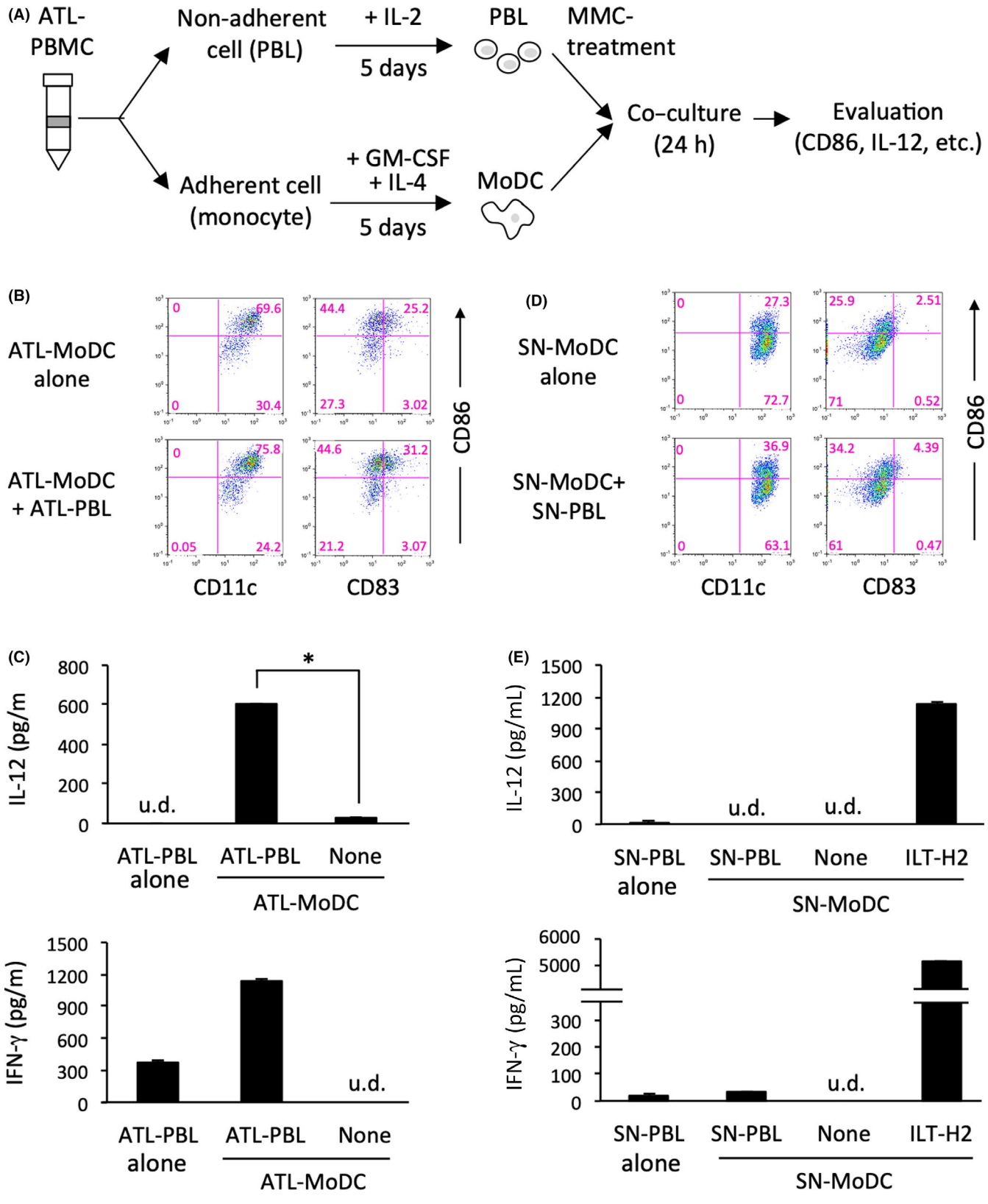


FIGURE 4 Short-term cultured primary adult T-cell leukemia (ATL) cells can induce Tax cross–presentation. A, Cell surface expression of CD3, CD4, CD25, and CCR4 on primary peripheral blood mononuclear cells (PBMC) from an HLA-A2-negative patient with chronic ATL (ATL #1) were analyzed by flow cytometry. B, PBMC of the patient ATL #1 were cultured for 3 days in the presence of interleukin (IL)-2 without fractionation (whole) or following depletion of $CD8^+$ cells (CD8[−]), and intracellular Tax expression before and after culture was analyzed by flow cytometry. C, The 3 day-cultured PBMC prepared in (B) were mitomycin C (MMC)-treated and co–cultured with (black bar) or without (white bar) THP-1 cells in the presence of azidothymidine (AZT; final 10 μM) for 24 h, fixed, and further co–cultured with HLA-A2-restricted Tax-specific CTL. Interferon (IFN)-γ concentrations in the supernatants are shown as mean and SD of duplicate samples. n.s., not significant. $^*P < .01$. D, Cell surface phenotype of primary PBMC from another HLA-A2-negative patient with chronic ATL (ATL #2) was analyzed as described in (A). E, PBMC of the patient ATL #2 were cultured for 3 days in the presence of IL-2, with DMSO (.08%), SAHA (1 μM), or VPA (1 mM) for the last 18 h. Intracellular Tax expression before or after culture was analyzed by flow cytometry. F, The 3 day-cultured PBMC prepared in (E) were MMC-treated, co–cultured with THP-1 cells in the presence of AZT, fixed, and further co–cultured with HLA-A2-restricted Tax-specific CTL as described in (C). IFN-γ concentrations in the supernatants are shown as mean and SD of duplicate samples. u.d., undetectable. $^*P < .01$

FIGURE 5 Short-term cultured primary adult T-cell leukemia (ATL) cells can activate autologous monocyte-derived dendritic cells (MoDC). A, Schematic of the autologous co–culture experiment. Primary peripheral blood mononuclear cells (PBMC) from the ATL patient described in Figure 4 were divided into adherent monocyte-enriched and non–adherent PBL fractions. Adherent cells were differentiated to MoDC in the presence of interleukin (IL)-4 and granulocyte macrophage-colony stimulating factor (GM-CSF) for 5 days and co–cultured with mitomycin C (MMC)-treated autologous peripheral blood lymphocytes (PBL) that had been cultured in the presence of IL-2 for 5 days. Azidothymidine (AZT) was included in the medium throughout. B, C, After 24 h of co–culture of ATL patient's MoDC (ATL-MoDC) and MMC-treated autologous PBL (ATL-PBL), CD86 and CD83 expression in the $CD11c^+$ MoDC (B) and IL-12 and interferon (IFN)-γ production in the supernatants (C) were analyzed by flow cytometry and ELISA, respectively. u.d., undetectable. $^*P < .01$. D, E, MoDC were similarly induced from primary PBMC of an uninfected individual (SN-MoDC) and co–cultured with MMC-treated autologous PBL (SN-PBL). CD86 and CD83 expression (D) and IL-12 and IFN-γ production (E) were evaluated. MMC-treated ILT-H2 cells were used as a positive control. ELISA results show mean and SD of duplicate samples. u.d., undetectable

upon in vitro culture. The present study indicates that such Tax-expressing HTLV-1-infected cells derived from ATL patients have the potential to induce Tax antigen cross-presentation, co-stimulatory molecule expression, and IL-12 production in co-cultured APC, all of which are required to raise $CD8^+$ Tax-specific CTL responses.

Although MMC-treated HTLV-infected cells retain infectivity, Tax antigen was still presented on MHC-I in APC following co-culture



with MMC-treated ILT cells in the presence of AZT, while HTLV-1 proviral integration was completely blocked (Figure 2). These results clearly indicated that the newly synthesized viral antigens following de novo HTLV-1 infection were dispensable for Tax antigen presentation in APC.

Antigen cross–presentation on MHC-I is mediated by some subsets of DC through cytosolic and vacuolar pathways, although the precise mechanisms are not fully understood.[44,45] A previous study indicated that poly-IC promotes antigen cross–presentation by stimulating toll-like receptor (TLR) 3 through innate immune pathways in DC.[46] In HTLV-1 infection, experimentally prepared cell-free HTLV-1 virions induce type-I IFN responses in plasmacytoid (p)DC as a result of TLR7 stimulation by viral RNA.[47] Assil et al (2019) indicated that pDC sense glycan-rich structures such as biofilm-like structures on the surface of HTLV-1-infected cells, which promotes virus-induced IFN responses.[48] In the present study, similar innate immune responses might be involved in the mechanisms of Tax cross–presentation induced by MMC-treated HTLV-1-infected cells.

We observed some differences among the ILT in their ability to activate APC. ILT-156 efficiently induced Tax cross–presentation but not CD86 or IL-12 expression, while ILT-H2 successfully induced all of these effects. This may be partly attributed to the cytokine profile of ILT cells. ILT-156 produced much higher levels of IL-10 than ILT-H2, while ILT-H2 but not ILT-156 cells produced IFN-γ (Figure 3C). The importance of IFN-γ in induction of IL-12 is well known.[49] Conversely, IL-10 suppresses IL-12 production,[50] explaining why ILT-H2 much more efficiently induced IL-12 production than ILT-156 (Figure 3C). ILT-156 also failed to induce CD86 in APC (Figure 3B). These defects were improved by HDAC inhibitors, although the mechanisms remain to be clarified (Figure 3C).

Histone deacetylase inhibitors mediate epigenetic anti–tumor effects such as induction of tumor suppressor genes.[51,52] In HTLV-1 infected cells, HDAC inhibitors have been shown to augment viral expression and apoptosis in infected cells.[53] In the present study, HDAC inhibitors enhanced the abilities of HTLV-1-infected cells to induce cross–presentation of Tax antigen, IL-12 production, and CD86 expression in APC, all of which are advantageous for raising $CD8^+$ Tax-specific CTL responses (Figure 3). The therapeutic effects of VPA were previously demonstrated using a baboon model that was naturally infected with simian T-cell leukemia virus, closely related to HTLV-1, in which systemic administration of VPA combined with AZT resulted in reduced PVL and increased CTL.[54] Reduction of bovine leukemia virus-induced lymphomas by intratumoral administration of VPA was also reported in sheep.[55] In humans, VPA treatment reduced PVL after transient elevation of PVL in HAM/TSP patients, while the effect on HAM/TSP symptoms was limited.[42,56] Our findings in the present study suggest an alternative use of HDAC inhibitors in vitro to augment immunogenicity of a cell-based vaccine to induce $CD8^+$ Tax-specific CTL responses.

The experiments using primary ATL cells indicated, however, that even in the absence of HDAC inhibitors, short-term cultured primary ATL cells were capable of activating APC to induce Tax cross–presentation, IL-12, and co–stimulatory molecules (Figures 4 and 5). Notably, only a small percentage of the short-term cultured PBMC from the ATL patient tested expressed detectable levels of Tax, which were enough to produce these effects. Because induction of CD86 was observed also in the combination of MoDC and MMC-treated PBL from an uninfected individual (Figure 5D), it could be a nonspecific response to phagocytosis, whereas IL-12 induction was observed only in the ATL samples (Figure 5C,E). Because the co–cultured MoDC and PBL were autologous, allogeneic reaction was excluded, suggesting that IL-12 induction was mediated by components of HTLV-1.

Because Tax-specific CTL are a critical anti–tumor effector in HTLV-1 infection, we focused on Tax antigen cross–presentation in this study. However, the short-term cultured PBMC from HTLV-1-infected individuals express various HTLV-1 antigens other than Tax and cellular antigens as well, which might also contribute to anti–tumor/virus defense. Besides Tax, HTLV-1 Env,[57] Pol,[10] p12, p13,[58] and HBZ[59] have been reported to be recognized by CTL, and a potential anti–tumor effect of HBZ-specific CTL has been suggested in an animal model.[60] Unwanted autoimmune response should also be carefully watched.

To confirm the in vivo efficacy of the PBMC-based vaccine on the CTL induction, we are currently conducting an in vivo study in non–human primates, investigating inoculation of autologous short-term cultured PBMC into naturally STLV-1-infected Japanese monkeys (manuscript in preparation).

In conclusion, HTLV-1-infected cells expressing Tax in cultured PBMC of ATL patients have the potential to activate APC raising $CD8^+$ Tax-specific CTL responses by inducing cross–presentation of Tax antigen, IL-12 production, and co–stimulatory molecule expression. These abilities in infected cells might vary among individuals but could be improved by HDAC inhibitors. Although the number of samples tested is limited, the present findings strongly suggest that the short-term cultured autologous PBMC from ATL patients expressing Tax could potentially act as a vaccine to induce Tax-specific CTL, which might also be extended to indolent ATL patients.

ACKNOWLEDGMENTS

We thank Professor Yuetsu Tanaka (University of the Ryukyus, Okinawa, Japan) for providing the anti–Tax mAb, Lt-4. We also thank Dan Matsuda, Toshiki Yanagisawa, and Masaya Nakamizo (medical students of Tokyo Medical and Dental University, Tokyo, Japan) for technical assistance. This study was supported by grants (MK) from the Japan Agency for Medical Research and Development (19cm0106365h0001), JSPS KAKENHI Grant Number JP20K07512, and the Japan Cancer Society (Project Mirai Cancer Research Grants).

DISCLOSURE

The authors have no conflict of interest.

ORCID

Atsuhiko Hasegawa https://orcid.org/0000-0002-2974-990X
Mari Kannagi https://orcid.org/0000-0002-3433-2658

REFERENCES

1. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood*. 1977;50:481-492.
2. Hinuma Y, Nagata K, Hanaoka M, et al. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc Natl Acad Sci USA*. 1981;78:6476-6480.
3. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA*. 1980;77:7415-7419.
4. Yoshida M. Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. *Annu Rev Immunol*. 2001;19:475-496.
5. Satou Y, Yasunaga J, Yoshida M, Matsuoka M. HTLV-I basic leucine zipper factor gene mRNA supports proliferation of adult T cell leukemia cells. *Proc Natl Acad Sci USA*. 2006;103:720-725.
6. Mesnard JM, Barbeau B, Cesaire R, Peloponese JM. Roles of HTLV-1 basic Zip Factor (HBZ) in viral chronicity and leukemic transformation. Potential new therapeutic approaches to prevent and treat HTLV-1-related diseases. *Viruses*. 2015;7:6490-6505.
7. Osame M, Usuku K, Izumo S, et al. HTLV-I associated myelopathy, a new clinical entity. *Lancet*. 1986;1:1031-1032.
8. Gessain A, Barin F, Vernant JC, et al. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet*. 1985;2:407-410.
9. Jacobson S, Shida H, McFarlin DE, Fauci AS, Koenig S. Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. *Nature*. 1990;348:245-248.
10. Parker CE, Daenke S, Nightingale S, Bangham CR. Activated, HTLV-1-specific cytotoxic T-lymphocytes are found in healthy seropositives as well as in patients with tropical spastic paraparesis. *Virology*. 1992;188:628-636.
11. Takamori A, Hasegawa A, Utsunomiya A, et al. Functional impairment of Tax-specific but not cytomegalovirus-specific CD8+ T lymphocytes in a minor population of asymptomatic human T-cell leukemia virus type 1-carriers. *Retrovirology*. 2011;8:100.
12. Ohashi T, Hanabuchi S, Kato H, et al. Prevention of adult T-cell leukemia-like lymphoproliferative disease in rats by adoptively transferred T cells from a donor immunized with human T-cell leukemia virus type 1 Tax-coding DNA vaccine. *J Virol*. 2000;74:9610-9616.
13. Hanabuchi S, Ohashi T, Koya Y, et al. Regression of human T-cell leukemia virus type I (HTLV-I)-associated lymphomas in a rat model: peptide-induced T-cell immunity. *J Natl Cancer Inst*. 2001;93:1775-1783.
14. Kato H, Koya Y, Ohashi T, et al. Oral administration of human T-cell leukemia virus type 1 induces immune unresponsiveness with persistent infection in adult rats. *J Virol*. 1998;72:7289-7293.
15. Mori N, Gill PS, Mougdil T, Murakami S, Eto S, Prager D. Interleukin-10 gene expression in adult T-cell leukemia. *Blood*. 1996;88:1035-1045.
16. Inagaki A, Ishida T, Ishii T, et al. Clinical significance of serum Th1-, Th2- and regulatory T cells-associated cytokines in adult T-cell leukemia/lymphoma: high interleukin-5 and -10 levels are significant unfavorable prognostic factors. *Int J Cancer*. 2006;118:3054-3061.
17. Sawada L, Nagano Y, Hasegawa A, et al. IL-10-mediated signals act as a switch for lymphoproliferation in Human T-cell leukemia virus type-1 infection by activating the STAT3 and IRF4 pathways. *PLoS Pathog*. 2017;13:e1006597.
18. Kagdi H, Demontis MA, Ramos JC, Taylor GP. Switching and loss of cellular cytokine producing capacity characterize in vivo viral infection and malignant transformation in human T- lymphotropic virus type 1 infection. *PLoS Pathog*. 2018;14:e1006861.
19. Kozako T, Yoshimitsu M, Fujiwara H, et al. PD-1/PD-L1 expression in human T-cell leukemia virus type 1 carriers and adult T-cell leukemia/lymphoma patients. *Leukemia*. 2009;23:375-382.
20. Masaki A, Ishida T, Suzuki S, et al. Human T-cell lymphotropic/leukemia virus type 1 (HTLV-1) Tax-specific T-cell exhaustion in HTLV-1-infected individuals. *Cancer Sci*. 2018;109:2383-2390.
21. Hishizawa M, Kanda J, Utsunomiya A, et al. Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study. *Blood*. 2010;116:1369-1376.
22. Utsunomiya A, Choi I, Chihara D, Seto M. Recent advances in the treatment of adult T-cell leukemia-lymphomas. *Cancer Sci*. 2015;106:344-351.
23. Harashima N, Kurihara K, Utsunomiya A, et al. Graft-versus-Tax response in adult T-cell leukemia patients after hematopoietic stem cell transplantation. *Cancer Res*. 2004;64:391-399.
24. Suehiro Y, Hasegawa A, Iino T, et al. Clinical outcomes of a novel therapeutic vaccine with Tax peptide-pulsed dendritic cells for adult T cell leukaemia/lymphoma in a pilot study. *Br J Haematol*. 2015;169:356-367.
25. Kannagi M, Hasegawa A, Nagano Y, Iino T, Okamura J, Suehiro Y. Maintenance of long remission in adult T-cell leukemia by Tax-targeted vaccine: a hope for disease-preventive therapy. *Cancer Sci*. 2019;110:849-857.
26. Kannagi M, Shida H, Igarashi H, et al. Target epitope in the Tax protein of human T-cell leukemia virus type I recognized by class I major histocompatibility complex-restricted cytotoxic T cells. *J Virol*. 1992;66:2928-2933.
27. Harashima N, Tanosaki R, Shimizu Y, et al. Identification of two new HLA-A*1101-restricted tax epitopes recognized by cytotoxic T lymphocytes in an adult T-cell leukemia patient after hematopoietic stem cell transplantation. *J Virol*. 2005;79:10088-10092.
28. Hasegawa A, Ohashi T, Hanabuchi S, et al. Expansion of Human T-cell leukemia virus type 1 (HTLV-1) reservoir in orally infected rats: inverse correlation with HTLV-1-specific cellular immune response. *J Virol*. 2003;77:2956-2963.
29. Komori K, Hasegawa A, Kurihara K, et al. Reduction of human T-cell leukemia virus type 1 (HTLV-1) proviral loads in rats orally infected with HTLV-1 by reimmunization with HTLV-1-infected cells. *J Virol*. 2006;80:7375-7381.
30. Hinuma Y, Gotoh Y, Sugamura K, et al. A retrovirus associated with human adult T-cell leukemia: in vitro activation. *Gann*. 1982;73:341-344.
31. Kurihara K, Harashima N, Hanabuchi S, et al. Potential immunogenicity of adult T cell leukemia cells in vivo. *Int J Cancer*. 2005;114:257-267.
32. Kinpara S, Hasegawa A, Utsunomiya A, et al. Stromal cell-mediated suppression of human T-cell leukemia virus type 1 expression in vitro and in vivo by type I interferon. *J Virol*. 2009;83:5101-5108.
33. Kulkarni A, Taylor GP, Klose RJ, Schofield CJ, Bangham CR. Histone H2A monoubiquitylation and p38-MAPKs regulate immediate-early gene-like reactivation of latent retrovirus HTLV-1. *JCI Insight*. 2018;3:e123196.
34. Takeda S, Maeda M, Morikawa S, et al. Genetic and epigenetic inactivation of tax gene in adult T-cell leukemia cells. *Int J Cancer*. 2004;109:559-567.
35. Mellman I, Steinman RM. Dendritic cells: specialized and regulated antigen processing machines. *Cell*. 2001;106:255-258.
36. Trombetta ES, Mellman I. Cell biology of antigen processing in vitro and in vivo. *Annu Rev Immunol*. 2005;23:975-1028.
37. Blander JM. Regulation of the cell biology of antigen cross–presentation. *Annu Rev Immunol*. 2018;36:717-753.
38. Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int J Cancer*. 1980;26:171-176.

39. Kannagi M, Harada S, Maruyama I, et al. Predominant recognition of human T cell leukemia virus type I (HTLV-I) pX gene products by human CD8+ cytotoxic T cells directed against HTLV-I-infected cells. *Int Immunol*. 1991;3:761-767.
40. Lee B, Tanaka Y, Tozawa H. Monoclonal antibody defining tax protein of human T-cell leukemia virus type-I. *Tohoku J Exp Med*. 1989;157:1-11.
41. Hu PQ, Tuma-Warrino RJ, Bryan MA, et al. *Escherichia coli* expressing recombinant antigen and listeriolysin O stimulate class I-restricted CD8+ T cells following uptake by human APC. *J Immunol*. 2004;172:1595-1601.
42. Lezin A, Gillet N, Olindo S, et al. Histone deacetylase mediated transcriptional activation reduces proviral loads in HTLV-1 associated myelopathy/tropical spastic paraparesis patients. *Blood*. 2007;110:3722-3728.
43. Jain P, Ahuja J, Khan ZK, et al. Modulation of dendritic cell maturation and function by the Tax protein of human T cell leukemia virus type 1. *J Leukoc Biol*. 2007;82:44-56.
44. Ho NI, Huis in 't Veld LGM, Raaijmakers TK, Adema GJ. Adjuvants enhancing cross–presentation by dendritic cells: the key to more effective vaccines? *Front Immunol*. 2018;9:2874.
45. Embgenbroich M, Burgdorf S. Current concepts of antigen cross–presentation. *Front Immunol*. 2018;9:1643.
46. Azuma M, Ebihara T, Oshiumi H, Matsumoto M, Seya T. Cross–priming for antitumor CTL induced by soluble Ag + polyI: C depends on the TICAM-1 pathway in mouse CD11c(+)/CD8alpha(+) dendritic cells. *Oncoimmunology*. 2012;1:581-592.
47. Colisson R, Barblu L, Gras C, et al. Free HTLV-1 induces TLR7-dependent innate immune response and TRAIL relocalization in killer plasmacytoid dendritic cells. *Blood*. 2010;115:2177-2185.
48. Assil S, Futsch N, Decembre E, et al. Sensing of cell-associated HTLV by plasmacytoid dendritic cells is regulated by dense beta-galactoside glycosylation. *PLoS Pathog*. 2019;15:e1007589.
49. Hayes MP, Wang J, Norcross MA. Regulation of interleukin-12 expression in human monocytes: selective priming by interferon-gamma of lipopolysaccharide-inducible p35 and p40 genes. *Blood*. 1995;86:646-650.
50. Aste-Amezaga M, Ma X, Sartori A, Trinchieri G. Molecular mechanisms of the induction of IL-12 and its inhibition by IL-10. *J Immunol*. 1998;160:5936-5944.
51. Song SH, Han SW, Bang YJ. Epigenetic-based therapies in cancer: progress to date. *Drugs*. 2011;71:2391-2403.
52. Nebbioso A, Carafa V, Benedetti R, Altucci L. Trials with 'epigenetic' drugs: an update. *Mol Oncol*. 2012;6:657-682.
53. Belrose G, Gross A, Olindo S, et al. Effects of valproate on Tax and HBZ expression in HTLV-1 and HAM/TSP T lymphocytes. *Blood*. 2011;118:2483-2491.
54. Afonso PV, Mekaouche M, Mortreux F, et al. Highly active antiretroviral treatment against STLV-1 infection combining reverse transcriptase and HDAC inhibitors. *Blood*. 2010;116:3802-3808.
55. Achachi A, Florins A, Gillet N, et al. Valproate activates bovine leukemia virus gene expression, triggers apoptosis, and induces leukemia/lymphoma regression in vivo. *Proc Natl Acad Sci USA*. 2005;102:10309-10314.
56. Mosley AJ, Meekings KN, McCarthy C, et al. Histone deacetylase inhibitors increase virus gene expression but decrease CD8+ cell antiviral function in HTLV-1 infection. *Blood*. 2006;108:3801-3807.
57. Jacobson S, Reuben JS, Streilein RD, Palker TJ. Induction of CD4+, human T lymphotropic virus type-1-specific cytotoxic T lymphocytes from patients with HAM/TSP. Recognition of an immunogenic region of the gp46 envelope glycoprotein of human T lymphotropic virus type-1. *J Immunol*. 1991;146:1155-1162.
58. Pique C, Ureta-Vidal A, Gessain A, et al. Evidence for the chronic in vivo production of human T cell leukemia virus type I Rof and Tof proteins from cytotoxic T lymphocytes directed against viral peptides. *J Exp Med*. 2000;191:567-572.
59. Rowan AG, Suemori K, Fujiwara H, et al. Cytotoxic T lymphocyte lysis of HTLV-1 infected cells is limited by weak HBZ protein expression, but non–specifically enhanced on induction of Tax expression. *Retrovirology*. 2014;11:116.
60. Sugata K, Yasunaga J, Mitobe Y, et al. Protective effect of cytotoxic T lymphocytes targeting HTLV-1 bZIP factor. *Blood*. 2015;126:1095-1105.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Ishizawa M, Ganbaatar U, Hasegawa A, et al. Short-term cultured autologous peripheral blood mononuclear cells as a potential immunogen to activate Tax-specific CTL response in adult T-cell leukemia patients. *Cancer Sci*. 2021;112:1161–1172. https://doi.org/10.1111/cas.14800