changed to lysine) makes Vpr significantly resistant to fumagillin (Fig. 3B). Since the E25K Vpr still inhibits growth of yeast cells (26), the mechanism of Vpr may be directly on Vpr rather than on a downstream pathway. The precise mechanism through which the E25K mutation renders Vpr resistant to fumagillin is not clear, but it is possible that fumagillin interacts directly (albeit too weakly to detect) with Vpr at residues surrounding E25.

Vpr is required for efficient replication of HIV-1 in non-dividing cells such as macrophages (4, 23, 24). During the HIV-1 life cycle, Vpr functions after entry and reverse transcription, yet prior to, or at the time of, proviral transcription (4). Thus we examined the effect of fumagillin on the proviral transcription upon the infection using an *env*-deficient HIV-1 vector that allows only a single round of infection. Wild type or frame-shifted Vpr-containing, *env*-deficient HIV-1 reporter vector in which nef has been replaced by the luciferase gene (NL-Luc-R+ or NL-Luc-R-, respectively, (4)) was used to infect primary human macrophages that were derived by culturing primary monocytes. Luciferase activity, determined 6 days after infection (Fig. 4A, B), was about 4 times higher than that from Vpr- virus, indicating that Vpr is required for efficient expression of virally-encoded genes in macrophages (4). When fumagillin or TNP470 was added at the time of infection, luciferase expression from the Vpr+ virus was inhibited in a dose-dependent manner (Fig. 4A, B). In contrast, the low level of luciferase activity from the Vpr- virus was not affected by fumagillin or TNP470, indicating that the inhibition of viral gene expression in Vpr+ infected cells is due to the inhibition of Vpr by these drugs rather than to some non-specific toxicity of them, if any. In this regard, we also could not see any sign of toxicity of these drugs for the macrophages under a microscope. Taken together, our results show that fumagillin or

TNP470 suppresses the Vpr-dependent viral gene expression that is required for the viral replication upon the infection.

Because it is now evident that Vpr's contribution to the pathogenesis of HIV-1 infection *in vivo* is crucial, Vpr has been proposed to be an attractive target for developing novel therapeutic strategies for AIDS therapy. Our results show that fumagillin and its derivatives can be used as a new type of AIDS therapeutic drug, which targets Vpr. In this context, it should be noted that fumagillin and TNP470 are already used clinically to treat Kaposi's sarcoma or microsporidiosis in AIDS patients with successful results (5, 6), although the effects of these drugs on the viral replication have not been reported. Thus, the day when the fumagillin-derived compounds can be used clinically to prevent HIV-1 replication may come sooner than expected.

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FIGURE LEGENDS

FIG. 1. Screening system to isolate Vpr inhibitors.

(A) Schematic presentation of Vpr screening system. Budding yeast cells (a multidrug sensitive yeast strain MLC30 obtained from Tokichi Miyakawa (Hiroshima Univ., Higashi-Hiroshima, Japan) (19)) harboring Vpr expression vector (*Xho*I-*Not*I fragments (25) of N-terminal FLAG-tagged HIV-1_{NL4-3}Vpr were blunted and inserted into *Bam*HI sites of copper inducible yeast expression vector, pYEX-BX (AMRAD BIOTECH, Victoria, Australia)) were embedded in agar plates containing inducer (0.05 mM $CuSO_4$ in SD medium (0.7% yeast nitrogen base (DIFCO), 2% glucose) containing 0.001% SDS and amino acids minus selective amino acids in 2% agar (Phytagar, GIBCO)). Paper filters (ϕ=6 mm) with broths or compounds to be tested were put on the plates. Only the yeasts surrounding filters that contain Vpr inhibitors were able to grow.

(B and C) Growing yeasts surrounding filters containing 10 μl of extracts from the culture broth of a fungus with Vpr inhibitory activity (B) or purified fumagillin (C; 2mg/ml, 10 μl). Plates were incubated for 4 days at 30 °C. Fumagillin was isolated from the culture broth of a producing fungal strain using bioassay-guided purification procedures. The structure of fumagillin was determined by the physico-chemical properties, detailed ^{1}H- and ^{13}C-NMR analysis, and mass spectroscopy (2).

(D) Chemical structures of fumagillin and TNP470. TNP470 was synthesized from fumagillin as described previously (18) and used in this study.

FIG. 2. Fumagillin and TNP470 inhibit Vpr activity in HeLa cells.

(A) Thirty minutes before the addition of zinc, fumagillin (FM) or TNP470 was added to MT-Vpr1 cells (25) at the concentrations described. Cells were cultured for a day in the presence or absence of zinc (Zn; 150 μM) and harvested for FACS analysis. Numbers in the figures represent the percentage of cells with 4C DNA contents.

(B) Mt-Vpr1 cells were synchronized at G1/S border (25), then released in the presence or absence of zinc (Zn; 150 μM) and/or fumagillin (FM; 10 ng/ml). Zinc and fumagillin were added at 2 h and 1 h before the release, respectively. Numbers in the figures represent the percentage of cells with 4C DNA contents.

(C) Cells as in (A) were cultured for 6 hours and harvested for western analysis to detect FLAG-Vpr expression (25). Drugs were added at 10 ng/ml.

FIG. 3. Mechanism of fumagillin to cancel the Vpr activity.

(A) Vpr inhibits growth of yeast cells independently from MetAP2 activity. MetAP2 deletion mutant strain (*Δmap2*, *map2::URA3*) (15) were obtained from Yie-Hwa Chang (St. Louis Univ. Sch. Med., MO). *Δmap2* cells (right) or its isogenic control cells (left) were cultured in the presence (closed circle) or absence (open circle) of the Vpr expression (*URA3* marker in pYEX-BX vector was changed to *HIS3*). The growth of yeast cells was monitored with the absorbance at 600 nm.

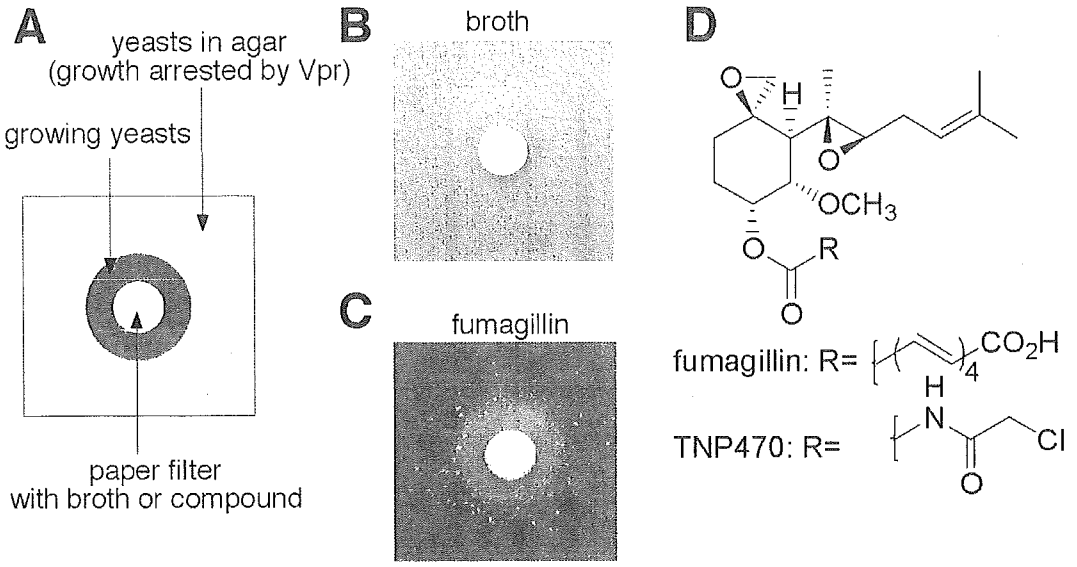
(B) E25K mutation makes Vpr resistant to fumagillin. Yeast cells with wild type Vpr (left) or E25K mutated Vpr were embedded in agar plates as in FIG.1. Paper filters with 20 μg of fumagillin were put on the plates and incubated for 3 days at 30 °C. Photographs were taken with translucent light to increase sensitivity.

FIG. 4. Fumagillin and TNP470 inhibit Vpr dependent proviral gene expression.

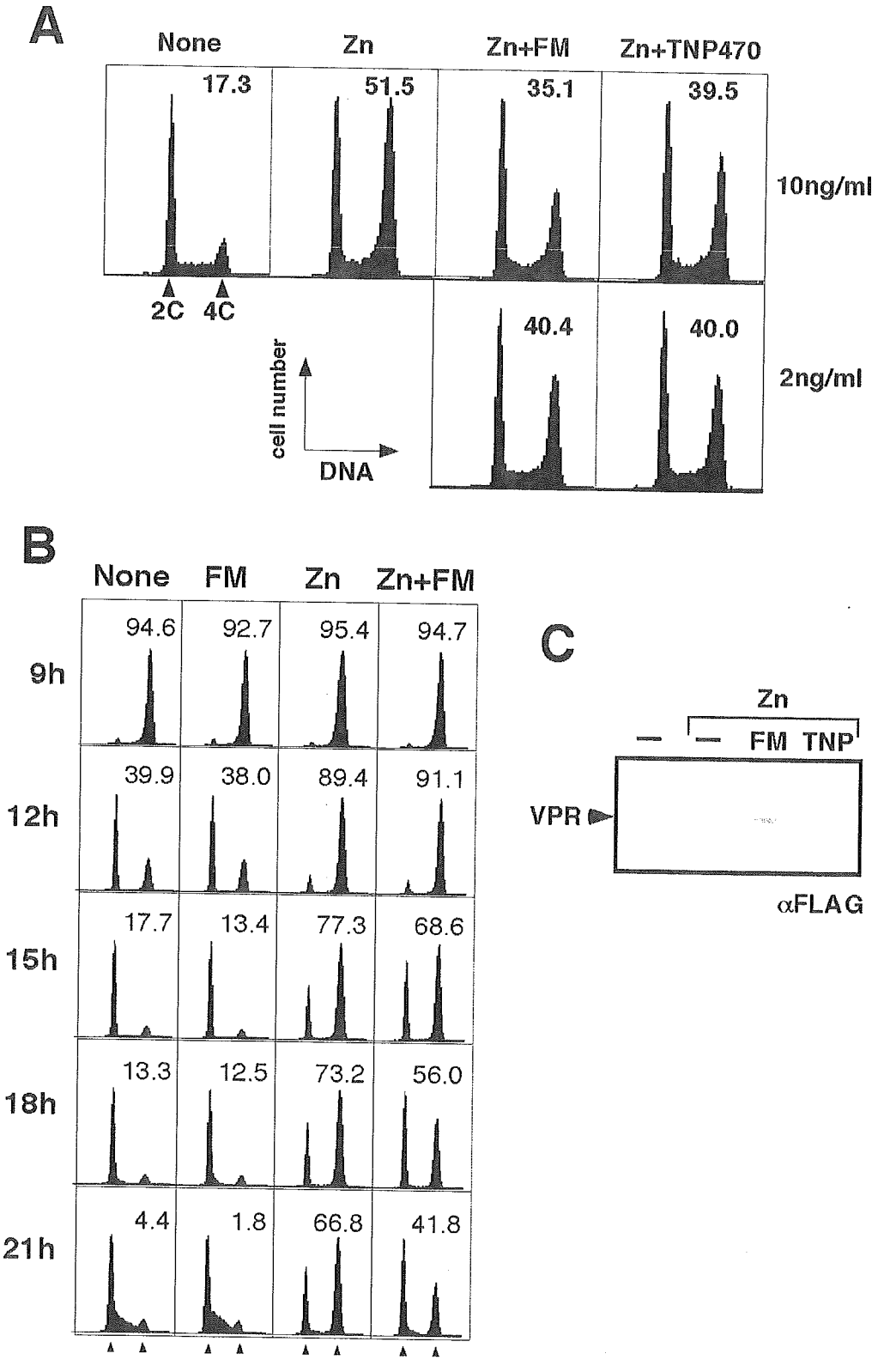
(A and B) To generate the single-round replication incompetent luciferase reporter virus stocks (NL-Luc-E$^-$R$^+$ or NL-Luc-E$^-$R$^-$) (4), 293T cells were co-transfected with the proviral DNAs (obtained from Ned Landau through the AIDS Research and Reference Reagent Program) and plasmids encoding vascular stomatitis virus envelope protein (pCMV-VSV-G-RSV-Rev). Macrophages obtained from peripheral blood mononuclear cell (PBMC) of healthy donors as described (3) were infected with the HIV-1 reporter

vector with wild type (closed circle; NL-Luc-E$^-$R$^+$(VSV-G)) or truncated (open circle; NL-Luc-E$^-$R$^-$(VSV-G)) Vpr and cultured in the presence of fumagillin (A) or TNP470 (B). The proviral gene expression was monitored by the luciferase activity 6 days after the infection using luciferase assay substrate (Promega).

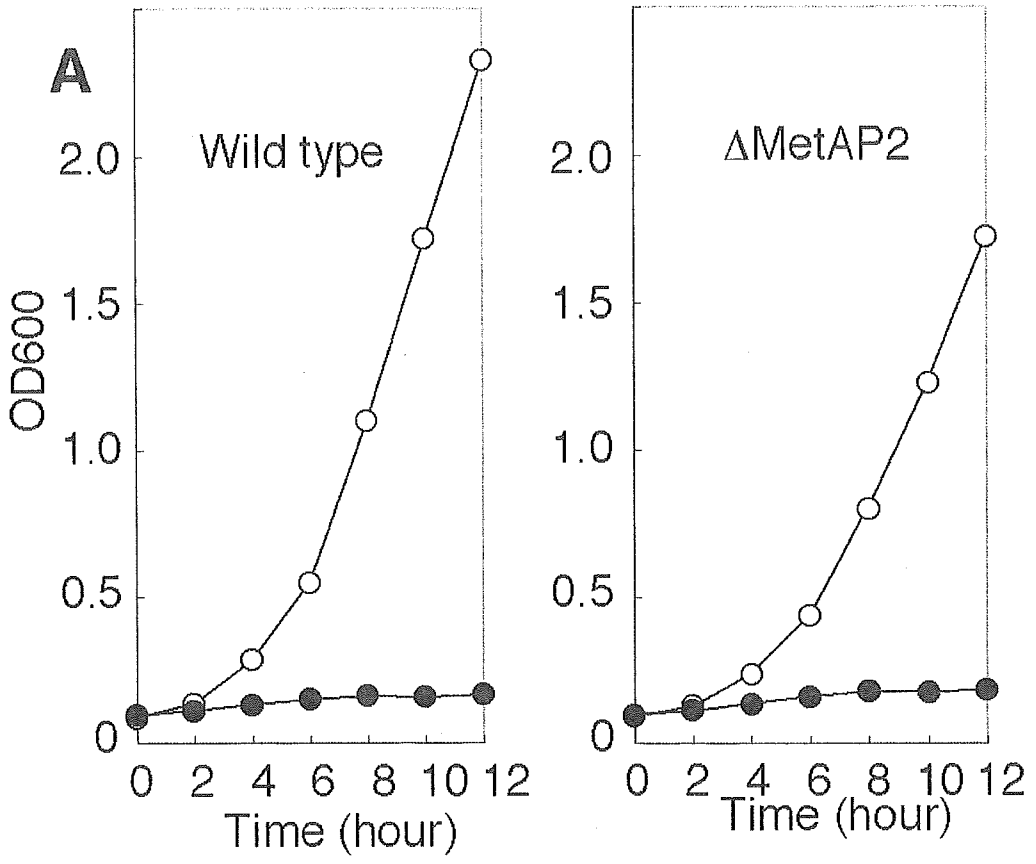
Watanabe, N. et al. FIG. 1

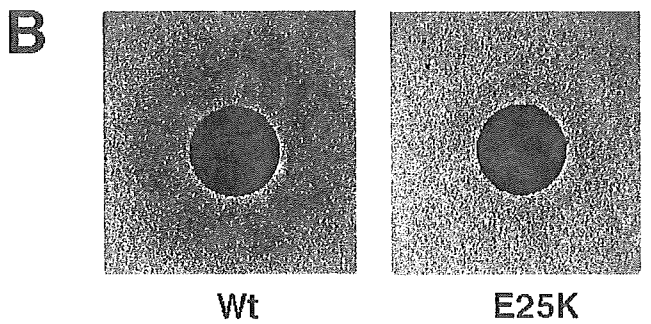


Watanabe, N. et al. FIG. 2

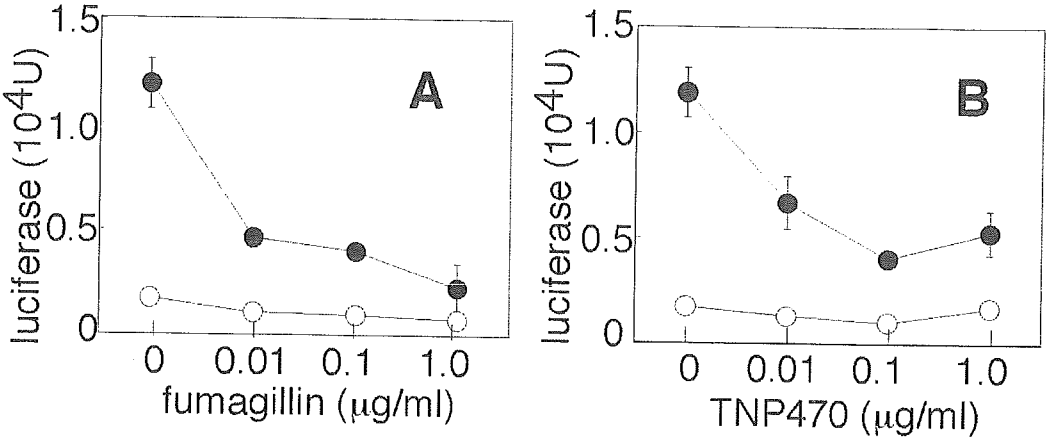


Watanabe, N. et al. FIG. 3





Watanabe, N. et al. FIG. 4



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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

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We previously reported that Tax-specific CD8$^+$ cytotoxic T lymphocytes (CTLs), directed to single epitopes restricted by HLA-A2 or A24, expanded in vitro and in vivo in peripheral blood mononuclear cells (PBMC) from some adult T-cell leukemia (ATL) patients after but not before allogeneic hematopoietic stem cell transplantation (HSCT). Here, we demonstrated similar Tax-specific CTL expansion in PBMC from another post-HSCT ATL patient without HLA-A2 or A24, whose CTLs equally recognized two newly identified epitopes, Tax88-96 and Tax272-280, restricted by HLA-A11, suggesting that these immunodominant Tax epitopes are present in the ATL patient in vivo.

Adult T-cell leukemia (ATL) caused by human T-cell leukemia virus type I (HTLV-I) is characterized by poor prognosis following chemotherapy (7, 18, 20, 23). However, the results of recent allogeneic hematopoietic stem cell transplantation (HSCT) for ATL patients are encouraging (9, 24). This indicates that a graft-versus-leukemia (GVL) response may be effective for ATL as well as other types of leukemia, although there is a risk of graft-versus-host (GVH) diseases (GVHD).

We previously found that peripheral blood mononuclear cells (PBMC) from ATL patients after but not before HSCT from HLA-identical donors exhibited vigorous HTLV-I-specific cytotoxic T lymphocyte (CTL) responses that were directed to a limited number of Tax epitopes, i.e., an HLA-A2-restricted Tax11-19 epitope in one patient and an HLA-A24-restricted Tax301-309 epitope in another (6). These patients have now been in complete remission for more than 3 years.

Since HTLV-I Tax is the dominant target antigen recognized by HTLV-I-specific CTLs (8, 10, 16), which are thought to be responsible for in vivo immune surveillance for HTLV-I leukemogenesis (11), the positive conversion of Tax-specific CTL responses in post-HSCT ATL patients suggested that these CTLs might be involved in a GVL response. In a rat model of HTLV-I-infected T-cell lymphomas, Tax oligopeptide at a dominant CTL epitope successfully induced antitumor immunity, implying that the dominant CTL epitope identified in ATL patients may also be a potential candidate for a tumor vaccine (5, 15).

In the present study, we analyzed T-cell responses in another post-HSCT ATL patient without HLA-A2 or A24 and identified two new HLA-A11-restricted epitopes.

PBMC from an acute-type ATL patient (patient 156, a 51-year-old male) at 145 days after HSCT and from his HLA-identical (HLA-A11/A26, B52/B61 DR6/DR15) sibling donor (donor 167, a 55-year-old male) were collected after signed informed consent. Patient 156 obtained complete remission within 2 months after HSCT and sustained remission for longer than 15 months, although chronic GVHD was observed from 8 months after HSCT. Donor 167 was negative for HTLV-I.

A spontaneously HTLV-I-infected T-cell line (ILT-156) established from the PBMC of patient 156 before HSCT and an exogenously HTLV-I-infected T-cell line (ILT-167) established from PBMC of the seronegative donor 167 were maintained in the presence of interleukin-15. An Epstein-Barr virus-transformed lymphoblastoid cell line (LCL-156) was established from PBMC of patient 156 before HSCT, as described elsewhere (6, 22).

CD8$^+$ PBMC isolated from post-HSCT patient 156 at 147 days after HSCT were cocultured with 1% formalin-treated ILT-156 cells, derived from pre-HSCT patient 156, twice with a 14-day interval in the presence of interleukin-2. The responder PBMC vigorously proliferating in culture at 17 days after initiation of culture produced significant levels of gamma interferon (IFN-γ) against ILT-156, but not against LCL-156, following overnight incubation (Fig. 1A). Cytotoxicities of the CTLs against ILT-156 cells were confirmed by ^{51}Cr release assay. Significant levels of IFN-γ response were observed against allogeneic HTLV-I-infected cells sharing only HLA-A11 (TCL-Kan) but not against the ones sharing only HLA-A26 (ILT-Nkz-2). IFN-γ production of the responder cells against ILT-156 cells was significantly inhibited by treatment of responder cells with anti-CD8 monoclonal antibody (MAb) or

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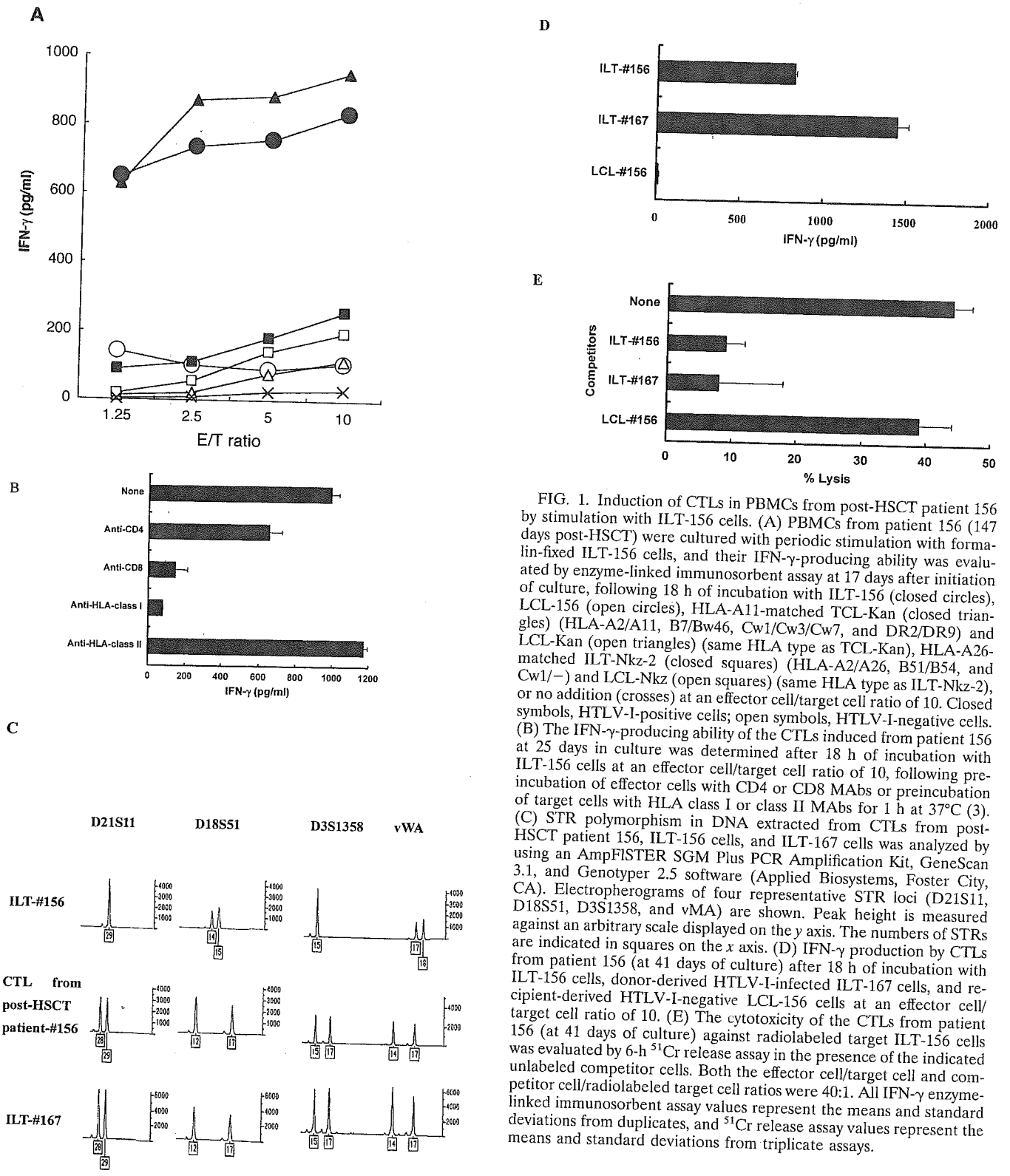

FIG. 1. Induction of CTLs in PBMCs from post-HSCT patient 156 by stimulation with ILT-156 cells. (A) PBMCs from patient 156 (147 days post-HSCT) were cultured with periodic stimulation with formalin-fixed ILT-156 cells, and their IFN-γ-producing ability was evaluated by enzyme-linked immunosorbent assay at 17 days after initiation of culture, following 18 h of incubation with ILT-156 (closed circles), LCL-156 (open circles), HLA-A11-matched TCL-Kan (closed triangles) (HLA-A2/A11, B7/Bw46, Cw1/Cw3/Cw7, and DR2/DR9) and LCL-Kan (open triangles) (same HLA type as TCL-Kan), HLA-A26-matched ILT-Nkz-2 (closed squares) (HLA-A2/A26, B51/B54, and Cw1/−) and LCL-Nkz (open squares) (same HLA type as ILT-Nkz-2), or no addition (crosses) at an effector cell/target cell ratio of 10. Closed symbols, HTLV-I-positive cells; open symbols, HTLV-I-negative cells. (B) The IFN-γ-producing ability of the CTLs induced from patient 156 at 25 days in culture was determined after 18 h of incubation with ILT-156 cells at an effector cell/target cell ratio of 10, following preincubation of effector cells with CD4 or CD8 MAbs or preincubation of target cells with HLA class I or class II MAbs for 1 h at 37°C (3). (C) STR polymorphism in DNA extracted from CTLs from post-HSCT patient 156, ILT-156 cells, and ILT-167 cells was analyzed by using an AmpFlSTER SGM Plus PCR Amplification Kit, GeneScan 3.1, and Genotyper 2.5 software (Applied Biosystems, Foster City, CA). Electropherograms of four representative STR loci (D21S11, D18S51, D3S1358, and vMA) are shown. Peak height is measured against an arbitrary scale displayed on the *y* axis. The numbers of STRs are indicated in squares on the *x* axis. (D) IFN-γ production by CTLs from patient 156 (at 41 days of culture) after 18 h of incubation with ILT-156 cells, donor-derived HTLV-I-infected ILT-167 cells, and recipient-derived HTLV-I-negative LCL-156 cells at an effector cell/target cell ratio of 10. (E) The cytotoxicity of the CTLs from patient 156 (at 41 days of culture) against radiolabeled target ILT-156 cells was evaluated by 6-h ^{51}Cr release assay in the presence of the indicated unlabeled competitor cells. Both the effector cell/target cell and competitor cell/radiolabeled target cell ratios were 40:1. All IFN-γ enzyme-linked immunosorbent assay values represent the means and standard deviations from duplicates, and ^{51}Cr release assay values represent the means and standard deviations from triplicate assays.

by treatment of target cells with anti-HLA-class I MAb (Fig. 1B), confirming that the responder cells induced from post-HSCT patient 156 contained CD8-positive, HLA-A11-restricted, HTLV-I-specific CTLs.

The hematopoietic system in post-HSCT patient 156 when tested had been reconstituted by that derived from donor 167, as determined by short tandem repeat (STR) polymorphism. By using similar methods, we assessed the origin of the CTLs from post-HSCT patient 156. As shown in Fig. 1C, the pattern of STRs of the CTLs was identical to that of ILT-167 cells but

not ILT-156 cells, clearly indicating that CTLs from post-HSCT patient 156 were derived from donor 167. We then examined whether the CTLs from post-HSCT patient 156 recognized potential GVH antigens expressed in ILT-156 cells but not in ILT-167 cells, besides HTLV-I. As shown in Fig. 1D, the CTLs induced from post-HSCT patient 156 equally recognized ILT-167 and ILT-156 but not LCL-156. Furthermore, cytotoxicity of the CTLs against radiolabeled ILT-156 was competed with unlabeled ILT-167 cells as well as ILT-156 cells significantly and to similar extents but was not competed with LCL-156 cells (Fig. 1E). These results indicated that CTLs from post-HSCT patient 156 were directed mainly to HTLV-I antigens commonly expressed in ILT-156 and -167 cells but not to potential GVH antigens expressed only in ILT-156 cells.

We next performed mapping analysis on the epitopes recognized by CTLs from post-HSCT patient 156 by using a panel of oligopeptides of HTLV-I Tax (6, 12), the major target antigen for HTLV-I-specific CTLs. LCL-156 cells, pulsed with a series of 15- to 24-mer oligopeptides corresponding to the amino acid sequence of the whole region of Tax, were incubated with CTLs from patient 156. Among 28 oligopeptides used, Tax81-104 (Fig. 2A) and Tax271-285 (Fig. 2B) selectively sensitized CTLs to produce IFN-γ. We then prepared five 9-mer peptides inside Tax81-104 and Tax271-285 sequences, which were predicted by computer analysis to bind HLA-A*1101 based on the anchor motifs in two databases (the BIMAS and SYFPEITHI databases) (14, 17). Among these peptides, we found that Tax88-96 (KVLTPPITH) and Tax272-280 (QSSSFIFHK) (Table 1) were dominantly recognized by the CTLs from post-HSCT patient 156.

Finally, we used phycoerythrin (PE)-conjugated HLA-A*1101/Tax88-96 and HLA-A*1101/Tax272-280 tetramers, which were prepared through the NIAID Tetramer Facility (Atlanta, GA), to directly detect HLA-A11-restricted Tax-specific CTLs. As shown in Fig. 3A, in the PBMC culture from post-HSCT patient 156 at 41 days from the initiation of culture, 5.7% of cells were positive for HLA-A*1101/Tax88-96 tetramer and CD8 and 5.8% of cells were positive for HLA-A*1101/Tax272-280 tetramer and CD8. When a mixture of both tetramers was used, 10.3% of the cells bound to these tetramers. These data clearly indicate that CTLs recognizing each epitope equally expanded in the PBMC culture derived from post-HSCT patient 156 in response to stimulation with pre-HSCT cell line ILT-156.

We further applied tetramer staining for uncultured PBMCs from post-HSCT patient 156 (Fig. 3B) and donor 167 (Fig. 3C). Although low levels of nonspecific stain were observed in the PBMCs of the seronegative donor 167, significantly higher percentages of the PBMCs of post-HSCT patient 156 were stained with CD8 MAbs and the tetramers: 0.48% for HLA-A*1101/Tax88-96 tetramer, 0.71% for HLA-A*1101/Tax272-280 tetramer, and 1.87% for both tetramers.

A number of CTL epitopes restricted by HLA-A2, -B14, and -B15 have been identified in HTLV-I Tax; most were found in the context of HTLV-I-specific CTLs derived from HTLV-I-associated myelopathy/tropical spastic paraparesis patients and asymptomatic HTLV-I carriers (2, 12, 13, 16, 25). However, to our knowledge this is the first report demonstrating HLA-A*1101-restricted Tax epitopes recognized by HTLV-I-specific CTLs. The phenotypic frequencies of HLA-A11 are 10% in

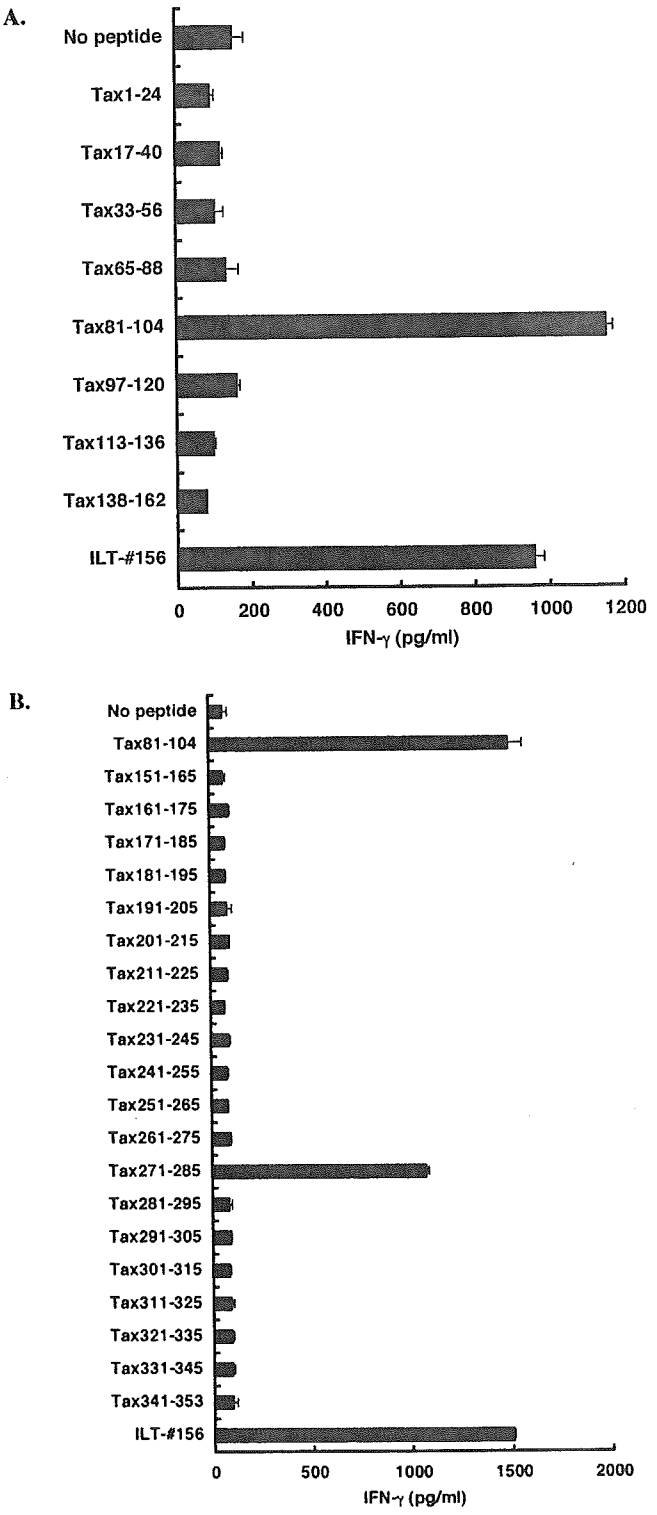

FIG. 2. Mapping of HTLV-I Tax epitopes recognized by CTLs from post-HSCT patient 156. LCL-156 cells were pulsed with 10 μM of a series of 24-mer synthetic oligopeptides covering the N-terminal half (A) and a series of 15-mer oligopeptides covering the C-terminal half (B) of the Tax amino acid sequence, and their susceptibility to CTLs of post-HSCT patient 156 was measured by IFN-γ enzyme-linked immunosorbent assay following 18 h of incubation at an effector cell/target cell ratio of 10. Values represent the means and standard deviations from duplicate assays.

TABLE 1. Reactivity of the Tax-specific CTLs to 9-mer oligopeptides with binding motifs to HLA-A*1101 within Tax81-104 and Tax271-285[a]

Peptide	Sequence	IFN-γ production (pg/ml)[b]
None		13.5 ± 4.4
Tax81-104	Q R T S K T L K V L T P P I T H T T P N I P P S	1,080.2 ± 92.6
Tax82-90	R T S K T L K V L	19.6 ± 8.1
Tax88-96	K V L T P P I T H	1,201.6 ± 55.9
Tax271-285	L Q S S S F I F H K F Q T K A	957.2 ± 47.0
Tax270-278	V L Q S S S F I F	104.4 ± 6.4
Tax272-280	Q S S S F I F H K	1,255.3 ± 13.4
Tax276-284	F I F H K F Q T K	79.1 ± 46.8

[a] CTLs induced from post-HSCT patient 156 at 41 days after initiation of culture were examined for IFN-γ-producing ability by enzyme-linked immunosorbent assay against LCL-156 cells pulsed with 10 μM of indicated Tax peptides (>80% purity) at an effector cell/target cell ratio of 10. All 9-mer peptides within Tax81-104 and Tax271-285 listed were selected based on the binding motif for HLA-A*1101 by using two databases (the BIMAS and SYFPEITHI databases) (14, 17) for HLA binding peptide prediction.

[b] Values represent the means and standard deviations from duplicate assays.

Caucasians, 33% in Chinese, 20% in Japanese, and 4% in black North Americans (19, 21). The newly identified HLA-A11-restricted epitopes together with previously identified epitopes can thus be applied to a large portion of the world's population.

A major challenge in the field of allogeneic HSCT is to prevent the alloreactivity that leads to GVHD while preserving a GVL effect (4). Although it is still not clear whether Tax could be a GVL target in ATL, our present study and earlier studies (6) suggest the presence of Tax antigen presentation in vivo in ATL patients and the potential contribution of these CTLs to GVL effects.

In summary, we identified two HLA-A*1101-restricted HTLV-I-specific CTL epitopes that were recognized by CTLs induced from an ATL patient after HSCT. The identified epitopes broaden the adaptable population for potential immunotherapy for ATL as well as for the monitoring of HTLV-I-specific CTL responses.

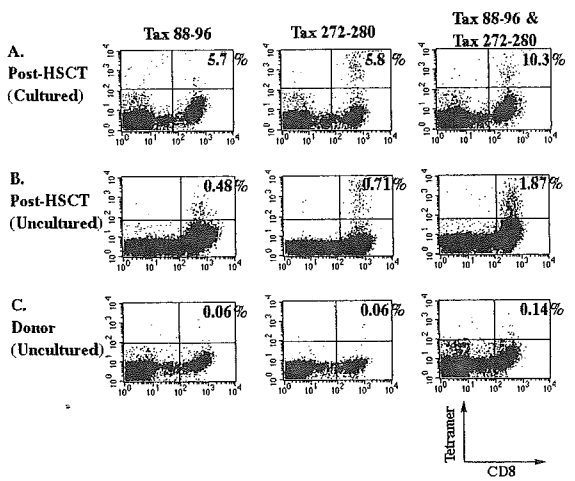

FIG. 3. Detection of Tax88-96 and Tax272-280-specific CTLs by tetramers in PBMCs from post-HSCT patient 156. CTLs from post-HSCT patient 156 at 41 days after initiation of culture (A), uncultured PBMCs from post-HSCT patient 156 (B), and uncultured PBMCs from donor 167 (C) were stained with PE-Cy5-labeled CD8 MAbs (HIT8a; BD PharMingen) together with PE-conjugated HLA-A*1101/Tax88-96 (left), HLA-A*1101/Tax272-280 (center), or a mixture of both tetramers (right). Both tetramers were provided by the National Institute of Allergy and Infectious Diseases Tetramer Facility, Emory University, and were used at a dilution of 1:800. Numbers in the upper right corners indicate percentages of CD8-positive cells bound to the tetramer as analyzed on a flow cytometer (1). A total of 100,000 events were collected in each case.

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Potential immunogenicity of adult T cell leukemia cells *in vivo*

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Experimental vaccines targeting human T cell leukemia virus type-I (HTLV-I) Tax have been demonstrated in a rat model of HTLV-I-induced lymphomas. However, the scarcity of HTLV-I-expression and the presence of defective HTLV-I-proviruses in adult T cell leukemia (ATL) cells have raised controversy about the therapeutic potential of HTLV-I-targeted immunotherapy in humans. We investigated the expression of HTLV-I antigens in fresh ATL cells by using both *in vitro* and *in vivo* assays. In flow cytometric analysis, we found that 3 of 5 acute-type and six of fifteen chronic-type ATL patients tested showed significant induction of HTLV-I Tax and Gag in their ATL cells in a 1-day culture. Concomitantly with HTLV-I-expression, these ATL cells expressed co-stimulatory molecules such as CD80, CD86 and OX40, and showed elevated levels of antigenicity against allogeneic T cells and HTLV-I Tax-specific cytotoxic T-lymphocytes (CTL). Representative CTL epitopes restricted by HLA-A2 or A24 were conserved in 4 of 5 acute-type ATL patients tested. Furthermore, spleen T cells from rats, which had been subcutaneously inoculated with formalin-fixed uncultured ATL cells, exhibited a strong interferon gamma-producing helper T cell responses specific for HTLV-I Tax-expressing cells. Our study indicated that ATL cells from about half the patients tested readily express HTLV-I antigens including Tax *in vitro*, and that ATL cells express sufficient amounts of Tax or Tax-induced antigens to evoke specific T cell responses *in vivo*.

Key words: cancer vaccine; human T cell leukemia virus type-I (HTLV-I); viral expression; co-stimulatory molecules; T cell immune response

Human T cell leukemia virus type-I (HTLV-I) is etiologically linked to adult T cell leukemia (ATL).[1–3] It is estimated that about 1 million people are infected with HTLV-I in Japan and 1–5% of infected subjects develop ATL.[4,5] Most other HTLV-I-carriers are asymptomatic throughout their lives and another small fraction of HTLV-I-carriers develop a chronic progressive neurological disorder termed HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[6,7] and other inflammatory disorders. Once patients develop acute-type ATL, leukemic cells resist anti-tumor chemotherapy, and the median survival time is 6.2 months.[8] Allogeneic hematopoietic stem cell transplantation (HSCT) has been applied recently in acute ATL patients and successful efficacy was obtained in some cases.[9,10] These effects may be attributed to a graft *vs.* leukemia reaction mediated by the donor-derived T cell immunity. There is also, however, a risk of graft *vs.* host reaction and its undesirable side effects are sometimes lethal. On this account, further improvement or new approaches are required for ATL treatment.

The precise mechanisms of HTLV-I-related diseases are not fully understood. HTLV-I viral protein Tax transactivates and interacts with many cellular proteins that regulate or dysregulate cell growth,[11] partly accounting for the mechanisms of HTLV-I-induced leukemogenesis.

In a rat model of HTLV-I-infected T cell lymphomas, uncontrollable expansion of tumor cells was highly associated with a functional defect or suppression of HTLV-I-specific T cell immunity including cytotoxic T lymphocytes (CTL).[12,13] Vaccination with autologous HTLV-I-infected cells,[12] Tax-encoding DNA,[14] or oligopeptides corresponding to a CTL-epitope[15] elicited anti-tumor effects in this model. HTLV-I Tax serves as an immunodominant target antigen for HTLV-I-specific CTL not only in rats but also in humans.[16,17] HTLV-I-specific CTL have been detected in the peripheral blood of HTLV-I-infected individuals[18] and can be induced from healthy carriers and HAM/TSP.[16,19,20] HTLV-I-specific CTL, however, is induced infrequently from ATL patients.[21,22] Moreover, Tax-specific CTL are capable of killing short-term cultured ATL cells.[22,23] These observations indicated that immunotherapy directed against Tax might be effective for ATL.

It is controversial, however, whether HTLV-I-specific immunotherapy has any therapeutic advantages for ATL patients with advanced disease because of the scarcity of HTLV-I-expression in ATL cells. ATL cells sometimes contain mutations and deletions in HTLV-I proviral genome,[24,25] and the ATL cells may not be able to express Tax. It is also known that viral expression in freshly isolated peripheral ATL cells is transiently suppressed.[26–28]

The reasons for insufficient HTLV-I-specific T cell response in ATL patients are also unclear. We found recently that a strong Tax-specific CTL response was induced in ATL patients after HSCT from HLA-identical donors,[29] indicating that the immune insufficiency in these patients before transplantation was not HLA-related. Pique *et al.*[30] reported that HTLV-I-specific CTL do exist in ATL patients but insufficiently expand. This suggests involvement of some immune suppression or tolerance. Alternatively, the levels of viral expression in ATL cells may be too low to evoke T cell immunity *in vivo*.

Because these cells may be a vaccine candidate, we investigated HTLV-I-expression of fresh ATL cells from 5 acute-type

Abbreviations: ATL, adult T cell leukemia; CTL, cytotoxic T lymphocytes; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; FSC, forward scatter; HAM/TSP, HTLV-I-associated myelopathy/tropical spastic paraparesis; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; HTLV-I, human T cell leukemia virus type-I; IFN-γ, interferon-gamma; IL, interleukin; LTR, long terminal repeat; mAb, monoclonal antibody; MHC-II; Class II major histocompatibility complex; MLR, mixed lymphocyte reaction; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PE, phycoerythrin; PHA, phytohemagglutinin; SSC, side scatter.

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