proliferation of human T-lymphotropic virus type I-infected cells in vivo. Cancer Res 57:4862–4867.

Gessain A, Barin F, Vernant JC, Gout O, Maurs L, Calender A, de The G. 1985. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 2:407–410.

Hanon E, Hall S, Taylor GP, Saito M, Davis R, Tanaka Y, Usuku K, Osame M, Weber JN, Bangham CR. 2000. Abundant tax protein expression in $CD4^+$ T cells infected with human T-cell lymphotropic virus type I (HTLV-I) is prevented by cytotoxic T lymphocytes. Blood 95:1386–1392.

Hino S, Yamaguchi K, Katamine S, Sugiyama H, Amágasaki T, Kinoshita K, Yoshida Y, Doi H, Tsuji Y, Miyamoto T. 1985. Mother-to-child transmission of human T-cell leukemia virus type-I. Jpn J Cancer Res 76:474–480.

Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto T, Kinoshita KI, Shirakawa S, Miyoshi I. 1981. 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 78:6476–6480.

Hisada M, Okayama A, Tachibana N, Stuver SO, Spiegelman DL, Tsubouchi H, Mueller NE. 1998a. Predictors of level of circulating abnormal lymphocytes among human T-lymphotropic virus type I carriers in Japan. Int J Cancer 77:188–192.

Hisada M, Okayama A, Shioiri S, Spiegelman DL, Stuver SO, Mueller NE. 1998b. Risk factors for adult T-cell leukemia among carriers of human T-lymphotropic virus type I. Blood 92:3557–3561.

Hisada M, Okayama A, Spiegelman D, Mueller NE, Stuver SO. 2001. Sex-specific mortality from adult T-cell leukemia among carriers of human T-lymphotropic virus type I. Int J Cancer 91:497–499.

Ijichi S, Izumo S, Eiraku K, Machigashira R, Kubota M, Nagai M, Ikegami N, Kashio N, Umehara I, Maruyama I, Osame M. 1993. An autoaggressive process against bystander tissues in HTLV-I-infected individuals: A possible pathomechanism of HAM/TSP. Med Hypotheses 41:542–547.

Ishihara S, Okayama A, Stuver S, Horinouchi H, Shioiri S, Murai K, Kubota T, Yamashita R, Tachibana N, Tsubouchi H, Mueller N. 1994. Association of HTLV-I antibody profile of asymptomatic carriers with proviral DNA levels of peripheral blood mononuclear cells. J Acquir Immune Defic Syndr 7:199–203.

Jacobson S, Shida H, McFarlin DE, Fauci AS, Koenig S. 1990. Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. Nature 348:245–248.

Jeffery KJ, Usuku K, Hall SE, Matsumoto W, Taylor GP, Procter J, Bunce M, Ogg GS, Welsh KI, Weber JN, Lloyd AL, Nowak MA, Nagai M, Kodama D, Izumo S, Osame M, Bangham CR. 1999. HLA alleles determine human T-lymphotropic virus-I (HTLV-I) provirus load and the risk of HTLV-I-associated myelopathy. Proc Natl Acad Sci USA 96:3848–3853.

Jeffery KJ, Siddiqui AA, Bunce M, Lloyd AL, Vine AM, Witkover AD, Izumo S, Usuku K, Welsh KI, Osame M, Bangham CR. 2000. The influence of HLA class I alleles and heterozygosity on the outcome of human T cell lymphotropic virus type I infection. J Immunol 165:7278–7284.

Kamihira S, Toriya K, Amagasaki T, Momita S, Ikeda S, Yamada Y, Tomonaga M, Ichimaru M, Kinoshita K, Sawada T. 1989. Antibodies against $p40^{tax}$ gene product of human T-lymphotropic virus type-I (HTLV-I) under various conditions of HTLV-I infection. Jpn J Cancer Res 80:1066–1071.

Kannagi M, Sugamura K, Sato H, Okochi K, Uchino H, Hinuma Y. 1983. Establishment of human cytotoxic T cell lines specific for human adult T cell leukemia virus-bearing cells. J Immunol 130:2942–2946.

Kannagi M, Sugamura K, Kinoshita K, Uchino H, Hinuma Y. 1984. Specific cytolysis of fresh tumor cells by an autologous killer T cell line derived from an adult T cell leukemia/lymphoma patient. J Immunol 133:1037–1041.

Kannagi M, Harada S, Maruyama I, Inoko H, Igarashi H, Kuwashima G, Sato S, Morita M, Kidokoro M, Sugimoto M, Funahashi S, Osame M, Shida H, Honjo T. 1991. 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 3:761–767.

Kannagi M, Matsushita S, Harada S. 1993. Expression of the target antigen for cytotoxic T lymphocytes on adult T-cell leukemia cells. Int J Cancer 54:582–588.

Kannagi M, Matsushita S, Shida H, Harada S. 1994. Cytotoxic T cell response and expression of the target antigen in HTLV-I infection. Leukemia 8:S54–S59.

Kashiwagi S, Kajiyama W, Hayashi J, Noguchi A, Nakashima K, Nomura H, Ikematsu H, Sawada T, Kida S, Koide A. 1990. Antibody to $p40^{tax}$ protein of human T cell leukemia virus 1 and infectivity. J Infect Dis 161:426–429.

Katahira Y, Yashiki S, Fujiyoshi T, Nomura K, Tara M, Mori M, Setoyama M, Kanzaki T, Shida H, Sonoda S. 1995. In vitro induction of cytotoxic T lymphocytes against HTLV-I-infected T-cells from adult T-cell leukemia patients, asymptomatic HTLV-I carriers and seronegative healthy donors. Jpn J Cancer Res 86:21–27.

Kikkawa E, Miyahara N, Narue TK, Shimada K, Azuma F, Hara H. Inoko H. 2003. Evaluation of the PCR-Luminex method for four-digital level genotyping of the HLA-A, HLA-B and HLA-DRB1 genes in the Japanese population. MHC 10:21–31.

Kinoshita K, Amagasaki T, Ikeda S, Suzuyama J, Toriya K, Nishino K, Tagawa M, Ichimaru M, Kamihira S, Yamada Y. 1985. Preleukemic state of adult T cell leukemia: Abnormal T lymphocytosis induced by human adult T cell leukemia-lymphoma virus. Blood 66:120–127.

Kinoshita K, Amagasaki T, Hino S, Doi H, Yamanouchi K, Ban N, Momita S, Ikeda S, Kamihira S, Ichimaru M. 1987. Milk-borne transmission of HTLV-I from carrier mothers to their children. Jpn J Cancer Res 78:674–680.

Kondo T, Nonaka H, Miyamoto N, Hanaoka M. 1985. Very low percentage flower cell carrier—Can it be an index for pre-leukemic stage for ATLL? Oncologia 12:139.

Kozako T, Arima N, Toji S, Masamoto I, Akimoto M, Hamada H, Che XF, Fujiwara H, Matsushita K, Tokunaga M, Haraguchi K, Uozumi K, Suzuki S, Takezaki T, Sonoda S. 2006. Reduced frequency, diversity, and function of human T cell leukemia virus type 1-specific $CD8^+$ T cell in adult T cell leukemia patients. J Immunol 177:5718–5726.

Kubota R, Kawanishi T, Matsubara H, Manns A, Jacobson S. 1998. Demonstration of human T lymphotropic virus type I (HTLV-I) tax-specific $CD8^+$ lymphocytes directly in peripheral blood of HTLV-I-associated myelopathy/tropical spastic paraparesis patients by intracellular cytokine detection. J Immunol 161:482–488.

Kubota R, Kawanishi T, Matsubara H, Manns A, Jacobson S. 2000. HTLV-I specific IFN-γ^+ $CD8^+$ lymphocytes correlate with the provirus load in peripheral blood of infected individuals. J Neuroimmunol 102:208–215.

Kuzushima K, Hayashi N, Kimura H, Tsurumi T. 2001. Efficient identification of HLA-A*2402-restricted cytomegalovirus-specific $CD8^+$ T-cell epitopes by a computer algorithm and an enzyme-linked immunospot assay. Blood 98:1872–1881.

Levin MC, Lehky TJ, Flerlage AN, Katz D, Kingma DW, Jaffe ES, Heiss JD, Patronas N, McFarland HF, Jacobson S. 1997. Immunologic analysis of a spinal cord-biopsy specimen from a patient with human T-cell lymphotropic virus type I-associated neurologic disease. N Engl J Med 336:839.

Maloney EM, Blattner WA. 2003. HTLV-I worldwide patterns and disease associations. Gann Monogr Cancer Res 50:339–361.

Manns A, Miley WJ, Wilks RJ, Morgan OS, Hanchard B, Wharfe G, Cranston B, Maloney E, Wells SL, Blattner WA, Waters D. 1999. Quantitative proviral DNA and antibody levels in the natural history of HTLV-I infection. J Infect Dis 180:1487–1493.

Matter M, Mumprecht S, Pinschewer DD, Pavelic V, Yagita H, Krautwald S, Borst J, Ochsenbein AF. 2005. Virus-induced polyclonal B cell activation improves protective CTL memory via retained CD27 expression on memory CTL. Eur J Immunol 35: 3229–3239.

Miyoshi I, Kubonishi I, Yoshimoto S, Shiraishi Y. 1981. A T-cell line derived from normal human cord leukocytes by co-culturing with human leukemic T-cells. Gann 72:978–981.

Nagai M, Usuku K, Matsumoto W, Kodama D, Takenouchi N, Moritoyo T, Hashiguchi S, Ichinose M, Bangham CRM, Izumo S, Osame M. 1998. Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: High proviral load strongly predisposes to HAM/TSP. J Neurovirol 4:586–593.

Nagai M, Kubota R, Greten TF, Schneck JP, Leist TP, Jacobson S. 2001. Increased activated human T cell lymphotropic virus type I (HTLV-I) Tax11-19-specific memory and effector CD8+ cells in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis: Correlation with HTLV-I provirus load. J Infect Dis 183:197–205.

Niewiesk S, Daenke S, Parker CE, Taylor G, Weber J, Nightingale S, Bangham CR. 1994. The transactivator gene of human T-cell leukemia virus type I is more variable within and between healthy carriers than patients with tropical spastic paraparesis. J Virol 68: 6778–6781.

Ogg GS, Jin X, Bonhoeffer S, Dunbar PR, Nowak MA, Monard S, Segal JP, Cao Y, Rowland-Jones SL, Cerundolo V, Hurley A, Markowitz M, Ho DD, Nixon DF, McMichael AJ. 1998. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. Science 279:2103–2106.

Okayama A, Stuver SO. 2003. Long-term follow-up of HTLV-I carriers. In: Two decades of adult T-cell leukemia and HTLV-I research. Gann Monogr Cancer Res 50:127–139.

Osame M, Usuku K, Izumo S, Ijichi N, Amitani H, Igata A, Matsumoto M, Tara M. 1986. HTLV-I associated myelopathy, a new clinical entity. Lancet 1:1031–1032.

Parker CE, Daenke S, Nightingale S, Bangham CR. 1992. Activated, HTLV-I-specific cytotoxic T lymphocytes are found in healthy seropositives as well as in patients with tropical spastic paraparesis. Virology 188:628–636.

Parker CE, Nightingale S, Taylor GP, Weber J, Bangham CR. 1994. Circulating anti-Tax cytotoxic T lymphocytes from human T-cell leukemia virus type I-infected people, with and without tropical spastic paraparesis, recognize multiple epitopes simultaneously. J Virol 68:2860–2868.

Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. 1980. 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 77:7415–7419.

Sawada T, Tohmatsu J, Obara T, Koide A, Kamihira S, Ichimaru M, Kashiwagi S, Kajiyama W, Matsumura N, Kinoshita K, Yano M, Yamaguchi K, Kiyokawa T, Takatsuki K, Taguchi H, Miyoshi I. 1989. High risk of mother-to-child transmission of HTLV-I in $p40^{tax}$ antibody-positive mothers. Jpn J Cancer Res 80:506–508.

Schneider J, Yamamoto N, Hinuma Y, Hunsmann G. 1984. Sera from adult T-cell leukemia patients react with envelope and core polypeptides of adult T-cell leukemia virus. Virology 15:1–11.

Sonoda S. 2003. Genetic risk of disease development among HTLV-I carriers. Gann Monogr Cancer Res 50:289–301.

Sonoda J, Koriyama C, Yamamoto S, Kozako T, Li HC, Lema C, Yashiki S, Fujiyoshi T, Yoshinaga M, Nagata Y, Akiba S, Takezaki T, Yamada K, Sonoda S. 2004. HTLV-1 provirus load in peripheral blood lymphocytes of HTLV-1 carriers is diminished by green tea drinking. Cancer Sci 95:596–601.

Tachibana N, Okayama A, Ishihara S, Shioiri S, Murai K, Tsuda K, Goya N, Matsuo Y, Essex M, Stuver S, Mueller N. 1992. High HTLV-I proviral DNA level associated with abnormal lymphocytes in peripheral blood from asymptomatic carriers. Int J Cancer 51:593–595.

Tajima K. 1990. The 4th nation-wide study of adult T-cell leukemia/lymphoma (ATL) in Japan: Estimates of risk of ATL and its geographical and clinical features. The T- and B-cell Malignancy Study Group. Int J Cancer 45:237–243.

Taylor GP, Tosswill JH, Matutes E, Daenke S, Hall S, Bain BJ, Davis R, Thomas D, Rossor M, Bangham CR, Weber JN. 1999. Prospective study of HTLV-I infection in an initially asymptomatic cohort. J Acquir Immune Defic Syndr 22:92–100.

van Essen D, Dullforce P, Brocker T, Gray D. 2000. Cellular interactions involved in Th cell memory. J Immunol 165:3640–3646.

Vine AM, Witkover AD, Lloyd AL, Jeffery KJ, Siddiqui A, Marshall SE, Bunce M, Eiraku N, Izumo S, Usuku K, Osame M, Bangham CR. 2002. Polygenic control of human T lymphotropic virus type I (HTLV-I) provirus load and the risk of HTLV-I-associated myelopathy/tropical spastic paraparesis. J Infect Dis 186:932–939.

Wodarz D, Bangham CR. 2000. Evolutionary dynamics of HTLV-I. J Mol Evol 50:448–455.

Wodarz D, Hall SE, Usuku K, Osame M, Ogg GS, McMichael AJ, Nowak MA, Bangham CR. 2001. Cytotoxic T-cell abundance and virus load in human immunodeficiency virus type 1 and human T-cell leukaemia virus type 1. Proc R Soc Lond B Biol Sci 268:1215–1221.

Yamamoto N, Schneider J, Koyanagi Y, Hinuma Y, Hunsmann G. 1983. Adult T-cell leukemia (ATL) virus-specific antibodies in ATL patients and healthy virus carriers. Int J Cancer 15:281–287.

Yao K, Hisada M, Maloney E, Yamano Y, Hanchard B, Wilks R, Rios M, Jacobson S. 2006. Human T lymphotropic virus types I and II western blot seroindeterminate status and its association with exposure to prototype HTLV-I. J Infect Dis 193:427–437.

Yashiki S, Fujiyoshi T, Arima N, Osame M, Yoshinaga M, Nagata Y, Tara M, Nomura K, Utsunomiya A, Hanada S, Tajima K, Sonoda S. 2001. HLA-A*26, HLA-B*4002, HLA-B*4006, and HLA-B*4801 alleles predispose to adult T cell leukemia: The limited recognition of HTLV type 1 tax peptide anchor motifs and epitopes to generate anti- HTLV type 1 tax $CD8^+$ cytotoxic T lymphocytes. AIDS Res Hum Retroviruses 17:1047–1061.

Yokota T, Cho MJ, Tachibana N, McLane M, Takatsuki K, Lee TH, Mueller N, Essex M. 1989. The prevalence of antibody to p42 of HTLV-I among ATLL patients in comparison to healthy carriers in Japan. Int J Cancer 43:970–974.

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Cyclosporin A Inhibits HTLV-I Tax Expression and Shows Anti-Tumor Effects in Combination With VP-16

Atsuo Ozaki,[1] Naomichi Arima,[2]* Kakushi Matsushita,[1] Kimiharu Uozumi,[2] Masaki Akimoto,[3] Heiichiro Hamada,[3] Hideaki Kawada,[1] Sawako Horai,[2] Yuetsu Tanaka,[4] and Chuwa Tei[3]

[1]*Department of Hematology and Immunology, Kagoshima University Hospital, Kagoshima, Japan*
[2]*Division of Host Response, Center for Chronic Viral Disease, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan*
[3]*Department of Cardiology, Respiratory and Metabolic Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan*
[4]*Department of Immunology, Graduate School and Faculty of Medicine, University of Ryukyu, Nishihara-cho, Nakagami-gun, Okinawa, Japan*

Adult T cell leukemia (ATL) is one of the most refractory malignant hematological diseases. Our previous studies demonstrated HTLV-1Tax protein involvement in clinical manifestation of the aggressive type of ATL and suggested the potential application of agents to inhibit Tax expression for ATL treatment. In the present study, we first examined Tax involvement in the resistance to VP-16-induced apoptosis using four HTLV-1 infected T cell clones and cTax DNA-transfected cells. Next, we examined whether cyclosporin A reduced expression of Tax and its related transfer factors on Western blot and CAT assay. We further investigated whether cyclosporin A in combination with VP-16 can induce apoptosis in HTLV-1 infected T cells. Tax-producing T cells, K3T and F6T, were resistant to VP-16 induced growth inhibition compared with that of the nonproducing cells, S1T and Su9T01. Experiments using S1T and Tax-expressing cDNA-transfected S1T demonstrated Tax-induced resistance to VP-16 induction of apoptosis by DNA ladder formation. Cyclosporin A reduced Tax expression in K3T by Western blot analysis and on CAT assay, showing maximal reduction of 61% and 60% compared to control culture using LTR CAT transfected Jurkat cells and K3T cells, respectively. Cyclosporin A also reduced the nuclear expression of two Tax-related transfer factors, ATF-1 and ATF-2 on Western blot. Cyclosporin A alone did not show any cytotoxicity by itself, but sensitized cells to VP-16 when combined with VP-16. Cyclosporin A may be a useful anti-ATL agent when combined with other anti-cancer agents possibly related to Tax inhibition.
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KEY WORDS: HTLV-I tax; apoptosis; cyclosporin A; immune suppressant; anti-tumor effect

INTRODUCTION

Adult T-cell leukemia (ATL) is a malignant disease of CD4-positive T cells associated with human T-cell lymphotropic virus type 1 (HTLV-1) infection [Yoshida et al., 1982; Seiki et al., 1983, 1984]. In contrast to many other RNA tumor viruses, HTLV-1 lacks a classical oncogene [Seiki et al., 1983, 1984]. The mechanism of CD4 T-lymphocyte transformation by HTLV-1 has not yet been identified. Recently, attention has focused on the oncogenic potential of Tax protein of HTLV-1. A recent study by Hasegawa et al. [2006] reported the thymus-derived leukemia-lymphoma in mice transgenic for Tax gene. Therefore, it is very likely that Tax protein is very related to ATL development in HTLV-1 carriers. Tax protein serves as a potent transcriptional activator of its own long terminal repeat (LTR) as well as select cellular growth related genes [Sodroski et al., 1984; Inoue et al., 1986; Maruyama et al., 1987; Siekevitz et al., 1987; Fujii et al., 1988]. Tax activation

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*Correspondence to: Naomichi Arima, Division of Host Response, Center for Chronic Viral Diseases, Kagoshima University Graduate School of Medical and Dental Sciences, Sakuragaoka 8-35-1, Kagoshima 890-8544, Japan.
E-mail: nao@m2.kufm.kagoshima-u.ac.jp

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of the LTR and these growth-related genes involves the transfer factor ATF/TREB and nuclear factor κB (NF-κB), respectively [Ballard et al., 1988; Leung and Nabel, 1988; Ruben et al., 1988; Hai et al., 1989; Kieran et al., 1990; Yoshimura et al., 1990].

Clinical features of ATL are characterized by heterogeneity described as smoldering, chronic, acute, and lymphoma types [Uchiyama et al., 1977; Shimoyama, 1991]. Acute and lymphoma types are categorized into aggressive ATL and are still very refractory to conventional chemotherapies [Uozumi and Arima, 2005]. In a previous study, we demonstrated that HTLV-1 Tax protein induces part of the clinical manifestations of acute type ATL through a nuclear transfer factor, NF-κB [Arima et al., 1999]. Particularly, Tax protein induces activation of cellular genes including growth related cytokines or its receptors such as IL-2, IL-2 receptor, and IL-6, via Tax activation of NF-κB and other nuclear factors [Arima et al., 1999]. Therefore, any method of inhibiting the Tax protein could be a potentially useful therapy for acute type ATL and probably for lymphoma type, another refractory type.

Cyclosporin A, one of calcineurin inhibitors, is well known as an immune suppressant that inhibits cytokine production and T cell activation [Kronke et al., 1984; Liu, 1993]. Thinking together with Tax—NF-κB pathway activation of growth related cytokine genes, these findings suggest that cyclosporin A also affects the ATL tumor cell itself. Further, the very recent success of allogenic stem cell transplantation for ATL has promoted the frequent usage of cyclosporin A to control graft versus host diseases [Utsunomiya et al., 2001; Okamura et al., 2003]. Under such circumstances, the question of whether cyclosporin A affects the growth of tumor cells or function of anti-cancer agents for ATL is very significant. In the present study, we examined whether cyclosporin A can inhibits HTLV-1 Tax production in HTLV-1-infected T cell clones and further if cyclosporin A can be used in treatment for ATL particularly Tax-related refractory type ATL.

MATERIALS AND METHODS

HTLV-1 Infected Cell Lines, and the Tax-Transfected Cell Line

HTLV-1 infected T cell lines (F6T, K3T, S1T, Su9T01) were established in our laboratory from peripheral blood mononuclear cells of ATL patients [Arima et al., 1991]. F6T and K3T cells are Tax producers, whereas S1T and Su9T01 cells are non-producers. The S1T cTax-neo clones consist of S1T cells stably transfected with *tax* and neomycin resistant gene expressing vector pcTaxWT, which contains wild-type *tax* cDNA and a neomycin-resistant gene in a construct [Kieran et al., 1990] by the electroporation method [Wano et al., 1988]. Viable cells in medium containing the antibiotics G418/neomycin (Sigma, St. Louis, MO) were cloned by limiting dilution. Multiple clones were then screened for Tax protein by in situ immunostaining and for *tax* mRNA by reverse transcriptase-polymerase chain reaction. A clone that markedly expressed Tax protein was selected for further study. Cells were maintained with 10% fetal calf serum complemented RPMI 1640 medium containing antibiotics.

Cell Growth Activity by MTT Assay

Cells were cultured at a concentration of 10^4/200 μl in medium with various concentrations of cyclosporin A or VP-16 for 72 hr in flat-bottomed micro titer plates (Coster, Cambridge, MA). Cyclosporin A (donated by Nippon Kayaku Co., Tokyo, Japan) was dissolved in 10 mM/L ethanol and frozen at −30°C until used. VP-16 (Etoposide, donated from Novartis Pharma Co., Tokyo, Japan), a key drug for ATL treatment, was stored at room temperature and diluted with medium when used.

One hundred microliter of MTT solution (5 μg/ml of 20% SDS) was added to each well and then the plates were incubated for additional 4 hr. The plates were read at 570 nm using a model 550 Micro Plate Reader (Bio-Rad, Hercules, CA).

Western Blot for Tax and ATF Proteins

Whole cell lysates were prepared as described previously [Arima et al., 2004]. The collected cells cultured with or without various concentrations of cyclosporin A were lysed in lysis solution [20 mM Tris pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF)]. Nuclear extract was prepared as previously described [Stein et al., 1989]. Briefly, cells were suspended in a lysis solution [10 mM HEPES, pH 7.9, 1 mM EDTA, 60 mM KCl, 0.04% NP-40, 1 mM dithiothreitol, 1 mM PMSF]. After centrifuging, nuclei were sedimented at 1,200*g* for 5 min, and resuspended in nuclear suspension buffer (250 mM Tris pH7.8, 60 mM KCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF). The nuclear extract was collected after three cycles of freezing and thawing in an ethanol-dry ice bath and followed by centrifugation 7,000*g* for 15 min.

Western blotting was performed with NuPage TM Electrophoresis System (Invitrogen, Carlsbad, CA). Briefly, whole cell lysate (40 μg) was resuspended in reduced sample buffer (Invitrogen), then electrophoresed on a 4–12% Bis-Tris Gel with MOPS running buffer, blotted to nitrocellulose, and sequentially probed with mouse monoclonal antibodies against Tax [Tanaka et al., 1995], ATF-1, ATF-2, and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA). Horseradish peroxidase (HRP)-conjugated anti-mouse IgG (Santa Cruz Biotechnology) was then added, and the specific band for each antibody was detected by autoradiography using enhanced chemiluminescence (ECL Plus; Amersham, Piscataway, NJ).

DNA Electrophresis Assay

The pattern of DNA fragmentation of cultured cells was analyzed as described previously [Bullock et al., 1993]. In brief, the cultured cells were precultured with the agents, and DNA of the cells was extracted by the chloroform/phenol method. DNAs were electrophoresed

on 4% Nusieve 3:1 agarose gel as described previously. DNA ladder was visualized by ethidium bromide.

CAT Assay for Tax Transcription Activity

To assay Tax transcription activity/HTLV-1 long terminal repeat (LTR), we established several reporter cells, Jurkat LTR CAT, and K3T LTR CAT. In brief, an HTLVL-1 LTR chloramphenicol acetyl transferase (CAT)-expressing plasmid (Pu3R-I) with a neomycin resistant gene was transfected to Jurkat cells and K3T cells by electroporation [Wano et al., 1988]. The reporter cells were selected with G418 containing solution and cloned by limiting dilution. CAT activity was assayed according to Neumann et al. [1987]. In brief, after 24 hr culture of cells with cyclosporin A, cell extracts were prepared and assayed for CAT activity. All results were normalized based on protein recovery and results expressed as radioactivity (dpm).

The protocol of the present study has been approved by a suitably constituted Ethics Committee of the institution within which the work was undertaken and that it conforms to the provisions of the Declaration of Helskinki in 1995.

RESULTS

VP-16 Induced Growth Inhibition of HTLV-1 Infected Cells in a Concentration-Responsive Manner

As shown in Figure 1, VP-16 induced growth inhibition of all HTLV-1 infected T cell clones tested in a concentration-dependent manner. However, K3T and F6T, Tax producers, showed resistance to VP-16 compared with Su9T01 and S1T, Tax nonproducers. These findings suggest that HTLV-1 Tax protein inhibits VP-16 induction of cell growth inhibition.

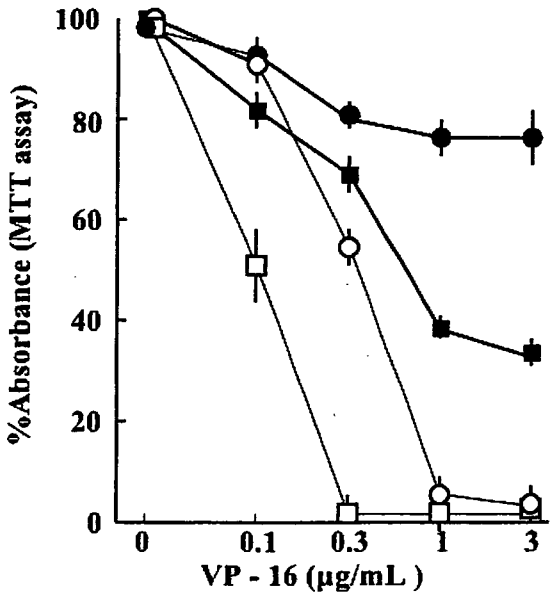

Fig. 1. HTLV-1 Tax inhibition of VP-16 induced the growth inhibition in Tax-producing HTLV-1 infected T cells. Ten thousands cells were cultured with VP-16 in 96-well flat bottom plates for 72 hr. Growth activities of the cells were determined by the MTT assay described in Materials and Methods. Closed circle represents F6T, closed square K3T, open circle Su9T01, and open square S1T. Data are expressed as mean ± SD of triplicate cultures. IC50 in S1T, Su9T01, K3T, or F6T is 0.10, 0.37, 0.65, or >3.0 μg/ml, respectively.

Tax Involved Resistance Against VP-16 Induced Apoptosis

To examine whether Tax is responsible for this resistance to VP-16, we used Tax-expressing DNA-transfected S1T cells that were previously established in our laboratory. As shown in Figure 2, VP-16 induced growth inhibition in S1T and S1Tneo at a lower concentration than that in S1TcTax cell. Next, to examine whether the apoptosis is involved in the VP-16 induced cell growth inhibition and whether Tax protein induces the resistance to VP-16, we performed DNA ladder formation. Figure 3 shows that VP-16 induces the DNA ladder formation in S1T cell in a concentration-responsive manner, and that the clear resistance to P-16 is observed in S1TcTax cells. These findings show that VP-16 induces apoptosis in S1T cell and that HTLV-1 Tax protein induces the resistance to VP-16.

Cyclosporin A Inhibits Tax and ATF Expression in HTLV-1 Infected Clones

Previous studies including ours demonstrated that HTLV-1 Tax protein is responsible for refractoriness to conventional chemotherapies or aggressiveness of this disease [Arima et al., 1999; Arima and Tei, 2003]. These

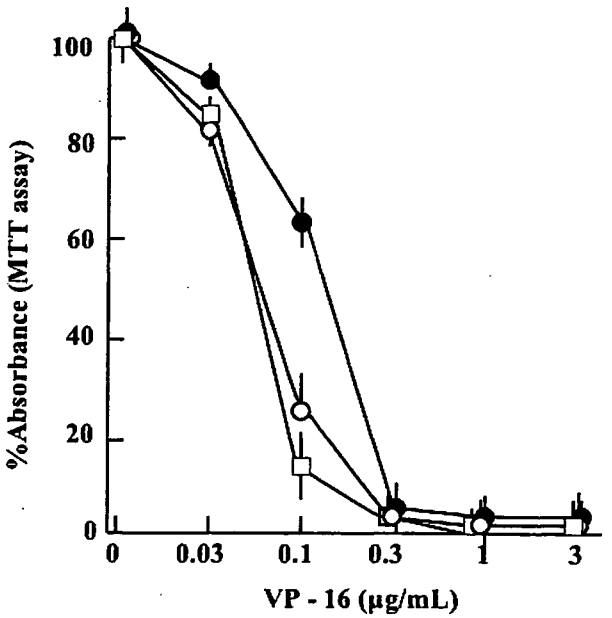

Fig. 2. HTLV-1 Tax inhibition of VP-16 induced the growth inhibition in Tax cDNA-transfected S1T cells. Ten thousand cells were cultured with VP-16 in 96-well flat bottom plates for 72 hr. Growth activities of the cells were determined by the MTT assay described in Materials and Methods. Closed circle represents S1TcTax neo cells, open circle S1T cells, and open square S1T neo cells. Data are expressed as mean ± SD of triplicate cultures. IC50 in S1T, S1T neo, or S1TcTax neo is 0.059, 0.052, or 0.122 μg/ml, respectively. There are significant differences between S1T and S1TcTax neo ($P < 0.01$) and between S1T neo and S1TcTax neo ($P < 0.01$ by student's t-test).

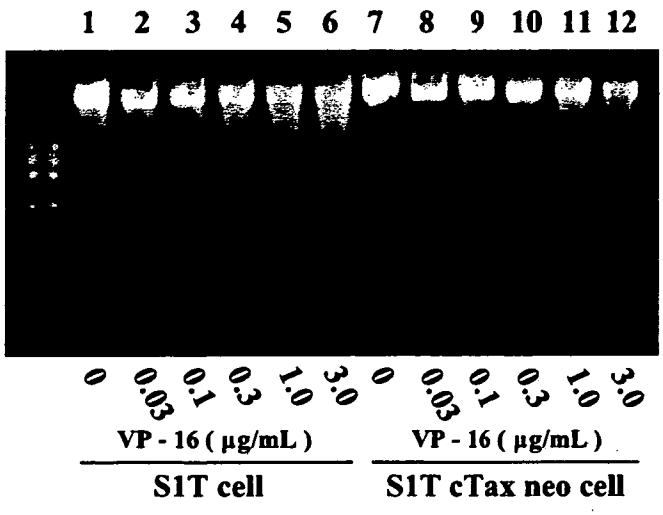

Fig. 3. HTLV-1 Tax inhibition of VP-16 induced DNA ladder formation in S1T cTax DNA-transfected S1T cells. The cells were precultured with various concentrations of VP-16 as described in Materials and Methods, and DNA was extracted by the chloroform/phenol method. DNA samples were electrophoresed on 4% Nusieve 3:1 agarose gel and visualized by ethidium bromide.

findings suggest that Tax expression is one of the therapeutic targets in ATL. Next, we performed Western blot to examine whether cyclosporin A inhibits Tax expression of Tax-producing HTLV-1 clone, K3T. As shown in Figure 4A, cyclosporin A clearly reduced Tax expression in a concentration-responsive manner. The reduction of Tax expression was seen from 0.1 μM cyclosporin A at lowest concentration of the agent. This concentration is consistent with pharmacological concentration in plasma. Further, we examined whether cyclosporin A reduces nuclear transfer factors, ATF-1 and ATF-2 expression in the nuclei of K3T cells. As shown in Figure 4B, cyclosporin A inhibited ATF-1 and ATF-2 expression in the nucleus, which is probably responsible for Tax expression. Next, we confirmed and estimated quantitatively Tax inhibition of cyclosporin A using K3T and Jurkat cells transfected with HTLV-1 LTR CAT DNA. Figure 5A demonstrates the inhibitory effect of cyclosporin A on CAT activity of K3T LTR CAT cell at a minimal concentration of 0.1 μM. Cyclosporin A showed a maximal 61% inhibition compared to control culture at a concentration of 0.5 μM in K3T LTR CAT cells. We also examined the cyclosporin A inhibitory effect on Tax expression in Jurkat T cells transfected with a HTLV-1 LTR CAT construct, Jurkat LTR CAT cells. Figure 5B also demonstrated a maximal 60% inhibition compared to control culture at concentrations ranging from 0.1 to 2 μM, indicating that this inhibition is not specific to K3T cells. These findings further suggest that cyclosporin A in combination with VP-16 induced apoptosis in ATL cells.

Cyclosporin A Sensitizes HTLV-1 Tax Producing Cells to VP-16

These findings rise a possibility that cyclosporin A sensitizes cells to VP-16 via reduction of Tax expression. To examine whether cyclosporin A in combination with VP-16 sensitizes cells to VP-16, we compared the sensitivity of Tax-producing HTLV-1 cells to VP-16 in the presence of 0.5 μM cyclosporin A by MTT assay. As shown in Figure 6, cyclosporin A significantly sensitized the cell to VP-16.

DISCUSSION

Among four clinical types of ATL, lymphoma and acute types are very aggressive and refractory to conventional chemotherapies. Many protocols were tried for the aggressive types, but ended in unsatisfactory results. The best of these protocols is the LSG-16 protocol by the Japanese Clinical Oncology Group (JCOG-LSG), although median survival time is only 13 months and the 5-year survival is 17.5% [Yamada et al., 2001]. Several factors are responsible for the poor prognosis of ATL; the advanced age of the patients, organ infiltration by tumor cells, immune dysfunction, and multi-drug resistance of tumor cells. Many studies have demonstrated a worse prognosis among elderly patients with hematological malignancies [Arimitage et al., 1989; Gisselbrecht et al., 1998]. The mean onset age of ATL patients during the recent 10-year period at our institute has been 62.9 years old with a range from 41 to 89 [Matsushita et al., 1999]. Generally, elderly people have dysfunctions of various organs, and the

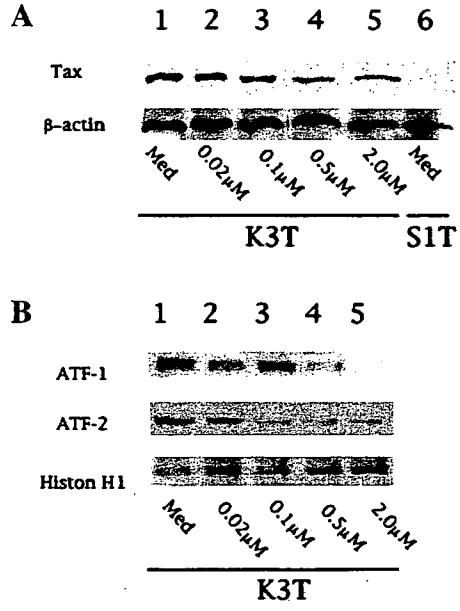

Fig. 4. Cyclosporin A inhibition of Tax protein expression (A), ATF-1, and ATF-2 expression (B). One million K3T cells were cultured with various concentrations of cyclosporin A for 3 days and whole cell lysates were extracted as described in Materials and Methods. The whole cell lysates (40 μg) for Tax and actin or nuclear extracts (15 μg) for ATF-1, ATF-2, and Histon were resuspended in reduced sample buffer (Invitrogen), then electrophoresed on a 4–12% Bis-Tris Gel with MOPS running buffer, blotted to nitrocellulose, and sequentially probed with mouse monoclonal antibodies against Tax, ATF-1, ATF-2, β-actin, and Histon H1. Horseradish peroxidase (HRP)-conjugated anti-mouse IgG was then added, and the specific band for each antibody was detected by autoradiography using enhanced chemiluminescence as described in Materials and Methods.

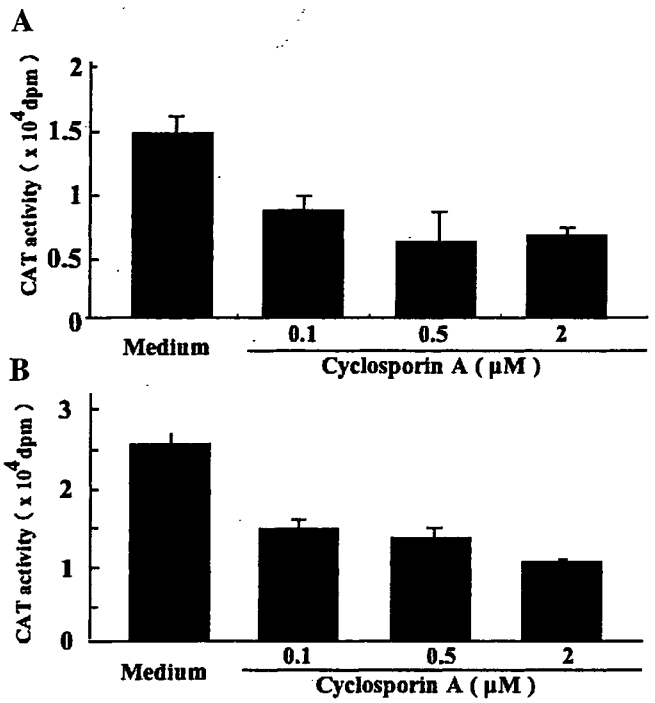

Fig. 5. Cyclosporin A inhibition of CAT activity in K3T cells (A) or Jurkat cells (B) transfected with HTLV-1 LTR CAT construct. After 24 hr culture of cells with various concentrations of cyclosporin A, cell extracts were prepared and assayed for CAT activity as described in Materials and Methods. All results were normalized based on protein recovery and results were expressed as percentage of radioactivity. All cultures with cyclosprin A were significantly lower than that without cyclosporin A ($P < 0.05\%$ by student's t-test) in either cells. No significant difference was seen between any cultures with the agent in either cell.

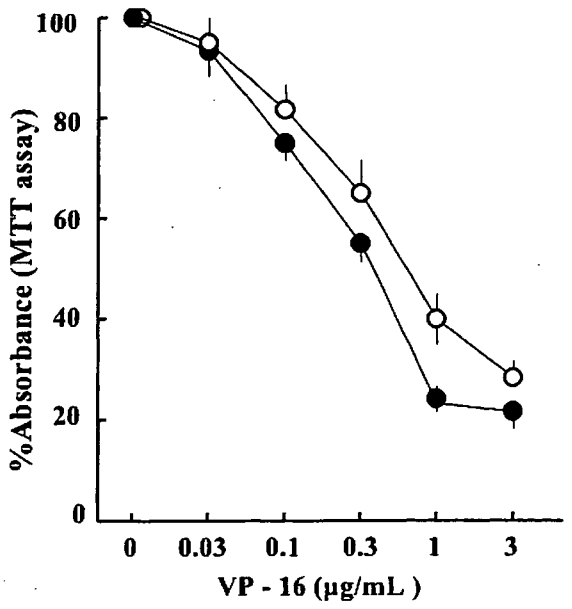

Fig. 6. Cyclosporin A sensitization of VP-16 growth inhibition of HTLV-1 Tax-producing cells. Ten thousand K3T cells were cultured with VP-16 in the presence (closed circle) or absence (open circle) of 0.5 μM cyclosporin A in 96-well flat bottom plates for 72 hr. The cell growth activities were determined by MTT assay as described in Materials and Methods. Data are expressed as mean ± SD of triplicate cultures. IC50 with or without cyclosporin A is 0.62 or 0.41 μg/ml, respectively ($P < 0.05$ by student' t-test).

intense chemotherapies are often interrupted due to the toxicities of these drugs in elderly people. The interruption of chemotherapy is a major factor in the refractoriness of ATL.

Tumor cell infiltration into organs promotes organ dysfunction and worsens the prognosis. Recent studies have demonstrated that adhesion molecules are considered responsible for infiltration into skin or other organs in ATL. Many studies demonstrated the involvement of lymphocyte function-associated antigen-1 (LFA-1), E-selectin, sialyl Lewis X antigen (Slex), ICAM-1, and LFA-3 in tumor infiltration into skin and other organs in ATL [Ishikawa et al., 1993; Furukawa et al., 1994]. Several adhesion molecules are shown to be related to HTLV-1 Tax expression [Tanaka et al., 1995; Hiraiwa et al., 1997]. L-selectin, which mediates the initial step of leukocyte adhesion to vascular endothelium, is also transactivated by HTLV-1 Tax in fresh leukemia cells of ATL [Takewaki et al., 1995]. These findings demonstrate that tumor cell infiltration is caused by HTLV-1 Tax at least in part and that refractoriness or a worse prognosis is caused by HTLV-1 Tax as a result.

Marked dysfunction of cellular immunity in ATL patients has been reported [Hamaoka, 1992]. Fatal lung infection is reported in more than 80% of infections in ATL [Yoshioka et al., 1985]. Dysfunction of T cell or dendritic cell is considered partly responsible for the fatal infections in ATL. The immune dysfunction in ATL is also likely related to cytokines at least in part. At present, cytokines such as interleukin-1α (IL-1α), IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-15, transforming growth factor-β (TGF-β), tumor necrosis factor α (TNF-α), granulo-monocyte-colony stimulating factor (GM-CSF), G-CSF, and parathyroid hormone related peptide (PTHrP) have been shown to be produced spontaneously in HTLV-1-infected T-cells or primary ATL cells [Arima, 1997]. Among them, overexpression of TGF-β is considered to promote immune suppression [Huang and Lee, 2003]. The second candidate for an immune suppressive cytokine-related substance is soluble IL-2 receptor α (sIL-2Rα). IL-2 is a major cytokine produced by T cells and activates T cell functions [Morgan et al., 1976; Taniguchi et al., 1983]. High concentration of sIL-2Rα promotes dysfunction of normal T cells via binding to and inactivating of IL-2.

Resistance to anti-cancer drugs is also a major cause of treatment failure in ATL. Several mechanisms of multidrug resistance have been demonstrated, such as p-glycoprotein, multidrug-resistance protein (MDR1), multidrug-resistance-associated protein (MRP) 1-5 [Borst et al., 2000], and lung resistance-related protein (LRP) [Scheper et al., 1993]. Recently, Lau et al. [1998] demonstrated that Tax protein induces MDR1 gene expression in ATL cells. We recently demonstrated that ATL cells acquired resistance to DXR, VP-16, and vindesine in association with LRP expression, and that LRP mRNA is induced by HTLV-1 Tax expression [Sakaki et al., 2002].

Another mechanism of Tax-related deterioration of prognosis is the dysfunction of cell cycle regulation.

Tax-related escape from negative cell cycle regulation or apoptosis induction in ATL cells is shown at two levels in either p53 or p16. One level is functional inactivation of the p53 protein by Tax binding to p53 [Mulloy et al., 1998; Pise-Masison et al., 1998]. Another level is *p53* gene mutation; Sakashita et al. [1992] demonstrated that more than 50% of *p53* genes in ATL cells were mutants. These two mechanisms also exist in p16 [Suzuki et al., 1996]. The gene alterations of *p16* and *p53* are suggested to be caused by Tax-induced dysfunction of the DNA repair system; inhibition of DNA-β polymerase expression [Jeang et al., 1990]. These findings strongly suggest that the Tax protein is also related at least in part to ATL resistance to conventional chemotherapies. Therefore, the Tax protein could be one of the targets of treatment for ATL.

Cyclosporin A was originally known as an intensive immune suppressant in organ transplantation medicine [Schreiber, 1991]. Cyclosporin A is a representative immunophilin, and conforms a complex with a cyclophiline. The complex binds a calcineurin and inhibits its dephosphorylation, resulting in inhibiting translocation of nuclear transfer factors of NF-AT and NF-κB. The inhibition of NF-AT and NF-κB nuclear translocation causes the inhibition of cytokine production such as IL-2, IL-5, IFNγ, and TNFα, and suppression of T and B cell function as a result [Schreiber, 1991]. At present, cyclosporin A is widely used for treatment of autoimmune diseases and GVHD in stem cell transplantation for malignant hematological diseases including ATL as a strong immune suppressant.

The precise molecular basis of the relation between cyclosporin A and HTLV-1 Tax remains unclear. In the present study, we first demonstrated that cyclosporin A inhibits Tax protein expression in a Tax-producing HTLV-1 infected T cell clone by Western blot. Further, we demonstrated that a pharmacological concentration of this agent reduced Tax production almost 40% on HTLV-1 LTR CAT assay. HTLV-1 Tax protein increases the rate of transcription from the HTLV-1 promoter [Felber et al., 1985]. The HTLV-1 promoter contains three copies of a 21-bp repeat, called the Tax-responsive element (TRE). Recently, cDNAs encoding the Tax-responsive element binding proteins (TREB) were cloned [Yoshimura et al., 1990], and two binding proteins, TREB7 and TREB36, which are essential for HTLV-1 promoter function were demonstrated to be the same as previously reported nuclear transfer factors, ATF-2 and ATF-1, respectively [Hai et al., 1989; Yoshimura et al., 1990]. ATF is known as a member of CREB/ATF family. At present, it is considered that Tax acts as a transcription activator through forming a complex with ATF and binding TRE in LTR, resulting in Tax production. These findings demonstrate that there exists a positive regulatory loop between Tax protein and ATF activation and cyclosporin A disrupts this positive regulatory loop as a result. Cyclosporin A inhibition of NF-κB and NF-AT activation is mediated by forming a complex with cyclophiline and inhibiting the calcineurin dephosphorylation of the nuclear factors by the complex as indicated above. It is possible that cyclosporin A inhibits ATF activation through a mechanism shared with these two nuclear factors. If it is the case, cyclosporin A would inhibit at least three nuclear factors, NF-AT, NF-κB, and additionally ATF. Interestingly, a recent study suggested the involvement of HTLV-1 encoded protein $p12^I$ in interaction between cyclosporin A and Tax expression. Albrecht et al. [2002] demonstrated that HTLV-1 $p12^I$ causes an increase in the release of calcium from the endothelial reticulum and leads to activation of calcineurin, dephosphorylation of cytosolically retained NF-AT and activation of NF-AT. These findings also suggest that cyclosporin A-induced reduction of ATF activation causes reduction of HTLV-1 $p12^I$ expression as well as HTLV-1 Tax expression, consequently inhibiting NF-κB, NF-AT, and ATF. If it is the case, cyclosporin A would initiate a negative regulatory loop involving HTLV-1 Tax, $p12^I$, and ATF.

The present study also demonstrated a higher sensitivity to VP-16 induced apoptosis in the presence of cyclosporin A. Cyclosporin A alone was not cytotoxic to HTLV-1 infected T cell clone, but sensitized the cell to VP-16 induced apoptosis. NF-κB has been demonstrated to act as an inhibitor of apoptosis induction [Kawakami et al., 1999]. Therefore, inhibition of NF-κB by cyclosporin A may be responsible for this effect on apoptosis induction. As indicated above, Tax protein causes drug resistance to ATL treatment via several mechanisms; activation of drug-resistance-related genes and growth-related cytokine or cytokine receptor genes. Cyclosporin A induced higher sensitivity to VP-16 may be involved in the reduction of Tax protein via Tax-related drug resistance including reduction of multi-drug resistance gene expression. However, recent studies by Gary [2003] have suggested that drug-resistance involves several mechanisms in addition to the multi-drug resistance. Qadir et al. [2005] demonstrated that cyclosporin A acts as a broad-spectrum multi-drug resistance modulator. These findings suggest that complex mechanisms or pathways are involved in cyclosporin A-related reduction of drug-resistance in HTLV-1 infected T cells. Tax unrelated mechanisms maybe also included. Although we have not examined all possibilities in drug resistance mechanisms in our HTLV-1 infected T cells, the present study demonstrated that cyclosporin A induces higher sensitivity to VP-16 via reduction of HTLV-1 Tax protein expression, at least in part.

In the present study, we demonstrated that cyclosporin A inhibits HTLV-1 promoter activity resulting in reduction of Tax protein expression. Since HTLV-1 Tax acts as a transactivator of many growth-related cellular genes and is clinically related to disease aggressiveness, it could be a target for ATL treatment. Cyclosporin A reduction of Tax expression may contribute to ATL treatment in two ways. One is sensitizing anti-tumor effect of VP-16, while the other is the anti-Tax effect itself in inactivating the ATL cells. In fact, cyclosporin A is widely used for GVHD treatment in allo-stem cell transplantation in ATL. Cyclosporin A may decrease the

frequent relapse of this malignancy by inhibition of HTLV-1 Tax expression. Clinical trials that include cyclosporin A for ATL treatment should be conducted.

REFERENCES

Albrecht B, D'Souza D, Ding W, Tridandapani S, Coggeshall KM, Lairmore MD. 2002. Activation of nuclear factor of activated T cells by human T-lymphotrophic virus type 1 accessory protein p12^{I}. J Virol 76:3494–3501.

Arima N. 1997. Autonomous and interleukin resposive growth of leukemia cells in adult T-cell leukemia (ATL): A review of the clinical significance and molecular basis of ATL cell growth. Leuk Lymphoma 26:479–487.

Arima N, Tei C. 2003. HTLV-1 Tax protein involved refractoriness of ATL: Molecular mechanisms and clinical significance. Recent Res Devel Haematol 1:103–115 Transworld Research Network, Kerala, India.

Arima N, Molitor JA, Smith MR, Kim JH, Daitoku Y, Greene WC. 1991. Human T-cell leukemia virus type 1 Tax induces expression of the rel-related family of κB enhancer-binding proteins: Evidence for a pretranslational component of regulation. J Virol 65:6892–6899.

Arima N, Matsushita K, Obata H, Ohtsubo H, Fujiwara H, Arimura K, Kukita T, Suruga Y, Wakamatsu S, Hidaka S, Tei C. 1999. NF-κB involvement in the activation of primary adult T-cell leukemia cells and its clinical implication. Exp Hematol 27:1168–1175.

Arima N, Arimura K, Tokito Y, Sakaki Y, Matsushita K, Orihara K, Akimoto M, Ozaki A, Kukita T, Hagiwara T, Hamada H, Tei C. 2004. HTLV-1 Tax protein inhibits apoptosis induction but not G1 arrest by pyrrolidinedithiocarbamate, an anti-oxidant, in adult T cell leukemia cells. Exp Hematol 32:195–201.

Arimitage JO, Vose JM, Linder J, Weisenburger D, Harrinton D, Casey J, Bierman P, Sorensen S, Hutchins M, Moravec DF, Howe D, Dowling MD, Mailliard J, Johnson S, Pevnick W, Packard WM, Okerbloom J, Hompson RF, Langdon RM, Soori G, Peterson C, Prtilo D. 1989. Clinical significance of immunophenotype in diffuse aggressive non-Hodgkin's lymphoma. J Clin Oncol 7:1783–1790.

Ballard DW, Bohnlein E, Lowenthal JW, Wano Y, Franza BR, Greene WC. 1988. HTLV-1 Tax induces cellular proteins that activate the κB element in the IL-2 receptor-α gene. Science 241:1652–1655.

Borst P, Evers Kool M, Wijnholds J. 2000. A family of drug transporters: The multidrug resistance-associated protein. J Natl Cancer Inst 92:1295–1302.

Bullock G, Tang C, Tourkina E, Ibrado AM, Lutzky J, Huang Y, Mahoney ME, Bhalla K. 1993. Effect of combined treatment with interleukin-3 and interleukin-6 on cell death or apoptosis in human myeloid leukemia cells. Exp Hematol 21:1640–1647.

Felber BK, Paskalis H, Kleinman-Ewing C, Wong-Staal F, Pavlakis GN. 1985. The pX protein of HTLV-1 is a transcriptional activator of its long terminal repeats. Science 229:675–679.

Fujii M, Sassone-Corsi P, Verma IM. 1988. c-fos promoter trans-activation by the Tax-1 protein of human T-cell leukemia virus type I. Proc Natl Acad Sci USA 85:8526–8530.

Furukawa Y, Tara M, Ohmori K, Kannagi R. 1994. Variant type of sialyl Lewis X antigen expressed on adult T cell leukemia cells is associated with skin involvement. Cancer Res 54:6533–6538.

Gary DK. 2003. Introduction to resistance to anticancer agents. Oncogene 22:2762–2764.

Gisselbrecht C, Gaulard P, Lepage F, Coiffier B, Briere J, Haioun C, Cazals-Hatem D, Bosly A, Xerri L, Tilly H, Berger E, Bouhabdallah R, Diebold J, for the Groupe d'Eludes des Lymphomes l'Adulte (GELA). 1998. Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Blood 92:76–82.

Hai T, Liu F, Coukos WJ, Green MR. 1989. Transcription factor ATF cDNA clones: An extensive family of leucine zipper proteins able to selectively from DNA-binding heterodimers. Genes Dev 3:2083–2090.

Hamaoka M. 1992. Progress in adult T cell leukemia research. Acta Pathol Jpn 321:171–185.

Hasegawa H, Sawa H, Jewis MJ, Orba Y, Sheehy N, Yamamoto Y, Ichinohe T, Tsunetsugu-Yokota Y, Katano H, Takahashi H, Matsuda J, Sat T, Kurata T, Nagashima K, Hall W. 2006. Thymus-derived leukemia-lymphoma in mice transgenic for the Tax gene of human T-lymphotropic virus type I. Nature Medicine 12:466–472.

Hiraiwa N, Hiraiwa M, Kannnagi R. 1997. Human T-cell leukemia virus-1 encoded Tax protein transactivates al-3 fucosyltransferase Fuc-T VII, which synthesizes sialyl Lewis X, a selectin legand expressed on adult T-cell leukemia cells. Biochem Biophys Res Commun 231:183–186.

Huang X, Lee C. 2003. From TGF-beta to cancer therapy. Curr Drug Targets 4:243–250.

Inoue J, Seiki M, Taniguchi T, Truss S, Yoshida M. 1986. Induction of interleukin-2 receptor gene expression by p40x encoded by human T-cell leukemia virus type I. EMBO J 5:2883–2888.

Ishikawa T, Imura A, Tanaka K, Shirane H, Okuma M, Uchiyama T. 1993. E-selectin and vascular cell adhesion molecule-1 mediate adult T-cell leukemia cell adhesion to endothelial cells. Blood 82:1590–1598.

Jeang KT, Widen SG, Semmes OJ, Wilson SH. 1990. HTLV-1 trans-activator protein, Tax, is a transrepressor of the human β-polymerase gene. Science 247:1082–1084.

Kawakami A, Nakashima T, Sakai H, Urayama S, Yamasaki S, Hida A, Tsuboi M, Nakamura H. 1999. Inhibition of caspase cascade by HTLV-1 tax through induction of NF-κB nuclear translocation. Blood 94:3847–3854.

Kieran M, Blank V, Logeat F, Vandekerckhove J, Lottspeich F, Le Bail O, Urban MB, Kourilsky P, Baeuerle PA, Israel A. 1990. The DNA binding subunit of NF-kappa B is identical to factor KBF1 and homologous to the rel oncogene product. Cell 62:1007–1018.

Kronke M, Leonard WJ, Depper JM, Arya SK, Wong-Staal F, Gallo RC, Waldmann TA, Greene WC. 1984. Cyclosporin A inhibits T-cell growth gene expression at the level of mRNA transcription. Proc Natl Acad Sci USA 81:5214–5219.

Lau A, Nightingale S, Taylor GP, Gant TW, Cann AJ. 1998. Enhanced MDR1 gene expression in human T-cell leukemia virus-I-infected patients offers new prospects for therapy. Blood 91:2467–2474.

Leung K, Nabel GJ. 1988. HTLV-1 transactivator induces interleukin-2 receptor expression through and NF-κB-like factor. Nature 333:776–778.

Liu J. 1993. FK506 and cyclosporine, molecular probes for studying intracellular signal transduction. Immunol Today 14:290–295.

Maruyama M, Shibuya H, Harada H, Hatakeyama M, Seiki M, Fujita T, Inoue J, Yoshida M, Taniguchi T. 1987. Evidence for aberrant activation of the interleukin-2 autocrine loop by HTLV-I encoded p40x and T3/Ti complex triggering. Cell 48:343–350.

Matsushita K, Matsumoto T, Ohtsubo H, Fujiwara H, Imamura N, Hidaka S, Kukita T, Tei C, Matsumoto M, Arima N. 1999. Long-term maintenance combination chemotherapy with OPEC/MPEC (vincristine or methotrexate, prednisolone, etoposide and cyclophosphamide) or with daily oral etoposide and prednisolone can improve survival and quality of life in adult T-cell leukemia/lymphoma. Leuk Lymphoma 36:67–75.

Morgan DA, Ruscetti FW, Gallo R. 1976. Selective in vitro growth of T-lymphocytes from normal human bone marrows. Science 193: 1007–1008.

Mulloy JC, Kislyakova T, Cereseto A, Casareto L, LoMonico A, Fullen J, Lorenzi MY, Cara A, Nicot C, Giam C, Franchini G. 1998. Human T-cell lymphotropic leukemia virus type 1 Tax abrogates p53-induced cell cycle arrest and apoptosis through its CREB/ATF functional domain. J Virol 72:8852–8860.

Neumann JR, Morency CA, Russian KO. 1987. A novel rapid assay for chloramphenicol acetyltransferase gene expression. Biotechniques 5:444–447.

Okamura J, Utsunomiya A, Tanosaki R, Uike N, Sonoda S, Kannagi M, Tomonaga M, Harada M, Kimura N, Masuda M, Kawano F, Yufu Y, Hattori H, Kikuchi H, Saburi Y, for ATL-NST study group. 2006. Non-myeloablative allogeneic hematopoietic stem cell transplantation (NST) for adult T-cell leukemia/lymphoma (ATL)-multicenter phase I clinical trial. Blood 102:2704a.

Pise-Masison CA, Choi KS, Radonovich M, Dittmer J, Kim SJ, Brady J. 1998. Inhibition of p53 transactivation function by the human T-cell lymphotropic virus type I Tax protein. J Virol 72:1165–1170.

Qadir M, O'Loughlin KL, Fricke SM, Williamson NA, Greco WR, Minderman H, Baer MR. 2005. Cyclosporin A is a broad-spectrum multidrug resistance modulator. Clin Cancer Res 11:2320–2326.

Ruben S, Poteat H, Tan TH, Kawakami K, Roeder R, Haseltine W, Rosen CA. 1988. Cellular transcription factors and regulation of IL-2 receptor gene expression by HTLV-1 Tax gene product. Science 241:89–92.

Sakaki Y, Terashi K, Yamaguchi M, Kawamata N, Tokito Y, Mori H, Umehara M, Yoshiyama T, Ohtsubo H, Arimura K, Arima N, Tei C.

2002. Human T-cell lymphotropic virus type I Tax activates lung resistance-related protein expression in leukemic clones established from an adult T-cell leukemia patient. Exp Hematol 30:340–345.

Sakashita A, Hattori T, Miller CW, Suzushima H, Asou N, Takatsuki K, Koeffler HP. 1992. Mutations of the p53 gene in adult T-cell leukemia. Blood 79:477–480.

Scheper RJ, Broxterman HJ, Scheffer GL, Kaaijk P, Dalton WS, van Heijningen TH, van Kalken CK, Slovak ML, de Vries EG, vann der Vlk P. 1993. Overexpression of a M(r) 110,000 vesicular protein in non-P-glycoprotein-mediated multidrug resistance. Cancer Res 53:1475–1479.

Schreiber SL. 1991. Chemistry and biology of the immunophilins and their immunosuppressive ligands. Science 251:283–287.

Seiki M, Hattori S, Hirayama Y, Yoshida M. 1983. Human adult T cell leukemia virus: Complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. Proc Natl Acad Sci USA 80:3618–3622.

Seiki M, Eddy R, Show TB. 1984. Nonspecific integration of the HTLV provirus genome into adult T-cell leukaemia cells. Nature 309:640–642.

Shimoyama M, for members of the Lymphoma Study Group. 1991. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukemia-lymphoma. Br J Haematol 79:428–437.

Siekevitz M, Feinberg MB, Holbrook N, Wong-Staal F, Greene WC. 1987. Activation of interleukin-2 and interleukin-2 receptor (Tac) promoter expression by the trans-activator (tat) gene product of human T cell leukemia virus, type I. Proc Natl Acad Sci USA 84:5389–5393.

Sodroski JG, Rosen CA, Haseltine HA. 1984. Trans-acting transcriptional activation of the long terminal repeat of human T lymphotropic viruses in infected cells. Science 225:381–385.

Stein B, Rhamsdorf HJ, Steffen A, Litfin M, Herrlich P. 1989. UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type 1, collagenase, c-fos, and metallothionein. Mol Cell Biol 9:5169–5189.

Suzuki T, Kitao S, Matsushime H, Yoshida Y. 1996. HTLVl-1 Tax protein interacts with cyclin-dependent kinase inhibitor p16 INK4A and counteracts its inhibitory activity towards C DK4. EMBO J 5:1607–1614.

Takewaki M, Yamaguchi K, Matsuoka M, Ishii T, Miyasaka M, Mori S, Takatsuki K, Watanabe T. 1995. Constitutive overexpression of the L-selectin gene in fresh leukemic cells of adult T-cell leukemia that can be transactivated by human T-cell lymphotropic virus type 1 Tax. Blood 86:3109–3117.

Tanaka Y, Fukudome K, Hayashi M, Takagi S, Yoshie O. 1995. Induction of ICAM-1 and LFA-3 by Tax1 of human T-cell leukemia virus type 1 and mechanism of down-regulation of ICAM-1 or LFA-1 in adult T-cell leukemia cell lines. Int J Cancer 60:554–561.

Taniguchi T, Matsui H, Fujita T, Takaoka C, Kashima N, Yoshimoto R, Hamura J. 1983. Structure and expression of a cloned cDNA for human interleukin-2. Nature 302:305–310.

Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. 1977. Adult T-cell leukemia: Clinical and hematologic features of 16 cases. Blood 50:481–492.

Uozumi K, Arima N. 2005. Recent advances and future perspectives in the treatment of adult T-cell leukemia/lymphoma. Recent research development in Haematology. Transworld Res Network 2:1–19 Kerala.

Utsunomiya A, Miyazaki Y, Takatsuka Y, Hanada S, Uozumi K, Yashiki S, Tara M, Kawano F, Saburi Y, Kikuchi H, Hara M, Sao H, Morishima Y, Kodera Y, Sonoda S, Tomonaga M. 2001. Improved outcome of adult T cell leukemia/lymphoma with allogenic hematopoietic stem cell transplantation. Bone Marrow Transplant 27:15–20.

Wano Y, Feinberg M, Hosking JB, Bogerd H, Greene WC. 1988. Stable expression of the tax gene of type 1 human T-cell leukemia virus in human T cells activates specific cellular genes involved in growth. Proc Natl Acad Sci USA 85:9733–9737.

Yamada Y, Tomonaga M, Fukuda H, Hanada S, Utsunomiya A, Tara M, Sano M, Ikeda S, Takatsuki K, Kozuru M, Araki K, Kawano F, Niimi M, Tobinai K, Hotta T, Shimoyama M, other members of the lymphoma study group of the Japan clinical oncology group (1994–1996). 2001. A new G-CSF-supported combination chemotherapy, LSG 15,for adult T-cell leukaemia-lymphoma: Japan Clinical Oncology Group Study 9303. Br J Haematol 113:375–382.

Yoshida M, Miyoshi I, Hinuma Y. 1982. Isolation and characterization of retrovirus (ATLV) from cell lines of adult T cell leukemia and its implication in the disease. Proc Natl Acad Sci USA 79:2031–2035.

Yoshimura T, Fujisawa J, Yoshida M. 1990. Multiple cDNA clones encoding nuclear proteins that bind to the tax-dependent enhancer of HTLV-1: All contain a leucine zipper structure and basic amino acid domain. EMBO J 9:2537–2542.

Yoshioka R, Yamaguchi K, Yoshinaga T, Takatsuki K. 1985. Pulmonary complications in patients with adult T-cell leukemia. Cancer 55:2491–2494.