—Review—

New Strategy of Adult T-cell Leukemia Treatment Targeted for Anti-tumor Immunity and a Longevity Gene-encoded Protein

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Adult T-cell leukemia-lymphoma (ATL) is an aggressive peripheral T-cell neoplasm with a poor prognosis, developing after long-term infection with human T-cell leukemia virus-1 (HTLV-1). Multiple factors (*e.g.*, virus, host cells, epigenetic aberrations, and immune factors) have been implicated in the development of ATL, although the underlying mechanisms of leukemogenesis have not been fully elucidated. Despite recent progress in both chemotherapy and supportive care for hematological malignancies, the prognosis of ATL is still poor; overall survival at 3 years is only 24 %. New strategies for the therapy and prophylaxis of ATL (*e.g.*, vaccines and novel molecular target agents) are still required. This article reviews new strategy of ATL treatment targeted for HTLV-1-specific cytotoxic T-lymphocytes (CTLs) and SIRT1, a longevity gene-encoded protein. HTLV-1-specific CTLs play a critical role in the host immune response against HTLV-1. We have described here the decreased frequency and function of HTLV-1-specific CD8+ T cells in ATL patients and the efficient induction of the HTLV-1-specific CTLs response in human leukocyte antigen-A*0201-transgenic mice by the HTLV-1/hepatitis B core chimeric particle and oligomannose-coated liposomes encapsulating HTLV-1 epitope without adjuvant, suggesting that the efficient antigen delivery system and CTL induction can be exploited to develop a prophylactic vaccine model against tumors and infectious diseases. Furthermore, our studies suggest that SIRT1, a longevity gene-encoded protein, is a crucial anti-apoptotic molecule in ATL cells, and that SIRT1 inhibitors may be useful therapeutic agents for leukemia, especially in patients with ATL. These studies targeted for anti-tumor immunity such as vaccine and SIRT1 may support the new prophylactic and therapeutic approach for ATL.

Key words——human T-cell leukemia virus-1 (HTLV-1); adult T cell leukemia/lymphoma; vaccine; SIRT1

INTRODUCTION

Adult T-cell leukemia/lymphoma (ATL),[1–3] an aggressive malignancy of mature peripheral T-lymphocytes, and human T-cell lymphotropic virus type I (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP),[4–6] a chronic inflammatory disease, are two of the most important diseases associated with long term infection of HTLV-1, which has infected approximately 10–20 million people worldwide, particularly in Equatorial Africa, the Caribbean basin, South America, Melanesia, and southern Japan.[7] The lifetime risks of developing ATL and HAM/TSP are estimated to be approximately 2.5 % to 5% and 0.3% to 2%, respectively. The different patterns of clinical disease are thought to be linked to host immunogenetic factors. HTLV-1 is also associated with a variety of autoimmune disorders including T cell alveolitis, myopathy, uveitis, arthritis, and Sjögren's syndrome,[8–11] although the precise relationship between these disorders and HTLV-1 infection remains unclear. Despite recent progress in both chemotherapy and supportive care for hematological malignancies, the median surviral time that followed the bes clinical results to date is approximately 13 months.[12–14] Therefore, new strategies for the therapy and prophylaxis of ATL (*e.g.*, vaccines and novel molecular target agents) are still required.

Antigen-specific cytotoxic T-lymphocytes (CTLs) induction is an attractive immunotherapeutic strategy against hematologic malignancies and other cancers.[15,16] The difficulty in inducing antigen-specific CTLs in individual patients vitiates a more widespread use of adoptive T cell therapy. HTLV-1-specific CTLs also play an important role in suppressing proliferation of HTLV-1-infected or transformed T-cells *in vitro*.[17,18] Therefore, impaired host CTL function abrogates protection against accumulation

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of HTLV-1-transformed cells, and circumventing this hurdle may yield an effective immune strategy against leukemogenesis.[19,20]

SIRT1, a nicotinamide adenine dinucleotide (NAD^+)-dependent class III histone deacetylase, has been highlighted as a key regulator of lifespan extension by caloric restriction.[21] SIRT1 plays a crucial role in a variety of physiological processes, such as metabolism, apoptosis, and aging, due to its ability to deacetylate numerous substrates, such as histones, p53, and nuclear factor-κB (NF-κB).[22] NF-κB, which is a key mediator of the inflammatory response and is crucial for cell survival and proliferation, is activated in HTLV-1-infected cells by the transcriptional regulator Tax, an HTLV-1-encoded regulatory protein.[23] SIRT1 is of great significance with regard to both antiaging and tumorigenesis.[24] However, there have been no reports regarding SIRT1 regulation in ATL.

The onset of ATL after HTLV-1 infection appears to require a long latency period because the median age at diagnosis ranges from 40 to 60 years in most endemic regions where mother-child viral transmission had been previously common.[25] ATL is important with regard to studies of both the host immune system in chronic infection and virus-induced malignancy in humans. In this study, we investigated new strategy of ATL treatment targeted for HTLV-1-specific CTLs and SIRT1.

DECREASED FREQUENCY AND FUNCTION OF HTLV-1-SPECIFIC CTL IN ATL

Frequency and Function of HTLV-1-specific CD8+ T-cell in HTLV-1-related Diseases

HTLV-1-specific CTL plays an important role in suppressing proliferation of HTLV-1-infected or transformed T cells *in vitro*[26,27] and thus may prevent development of ATL.[28,29] It is possible that CTL fails in only a fraction of HTLV-1 carriers with a specific immunogenetic background.[30] Yashiki *et al.* identified HTLV-1 Tax epitopes recognized by human leukocyte antigen (HLA) class I molecules using peripheral blood mononuclear cell (PMBC) of asymptomatic HTLV-1 carriers (ACs) *in vitro* and reported that the frequencies of HLA alleles lacking epitope anchor motifs, HLA-A*26, HLA-B*4002, HL-B*4006, and HLA-B*4801, were higher in ATL patients than in ACs. These findings suggested that insufficient generation of CTL allowed outgrowth of HTLV-1-transformed cells in the host.[31] These findings suggested a key role of anti-HTLV-1 Tax CTL in prevention of ATL leukemogenesis. However, *in vivo* conditions of anti-HTLV-1 CTL before and after ATL development have yet to be determined.

To characterize anti-HTLV-1 CTL in ACs and ATL patients, we analyzed the frequency and diversity of HTLV-1-specific CD8+ T-cells in PBMC of ACs and ATL patients using 16 distinct epitopes of HTLV-1 Tax or Env/HLA tetramers, which was developed on the base of our identified epitopes (Table 1), along with intracellular cytolytic effector molecules (interferon-gamma; IFN-γ, perforin, and granzymeB). ACs possessed Tax11–19/HLA-A*0201-specific tetramer+ cells by 73% and Tax301–309/HLA-A*2402-specific tetramer+ cells by 94% (Table 1). Some ACs recognized more than one epitope. In contrast, ATL recognized only Tax11–19 with HLA-A*0201 and Tax301–309 with HLA-A*2402 at frequencies of 33% and 52%. Overall frequency of subjects possessing Tax-specific CD8+ T-cells was significantly lower in ATL than ACs (57% *vs.* 96%, $p<0.001$) whereas the difference in Env-specific CD8+ T-cells was not statistically significant (Table 2). There were also significant differences in percentage of cells binding Tax11–19/HLA-A*0201 and Tax301–309/HLA-A*2402 tetramers between ACs and ATL (Fig. 1). Contrarily, perforin and granzymeB expression in anti-HTLV-1 CD8+ T-cells of ATL was significant lower than that of ACs.[32] Frequency of Tax-specific CD8+ T-cells in ACs were related with proviral load in HLA-A*0201. These results suggest that decreased frequency, diversity, and function of anti-HTLV-1 Tax CD8+ T-cell clones may be one of the risks of ATL development.[32,33]

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Table 1. Diversity of HTLV-1 Epitopes Recognized by CTL in ACs, ATL Patients, HAM/TSP Patients and CCs

Tetramers	HLA allele	HTLV-1 peptide	ACs	ATL	HAM/TSP	CCs
T11	A*0201	Tax 11–19	73% (8/ 11)[a]	33% (3/ 9)**	90% (9/ 10)	58% (7/ 12)
T123	A*0201	Tax 123–131	9% (1/ 11)	0% (0/ 9)	20% (2/ 10)	42% (5/ 12)
T155	A*0201	Tax 155–163	0% (0/ 11)	0% (0/ 9)	10% (1/ 10)	0% (0/ 12)
T178	A*0201	Tax 178–186	9% (1/ 11)	0% (0/ 9)	20% (2/ 10)	17% (2/ 12)
T307	A*0201	Tax 307–315	9% (1/ 11)	0% (0/ 9)	0% (0/ 10)	0% (0/ 12)
E175	A*0201	Env 175–183	0% (0/ 11)	0% (0/ 9)	0% (0/ 10)	8% (1/ 12)
E239	A*0201	Env 239–247	0% (0/ 11)	0% (0/ 9)	10% (1/ 10)	17% (2/ 12)
E442	A*0201	Env 442–450	0% (0/ 11)	0% (0/ 9)	0% (0/ 10)	17% (2/ 12)
T12	A*2402	Tax 12–20	11% (2/ 18)	0% (0/ 23)	13% (2/ 15)	26% (5/ 19)
T187	A*2402	Tax 187–195	11% (2/ 18)	0% (0/ 23)	7% (1/ 15)	16% (3/ 19)
T289	A*2402	Tax 289–297	0% (0/ 18)	0% (0/ 23)	13% (2/ 15)	16% (3/ 19)
T301	A*2402	Tax 301–309	94% (17/ 18)	52% (12/ 23)**	87% (13/ 15)	79% (15/ 19)
T311	A*2402	Tax 311–319	0% (0/ 18)	0% (0/ 23)	7% (1/ 15)	5% (1/ 19)
E11	A*2402	Env 11–19	6% (1/ 18)	0% (0/ 23)	40% (6/ 15)***	42% (8/ 19)***
E21	A*2402	Env 21–29	0% (0/ 18)	0% (0/ 23)	13% (2/ 15)	32% (6/ 19)***
E153	A*2402	Env 153–161	0% (0/ 18)	0% (0/ 23)	13% (2/ 15)	21% (4/ 19)
Tax CTL positives			22% (32/145)	9% (15/160)*	26% (33/125)	26% (41/155)
Env CTL positives			1% (1/ 87)	0% (0/ 96)	15% (11/ 75)*	25% (23/ 93)*
Total CTL positives			14% (33/232)	6% (15/256)*	22% (44/200)***	26% (64/248)**

[a] Epitopes detected by HTLV-1/HLA tetramers/number of tetramers tested. * $p<0.001$; ** $p<0.01$; *** $p<0.05$ *vs.* ACs.

Table 2. The Number of Subjects Positive for HTLV-1-specific CD8+ T Cells among ACs, ATL Patients, HAM/TSP Patients and CCs

HLA allele	Tetramers	ACs	ATL	HAM/TSP	CCs
A*02	Tax	82% (9/11)	22% (2/ 9)*	90% (9/10)	67% (8/12)
A*02	Env	0% (0/11)	0% (0/ 9)	10% (1/10)	25% (3/12)
A*24	Tax	94% (17/18)	27% (6/22)*	100% (15/15)	89% (17/19)
A*24	Env	6% (1/18)	0% (0/22)	40% (6/15)*	74% (14/19)*
Tax CTL positives		96% (25/26)	57% (16/28)*	100% (18/18)	81% (22/27)
Env CTL positives		4% (1/26)	0% (0/28)	50% (9/18)*	59% (16/27)*

The number of subjects positive for tetramers; tetramer positivity indicates subjects with percentages of HTLV-1/HLA tetramer+ CD8 T cells in the CD8+ CD45+ T lymphocytes >0.1%. * $p<0.001$ *vs.* ACs.

18 ACs and 12 ATL patients were cultured for further analysis of anti-HTLV-1 CD8+ T cells *in vitro*. The cultured cells were morphologically activated T cells and clustered in colony formation. We observed increases in the numbers of positive cells corresponding to Tax11–19 or Tax301–309 in ACs, while there was an increase of anti-HTLV-1 T cells in PBMCs from only one ATL patient.[32] Furthermore, two specificities of HTLV-1 Tax-specific CD8+ T cells for Tax289–295 and Tax311–319 with HLA-A*2402 were newly identified in the cultured PBMCs from ACs but not from ATL patients. Thus, it is difficult to increase anti-HTLV-1 CD8+ T cells from ATL patients. The low frequency of anti-Tax CD8+ T cells in ATL

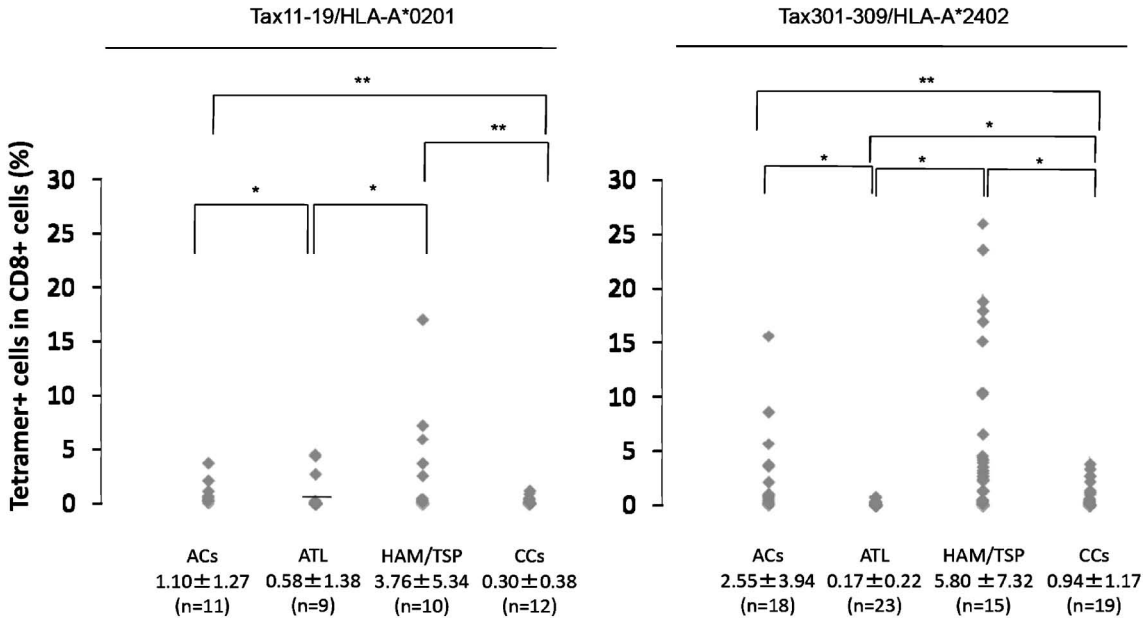

Fig. 1. Frequency of Tax11-19/HLA-A*0201 or Tax301–309/HLA-A*0201 Tetramer Binding CD8+ T Cells in ACs, ATL Patients, HAM/TSP Patients and CCs

The percentage of tetramer+ cells in CD8+ lymphocytes in ACs, HAM/TSP patients and CCs. Horizontal bars indicate the mean percentage of tetramer positive cells. The numbers below each subject are the means±S.D. $^{*}p<0.001$, $^{**}p<0.05$ (significant differences by Mann-Whitney U test).

patients may be involved in progression from HTLV-1 carrier to ATL, and also contribute to the aggressiveness of this disease, which is refractory to treatment.

Central memory T-cells (T_{CM}) (CD45RA−/CCR7+) defines a pool of cells that have been activated, proliferated, and maintained the ability to migrate to lymphnodes where they can stimulate dendritic cells and B cells, but may be less efficient at mediating immediate effector functions.[34] Effector memory T-cells (T_{EM}) (CD45RA−/CCR7−) have a rapid effector function, proliferate less well, but are capable of migrating to tissue sites of inflammation.[34] A terminally differentiated effector memory cell population (CD45RA+/CCR7−; T_{Diff}) has also been described, but not well characterized, and may have a limited proliferative capacity.[35] In HAM/TSP, HTLV-1 Tax11–19+ cells proliferate spontaneously *in vitro* and can be tracked using the Tax 11–19 MHC Class I tetramer. Immediately *ex vivo*, these cells were a mix of CD45RA−/CCR7− T_{EM} and CD45RA+/CCR7− T_{Diff} memory CTL. The subsequent proliferating Tax11–19 tetramer+ population expressed low levels of IL-7Ra, failed to respond to IL-7 and IL-15, and did not develop a T_{CM} phenotype. Thus, chronic exposure to viral antigen may result in a sustained pool of T_{EM} cells.

Furthermore, there is no report on the repertoire of HTLV-1-specific $CD8^+$ T-cells in HAM/TSP patients or carriers with autoimmune diseases (CCs), both characterized by an abnormal immune state. In our study, to characterize HTLV-1-specific $CD8^+$ T-cells in ACs, HAM/TSP patients and CCs, we examined the frequency and diversity of HTLV-1-specific $CD8^+$ T-cells using HTLV-1 tetramers. HTLV-1 Env-specific $CD8^+$ T-cells were significantly more frequent in HAM/TSP and CCs compared with ACs, while the frequency of HTLV-1 Tax-specific $CD8^+$ T-cells was not significantly different among them (Table 1). $CD8^+$ cells binding to HTLV-1 Tax tetramers in CCs were significantly reduced compared with HAM/TSP patients (Fig. 1). This study demonstrates the importance of $CD8^+$ T-cells recognizing HTLV-1 Env-tetramers in HAM/TSP patients and CCs, thereby suggesting that the diversity, frequency and repertoire of HTLV-1 Env-specific $CD8^+$ T-cell clones may be related to the hyperimmune response in HAM/TSP and CCs, although different immunological mechanisms may mediate the hyperimmunity in these conditions.[36,37]

Dysfunction of HTLV-1-specific CD8+ T-cell in ATL Patients *via* the PD-1/PD-L1 Pathway

T cell receptor costimulatory pathways assist in regulating T cell activation and tolerance.[38–40] The

B7-CD28 superfamily membership has been expanded to include costimulatory and inhibitory T cell receptors, including CD28 and programmed death-1 (PD-1).[39] Indeed, studies in PD-1-deficient mice have indicated that PD-1 serves as a negative regulator of immune responses.[41] The negative regulatory PD-1/programmed death-ligand 1 (PD-L1) pathway has been implicated in the induction of CTL exhaustion during chronic viral infection along with tumor escape from host immunity. Studies have also suggested that the PD-1/PD-L1 pathway in virus-specific CD8+ T cells may be operating in chronic HIV infection.[42] However, there is no report on PD-1 expression in tumor-associated antigen-specific CTLs in humans. To determine whether the PD-1/PD-L1 pathway could be involved in the establishment of persistent HTLV-1 infections and immune evasion of ATL cells in patients, we examined PD-1/PD-L1 ex-

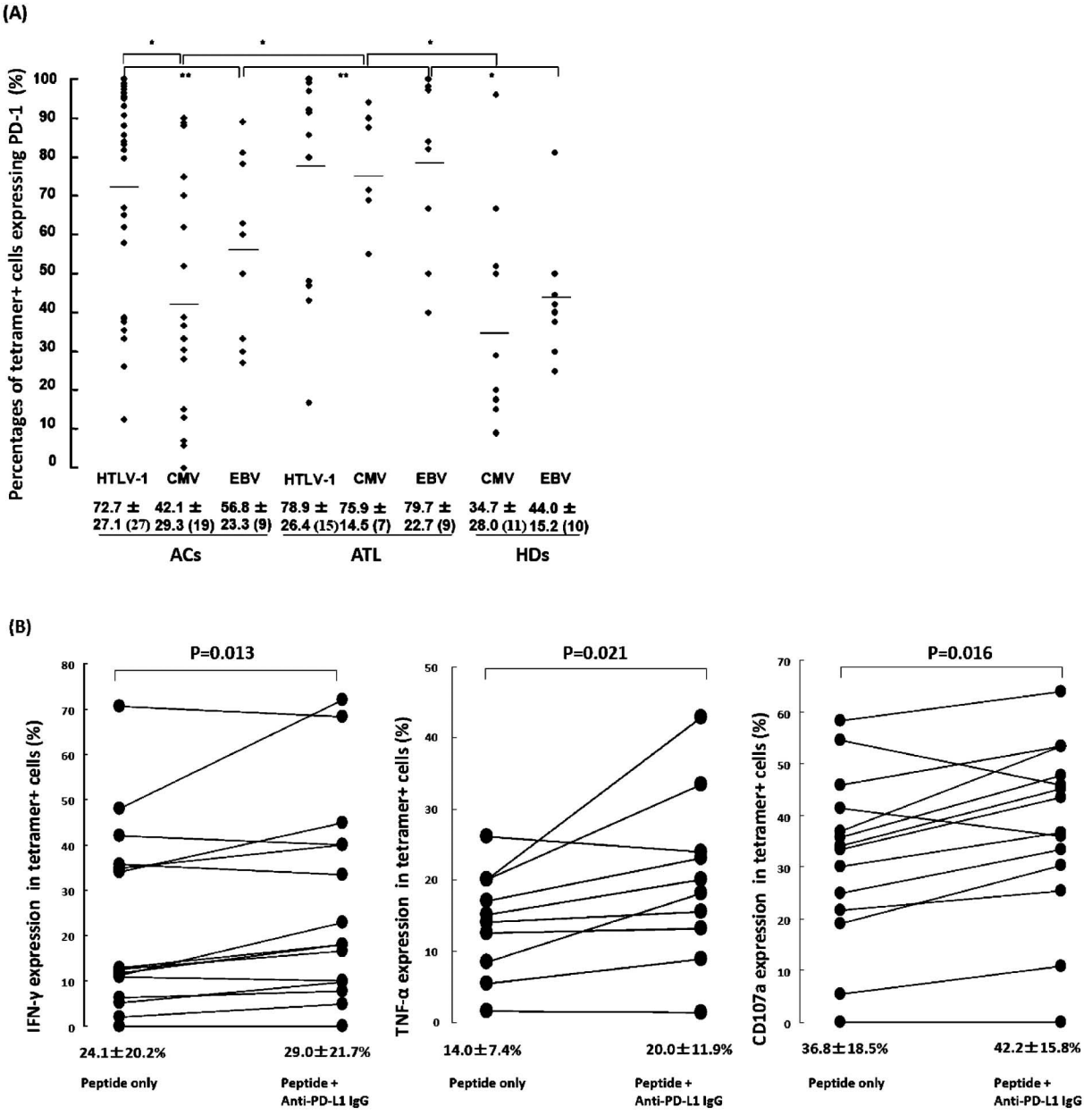

Fig. 2. PD-1 Expression on Virus-specific CD8+ T Lymphocytes and Blocking the PD-1/PD-L1 Pathway in HTLV-1-specific CD8+ Cells

(A) Percentage of PD-1 expression on CD8+ lymphocytes in AC, ATL, and HD. Horizontal bars indicate the mean percentage of PD-1-positive cells. The number below each subject are the means±S.D. (B) Percentage of intracellular cytolytic effector function (IFN-γ and TNF-α) and CD107a —an integral membrane protein in cytolytic granules—expression in HTLV-1-specific CD8+ T lymphocytes treated with peptide and with anti-PD-L1 IgG. The numbers are the means±S.D. Statistical comparisons were made using the Wilcoxon matched pairs test.

pression on cells from ACs and ATL patients in comparison to cells from healthy donors (HDs). PD-1 expression on HTLV-1-specific CTLs from ACs and ATL patients was dramatically elevated (Fig. 2A). In addition, PD-1 expression was significantly higher on CD8+ T-cells along with cytomegalovirus (CMV)- and Epstein-Barr virus (EBV)-specific CTLs in ATL patients compared to ACs and HDs. Primary ATL cells in 21.7% of ATL patients expressed PD-L1, while elevated expression was not observed in cells from ACs.[43] Finally, in functional studies we observed that an anti-PD-L1 antagonistic antibody upregulated HTLV-1-specific CD8+ T-cell response (Fig. 2B). These observations suggest that the PD-1/PD-L1 pathway plays a role in fostering persistent HTLV-1 infections, which may further ATL development and facilitate immune evasion by ATL cells.[43,44]

EFFICIENT INDUCTION OF HTLV-1-SPECIFIC CTL

Induction of HTLV-1-specific CTL by Chimeric Particle without Adjuvant

Antigen-specific CTL induction is an attractive immunotherapeutic strategy against hematologic malignancies and other cancers.[15,16] The difficulty in inducing antigen-specific CTLs in individual patients vitiates a more widespread use of adoptive T cell therapy. Whereas free synthetic peptides have proven to be relatively poor immunogens, viral-like particles such as hepatitis B core (HBc) have been consistently shown to induce strong antibody responses and CTLs, even without adjuvant.[45,46] The HBcAg, a potent immunogen eliciting strong humoral, T-helper and CTL responses and amenable to a variety of heterologous epitopes without adjuvant,[47] is a potentially effective carrier protein for T-cell mediated vaccine development.[48] In the present study, we fused HTLV-1 Tax11–19 peptide, recognized by HLA-A*0201-restricted HTLV-1-specific CD8+ T-cells with high frequency,[32] to the HBcAg to synthesize an HTLV-1/HBc chimeric particle (Fig. 3A, B).

The immunization of HLA-A*0201 transgenic mice with the chimeric particle induced antigen-specific IFN-γ reaction, whereas immunization with epitope peptide only induced no reaction as assessed by enzyme-linked immunospot assay (Fig. 3C). Immunization with the chimeric particle also induced HTLV-1-specific CD8+ T-cells in spleen and inguinal lymph

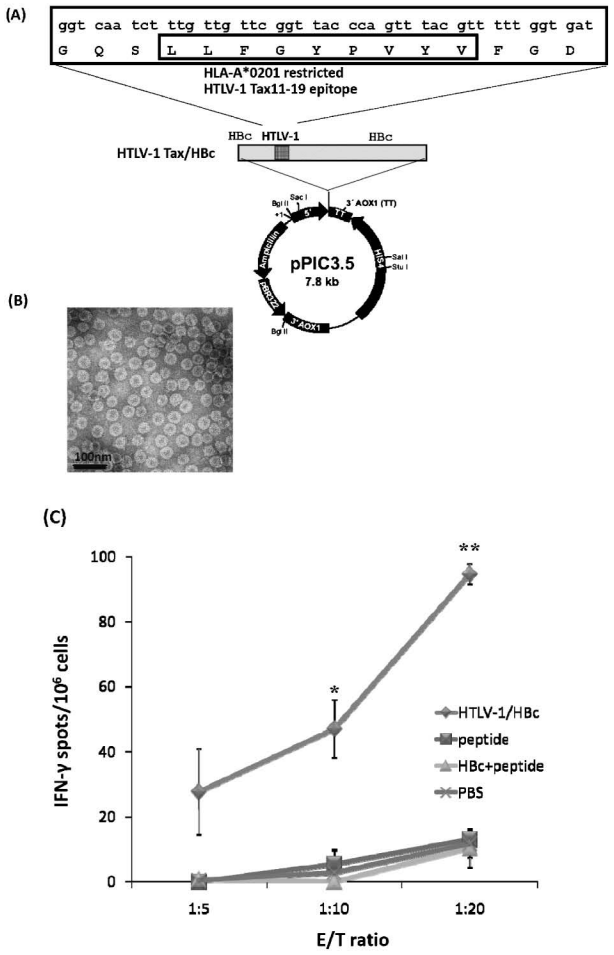

Fig. 3. Expression of Recombinant HTLV-1/HBc Chimeric Particle

(A) The construction of HTLV-1/HBc chimeric protein. (B) Electron micrograph of the HTLV-1/HBc chimeric particle. Original magnification × 100000. (C) HLA-A*0201 transgenic mice were intradermally immunized twice with HTLV-1/HBc chimeric particle (20 μg), HTLV-1 peptide (1 μg: LLFGYPVYV), HTLV-1 peptide (1 μg) plus HBc particle (20 μg), or phosphate buffered saline (PBS) at day 0 and day 14. Seven days after the last immunization, the spleens and inguinal lymph nodes were collected. The inguinal lymph node cells (2×10^6/well) were stimulated with Tax11–19 peptide *in vitro*. Then 6 days later, the frequency of cells producing IFN-γ per 5, 10, 20 $\times10^4$ inguinal lymph node cells upon stimulation with syngenic bone marrow derived DCs (1×10^4/well), pulsed with or without each peptide, was determined by ELISPOT assay. IFN-γ spots are expressed as the number of peptide-loaded to peptide-unloaded target cells. $^*p<0.05$, $^{**}p<0.01$ *vs.* PBS group. The experiments were performed in triplicates. Results represent means±S.D. HTLV-1-specific CD8+ T-cell induction from spleen cells.

nodes.[49] Furthermore, upon exposure of dendritic cells (DCs) from HLA-A*0201-transgenic mice to the chimeric particle, the expression of CD86, HLA-A02, Toll-like receptor 4 (TLR4) and Major Histocompatibility Complex (MHC) class II was increased (Fig. 4). Additionally, our results show that HTLV-1-specific CD8+ T cells can be induced by peptide with HTLV-1/HBc particle from ATL patient, but not by peptide only and these HTLV-1-

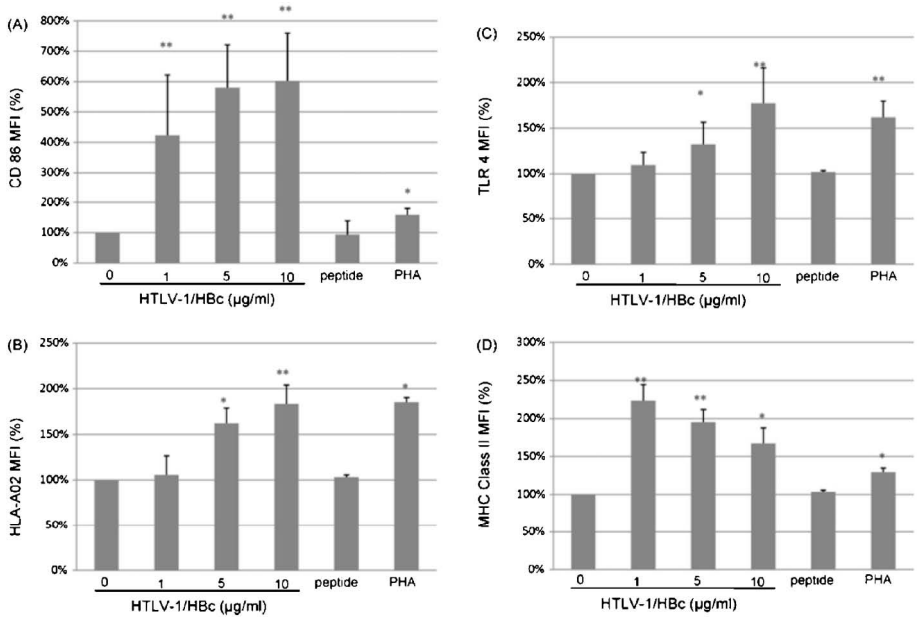

Fig. 4. Maturation of DCs Induced by HTLV-1/HBc Chimeric Particle

Maturation of DCs induced by HTLV-1/HBc chimeric particle is illustrated by the expression of CD86 (A), HLA-A02 (B), TLR4 (C) and MHC class II (D) on the surface of DCs after incubation with antigens. The immature DCs (iDC) were incubated with the indicated concentrations of HTLV-1/HBc chimeric particle: 10 μg/ml of HTLV-1 peptide or 10 μg/ml of phytohemagglutinin (PHA) at 37℃. Data are expressed as the mean fluorescence intensity for each molecule compared to unpulsed (0 μg/ml) iDC controls. Results represent means±S.D. for four independent experiments. $^{*}p<0.05$, $^{**}p<0.01$ *vs.* unpulsed iDC controls.

specific CD8+ T cells were able to lyse cells presenting the peptide.[49] It is also known that T helper epitopes in the HBc protein augment CTL development and enhance CD8+ memory T-cell survival. Others have reported on the efficient processing of lymphocytic choriomeningitis virus-derived p33/HBc chimera *via* cross-presentation, although only weak CTL responses were induced in C57BL/6 mice.[48] Thus, while VLPs alone are inefficient at inducing CTL responses, they become very potent vaccines when combined with antigen presenting cell (APC) activating substances like anti-CD40 mAbs or nonmethylated CG motif-rich DNA (CpGs). These results suggest that HTLV-1/HBc chimeric particle is capable of inducing strong cellular immune responses without adjuvants *via* effective maturation of DCs and is potentially useful as an effective carrier for therapeutic vaccines in tumors, or in infectious diseases by substituting the epitope peptide.[49,50]

Oligomannose-coated Liposomes Efficiently Induce HTLV-1-specific CTL

Oligomannose-coated liposomes (OMLs) can be incorporated into F4/80-positive macrophages or intraperitoneal CD11b-positive DCs resulting in the induction of a protective response following injection into the peritoneal cavity.[51,52] OMLs may also activate peritoneal macrophages to upregulate the expression of costimulatory molecules and preferentially secrete IL-12, which would result in the activation of both CD4-positive and CD8-positive T cells.[53] Furthermore, OMLs employed in effective antigen-delivery could induce both Th subsets and CTL against ovalbumin antigens encapsulated in the liposomes.[54] OMLs using human monocytes/macrophages as a cellular vehicle have the potential to target peritoneal micrometastasis in the omentum of gastric cancer patients.[55] Therefore, OMLs can also be used as an effective antigen delivery system for cancer immunotherapy activating both CTL and Th subsets.[56,57] Here, we examined the efficient induction of HTLV-1-specific CD8+ T cell response by OMLs encapsulating the HLA-A*0201-restricted HTLV-1 Tax-epitope (OML/Tax).

Immunization of HLA-A*0201 transgenic mice with OML/Tax induced an HTLV-1-specific IFN-γ reaction, whereas immunization with epitope peptide alone induced no reaction (Fig. 5A). Upon exposure of DCs to OML/Tax, the levels of CD86, MHC class I, HLA-A02 and MHC class II expression were increased.[58] In addition, our results showed that HTLV-1-specific CD8+ T cells can be efficiently induced by OML/Tax from HTLV-1 carriers compared

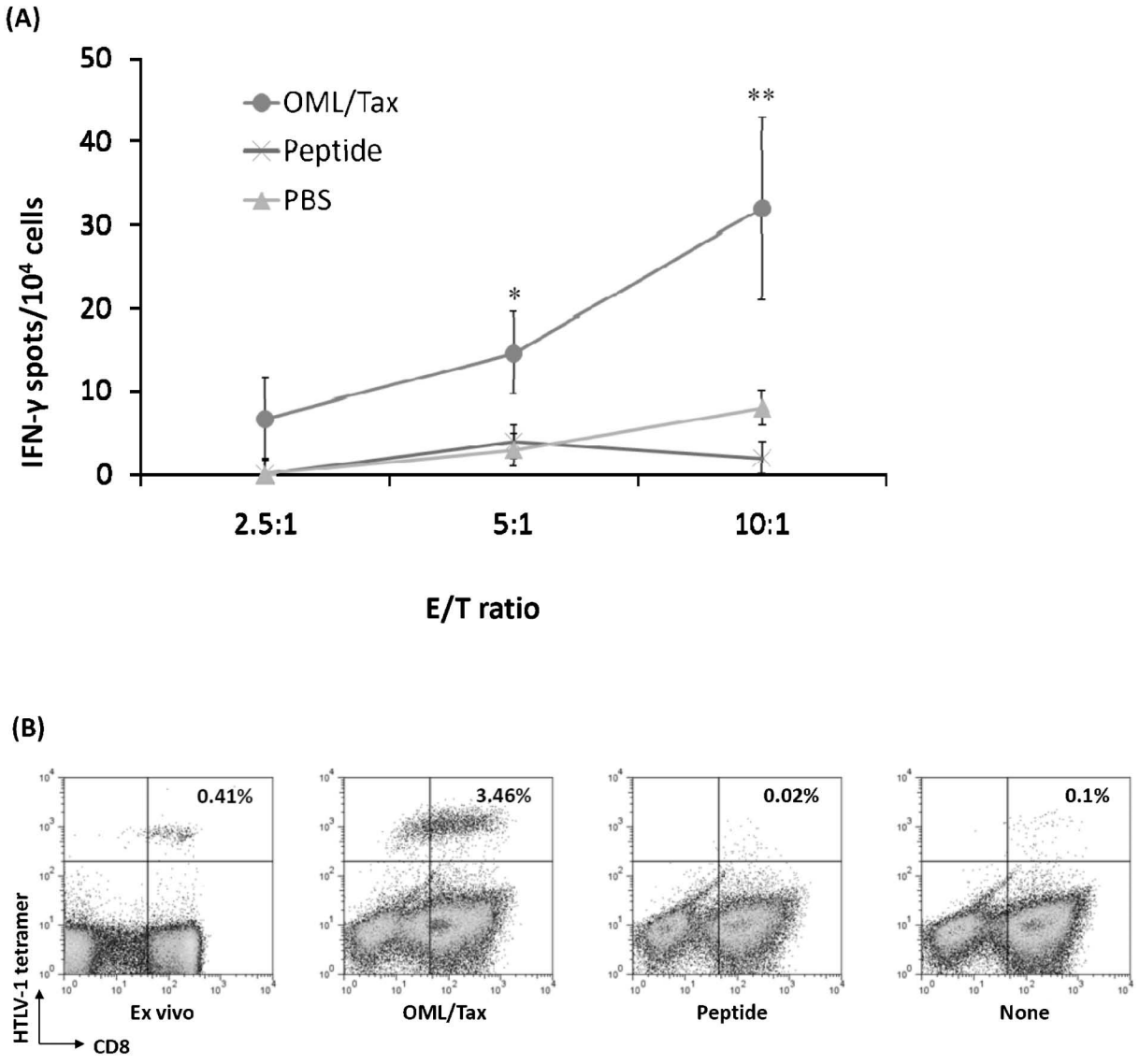

Fig. 5. Induction of Cellular Immunity by OML/Tax

(A) Five HLA-A*0201 transgenic mice per group were intradermally immunized twice with OML/Tax, HTLV-1 peptide (LLFGYPVYV), or PBS on days 0 and 14. Seven days after the last immunization, the spleens and inguinal lymph nodes were collected. The inguinal lymph node cells (2×10^6/well) were stimulated with HTLV-1 peptide *in vitro*. Six days later, the frequencies of cells producing IFN-γ per 2.5, 5 and 10×10^4 inguinal lymph node cells upon stimulation with syngeneic bone marrow derived-DCs (1×10^4/well), pulsed with or without each peptide, were determined by ELISPOT assay. IFN-γ spots are expressed as the number of peptide-loaded to peptide-unloaded target cells. $^*p<0.05$, $^{**}p<0.01$ *vs.* PBS group. The experiments were performed in triplicates. The values are the average of five mice. Results represent means±S.D. (B) Freshly isolated or cryopreserved PBMCs from HTLV-1 carriers were cultured with OML/Tax, with peptide alone, or without antigen. The tetramer assay was performed in fresh (*ex vivo*) or cultured PBMCs. Numbers in the upper right quadrants represent the percentages of tetramer+CD8+ T cells in T lymphocytes.

with epitope peptide alone (Fig. 5B), and these HTLV-1-specific CD8+ T cells were able to lyse cells presenting the peptide. These results were explained by the Th1-skewing of the cytokine profiles due to the advantage of OML-mediated immunization. Mizuuchi *et al*. have recently reported the induction of CTLs specific to the HLA-A24-restricted epitopes of Survivin2B by MLPC with OML-coated survivin2B peptide and those of human papillomavirus type16 E6 and E7 by MLC with OML-coated papillomavirus DNA (Mizuuchi *et al*. unpublished data). A previous study also showed that OMLs were preferentially incorporated into macrophages.[52] As the macrophage mannose receptor (CD206) is mainly expressed on macrophages,[59] the action of OMLs is thought to be caused by their facilitation of antigen delivery to macrophages as a result of interaction between CD206 and oligomannose exposed on the liposomes. In addition, a recent study showed that specific ICAM-3 grabbing nonintegrin-related 1 and complement

receptor type 3 played a crucial role in the uptake of OMLs by macrophages.[53] Uptake of the HTLV-1 antigen-encapsulating OMLs by macrophages would have been an initial key event in the induction of the antigen-specific Th1 immune response. These results suggest that OML/Tax is capable of inducing antigen-specific cellular immune responses without adjuvants and may be useful as an effective vaccine carrier for prophylaxis in tumors and infectious diseases by substituting the epitope peptide.[58]

NEW STRATEGY OF ATL TREATMENT TARGETED FOR SIRT1

SIRT1, an NAD^+-dependent histone/protein deacetylase, plays a crucial role in various physiological processes, such as aging, metabolism, neurogenesis and apoptosis, due to its ability to deacetylate numerous substrates, such as histone and NF-κB,[22] which is implicated as an exacerbation factor in ATL. In this study, we set out to assess how SIRT1 is expressed and the action of a SIRT1 inhibitor in primary ATL cells and leukemic cell lines.

SIRT1 expression in ATL patients was significantly higher than that in healthy controls, especially in the acute type (under submission).[60] Sirtinol, a SIRT1 inhibitor, induced significant growth inhibition or apoptosis in cells from ATL patients and leukemic cell lines, especially HTLV-1-related cell lines (S1T and MT-2). Sirtinol-induced apoptosis was mediated by activation of the caspase family, and inactivation of NF-κB, reducing IκBα phosphorylation. These results suggest that SIRT1 is a crucial antiapoptotic molecule in ATL cells, and that SIRT1 inhibitors may be useful therapeutic agents for leukemia, especially in patients with ATL.[61]

CONCLUSION

ATL is important with regard to studies of both the host immune system in chronic infection and virus-induced malignancy in humans. During the past 3 decades since the clinical discovery of ATL, the results of conventional cytotoxic chemotherapy remain dismal because of low response rates and lack of long-term efficacy. Experimental therapeutics for ATL is now under investigation using hematopoietic stem cell transplantation and molecular targeting by CCR4 antibody. Besides these treatments, the study targeted for anti-tumor immunity such as vaccine and a longevity gene-encoded protein such as SIRT1 may support the new prophylactic and therapeutic approach for ATL (Fig. 6).

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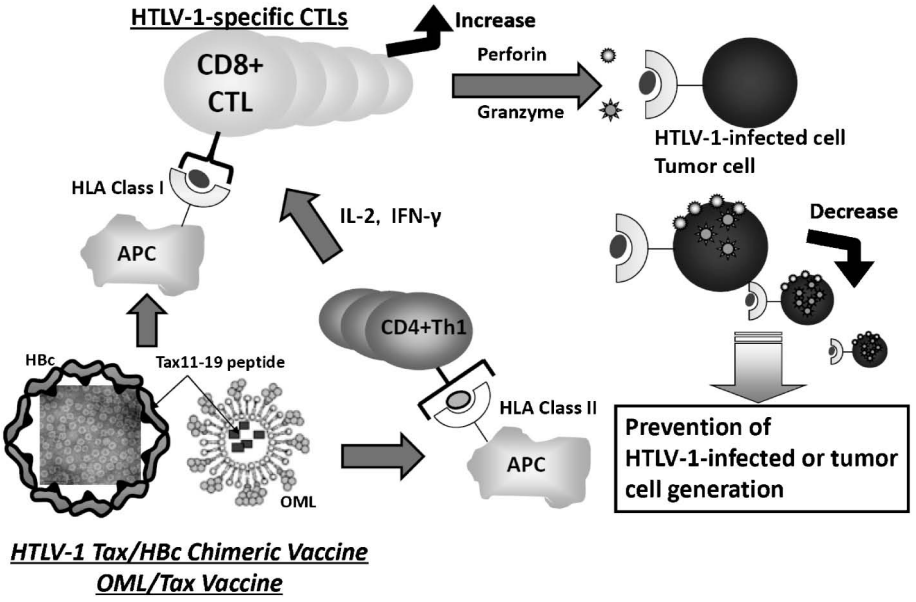

Fig. 6. Strategies for the Therapy and Prophylaxis of ATL Targeted for Anti-tumor Immunity

HTLV-1/HBc chimeric particle and OML/Tax is capable of inducing strong cellular immune responses without adjuvants and is potentially useful as an effective carrier for prophylaxis and therapeutic in infectious diseases and tumors such as HTLV-1-infected cells and ATL cells. APC, antigen presenting cell. Th, T cell helper.

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