discontinuation of CsA, which indicated the difficulty in the tapering or discontinuation of CsA in RIST. Interestingly, 3 patients who had a relapse responded to a rapid discontinuation of the immunosuppressive agent CsA. Although the difference was not statistically significant, the patients who developed aGVHD tended to show a better OS than those who did not ($P = .06$). These observations thus suggest the presence of a graft-versus-ATL effect in RIST. The dramatic decrease in the HTLV-1 proviral load to an undetectable level after RIST in more than half the patients was unexpected. Similar results, which demonstrated an antiviral effect by SCT for ATL, have been previously described in case reports.[23,24] Two patients who received grafts from HTLV-1$^+$ donors also became negative for viral load after RIST. The uninfected normal donor T cells present in the graft might have overwhelmed the HTLV-1–infected T cells in the unique environment after transplantation. In one patient (UPN6) who received a graft from an HTLV-1$^+$ carrier donor, an increase in the HTLV-1 proviral load without disease relapse was observed beyond 1 year after RIST. The proviral load gradually returned to the donor level after the second year. A temporary proliferation of HTLV-I–infected (nonclonal) donor cells might have occurred due to some unknown etiology.

We have herein shown that RIST is a feasible treatment procedure for ATL patients over 50 years of age. The possible presence of a graft-versus-ATL effect as well as anti–HTLV-1 activity for RIST were also observed. Ganciclovir and prophylactic oral acyclovir were the antiviral agents used in the study. They are effective only for herpes virus and not for retrovirus, and therefore, they possess a negligible anti–HTLV-1 activity. In a separate analysis in this study, Harashima et al found the presence of an HLA class I restricted proliferation of CD8$^+$ cytotoxic T lymphocytes (CTLs), which exhibited a specific reactivity to a certain epitope of the HTLV-1 regulatory protein Tax.[25] These Tax-specific CTLs might therefore play a critical role in eradicating ATL cells in vivo. These results indicate that RIST may be applicable as a new modality for the future treatment for other virus-induced diseases that have a poor prognosis.

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Allogeneic hematopoietic stem cell transplantation provides sustained long-term survival for patients with adult T-cell leukemia/lymphoma

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Adult T-cell leukemia/lymphoma (ATLL) is a distinct peripheral T-cell neoplasm that is highly resistant to chemotherapy. Several groups, including ours, have reported encouraging results of allogeneic hematopoietic stem cell transplantation (allo-HSCT) for patients with ATLL. To confirm our previous report and to establish the basis for a phase II clinical study, we analyzed 40 allo-HSCT for acute and lymphoma types of ATLL in seven institutions in Japan between 1997 and 2002. All evaluable cases entered complete remission (CR) after allo-HSCT and the median survival time was 9.6 months for all patients. The estimated 3-year overall and relapse-free survival, and disease relapse were 45.3, 33.8 and 39.3%, respectively. Among 10 cases with ATLL relapse, five cases achieved CR again: three by the reduction or cessation of immunosuppressive agents, which suggested a graft-versus-ATLL (GvATLL) effect. However, univariate or multivariate analysis did not show any benefit of graft-versus-host disease (GVHD) on the prevention of relapse. These results suggested that allo-HSCT was effective for some patients with aggressive ATLL, and that the GvATLL effect could be achieved even without GVHD. A new phase II trial to test the efficacy of allo-HSCT for ATLL is warranted.

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Introduction

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell neoplasm associated with the infection of a specific retrovirus, human T-cell lymphotropic virus type-I (HTLV-I).[1–4] ATLL is quite different from other types of non-Hodgkin's lymphoma (NHL): restricted endemic areas including the west coast of Japan, with distinct clinical features such as high frequency of hypercalcemia, a strong predisposition to infection and poor response to chemotherapy.[4,5] Multicenter clinical trials for ATLL conducted by the Japan Clinical Oncology Group (JCOG) have shown that standard-dose chemotherapy for NHL was unable to cure any patients with acute or lymphoma types of ATLL, which is more aggressive than the chronic or smoldering type.[5–7] The best chemotherapy result for those patients was obtained from the phase II trial of a new multidrug regimen containing nine agents with the support of granulocyte colony-stimulating factor (JCOG9303, LSG15 protocol).[8] The overall survival (OS) rate of the 93 patients in this trial was estimated at 31% at 2 years. In these circumstances, high-dose chemotherapy/radiotherapy with hematopoietic stem cell transplantation (HSCT) was applied to those patients, particularly in Japan. The results of allogeneic, not autologous, HSCT reported by several groups, including ours, suggested that it could provide durable clinical responses for some patients with ATLL.[9–14] However, the number of patients in each report was so small that the efficacy of allogeneic HSCT (allo-HSCT) for ATLL is still controversial. In this report, we aimed to extend our previous study to establish the basis for a phase II clinical trial of allo-HSCT for aggressive ATLL by retrospectively collecting a larger number (40) of transplants from seven institutions in Japan. Our results suggested that allo-HSCT is effective for some patients with ATLL, and that there may be a graft-versus-ATLL (GvATLL) effect that cannot be obtained by chemotherapy alone or high-dose therapy with autograft.

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Patients and methods

Study design, data collection and transplantation procedure

This is a retrospective analysis of myeloablative allo-HSCT for patients with ATLL performed at seven institutions in Japan from June 1997 to April 2002. Data on donors and recipients were collected using questionnaires distributed to each participating center in this study. Patients included in this study received intensive conditioning regimens prior to stem cell transplantation. The early results of the four out of 40 cases were reported previously.[14]

Since transplantation was performed following the protocol of each institution, the conditioning regimen or prophylaxis for GVHD varied among institutions (Table 1); however, all but one preconditioning regimens had myeloablative intensity (containing more than 12 Gy of total body irradiation (TBI, 22 cases), 16 mg/kg of busulfan (17 cases) and 140 mg/m^2 of melphalan (one case)).

Definitions of clinical end points and responses

The day of engraftment was defined as the first of 3 consecutive days on which the neutrophil count exceeded 0.5×10^9/l.

Table 1 Patient characteristics and transplant condition

Sex (male/female)	22/18
Median age at transplantation (range)	44 (28–53)
Subtypes of ATLL	
Acute	30
Lymphoma	10
Disease status at transplantation	
CR1	14
CR2	1
PR	13
NC	3
PD	9
Performance status at transplantation	
0	3
1	33
2	3
3	1
Donor	
HLA-matched related	27
HLA-mismatched related	5
HLA-matched unrelated	8
Anti-HTLV-I antibody of donor	
Positive	9
Negatine	27
NE	4
Source of stem cells	
Bone marrow	21
Peripheral blood stem cell	19
Conditioning regimen	
TBI-containing	18
Non-TBI-containing	22
GVHD prophylaxis	
CsA+MTX	28
TCR+MTX	11
TCR	1

NE, not evaluable; CsA, ciclosporin; TCR, tacrolimus; MTX, methotorexate.

Patients who died before day 28 were considered unevaluable for engraftment. Relapse-free survival (RFS) was defined as the time interval from transplantation to the first event (either relapse or death in complete remission (CR)). Acute graft-versus-host disease (aGVHD) was diagnosed and graded at each transplantation center according to the standard criteria with grades 0, I, II, III and IV.[15] Chronic GVHD (cGVHD) was defined according to standard criteria of absent, limited or extensive.[16] One patient, who received transplantation at the stage of progressive disease (PD), died of infection on day 8 after transplantation, and this case was excluded from the analysis of engraftment, response to transplantation and GVHD. The response criteria used in this report followed those established by JCOG.[8] CR was defined as the disappearance of all clinical and radiographic evidence of disease and the normalization of lactate dehydrogenase (LDH). As HTLV-I carriers frequently have a small percentage of abnormal lymphocytes, CR was judged with less than 5% of such cells if the absolute lymphocyte count was less than 4×10^9/l. Partial response (PR) was defined as a $\geqslant$50% reduction of the measurable disease with more than 75% reduction in the absolute abnormal lymphocyte count. LDH had to be decreased to $<$1.5 of the normal upper limit. PD was defined by $\geqslant$25% increase of the measurable disease or the appearance of new lesions while under treatment. No change (NC) was defined by an intermediate response between PR and PD.

Statistical analysis

The Kaplan–Meier method was used to estimate OS and RFS. The 95% confidence intervals of 3-year OS and RFS were calculated. For analyzing the relapse after transplantation, the cumulative distribution function of relapse was calculated. The differences in the OS and RFS rates between the groups of transplantation-related factors were compared by the log-rank test. Among those who reached CR after transplantation, the differences in the proportion of ATLL relapse between the groups of transplantation-related factors were compared by the χ^2 test. Furthermore, simultaneous effects of prognostic factors on survival (OS and RFS) and relapse were analyzed using multivariate regression analysis based on the Cox's proportional hazards model and the linear logistic model, respectively. The most appropriate models were selected based on Akaike's information criteria (AIC). All analyses were performed using SAS (Statistical Analysis System Inc., Cary, NC, USA) software.[17]

Results

Patient characteristics and transplant conditions

Data on 40 transplantations were collected. The clinical characteristics of patients are summarized in Table 1. All patients received standard-dose chemotherapy before the procedure of transplantation, and 28 patients (70%) showed a clinical response to the previous chemotherapy. Nine related donors showed a positive result for the anti-HTLV-I antibody. The peripheral blood mononuclear cells of these donors were subjected to Southern blot analysis to examine the monoclonal integration of HTLV-I provirus into the genome, and all nine donors were confirmed as carriers of HTLV-I.

Engraftment and response to transplantation

The median number of cells transplanted was 3.10. (1.20–9.20) $\times 10^8$/kg of nucleated cells for bone marrow transplantation (21 cases) and 3.94 (1.20–8.30) $\times 10^6$/kg of CD34-positive cells for peripheral blood stem cell transplantation (19 cases). All evaluable patients (39 recipients) achieved engraftment and the median time of neutrophil recovery to a level more than 0.5×10^9/l was 15 days (range 11–36). No case suffered from late graft rejection. All patients who received transplantation during PR disease status (13 cases) achieved CR after transplantation. Of 11 patients with resistant disease (three NC and eight PD) at the time of transplantation, 10 patients (except one PD case) achieved CR.

GVHD and transplantation-related early toxicity

aGVHD developed in 26 of 39 patients (66.7%), and 15 out of 31 patients developed cGVHD (Table 2). There were 21 deaths after transplantation, and 16 were related to adverse events of transplantation (Table 2). Within 6 months after transplantation, 15 patients died: 13 were lost to adverse events of transplantation.

Table 2 Transplantation outcome

Alive/dead	19/21
Cause of death	
<6 months after transplanatation	
GVHD	6
Infection	3
TMA	3
Hepatitis	1
Disease progression	2
⩾6 months after transplantation	
Infection	2
Disease progression	2
Bronchiolitis obliterance	1
Unknown[a]	1
Relapse of ATLL	10
Acute GVHD	
Grade 0	13
Grade I	8
Grade II	11
Grade III	4
Grade IV	3
Chronic GVHD	
Limited	4
Extensive	11

[a]Sudden death.
TMA, thrombotic microangiopathy.

Survival and relapse of ATLL

The median survival time of all cases after transplantation was 9.6 months (range 0.3–63.6 months), and the median observation time of 19 surviving patients was 22.7 months (3.7–63.6) (Table 2 and Figure 1). Estimated OS and RFS rates at 3 years were 45.3% (95% confidence interval (CI): 31.8–58.8%) and 33.8% (95% CI: 17.2–49.4%), respectively (Figures 1 and 2). ATLL relapse occurred in 10 patients after transplantation (Table 2). The estimated risk of disease relapse at 3 years was 39.3% (95% CI: 19.7–58.9%) (Figure 3). However, five achieved another CR: three by reduction or cessation of immunosuppressive agents, one by standard-dose chemotherapy and one by local radiation therapy. Among these five patients, three maintained CR over 1 year.

Univariate and multivariate analysis for survival and relapse

The univariate analysis for survival identified several pretransplantation and posttransplantation factors (Table 3). The performance status before transplantation had a significant impact on OS and RFS, but the disease status at transplantation did not. The existence of aGVHD or cGVHD did not affect the ATLL relapse, and aGVHD (grades I–IV) had a significant negative impact on OS and RFS. Patients with grade 0 or I aGVHD showed no significant difference on their OS or RFS from those with stronger aGVHD. Transplantation from a carrier donor was associated with better survival than from an HTLV-I-negative donor. Multivariate analysis selected several factors for survival and relapse (Table 4). Among factors selected, the presence of aGVHD had a significant negative impact on both OS and RFS. For RFS, transplant from related donor showed better results. Transplantation from a carrier donor had

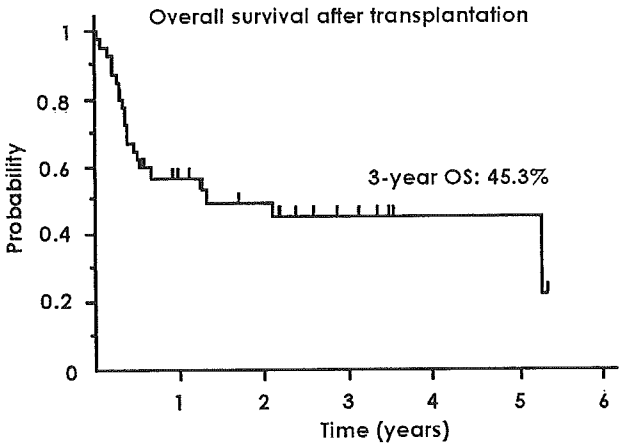

Figure 1 OS after transplantation. The estimated 3-year OS rate was 45.3%.

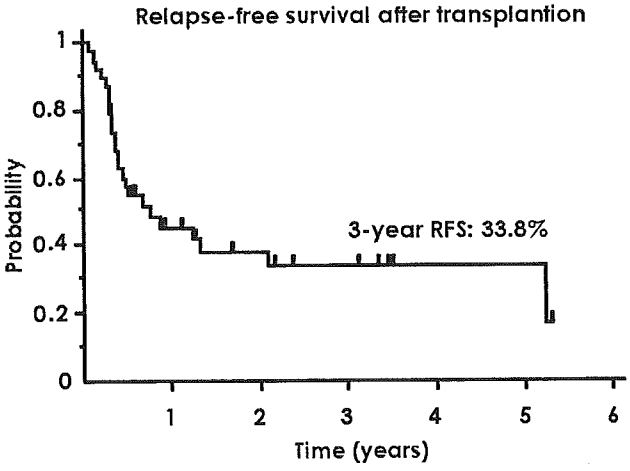

Figure 2 RFS after transplantation. The estimated 3-year RFS rate was 33.8%.

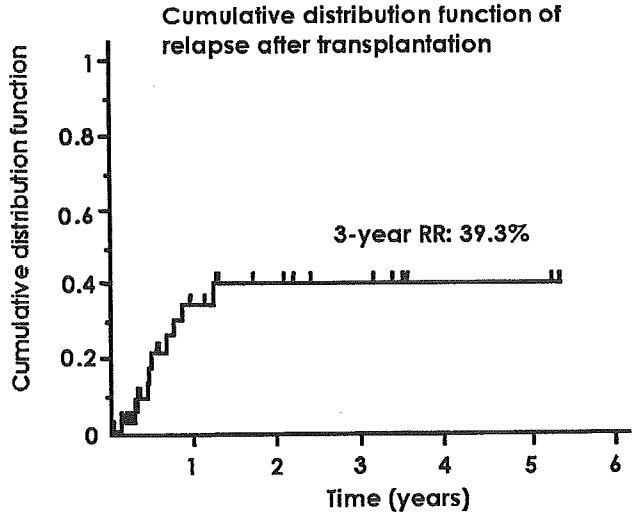

Figure 3 Cumulative distribution function of relapse after transplantation.

Table 3 Univariate analysis for OS, RFS and relapse

	No. of patients (for OS and RFS)	*OS (median survival, days)*		*RFS (median survival, days)*		*No. of patients (for Relapse)*	*No. of relapse*	
At diagnosis								
Subtype of ATLL								
Acute	30	208	$P=0.6762$	208	$P=0.7613$	29	6	$P=0.1574$
Lymphoma	10	405		174		9	4	
Transplantation-related factors								
Time from diagnosis to transplantation								
<220 days	20	192	$P=0.8344$	168	$P=0.6371$			
⩾220 days	20	405		247				
Age								
<43 years	15	216	$P=0.5148$	216	$P=0.2198$			
⩾43 years	25	340		134				
PS								
0	3	615	$P<0.0001$	308	$P<0.0001$			
1	33	362		201				
2	3	122		122				
3	1	8		NE				
Conditioning regimen								
TBI-based	22	405	$P=0.9234$	216	$P=0.4448$	20	6	$P=0.6587$
Busulfan-based	17	237		188		17	4	
Status 1								
CR1 or CR2	16	216	$P=0.6548$	262	$P=0.9745$	16	4	$P=0.8752$
Others	24	463		161		22	6	
Status 2								
CR1, CR2 or PR	29	406	$P=0.1586$	224	$P=0.4110$	29	9	$P=0.2357$
Others	11	135		128		9	1	
aGVHD1								
O	13	463	$P=0.0315$	340	$P=0.0100$	13	3	$P=0.7437$
Others	26	151		128		25	7	
aGVHD2								
0 or I	21	449	$P=0.1087$	262	$P=0.0918$	21	6	$P=0.7256$
Others	18	151		135		17	4	
cGVHD								
(+)	15	456	$P=0.9157$	372	$P=0.5849$	15	6	$P=0.1927$
(−)	16	450		216		16	3	
Relapse								
(+)	10	405	$P=0.6718$					
(−)	29	278						
Donor source								
Related	32	351	$P=0.1308$	208	$P=0.0227$	32	6	$P=0.0144$
Unrelated	8	168		139		6	4	
Donor HTLV-I								
(+)	9	463	$P=0.0462$	436	$P=0.1257$	9	4	$P=0.2484$
(−)	27	171		123		25	6	

significantly higher incidence of relapse than that from a noncarrier donor.

Discussion

This retrospective study reported the outcome of allo-HSCT for 40 patients with ATLL who received intensive treatment (myeloablative intensity in most cases) as a conditioning regimen before transplantation. The OS and RFS rates at 3 years were 45.3 and 33.8%, respectively, and these results were comparable with our previous report of 10 cases.[14] Patients in this study were diagnosed with either the acute or lymphoma type of ATLL, which usually shows an aggressive clinical course.[7] Among 11 evaluable patients who received transplantation at the point of resistant disease (NC and PD), all but one

Table 4 Multivariate analysis for OS, RFS and relapse

Factors selected	*P-value*	*Hazard ratio*
For OS		
PS = 2, 3 or 4	0.174	3.39
aGVHD (+) (any grade)	0.068	4.43
Related donor	0.238	0.41
Donor HTLV-I (+)	0.553	0.61
For RFS		
Age ⩾43	0.123	2.34
PS = 2, 3 or 4	0.528	1.70
aGVHD (+) (any grade)	0.033	4.10
Related donor	0.022	0.24
For relapse		
Related donor	0.040	0.09
Donor HTLV-I (+)	0.076	6.00

patient achieved CR after transplantation, suggesting the efficacy of myeloablative conditioning to ATLL. In this analysis, there was no difference in the incidence of relapse or survival among patients treated with busulfan-based regimens and TBI-containing regimens. It seemed that chemotherapy was as effective as TBI for ATLL in terms of a conditioning regimen for allo-HSCT.

The major difference between autologous and allo-HSCT was the incidence of relapse of the primary disease after transplantation. We reported previously that relapse occurred in all four evaluable cases after autologous HSCT.[9] In contrast, 10 out of 39 cases (25.6%) suffered ATLL relapse after allo-HSCT. The immunological reaction induced only by allo-HSCT could control the residual ATLL after high-dose therapy, which may be called the GvATLL effect. In this study, among 10 patients in whom ATLL relapsed after transplantation, five patients achieved second CR. Interestingly, three achieved CR only by the reduction or cessation of immunosuppressive agents. This observation further supported the idea that the GvATLL effect actually worked and contributed to long-term RFS. One of the clinical findings supporting the idea of graft-versus-leukemia (or lymphoma) effect is the benefit of GVHD in the prevention of disease relapse,[18–20] for example, as shown in NHL in which grades II–IV aGVHD were associated with a lower incidence of disease progression after transplantation from an unrelated donor.[21] In this study, however, we found no positive effect of GVHD on freedom from relapse, or long-term survival by univariate and multivariate analysis. On the other hand, the existence of aGVHD, even grade I, negatively affected the OS and RFS. Of note, the negative impact of GVHD on the survival did not change by the analysis with Cox's proportional hazard model (in the analysis of aGVHD1, $P = 0.06$ for OS and $P = 0.02$ for RFS), which treats GVHD as a time-dependent covariate, although nine cases lacking some clinical data were missed in this analysis. The possible GvATLL effect (second CR obtained by the reduction or cessation of immunosuppressive agents) but no benefit of aGVHD and cGVHD on the survival or the prevention of relapse (demonstrated by the univariate and multivariate analysis) suggests that the GvATLL effect could work on those patients even without clinically obvious GVHD. This hypothesis is in concordance with our recent observation[22] that showed the development of cytotoxic T cells specific for Tax, one of the HTLV-I products, in patients after nonmyeloablative allo-HSCT for ATLL. In some patients, the HTLV-I-infected cell-specific immune reaction may contribute to the eradication of ATLL.

A total of 21 patients died after allo-HSCT, and 15 of those 21 patients were lost within 6 months after HSCT mostly due to treatment-related adverse events. We assume that both intensive conditioning before transplantation and the immunological reaction between graft and host after transplantation were effective for ATLL; however, these two factors also had a negative impact on the survival of patients at the same time. As shown in the univariate analysis, the general status of patients but not the disease status at transplantation was significantly related with the survival after transplantation. The indication of myeloablative allo-HSCT for ATLL needs to be determined based on the general status rather than disease status to reduce TRM. Furthermore, because patients with ATLL are immunocompromised,[4,7] the strengthening of supportive care for infection and the development of less toxic conditioning could contribute to a better outcome. In this respect, nonmyeloablative conditioning will also be tested for ATLL. As shown by multivariate analysis, it seems important to control aGVHD for patients with ATLL. Transplantation from a related donor will lead to the reduction of aGVHD and TRM.

The median age of 44 years old of the patients in this series is apparently less than that of general ATLL patients (about 60 years old in Japan). Although there was some bias in case selection, our results demonstrated that allo-HSCT can provide apparent long-term RFS in some patients, the GvATLL effect was actually observed. In a patient who was transplanted from a seronegative donor, the original ATLL population in peripheral blood was not detected by Southern blot analysis 6 years after transplantation. However, anti-HTLV-I antibody was positive (data not shown). It is necessary to analyze the precise virological status of recipients after allo-HSCT using techniques such as PCR to understand how it works on ATLL. Further prospective controlled studies are needed to assess the efficacy of allo-HSCT for ATLL and the GvATLL effect.

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Review Article

Tumor immunity against adult T-cell leukemia

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Human T-cell leukemia virus type-I (HTLV-I) causes adult T-cell leukemia (ATL) in a small population of infected individuals after a long incubation period. Although the process of clonal evolution of ATL cells may involve multiple steps, ATL cells from half of the ATL cases still retain the ability to express HTLV-I Tax, a key molecule of HTLV-I leukemogenesis. A recent finding of reactivation of Tax-specific cytotoxic T lymphocytes (CTL) in ATL patients after hematopoietic stem cell transplantation suggests the presence of Tax expression *in vivo* and potential contribution of the CTL to antitumor immunity. This is consistent with the results of a series of animal experiments indicating that Tax-specific CTL limit the growth of HTLV-I-infected cells *in vivo*, although the animal model mimics only an early phase of HTLV-I infection and leukemogenesis. Establishment of an insufficient HTLV-I-specific T-cell response and an increased viral load in orally HTLV-I-infected rats suggests that host HTLV-I-specific T-cell response at a primary HTLV-I infection can be a critical determinant of persistent HTLV-I levels thereafter. These findings indicate that Tax-targeted vaccines may be effective for prophylaxis of ATL in a high-risk group, and also for therapy of ATL in at least half the cases. (*Cancer Sci* 2005; 96: 249–255)

It is estimated that approximately one million people are infected with human T-cell leukemia virus type I (HTLV-I) in Japan. Although most HTLV-I carriers are asymptomatic throughout their lives, 1–5% of infected subjects develop adult T-cell leukemia (ATL),[1–3] and another small fraction of HTLV-I carriers develop a chronic progressive neurological disorder termed HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and other inflammatory disorders.[4,5]

Adult T-cell leukemia is characterized by tumor cells with mostly $CD4^+$ and $CD25^+$ mature T-lymphocyte phenotypes, onset during middle age or later, immune suppression and poor prognosis.[6] There are four clinical subtypes of ATL: acute, lymphoma, chronic and smoldering types, based on Shimoyama's diagnostic criteria.[7] Monoclonal integration of HTLV-I provirus in ATL cells indicates that ATL arises from a single HTLV-I-infected cell that undergoes malignant phenotypic progression.[8] However, oligoclonal expansion of HTLV-I-infected cells *in vivo* is also observed in HAM/TSP patients and some asymptomatic HTLV-I carriers,[9] suggesting that HTLV-I-infected cells generally have proliferative potential.

The HTLV-I viral protein Tax is a multifunctional protein, interacting with many cellular proteins regulating cell growth and apoptosis resistance. Tax activates Nuclear factor κ B (NFκB), cAPM response element binding protein (CREB), serum response factor (SRF), activator protein 1 (AP-1), and represses p53 or other tumor suppressor proteins either by direct or indirect mechanisms, partly accounting for HTLV-I-induced leukemogenesis.[10]

However, the level of HTLV-I expression in freshly isolated peripheral ATL cells is extremely low. This paradoxical observation provides controversy concerning the role of Tax in HTLV-I leukemogenesis. It has been reported that fresh ATL cells exhibit constitutive activation of NFκB,[11] one of the transcription factors induced by Tax, while Tax is undetectable in these cells. This implies that either subdetectable levels of Tax or some other mechanism substituting for Tax function activates NFκB in ATL cells.

Expression of viral antigen

It has been noted that HTLV-I expression is inducible in ATL cells from some ATL patients after several hours of culture.[12] A recent study using flow cytometry indicated that similar induction of HTLV-I Tax and Gag antigens occurs in ATL cells in approximately half of the ATL cases tested (Fig. 1).[13] Earlier studies demonstrated that HTLV-I mRNA is detectable at low levels in ATL cells without culture, and is also detectable in ATL lymph nodes *in situ*.[14,15] Moreover inoculation of uncultured formalin-treated ATL cells into naive rats resulted in induction of a HTLV-I-specific T-cell response.[13] These observations suggest that ATL cells in approximately half of all ATL cases may express very low levels of HTLV-I antigens, which are further enhanced by *in vivo* culture. Similar transcriptional repression of HTLV-I expression *in vitro* and its induction in *in vitro* culture have been observed in the peripheral blood mononuclear cells (PBMC) of HAM/TSP patients and HTLV-I-carriers.[16–18]

The mechanism of this transient transcriptional repression of HTLV-I in the peripheral blood is unknown. Methylation of the CpG motif found in the 5′-long-terminal repeat (LTR) may be partly involved, but do not fully explain the phenomenon.[19–21]

Adult T-cell leukemia cells from the other half of cases fail to express HTLV-I antigens even after *in vitro* culture. This irreversible silencing of HTLV-I could be due to various genomic changes in the HTLV-I provirus of ATL cells, such as deletions at the 5′-LTR and *gag/pol* regions.[22,23]

Thus, ATL cases may be categorized into two groups in the context of their HTLV-I expression in ATL cells; HTLV-I expression is inducible in approximately half of all ATL cases, while irreversible in the other half of cases. The inducible type of viral suppression in the PBMC may be a common phenomenon in HTLV-I-infected individuals irrespective of the disease.

Anti-tumor immunity in HTLV-I infection

Cytotoxic T-lymphocyte response in human HTLV-I-infected individuals. Although no consistent differences have been observed among HTLV-I strains isolated from ATL and HAM/TSP patients,[24,25] immunological studies have found a clear difference in HTLV-I-specific T-cell responses between these

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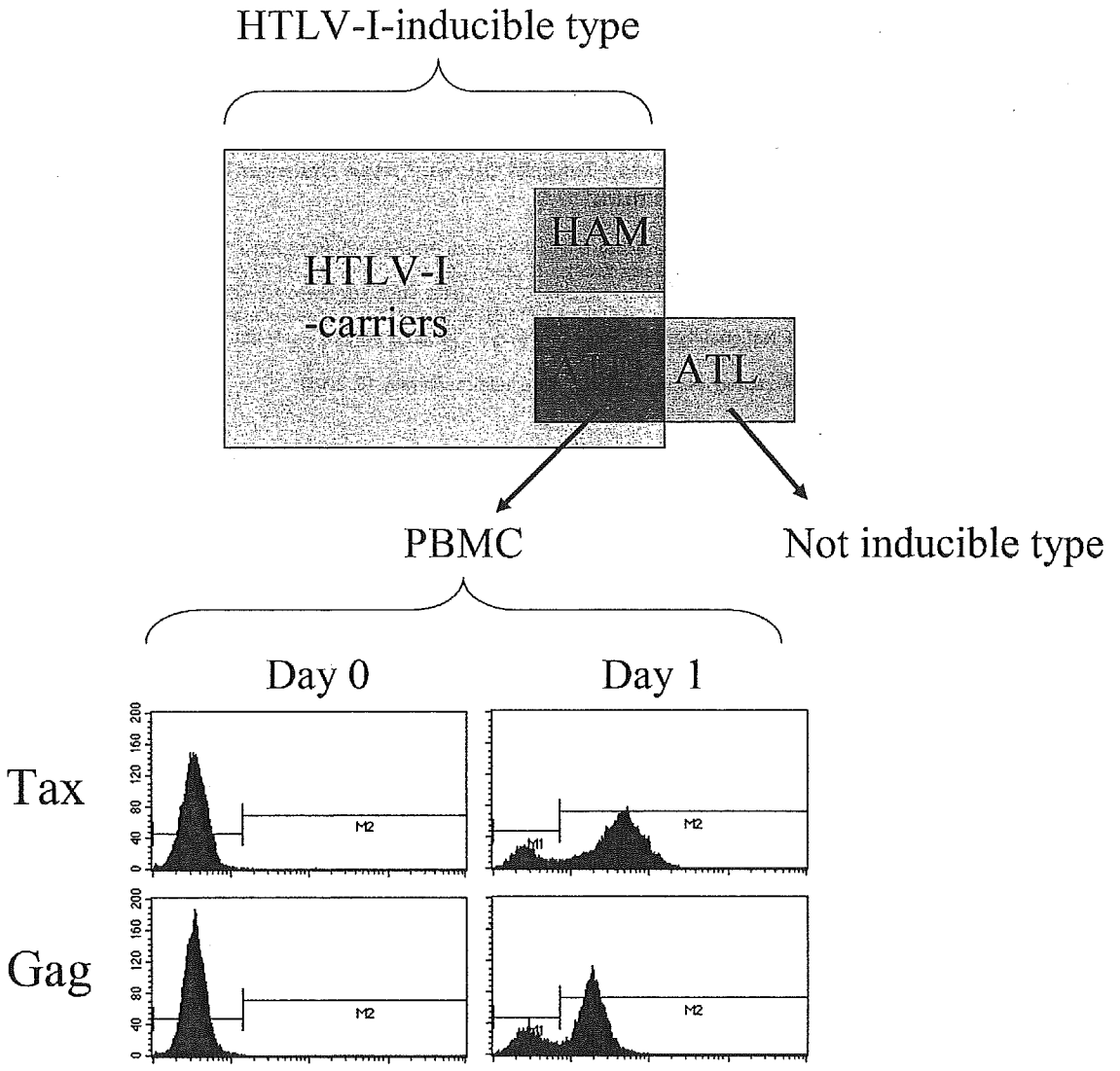

Fig. 1. Induction of human T-cell leukemia virus type-I (HTLV-I) antigens in adult T-cell leukemia (ATL) cells. ATL cells isolated from peripheral blood of approximately half of the ATL cases expressed a significant amount of HTLV-I Tax and Gag antigens in 1-day culture, as detected by flow cytometry.[13] A similar phenomenon is seen commonly in asymptomatic HTLV-I-carriers and HTLV-1-associated myelopathy/tropical spastic paraparesis patients. HTLV-I antigens were not inducible in the other half of the ATL cases.

two diseases. HTLV-I-specific cytotoxic T-lymphocytes (CTL) are highly activated in HAM/TSP patients and are sometimes readily detectable in PBMC without any stimulation *in vitro*. Similar CTL can be induced in PBMC culture from many asymptomatic HTLV-I carriers when stimulated with autologous HTLV-I-infected cells *in vitro*. However, HTLV-I-specific CTL are only rarely induced in ATL patients.[26–29] A recent report demonstrated that HTLV-I-specific CTL are present in ATL patients but expand insufficiently,[30] suggesting involvement of some immune suppression or tolerance.

Human T-cell leukemia virus type I core, envelope, polymerase and Tax proteins are recognized by HTLV-I-specific CTL.[28,31,32] In addition, oligopeptides of Tof and Rof were shown to induce CTL from HTLV-I-infected individuals.[33] Among these antigens, HTLV-I Tax, a critical viral protein for T-cell immortalization, is the most popular target for HTLV-I-specific CTL found in HTLV-I-infected individuals.[28,31] HTLV-I Tax-specific CTL are capable of killing short-term cultured ATL cells expressing viral antigens *in vitro*.[34]

Experimental tumor vaccine. To understand the influence of host immunity to HTLV-I leukemogenesis *in vivo*, a series of experiments using rat models for HTLV-I-infected T-cell lymphomas were carried out. In these models, a syngeneic HTLV-I-transformed T-cell line underwent phenotypic evolution to cause fatal lymphomas in immune-suppressed rats.[35] However, this cell line did not cause tumors in immune-competent rats. Immunological analysis revealed that the antitumor effects in immune-competent rats were mediated by CTL predominantly directed to HTLV-I Tax.[36] It is intriguing that the major target of HTLV-I-specific CTL is Tax both in rats and humans.

Antitumor effects of Tax-specific CTL were further confirmed by vaccine experiments, in which T-cells from immune-competent rats vaccinated with Tax-encoded DNA could eradicate fatal T-cell lymphomas in athymic rats when transferred.[37] Similar results were obtained by vaccination with oligopeptides corresponding to the major CTL epitope.[36]

Immune response in post-hematopoietic stem cell transplantation ATL patients

Although the rat models described above may mimic some aspects of HTLV-I leukemogenesis, they differ from full-blown

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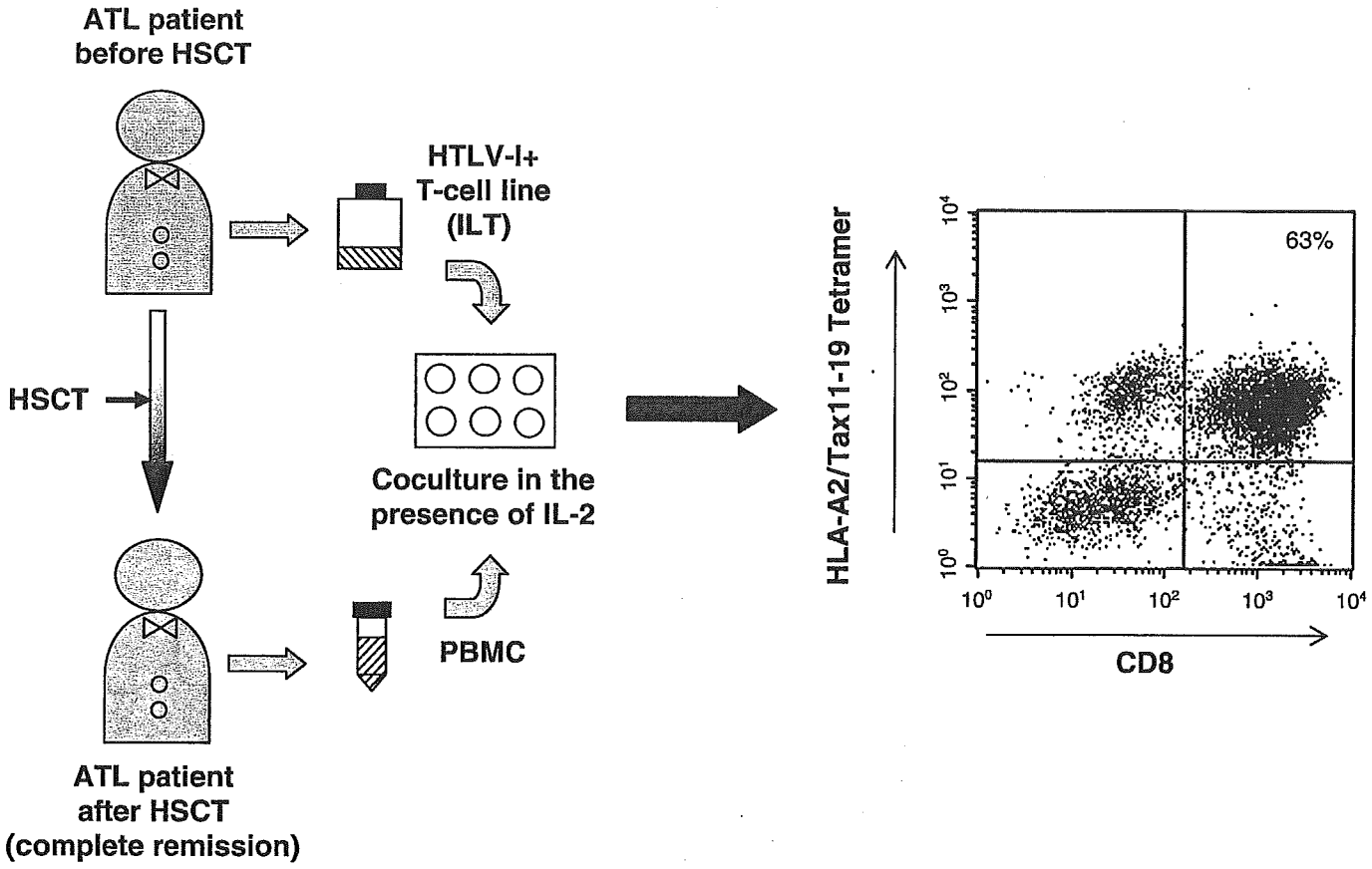

Fig. 2. Induction of Tax-specific cytotoxic T lymphocytes (CTL) from an adult T-cell leukemia (ATL) patient receiving hematopoietic stem cell transplantation (HSCT) from a human leukocyte antigen (HLA)-identical donor. A spontaneous human T-cell leukemia virus type-I-infected T-cell line (ILT) was established from an ATL patient before HSCT. These cells were formalin-treated, then co-cultured with peripheral blood mononuclear cells from the same ATL patient after HSCT. After several weeks of culture, 63% of the cells were HLA-A2-restricted Tax11-19-specific $CD8^+$ CTL as detected with phycoerythrin-conjugated tetramers.[39]

ATL in humans as ATL develops in immune-competent individuals following over 40 years of incubation, whereas rat lymphoma consists of HTLV-I-transformed cells and develops only in immune-suppressed hosts.

Allogeneic hematopoietic stem cell transplantation (HSCT) has been used to treat ATL and achieved long-lasting complete remission in some ATL patients.[38] Graft-versus-host (GVH) or graft-versus-leukemia (GVL) responses are presumed to contribute to antitumor effects in these patients. Because the GVH/GVL response is mediated primarily by T cells, we investigated T-cell responses in ATL patients who obtained complete remission following non-myeloablative allogeneic peripheral blood HSCT from human leukocyte antigen (HLA)-identical sibling donors (Fig. 2).[39] In that study, as the target of the GVH response, mitogen-stimulated IL-2-dependent T-cell lines (ILT) were established from ATL patients before HSCT, which express antigens originating from the recipient. These cells were also infected spontaneously with HTLV-I. When the PBMC from the same patients after HSCT were stimulated in culture with formalin-treated ILT cells *in vitro*, $CD8^+$ CTL capable of killing ILT cells proliferated vigorously. Further analysis revealed that most of these CTL predominantly recognized a limited number of Tax epitopes; Tax 11-19 restricted by HLA-A2 in one patient and Tax 301-309 restricted by HLA-A24 in another. However, PBMC from these ATL patients before HSCT did not show such CTL responses.

Similar oligoclonal expansion of Tax 11-19-specific CTL was reported previously in HLA-A2 + HAM/TSP patients.[40] This phenomenon is explained by a highly activated host CTL response against abundant HTLV-I antigens in HAM/TSP patients. Thus, the Tax-specific CTL expansion observed in the post-HSCT ATL patients implies that these patients may be in a similar status to HAM/TSP patients in the context of their activated levels of T-cell response and/or Tax antigen presentation *in vivo*. Significant reduction in the proviral load in these patients following HSCT might be partly due to such a strong anti-Tax CTL response.

Various minor histocompatibility antigens (mHA) have been postulated to act as the target of the GVH/GVL response in HSCT.[41] In cultures of post-HSCT ATL patients, a minor population of CTL induced against ILT cells was directed to an unknown antigen other than Tax, probably related to the GVH response. The role of the anti-Tax CTL response in relation GVL effects remains to be clarified. However, the strong HTLV-I-specific response observed in the patients after complete remission suggests that HTLV-I-specific CTL as well as the GVH effectors might participate in the maintenance of remission.

Immunological risk factors for ATL development

The insufficient HTLV-I-specific T-cell response observed generally in ATL patients could be either a consequence of ATL or a risk associated with ATL development. If this were a risk associated with ATL, a wide survey of HTLV-I-specific T-cell responses among HTLV-I carriers would be useful to identify a high-risk group, to whom prophylactic strategies should be applied.

Epidemiological risk factors for ATL. In cohort studies of HTLV-I carriers, it appears that the risk factors for ATL might include

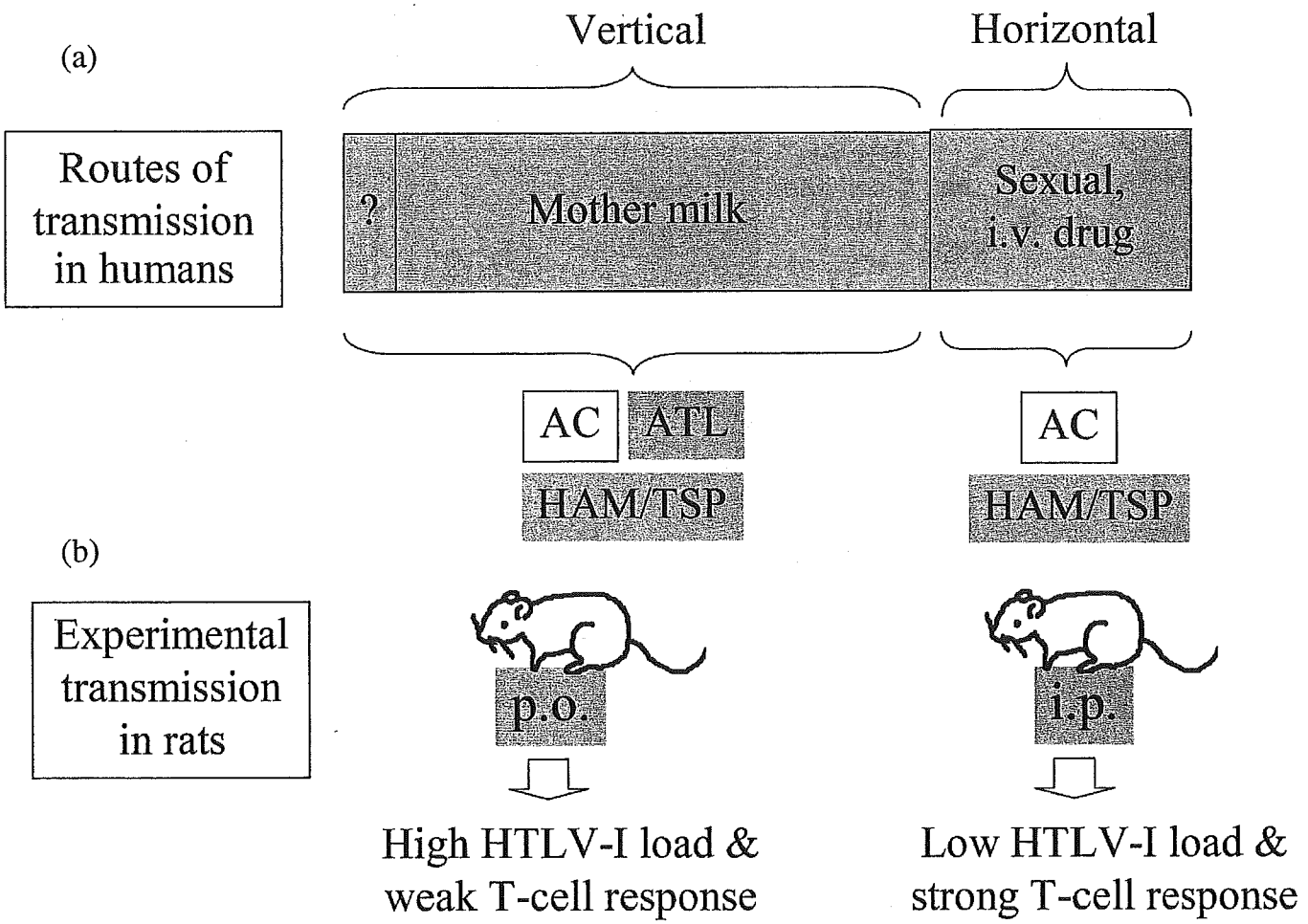

Fig. 3. Relationship between the routes of human T-cell leukemia virus type-I (HTLV-I) infection and diseases in humans or outcome in rat experiments. (a) Adult T-cell leukemia arises from a vertically infected population, whereas HTLV-1-associated myelopathy/tropical spastic paraparesis arises from both vertically and horizontally infected populations. Irrespective of the route of infection, most of the infected individuals are asymptomatic HTLV-I-carriers (AC). (b) Adult rats infected orally with HTLV-I had an increased viral load and a weak HTLV-I-specific T-cell response, whereas intraperitoneally infected rats had a low viral load and a strong HTLV-I-specific T-cell response.[48,49]

vertical HTLV-I infection, and increasing numbers of abnormal lymphocytes.[3,42,43] HTLV-I transmits from mother to child mainly through breast milk, and from male to female by sexual contact. Blood transfusion or intravenous drug use also causes HTLV-I transmission.[44–46] Among these, mother-to-child transmissions are the major natural route in Japan. The higher incidence of ATL in males is attributed to the relatively higher ratio of vertical infection in males. The presence of typical HLA haplotypes for ATL in an endemic area[47] also implies that vertical infection might transmit some determinants of HTLV-I leukemogenesis.

Oral infection as a determinant of insufficient T-cell response in rats. In a rat model, the routes of infection strongly affect HTLV-I-specific immunity (Fig. 3).[48] Among immune-competent adult rats infected with HTLV-I through various routes, rats inoculated orally showed very low levels of HTLV-I-specific T-cell response, whereas significant responses were detected in rats infected through other routes.[49] In contrast, HTLV-I proviral load in the spleen cells, examined several months after infection, was significantly higher in orally infected rats. Because HTLV-I proviruses are associated with infected cells, the increase in proviral load indicates the increase in infected cell number.

Together with the fact that oral infection through mothers' milk is a major route of vertical HTLV-I infection in humans,[44] the results of the rat experiments of oral HTLV-I infection strongly suggest that the epidemiological risks of ATL (i.e. vertical HTLV-I infection and high viral load) link to the immunological risk (i.e. low T-cell responses to HTLV-I).

Balance between host immunity and HTLV-I in natural HTLV-I infection

Positive or negative correlation between host immunity and the virus. In the rat experiment described above, there was an inverse correlation between HTLV-I proviral load and HTLV-I-specific T-cell proliferation,[49] indicating that HTLV-I-specific T-cell responses might contribute to limiting expansion the of HTLV-I-infected cells *in vivo*, and that oral infection may be a reason for insufficient T-cell immunity to HTLV-I.

Infants born to HTLV-I-carrying mothers are fed approximately 1×10^8 HTLV-I-infected cells before weaning,[45] and a number of infantile carriers stay seronegative for HTLV-I for a certain period of time,[50] probably due to some immunological tolerance during this period. Most of these children show seroconversion by the age of 3 years.[51] Although T-cell immune responses to HTLV-I in children are not known, many adult HTLV-I carriers show HTLV-I-specific CTL responses, suggesting that the T-cell response might recover spontaneously later in life, just as happens in vertical hepatitis B virus infection.

In contrast to the results of rat experiments, HTLV-I proviral load in human adult HTLV-I carriers correlate positively with

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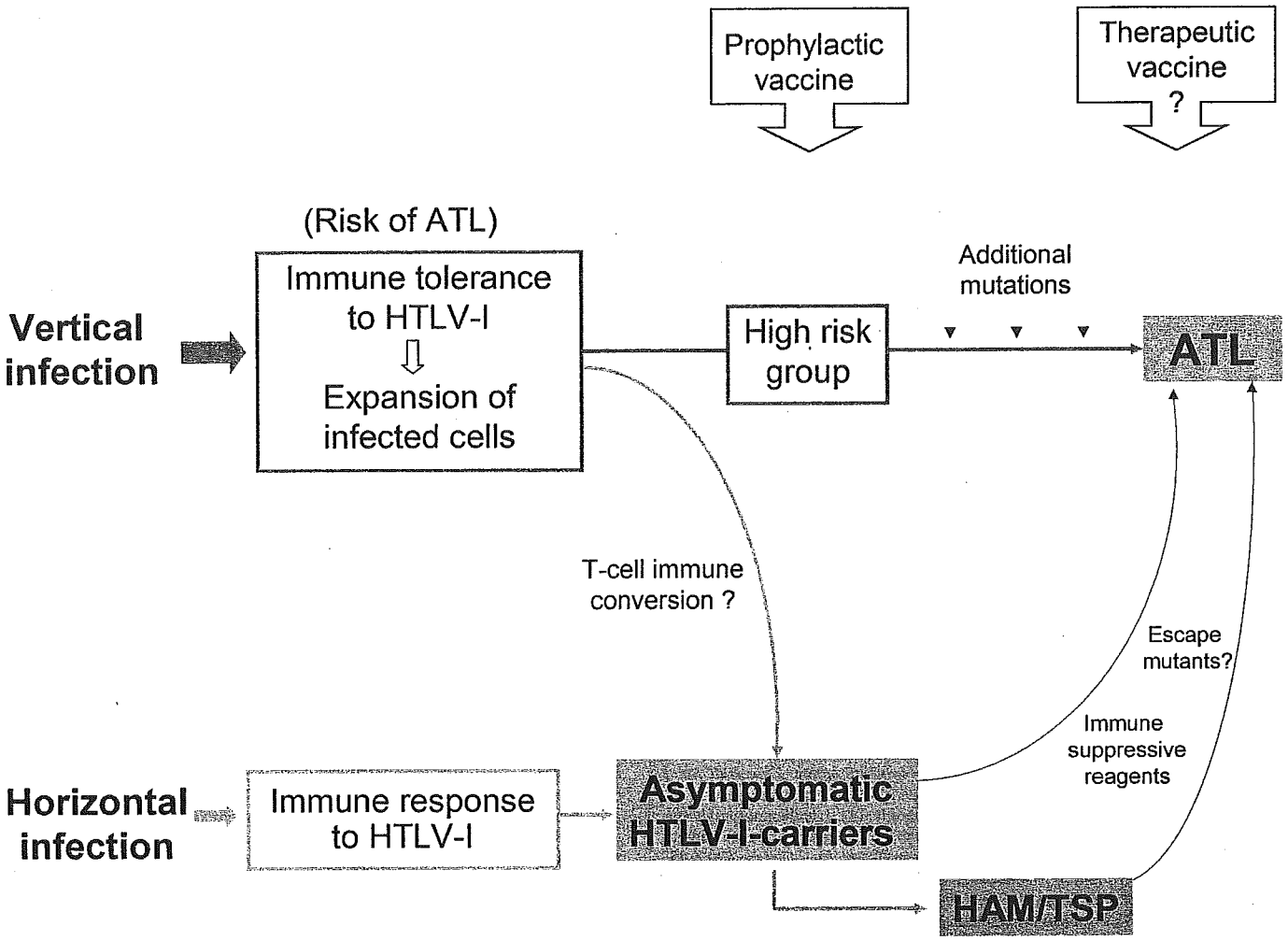

Fig. 4. Hypothesis on the relationship among host T-cell immunity, risk of adult T-cell leukemia (ATL), and the route of infection in humans. Vertically infected human T-cell leukemia virus type-I (HTLV-I) carriers harbor risks of ATL (i.e. insufficient HTLV-I-specific T-cell response and expansion of infected cells). HTLV-I-specific T-cell responses eventually recover spontaneously in most of these carriers and the risk of ATL decreases. However, if a small population remains in the high-risk group, insufficient T-cell immunity in these individuals may allow clonal evolution of infected cells toward ATL. HTLV-I carriers infected through horizontal routes would have a lower risk of ATL.

the HTLV-I-specific T-cell response.[52,53] This discrepancy between rats and humans may be partly explained by the difference in the period of HTLV-I infection; several months in the rat experiments but many years in human HTLV-I carriers. If the T-cell response recovered after a long period of insufficient response to HTLV-I, the magnitude of the recovered response would correlate positively with the elevated levels of pre-existing proviral load *in vivo*.

Hypothetical relationship among host immunity, disease development and the route of infection. Figure 4 gives a schematic demonstration of our current hypothesis on the immunological risks of ATL in the natural course of HTLV-I infection. Vertically infected HTLV-I carriers harbor risks of ATL (i.e. insufficient HTLV-I-specific T-cell response and expansion of infected cells). However, such risks may be reduced in many HTLV-I carriers by spontaneous recovery of the HTLV-I-specific T-cell response. If there is a small group of adult HTLV-I carriers still showing insufficient T-cell responses to HTLV-I despite an abundant viral load, this might be a high-risk group for ATL, to whom prophylactic vaccines targeting Tax may be beneficial.

Favorable levels of T-cell response and a lower risk of ATL are expected in individuals infected through horizontal routes, although a small fraction of this group might develop HAM/TSP. The genetic determinants of HAM/TSP are not known. In Japan, many HAM/TSP cases arise from the vertically infected population, suggesting that T-cell immune conversion has occurred at some stage. The oligoclonal expansion of HTLV-I-infected cell clones often seen in HAM/TSP patients indicates that these clones might have been in the process of leukemogenesis.[9] Nevertheless, the incidence of ATL among HAM/TSP patients is limited, probably due to the activated host HTLV-I-specific T-cell immunity. In this respect, administration of immunosuppressive reagents to HAM/TSP patients might increase the risk of ATL development. In addition, the post-HSCT patients with T-cell immune conversion should be followed up carefully, although development of HAM/TSP in post-HSCT ATL patients has not been reported so far.

Prophylaxis and therapy for ATL

The findings of the immunological studies described above suggest that Tax-targeted vaccines may be beneficial for prophylactic use against the high-risk group. For this purpose, a handy method to detect HTLV-I-specific T-cell immune response would be required for a wide survey among HTLV-I carriers.

A number of combination chemotherapy protocols have been applied, and the median survival time of a recent protocol

(JCOG9303) was 13 months.[54] In addition, several kinds of experimental therapies have also been applied to ATL. A small number of ATL patients respond to intravenous administration of anti-CD25 monoclonal antibody.[55] The combination therapy of azidothymidine (AZT) and interferon α achieved a high response rate but did not prevent relapse of ATL.[56,57] The mechanisms for the antitumor effects of these antiretroviral drugs are not clear, as HTLV-I proliferation occurs mainly by proliferation of infected cells, not by viral replication. A recent study indicated that AZT and interferon α suppress NFκB activity and induce TRAIL expression, respectively.[58] A combination of arsenic and interferon α[59] and some other NFκB-targeted therapies have been proposed.

Recently, allogeneic but not autologous HSCT achieved long-lasting complete remission in some ATL patients.[38,60] However, there is also a risk of GVH disease, which is sometimes lethal. If Tax-specific CTL induced in post-HSCT ATL patients makes any contribution to GVL effects, Tax-targeted immunotherapy might be worth trying either with or without HSCT, and selective GVL effects would be expected. Indication of Tax-targeted immunotherapy, however, should be limited to those cases whose ATL cells retain the ability to express Tax.

Conclusion

Although the peripheral HTLV-I-infected cells do not express detectable levels of HTLV-I antigens, they retain the ability to express Tax in most HTLV-I-infected individuals, including asymptomatic HTLV-I carriers, HAM/TSP patients and approximately half of all ATL cases. Immunological findings support the contribution of Tax-specific CTL to antitumor immunity in these hosts, encouraging immunological prophylaxis and therapy for ATL.

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LETTER TO THE EDITOR

Prolonged remission of adult T-cell leukemia/lymphoma treated with interferon-γ following autologous peripheral blood stem cell transplantation

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Adult T-cell leukemia/lymphoma (ATL) is refractory to conventional chemotherapies. Intensive combination chemotherapy has been evaluated for ATL, but complete remission (CR) rate and median survival of patients with ATL has been reported as only 33% and 13 months, respectively [1]. Autologous stem cell transplantation (auto-SCT) in ATL has recently been reported in a limited number of patients. Although most ATL patients treated with auto-SCT achieved CR, clinical outcomes reported to date have been accompanied by early recurrence [2]. However, auto-SCT can induce remission in patients with ATL. To obtain better outcomes, maintenance therapy may prove useful for patients with ATL following auto-SCT. We report the case of a patient with ATL who was treated with interferon (IFN)-γ following autologous peripheral blood stem cell transplantation. The patient remained in remission for 26 months post-transplantation. This therapy may prove worthwhile for patients with ATL who are not eligible for allogeneic stem cell transplantation, to achieve prolonged remission.

A 62-year-old Japanese male was referred to our hospital due to systemic lymphadenopathy in August 1999. White blood cell (WBC) count was 3.3×10^9/l, with 1% abnormal lymphocytes displaying lobulated nuclei. Pathological examination of biopsy specimens from an inguinal lymph node revealed T-cell malignancy. Antibody for human T-lymphotrophic virus type 1 (HTLV-1) was positive, and monoclonal integration of HTLV-1 proviral DNA in malignant cells was demonstrated using Southern blot analysis. Clinical diagnosis of lymphoma-type ATL was made. The patient received six courses of combination chemotherapy using cyclophosphamide, pirarubicin, etoposide and prednisolone, and achieved partial remission in December 1999. Thereafter, he was treated with oral low-dose etoposide (25 mg, p.o. daily) and achieved CR in May 2000. However, abnormal cells in peripheral blood increased gradually during treatment with oral etoposide. In November 2000, WBC count was 4.1×10^9/l, with 2% abnormal lymphocytes. High-dose chemotherapy combined with autologous peripheral blood stem cell transplantation (auto-PBSCT) was therefore adopted to further reduce ATL cells. Following mobilization (etoposide 750 mg once daily i.v. for 3 days followed by filgrastim 300 μg once daily s.c.), peripheral blood stem cells were harvested using a Cobe Spectra Cell Separator (Cobe BCT, Lakewood, CO, USA). The patient underwent PBSCT in February 2001 after conditioning with melphalan 130 mg/m^2 i.v. on day −1, cyclophosphamide 60 mg/kg i.v. on days −4 and −3, etoposide 250 mg/m^2 twice daily i.v. on days −4, −3 and −2, and dexamethasone 40 mg/body i.v. on days −4, −3, −2 and −1. A total of 18×10^6/kg CD34$^+$ cells were transplanted. The post-transplant course was basically uneventful. Abnormal lymphocytes in peripheral blood decreased gradually, and had disappeared by May 2001 (day 33 post-transplantation).

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Thereafter, intermittent administration of IFN-γ was performed to prevent relapse of ATL. IFN-γ was administered at a dose of 1×10^6 IU i.m. daily for 10 days followed by the same dose IFN-γ every 3 weeks. The patient developed low-grade fever on the day of each dosing, and this was treated using diclofenac sodium. The patient was then maintained on IFN-γ alone until March 2003 (total 30 doses). No signs of relapse had been observed in the patient by then. ATL relapsed suddenly with cervical lymphadenopathy in April 2003, 26 months after auto-PBSCT. WBC count was 4.2×10^9/l with 18% abnormal lymphocytes. IFN therapy was stopped and the patient was then treated with combination chemotherapy or deoxycoformycin monotherapy. However, the durability of responses was very short, and the patient died of cachexia in October 2003.

The relative copy number of HTLV-1 proviral DNA in peripheral blood mononuclear cells (PBMC) from the patient was measured before and after auto-PBSCT using real-time polymerase chain reaction amplification of HTLV-1 pX DNA, as previously described [3]. Our patient showed 89% reduction of HTLV-1 proviral DNA at 3 months, and maintained a level of 92% reduction at 6 months after auto-PBSCT (Table I). The mean level of HTLV-1 proviral DNA for asymptomatic HTLV-1 carriers is reported as 64.9 ± 73.9/1000 PBMC [3]. Proviral DNA in this period was successfully kept lower than mean HTLV-1 proviral DNA in asymptomatic HTLV-1 carriers. Thereafter, the patient had been given IFN-γ, and HTLV-1 proviral DNA levels had not differed from levels in asymptomatic HTLV-1 carriers until 24 months after PBSCT. However, when ATL relapsed at 26 months after auto-PBSCT, levels increased to the same as before PBSCT.

Allogeneic stem cell transplantation (allo-SCT) has recently been shown to be useful in a very small number of ATL patients [3]. However, due to the immunological fragility and advanced age of onset typically present with this disease, most patients with ATL are ineligible for allo-SCT. No suitable donor for allo-SCT was available for our patient. Tsukasaki et al. reviewed eight cases of Japanese ATL patients who had received auto-SCT [2]. Although the study included four patients who received transplants when in the refractory phase, CR was achieved in almost all patients after auto-SCT. However, all patients relapsed relatively soon. The mean duration of remission after auto-SCT in the eight patients was only 6.7 months. Our patient also achieved CR and a marked decrease in relative copy number was observed for HTLV-1 provirus DNA in PBMC until 6 months after auto-PBSCT. We considered that maintenance therapy following auto-PBSCT was required to prevent relapse of ATL.

Table I. HTLV-1 proviral DNA in PBMC.

	Months after auto-PBSCT				
Before conditioning treatment	1	3	6	24	26
214.9	309.3	24.0	16.4	69.1	274.9

Data are the relative copy number of HTLV-1 proviruses per 1000 peripheral blood mononuclear cells.

IFNs represent a group of significant biological response modifiers with antitumor activity against a variety of tumors. On the basis of the observations made in the HTLV-1 Tax-transgenic mouse model, it has been shown that IFN-γ-mediated inhibition of tumor angiogenesis is critically involved in the mechanisms of antitumor effect evoked by IFN-γ on HTLV-induced tumors [4]. The antitumor effects of IFN-γ have been documented in five patients with ATL, with one CR and two partial responses reported [5]. Moreover, IFN-γ is highly clinically useful for patients with ATL in whom lesions are confined to the skin. In these patients, symptoms that progressed to leukemia or lymphoma were observed in two of ten patients who showed response to IFN-γ and in eight of nine patients who showed no response [6]. Based on these findings, we hypothesized that use of IFN-γ following auto-PBSCT might be effective in inhibiting the growth of ATL cells. In the present report, the level of HTLV-1 proviral DNA was no different from levels in asymptomatic HTLV-1 carriers for 15 months after IFN-γ was started in our patient.

The use of IFN-α has recently been reported as effective following auto-PBSCT for ATL [7]. Although the conditioning regimen differed from the present, the patient in that case survived in remission for more than 12 months after auto-PBSCT. They suggested that IFN-α following auto-PBSCT reduces tumor aggressiveness via induction of IFN-γ producing CD3/CD8 double-positive cells activity. Thus, we used IFN-γ in our patient with auto-PBSCT. Similarly, our patient who was given IFN-γ remained in remission with full performance status until 26 months after auto-PBSCT.

The combination of zidovudine and IFN-α has been evaluated in several small series of patients. It was suggested that a regimen of zidovudine and IFN-α could induce a rapid and durable response in patients with advanced ATL [8]. However, the same report showed that, in most patients who received this therapy, ATL eventually recurred even on therapy, suggesting that this treatment could not cure ATL. Unfortunately, our patient also relapsed on the administration of IFN-γ. However, we

conclude that IFN therapy following auto-PBSCT for ATL also may be associated with prolonged remission. Although IFN-γ was maintained for 17 months of therapy in our case, optimum doses and the duration of therapy remain unclear. Further investigation of IFN as a maintenance therapy following auto-PBSCT is needed to determine how best to achieve better outcomes.

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