平成24年度　第1回　鵜池班班会議

日時：平成24年7月20日（金曜日）10：00〜16：00

場所：JR博多シティ会議室（JR博多駅10F）

福岡市博多区博多駅中央街1番1号（Tel 092-292-9258）

プ　ロ　グ　ラ　ム

（10：00〜10：15）

・開会の挨拶（新班員紹介）　九州がんセンター 血液内科　鵜池直邦

1）ATL-NST-3, 4, 5の経過および進捗状況報告　九州がんセンター 血液内科　崔　日承

2）ATL-WG「CNS病変」について

今村病院分院　宇都宮與、九州がんセンター　崔　日承、鵜池直邦

3）Tax特異的樹状細胞療法（第I相試験）仮登録例の報告

九州がんセンター血液内科　末廣陽子

4）第3期および第4期の進捗報告　本臨床研究支援ユニット　佐藤　恒、田村正一郎

（10：15〜11：05）（座長：豊嶋崇徳先生）

5）NST-3およびNST-4登録者におけるHTLV-1プロウイルス欠損とtax遺伝子変異の解析

京都大学ウイルス研究所　安永純一朗

6）慢性型ATLLのゲノム異常　愛知県がんセンター研究所　瀬戸加大

7）primary ATL/NOGマウスにおけるautologous Tax-CTL adoptive transfertherapy

名古屋市立大学 大学院医学研究科 腫瘍・免疫内科学　正木彩子、石田高司

8）骨髄移植後のATL患者で認められたHTLV-1特異的$CD4^+$ヘルパーT細胞応答

東京医科歯科大学免疫治療学分野 長谷川温彦、玉井洋太郎、高森絢子、笹田亜麻子、神奈木真理

9）TSLC1/CD7を用いた同種造血幹細胞移植後のATL細胞のモニタリング

東京大学医科学研究所 血液腫瘍内科　石垣知寛、内丸　薫

（11：05〜11：55）（座長：田野崎隆二先生）

10）RIST施行後2年5ヵ月で消化管と胸膜に再発し、化学療法で再寛解が得られている症例（NST4-09R）　慈愛会 今村病院分院 血液内科　竹内昇吾、宇都宮與

11）北海道におけるATLに対する全同種移植症例の後方視的解析

北海道大学病院 検査輸血部　重松明男

12）ATLLに対する臍帯血移植

久留米大学医学部 内科学講座 血液・腫瘍内科部門　奥英二郎、長藤宏司

13）同種末梢血幹細胞移植後にドナー由来のHTLV-1感染細胞が増殖した成人T細胞白血病

長崎大学病院 血液内科　田口正剛

14）ATL患者の治療選択における同種造血幹細胞移植の意義の検討

大野伸広[1]、田野崎隆二[2]、小林誠一郎[1]、渡辺信和[1]、内丸　薫[1]

[1]東京大学医科学研究所、[2]国立がん研究センター中央病院

(11：55〜12：45)（座長：宇都宮與先生）

15）抗 CCR4 抗体を用いた急性型 ATL3 症例　大阪南医療センター 血液内科　前田裕弘

16）ポテリジオの治療後に合併した肺炎　九州大学病院 血液腫瘍内科　加藤光次、豊嶋崇徳

17）当院での CCR4 抗体使用経験　大分県立病院 血液内科　宮﨑泰彦

18）当科におけるポテリジオの使用経験　長崎大学病院 血液内科　田口　潤、宮﨑泰司

19）抗 CCR4 抗体投与前のポテリジオテストに関する注意喚起：非血縁同種移植後再発急性型 ATL の一例　東京大学医科学研究所 血液腫瘍内科　小林誠一郎、内丸　薫

昼食（12：45〜13：30）

(13：30〜16：00)（座長：石田高司先生）

〜ATL 移植症例に対する抗 CCR4 抗体投与についての臨床研究の提案と検討〜

20）ポテリジオ；同種造血細胞移植後免疫状態に及ぼす影響の考察

名古屋市立大学　大学院医学研究科　腫瘍・免疫内科学　石田高司

21）「CCR4 陽性成人 T 細胞性リンパ腫における 同種造血幹細胞移植後の早期再発・再燃に対する Mogamulizumab 投与の安全性を検討する多施設共同研究」の提案

国立がん研究センター中央病院 造血幹細胞移植科　井上明威、福田隆浩

22）抗 CCR4 抗体療法における ATL 細胞、Treg、および CCR4 陽性細胞のフローサイトメトリーによるモニタリング　東京大学医科学研究所 病態解析領域　渡辺信和

総合討論；「ATL の移植療法・免疫療法の成績向上のために」

新規抗 CCR4 抗体をどう組み合わせていくか

・閉会の挨拶

平成24年度　第２回　鵜池班班会議

日時：平成24年12月21日（金曜日）10：00～16：00

場所：福岡センタービル（10階会議室）

福岡市博多区博多駅前2-2-1　TEL 092-441-3769

プ　ロ　グ　ラ　ム

（10：00～10：15）

・開会の挨拶　九州がんセンター 血液内科　鵜池直邦

1）ATL-NST-5（臍帯血移植）の進捗状況と付随研究　九州がんセンター 血液内科　崔　日承

2）ATL-DC1（免疫療法）の進捗状況と付随研究　九州がんセンター血液内科　末廣陽子

3）データセンターからの進捗報告　日本臨床研究支援ユニット　田村正一郎

（10：15～11：15）（座長：石田高司先生）

4）ATL 細胞に対する自然免疫の効果

東京医科歯科大学免疫治療学分野 神奈木真理、金原秀一、高森絢子、笹田亜麻子、長谷川温彦

5）HBZ に対する免疫応答の解析

名古屋市立大学大学院医学研究科 腫瘍・免疫内科学　成田朋子、石田高司

6）移植後再発 ATLL の治療に関する前向きコホート研究

九州大学病院 血液腫瘍内科　加藤光次、赤司浩一

7）ATL における同種造血幹細胞移植後の微少残存病変および免疫機能モニタリング試験

国立がん研究センター中央病院 造血細胞移植科　井上明威、福田隆浩

（11：15～12：00）（座長：伊豆津宏二先生）

8）ATL の死亡率の推移　愛知県がんセンター 疫学予防部　千原　大

9）RIST 施行後11年目にドナー由来バーキットリンパ腫を発症した ATL 症例

慈愛会 今村病院分院 血液内科　竹内昇吾、宇都宮與

10）亜ヒ酸・インターフェロン、ジドブジンを用いた ATL への治療　吉満　誠、有馬直道

11）移植後早期にドナー由来の異型 T リンパ球の増加を認めた ATL の２症例

国立がん研究センター中央病院 造血細胞移植科 高野久仁子、福田隆浩

大分大学医学部総合内科学第２　緒方正男

12）佐世保市立総合病院における ATL に対する同種臍帯血移植の現況

佐世保市立総合病院 血液内科　森内幸美

昼食（12：00～13：00）

（13：00～14：00）

特別講演（座長：赤司浩一先生）

「モガムリズマブによる ATL 治療と制御性 T 細胞」

岡山大学医学部 血液・腫瘍内科　松岡賢市先生

（14：00～14：40）（座長：松岡雅雄先生）

13）既治療 ATL に対する Tax を標的とした樹状細胞ワクチン治療例（ATL-DC1）

九州大学病院 遺伝子細胞療法部　飯野忠史 、九州がんセンター血液内科 末廣陽子

東京医科歯科大学免疫治療学分野　神奈木真理

14)「ATL の同種造血細胞移植後の再発・再燃に対する治療における免疫モニタリング」について
九州がんセンター 血液内科　崔　日承

(14：40〜15：40)(座長：菱澤方勝先生)

15) フローサイトメトリーによる ATL 細胞の解析　－がん医療水準均てん化事業・講習会の報告－
東京大学医科学研究所　病態解析領域　渡辺信和

16) 抗 CCR4抗体を投与した ATL4 症例の経過　阪南医療センター 血液内科　前田裕弘

17) モガムリズマブ使用後に同種骨髄移植をおこなった ATL の一例
京都大学医学部附属病院 血液・腫瘍内科　菱澤方勝

18) ポテリジオ投与時に NK 細胞活性をモニタリングした ATLL の 2 症例
大阪鉄道病院　高　起良

(15：40〜16：00)

総合討論

・閉会の挨拶

Ⅲ　研究成果の刊行物・別冊

Bone Marrow Transplantation (2011) 46, 116–118

www.nature.com/bmt

ORIGINAL ARTICLE

Long-term outcomes after hematopoietic SCT for adult T-cell leukemia/lymphoma: results of prospective trials

I Choi[1], R Tanosaki[2], N Uike[1], A Utsunomiya[3], M Tomonaga[4], M Harada[5], T Yamanaka[6], M Kannagi[7] and J Okamura[6], on behalf of the ATLL allo-HSCT Study Group

[1]*Department of Hematology, National Kyushu Cancer Center, Fukuoka, Japan;* [2]*Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo, Japan;* [3]*Department of Hematology, Imamura Bun-in Hospital, Kagoshima, Japan;* [4]*Molecular Medicine Unit, Department of Hematology, Atomic Bomb Disease Institute, School of Medicine, Nagasaki University, Nagasaki, Japan;* [5]*Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan;* [6]*National Kyushu Cancer Center, Institute for Clinical Research, Fukuoka, Japan and* [7]*Medical Research Division, Department of Immunotherapeutics, Tokyo Medical and Dental University, Tokyo, Japan*

We have previously conducted clinical trials of allogeneic hematopoietic SCT with reduced-intensity conditioning regimen (RIC) for adult T-cell leukemia/lymphoma (ATLL)—a disease caused by human T-lymphotropic virus type 1 (HTLV-1) infection and having a dismal prognosis. Long-term follow-up studies of these trials revealed that 10 of the 29 patients have survived for a median of 82 months (range, 54–100 months) after RIC, indicating a possible curability of the disease by RIC. However, we have also observed that the patterns of post-RIC changes in HTLV-1 proviral load over time among the 10 survivors were classified into three patterns. This is the first report to clarify the long-term outcomes after RIC for ATLL patients.
Bone Marrow Transplantation (2011) **46,** 116–118; doi:10.1038/bmt.2010.92; published online 19 April 2010
Keywords: adult T-cell leukemia/lymphoma; allogeneic hematopoietic SCT; reduced-intensity conditioning regimen; HTLV-1 proviral load

Introduction

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell malignancy that is caused by human T-lymphotropic virus type 1 (HTLV-1) infection and commonly affects individuals at an average age of 60 years. It has been reported that the 4-year survival rate was only 10.3%; in particular, patients with an acute or lymphoma subtype showed a dismal prognosis with a 4-year survival rate of approximately 5.0%.[1] Several retrospective studies for ATLL patients younger than 50 years have suggested the possible usefulness of allogeneic hematopoietic SCT (allo-HSCT) with a conventional conditioning chemotherapy regimen. However, the treatment-related mortality by conventional allo-HSCT was high (40–60%), probably due to the disease-specific immune deficiency at diagnosis.[2–4] This unacceptable level of mortality, even in the case of young patients, critically deters the applicability of conventional allo-HSCT for the general population of ATLL.

To permit the application of allo-HSCT for ATLL in patients aged more than 50 years, we can consider allo-HSCT for ATLL conditioned with reduced-intensity regimen (hereafter, allo-HSCT conditioned with reduced-intensity regimen is referred to as 'RIC'). Few retrospective studies have reported the results of RIC for ATLL so far; Shiratori *et al.*[5] followed up 15 patients after allo-HSCT (including 10 who received RIC) whose median age was 57 years and reported that the OS rate at 3 years reached 73%. Kato *et al.*[6] investigated the results of 33 patients with allo-HSCT from unrelated donors but this study included only 6 patients receiving RIC. However, our study group had previously activated the first clinical trials of RIC in 2001. These were two trials to clarify the feasibility of RIC: one studied RIC administered with immunosuppressant antithymocyte globulin (ATG) and the other studied RIC without ATG. The results have been already published elsewhere[7,8] and the treatment-related mortality in both trials collectively decreased to the 20% level, showing that RIC is a promising procedure for ATLL patients more than 50 years of age. In this report, we present the results of long-term follow-up of the two trials and discuss the longitudinal patterns of changes in HTLV-1 proviral load in survivors.

Correspondence: Dr J Okamura, National Kyushu Cancer Center, Institute for Clinical Research, 3-1-1 Notame, Minami-ku, Fukuoka 811-1395, Japan.
E-mail: jyokamur@nk-cc.go.jp
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Patients and methods

The patient characteristics have been described in the previous reports.[7,8] Briefly, patients were eligible if they had ATLL of acute or lymphoma type and were aged between

50 and 70 years. The patients were required to be in either CR or PR at the time of trial registration, and to have a HLA-identical sibling donor. The conditioning regimen consisted of fludarabine (30 mg/m^2 per day) for 5 days and BU (1 mg/kg orally per day) for 2 days. The patients in the first study also received low-dose ATG (2.5 mg/kg per day) for 2 days, whereas those in the second study did not. On day 0, G-CSF-mobilized peripheral blood grafts from their HLA-identical sibling donors were transplanted. To prevent GVHD, we continuously infused CYA (3 mg/kg per day) starting on day −1. The degree of donor–recipient chimerism in peripheral blood mononuclear cells was examined according to the previously reported method.[9] The HTLV-1 proviral load was estimated using blood samples obtained before and at 1, 2, 3, 6, 12 months and every year after transplantation. HTLV-1 proviral DNA was measured by the quantitative PCR amplification of HTLV-1 pX DNA.[10] The detection limit of the HTLV-1 proviral load was 0.5 copies per 1000 cells. The OS curve was estimated by the Kaplan–Meier method.

Results and discussion

Long-term survivors after RIC

In all, 15 and 14 patients were registered in the first and second studies, respectively. Eleven (six and five in the first and second studies, respectively) and eight (four in each study) patients died because of ATLL and the treatment, respectively. The last treatment-related death occurred 26 months after RIC. Characteristics of the remaining 10 patients (5 in each study) are summarized in Table 1. They are currently alive with a median follow-up period of 82 months after RIC (range, 54–100 months). Of the surviving patients, six and four patients had the acute and lymphoma types of ATLL. Of 10 patients, 5 received the grafts from HTLV-1-positive sibling donors. The OS rate at 60 months (5 years) was 34% (95% confidence interval, 18–51). No death was reported beyond 36 months after RIC (Figure 1).

Of the 10 survivors, 3 developed nonhematological relapse in the skin and/or lymph nodes within a half year after RIC (Table 1). However, remission was achieved again in these patients after the discontinuation of CYA, immunosuppressive agent, and the administration of additional treatments. In one of these patients, remission was achieved with the cessation of CYA alone. Two other patients were treated with systemic chemotherapy as well as local irradiation or donor lymphocyte infusion after the discontinuation of CYA, and thereafter obtained remission. These three patients survived for 100, 88 and 54 months after RIC, respectively. Because disease recurrence is usually fatal, the clinical course for the three patients was unique. It is suggested that the newly established immunological environment after RIC might have contributed to the eradication of ATLL lesions after early relapse.

All the 10 survivors developed acute GVHD (9 grades I–II and 1 grade III). Chronic GVHD was observed in all but one patient. Although immunosuppressive treatment was discontinued in 9 of the 10 patients, 1 patient is still receiving treatment due to active chronic GVHD. The development of chronic GVHD may suggest the presence of the graft-vs-ATLL effect. Of note is that 8 of 10 survivors received RIC when they were in PR after induction chemotherapy.

Kinetic patterns of HTLV-1 proviral load in long-term survivors

Serial changes in the HTLV-1 proviral load after RIC in the 10 long-term survivors are shown in Figure 2. The changes in the proviral load are heterogeneous but can be roughly classified into three patterns. In the first pattern, the proviral load became undetectable after RIC and continued to remain so; this pattern was seen in three patients. In the second pattern, the proviral load had become undetectable but returned to detectable levels thereafter; this pattern was also seen in three patients, all of whom had received RIC from HTLV-1-negative donors. Finally, in the third pattern, the proviral load had remained at the carrier level in four patients; these patients received the grafts from donors who were HTLV-1 carriers. All the 10 survivors continue to show complete donor chimera during the observation period regardless of the HTLV-1 proviral load level.

We noted that one survivor who was donated graft from an HTLV-1 carrier showed a strikingly high proviral load (nearly 1000 copies) during the first year after RIC; this

Table 1 Characteristics of long-term survivors

Age (years)	*Gender*	*ATL subtype*	*Donor status of HTLV-1*	*Status at RIC*	*Acute GVHD*	*Chronic GVHD*	*Relapse*	*Treatment after relapse*	*Current Karnofsky PS score (%)*	*Survival after RIC (months)*
62	Male	Acute	(+)	PR	I	Yes	Lynd, skin (day 28)	d/c CsA	>90	100
66	Female	Acute	(+)	PR	II	Yes	No		>90	98
51	Male	Acute	(−)	PR	II	Yes	No		>90	98
53	Male	Lymph	(−)	PR	II	Yes	No		>90	91
54	Male	Lymph	(−)	CR	II	Yes	Lynd (day 171)	d/c CsA, Rx, Cx	>90	88
55	Male	Lymph	(+)	PR	II	Yes	No		>90	75
62	Male	Acute	(+)	CR	II	Yes	No		>90	74
50	Female	Lymph	(−)	PR	I	Yes	No		>90	62
56	Male	Acute	(−)	PR	II	Yes	Skin (day 29)	d/c CsA, DLI, steroid	>90	54
53	Female	Acute	(+)	PR	III	No	No		>90	54

Abbreviations: Cx = chemotherapy; d/c = discontinued; DLI = donor lymphocyte infusion; lynd = lymph node; PS = performance status; RIC = hematopoietic stem cell transplantation conditioned with reduced-intensity regimen; Rx = radiation therapy.

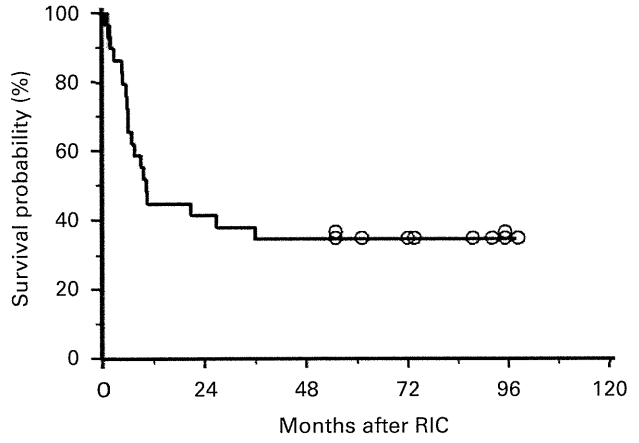

Figure 1 Kaplan–Meier curves for OS following RIC for ATLL. Circles show survivors (censored cases).

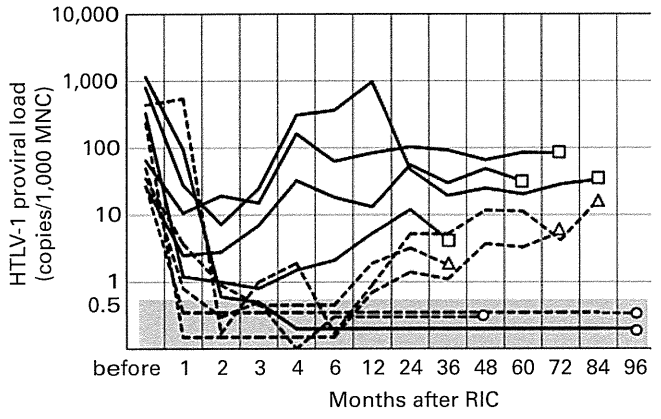

Figure 2 The longitudinal patterns of HTLV-1 proviral load after RIC in 10 long-term survivors. The HTLV-1 proviral load was measured by assaying serial blood samples after RIC by real-time PCR amplification of pX DNA and is expressed as copies per 1000 mononuclear cells (MNC). A load of less than 0.5 copies per 1000 MNC was considered undetectable, which is shown by the shaded area. A solid line indicates a patient who received a transplant from an HTLV-1 carrier donor whereas a dotted line indicates a patient from an HTLV-1-negative donor. Each circle, triangle or square indicates the latest measurement for the patient. Circle shows a pattern that the proviral load became undetectable after RIC and continued to remain so. Triangle shows a pattern that the proviral load had become undetectable but returned to detectable levels thereafter. Square shows a pattern that the proviral load had remained at the carrier level.

load then gradually decreased to the carrier level in the second year and the patient is currently surviving without any relapse. A temporary proliferation of HTLV-1-infected (nonleukemic) donor cells, as confirmed by a chimerism analysis, might have occurred due to some unknown etiology.

Conclusion

The long-term follow-up in our prospective studies has shown that one-third of the patients have survived and remain free of ATLL. We have also observed the different patterns of changes in proviral load; the pattern of changes in patients who received the grafts from HTLV-1-positive donors was different from that in patients who received the grafts from HTLV-1-negative donors. In conclusion, this is the first report on the long-term outcomes of ATLL patients who received allo-HSCT, and we have confirmed that RIC from matched sibling donors is a feasible treatment modality for ATLL, and that this treatment has a possible curative effect in patients with ATLL.

Conflict of interest

The authors declare no conflict of interest

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Review Article

Double control systems for human T-cell leukemia virus type 1 by innate and acquired immunity

Mari Kannagi,[1,3] Atsuhiko Hasegawa,[1] Shuichi Kinpara,[1] Yukiko Shimizu,[1] Ayako Takamori[1] and Atae Utsunomiya[2]

[1]Department of Immunotherapeutics, Tokyo Medical and Dental University, Graduate School, Tokyo; [2]Department of Hematology, Imamura Bun-in Hospital, Kagoshima, Japan

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Human T-cell leukemia virus type 1 (HTLV-1) is the causative retrovirus of adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-1-specific T-cell responses elicit antitumor and antiviral effects in experimental models, and are considered to be one of the most important determinants of the disease manifestation, since they are activated in HAM/TSP but not in ATL patients. The combination of low T-cell responses and elevated HTLV-1 proviral loads are features of ATL, and are also observed in a subpopulation of HTLV-1 carriers at the asymptomatic stage, suggesting that these features may be underlying risk factors. These risks may potentially be reduced by vaccination to activate HTLV-1-specific T-cell responses. HAM/TSP and ATL patients also differ in their levels of HTLV-1 mRNA expression, which are generally low *in vivo* but slightly higher in HAM/TSP patients. Our recent study indicated that viral expression in HTLV-1-infected T-cells is suppressed by stromal cells in culture through type-I IFNs. The suppression was reversible after isolation from the stromal cells, mimicking a long-standing puzzling phenomenon in HTLV-1 infection where the viral expression is very low *in vivo* and rapidly induced *in vitro*. Collectively, HTLV-1 is controlled by both acquired and innate immunity *in vivo*: HTLV-1-specific T-cells survey infected cells, and IFNs suppress viral expression. Both effects would contribute to a reduction in viral pathogenesis, although they may potentially influence or conflict with one another. The presence of double control systems for HTLV-1 infection provides a new concept for understanding the pathogenesis of HTLV-1-mediated malignant and inflammatory diseases. (*Cancer Sci* 2011; 102: 670–676)

It has been three decades since the discovery of human T-cell leukemia virus type 1 (HTLV-1) as the causative retrovirus of adult T-cell leukemia (ATL).[1,2] ATL develops during middle age or later mainly in a small portion of vertically HTLV-1-infected populations.[3,4] HTLV-1 also causes HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in another small population of infected individuals.[5,6] Some other inflammatory diseases such as uveitis and arthritis are also associated with HTLV-1 infection.[7,8] New therapeutic approaches such as hematopoietic stem cell transplantation (HSCT),[9,10] an antibody therapy targeting CCR4,[11] and antiviral therapy with interferon-alpha and zidovudin[12] partly improved the prognosis of ATL. However, ATL still shows high mortality, and HAM/TSP remains to be an intractable disease.

Enormous amounts of research findings have been accumulated regarding the virus-mediated pathogenesis. HTLV-1 Tax, a virus-encoded regulatory gene product, mediates cell activation, proliferation and resistance to apoptosis by transactivation through NF-κB, cAMP response element binding protein (CREB) and serum response factor (SRF), and by inactivation of tumor suppressors,[13–15] which would be involved in leukemogenesis and inflammation in HTLV-1 infection. Another minus-strand HTLV-1-encoded gene product, HTLV-1 basic leucine zipper factor (HBZ), is continuously expressed in infected cells *in vivo* regardless of the disease and may also be involved in the growth ability of infected cells.[16]

However, many unsolved questions still remain regarding the pathogenesis of HTLV-1 infection, for example, how the same virus causes totally different diseases such as ATL and HAM/TSP, why only small portions of HTLV-1-infected populations develop diseases, and why it takes more than 40 years to develop ATL. The answers to these questions would provide hints for predicting disease risks as well as aiding the development of prophylactic and therapeutic strategies.

HTLV-1-specific T-cell responses that contribute to antiviral and antitumor surveillance could be one of the most important determinants of the diseases. In fact, HTLV-1-specific T-cells are activated in HAM/TSP but not in ATL.[17–19] Oral HTLV-1 infection induces T-cell tolerance to HTLV-1 and increased proviral loads,[20,21] consistent with the epidemiological finding that vertical HTLV-1 infection is one of the risk factors for ATL.[3] Therefore, the individual status of HTLV-1-specific T-cell responses is expected to be an indicator of risk for ATL.[22] Although the pathological significance of HTLV-1-specific T-cells in HAM/TSP remains controversial,[23,24] advantages for HLA-A02-positive individuals in protection against HAM/TSP have been reported, and interpreted through the association of this HLA with strong CTL responses to a major epitope of HTLV-1 Tax.[25]

Elevation of proviral loads is also a risk factor for ATL. Given the fact that HTLV-1-specific CTLs have antiviral effects, these CTLs are likely to be one of the determinants of proviral loads.[26] However, proviral loads are also increased in HAM/TSP patients, and the correlations between proviral loads and HTLV-1-specific T-cell responses vary among studies,[27,28] suggesting the presence of additional factors for determining individual proviral loads.

Another curious finding in HTLV-1 infection is the scarcity of viral antigen expression in the peripheral blood, although the viral mRNA is barely expressed.[29] The transcription of HTLV-1 is mainly regulated by CRE-like repeats in the HTLV-1 LTR.[30] Involvement of inducible cAMP early repressor (ICER) and transducers of regulated CREB 2 (TORC2) in the inhibition of HTLV-1 transactivation has been suggested.[31,32] However, the mechanism involved in suppressing viral expression only *in vivo* has remained obscure. It is a paradox that HTLV-1 Tax contributes to the pathogenesis while Tax protein is undetectable *in vivo*. Expression of HBZ in the absence of Tax may partly

[3]To whom correspondence should be addressed. E-mail: kann.impt@tmd.ac.jp

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explain the growth advantage of infected cells,[33] but not all of HTLV-1-mediated leukemogenesis. In addition, it does not make sense that Tax-specific T-cell responses are maintained if Tax is not expressed *in vivo*. The paradox will remain until the state of viral expression and the mechanisms for suppressing HTLV-1 expression *in vivo* are clarified.

We recently found that innate immune responses, especially type-I interferons (IFNs), suppress HTLV-1 expression.[34] This integrates the issue of viral expression and the host defense system against HTLV-1, which includes innate immunity as well as acquired immunity. The presence of double control systems explains some of the paradox in persistent HTLV-1 infection, and adds new aspects to the pathogenesis of HTLV-1-mediated diseases.

Control of HTLV-1 by HTLV-1-specific T-cell responses

Antitumor surveillance by HTLV-1-specific T-cells. $CD8^+$ HTLV-1-specific CTL responses are found in many HAM/TSP patients and asymptomatic carriers (AC), but rarely in ATL patients.[17–19,35,36] These CTLs kill HTLV-1-infected cells *in vitro*, and mainly recognize HTLV-1 Tax.[18,37] The HTLV-1 envelope is also a popular target, especially for $CD4^+$ CTLs.[38] Other viral antigens, including polymerase,[39] ROF (p12) and TOF (p30/p13),[40] and HBZ,[41] have also been shown to be targets of CTLs. Elimination of $CD8^+$ cells among PBMCs from HAM/TSP patients induces HTLV-1 expression during subsequent cell culture,[42] clearly indicating that $CD8^+$ HTLV-1-specific CTLs contribute to the control of HTLV-1-infected cells.

A series of animal model experiments indicated that HTLV-1-specific T-cell responses limit the expansion of HTLV-1-infected cells *in vivo*. Oral HTLV-1 infection induced insufficiency of HTLV-1-specific T-cell responses in rats, and the HTLV-1 proviral loads were inversely correlated with HTLV-1-specific T-cell responses.[21] Re-immunization of these rats with mitomycin C-treated HTLV-1-infected cells restored HTLV-1-specific T-cell responses and reduced the proviral loads[43] (Fig. 1). In another rat model of HTLV-1-induced tumors, the otherwise fatal HTLV-1-infected lymphomas in T-cell-deficient rats were eradicated by transfer of T-cells from syngeneic rats that had been vaccinated with a Tax-encoding DNA or peptides corresponding to a major epitope for Tax-specific CTLs.[44,45]

Recent clinical reports have indicated that HTLV-1-carrying recipients after liver transplantation developed ATL under the administration of immunosuppressants.[46,47] In contrast, Tax-specific CTL responses were strongly activated in some ATL patients who obtained complete remission after HSCT, but were not observed in the same patients before transplantation.[48] These findings suggest that HTLV-1-specific T-cells, including Tax-specific CTLs, play important roles in antitumor surveillance against HTLV-1 leukemogenesis.

Insufficient HTLV-1-specific T-cell responses as a potential risk for ATL. Most HTLV-1-infected individuals are asymptomatic, and only about 5% develop ATL and <1% develop HAM/TSP.[3,49] The epidemiological risk factors for ATL include vertical transmission and increases in the number of abnormal lymphocytes or HTLV-1 proviral loads.[3,50,51] HTLV-1 proviral loads are also elevated in HAM/TSP patients.[52]

Immunological studies have suggested that insufficiency in host T-cell responses against HTLV-1 might be another risk factor for ATL.[22] A small-scale survey measuring Tax protein-specific IFN-γ production revealed a wide variety in the strengths of HTLV-1-specific T-cell responses among HTLV-1 carriers.[53] The combinations of HTLV-1-specific T-cell responses and proviral loads categorize HTLV-1 carriers into the following four groups: (i) low proviral loads with HTLV-1-specific T-cell responses; (ii) elevated proviral loads with HTLV-1-specific T-cell responses; (iii) low proviral loads with low T-cell responses; and (iv) elevated proviral loads with low T-cell responses (Fig. 2).

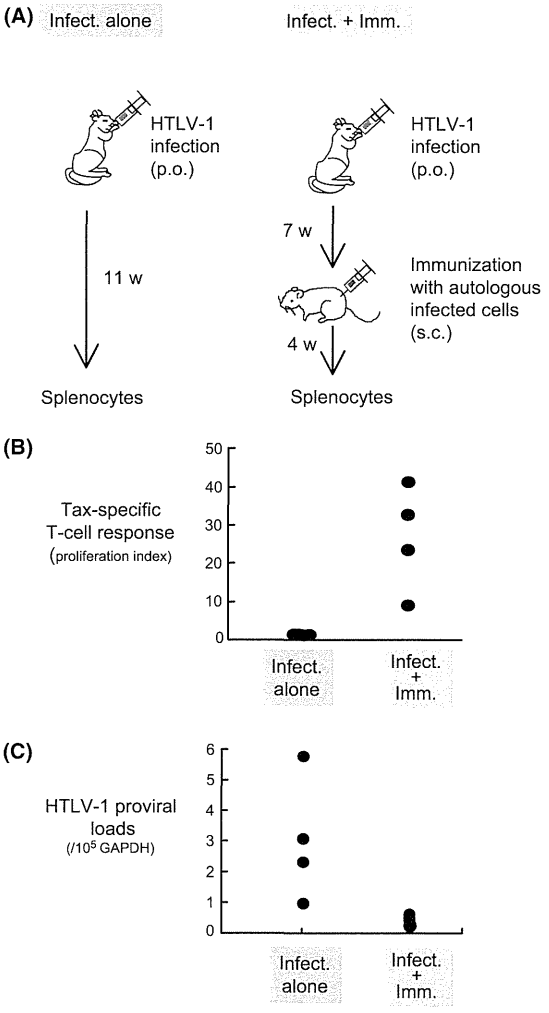

Fig. 1. Recovery of human T-cell leukemia virus type 1 (HTLV-1)-specific T-cell responses and reduction of proviral loads by re-immunization. Eight rats orally infected with HTLV-1 were divided into two groups. (A) One group was left untreated (Infect. alone) and the other was subcutaneously immunized with mitomycin C-treated HTLV-1-infected syngeneic rat T-cells (Infect. + Imm.) at 4 weeks. Spleen T-cells were harvested at 7 weeks after infection. (B,C) T-cells from the re-immunized rats (Infect. + Imm.) show elevated levels of Tax-specific T-cell proliferative responses (B) and lower proviral loads (C), compared with untreated rats (Infect. alone).[43]

Regarding these groups, ATL patients exhibit elevated proviral loads with low T-cell responses, while many, but not all, HAM/TSP patients show elevated proviral loads with high HTLV-1-specific T-cell responses. ACs are found in all four categories. It is noteworthy that small subgroups of ACs and smoldering ATL patients share a common feature with ATL patients. This indicates that the insufficiency of HTLV-1-specific T-cell responses is not merely the result of malignancy but is an underlying problem before the stage without apparent lymphoproliferation. Further follow-up studies are required to clarify whether the extent of the combination of elevated proviral loads with low T-cell responses could be a diagnostic indicator for risk of ATL.

Dissociation between proviral loads and T-cell responses. Although HTLV-1-specific T-cells have the potential to control infected cells, there are no clear correlations between

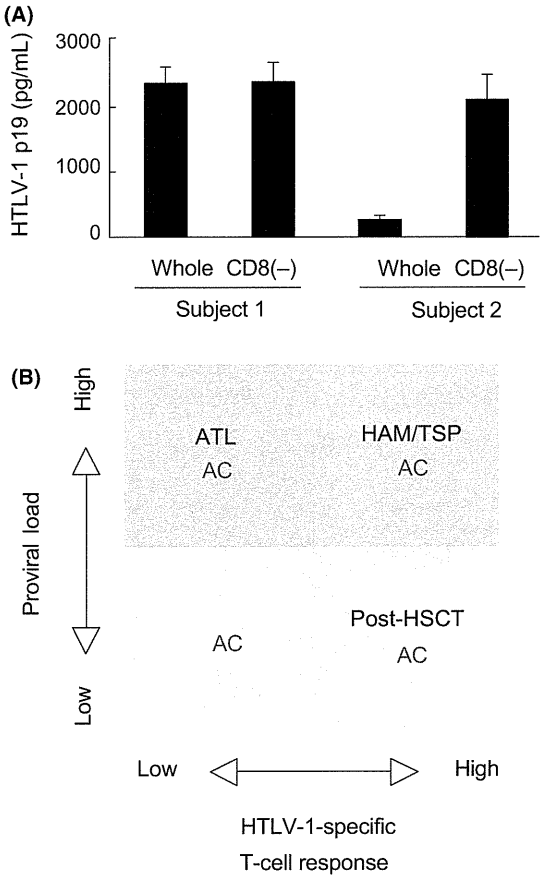

Fig. 2. Diversities in Tax-specific T-cell responses and dissociation with proviral loads in human T-cell leukemia virus type 1 (HTLV-1)-infected individuals. (A) Diversity in $CD8^+$ T-cell functions in two representative HTLV-1-infected individuals at the asymptomatic stage. Abundant amounts of HTLV-1 p19 were produced in PBMC cultures with or without $CD8^+$ T-cells in subject 1, but only after $CD8^+$ T-cell depletion in subject 2.[53] (B) A general image for the categories of HTLV-1-infected individuals at various stages according to the combinations of HTLV-1-specific T-cell responses (*x*-axis) and proviral loads (*y*-axis) is shown schematically. AC, asymptomatic carriers; ATL, adult T-cell leukemia; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; HSCT, hematopoietic stem cell transplantation.

proviral loads and HTLV-1-specific T-cell responses among HTLV-1-infected individuals. This is not surprising because both the proviral loads and T-cell responses are high in HAM/TSP patients. The proviral loads may be negatively correlated with T-cell responses only within an individual but not among individuals. Several other reports have indicated various findings concerning this issue. For example, a study measuring IFN-γ-producing $CD8^+$ HTLV-1-specific CTLs indicated a positive correlation with proviral loads in HAM/TSP patients but not in ACs,[28] while a study evaluating $CD8^+$ CTL function by *ex vivo* clearance of infected cells showed negative correlations with low proviral loads within an AC or a HAM/TSP group,[42] and another study indicated an association of higher frequency of tetramer-binding Tax-specific CTLs with low proviral loads in ACs.[27] Such inconsistent results suggest the presence of certain other determinants of proviral loads in addition to HTLV-1-specific CTLs.

The HTLV-1 proviral loads reflect the number of infected cells in the peripheral blood. Expansion of HTLV-1-infected cells *in vivo* occurs through both *de novo* infection and proliferation of infected cells.[54] The number of $CD4^+$ $FoxP3^+$ cells,[55] the frequency of iNKT cells,[56] or MHC-I favorable for HBZ-specific T-cell responses[41] have been suggested to influence HTLV-1 proviral loads.

In HTLV-1-infected rats, however, the proviral loads are inversely correlated with HTLV-1-specific T-cell responses.[21] One reason for the discrepancy between humans and rats may be the genetic heterogeneity in humans. It appears that, under the homogeneous genetic background in the experimental rat system, the influence of insufficient HTLV-1-specific T-cell responses may appear more clearly than in humans, allowing *de novo* infection and proliferation of HTLV-1-infected cells *in vivo*. The dissociation of proviral loads and HTLV-1-specific T-cell responses in humans suggests that additional determinants of proviral loads may vary genetically among individuals. As described in the next section, we suppose that innate immunity could be a candidate for this effect.

Control of HTLV-1 by innate immunity

Status of HTLV-1 expression *in vivo*. Since HTLV-1-specific antibodies and T-cells are maintained in HTLV-1-infected individuals, viral expression must occur somewhere *in vivo*. This notion is further supported by the emergence of Tax-specific CTL responses in HTLV-1-uninfected donor-derived hematopoietic systems reconstituted in recipient ATL patients after HSCT.[48,57] However, HTLV-1 mRNA but not viral proteins are detectable in PBMCs freshly isolated from HTLV-1-infected individuals. The levels of HTLV-1 mRNA are higher in HAM/TSP patients than in ACs,[58] but viral proteins are still undetectable. Only a few reports have indicated HTLV-1 protein expression *in situ*.[59]

HTLV-1 expression in ATL cells immediately after isolation from the peripheral blood is very low, and becomes significantly induced after culture for some hours *in vitro*.[60,61] This phenomenon is observed in about one half of ATL patients regardless of the disease severity.[62] Viral induction after *in vitro* culture does not occur in the other one half of ATL patients, probably because of genetic and epigenetic changes in the viral genome.[63–65] Rapid induction of viral expression after *in vitro* culture has also been observed in PBMCs from HAM/TSP patients and ACs,[66] indicating that there must be a common mechanism for transiently suppressing HTLV-1 expression *in vivo* regardless of the diseases.

Suppression of HTLV-1 expression by type-I IFN responses. Recently, we found that type-I IFN responses are involved in the suppression of HTLV-1 expression.[34] When HTLV-1-infected T-cell line cells were co-cultured with stromal cells such as epithelial cells and fibroblasts, HTLV-1 mRNA and proteins were markedly decreased in HTLV-1-infected cells. Similarly, induction of HTLV-1 expression in cultures of primary ATL cells was also suppressed by co-culture with stromal cells. Type-I IFNs were involved in the stromal cell-mediated suppression of HTLV-1 expression, because it was partly neutralized by anti-IFN-α/β receptor antibodies. Since efficient HTLV-1 expression is dependent on transactivation of its own LTR by Tax protein,[30,67] limitation of this protein below a certain level will lead to the maintenance of HTLV-1 expression at low levels. Stromal cells reduced viral expression via type-I IFNs, but did not reduce cell growth and even supported it by unknown mechanisms.[34,68]

It has been reported that plasmacytoid dendritic cells (pDCs), a major producer of type-I IFNs, are susceptible to HTLV-1 infection.[69,70] In ATL patients, pDCs are decreased in number and also lack the ability to produce IFN-α.[69] A recent report indicated that pDCs generate type-I IFNs mainly through TLR7 recognition of HTLV-1 RNA.[71] The precise mechanisms of the HTLV-1-mediated IFN responses remain to be clarified.

In addition to recombinant IFN-α and IFN-β, recombinant IFN-γ was also capable of reducing HTLV-1 expression to

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lesser extents in HTLV-1-infected cell lines.[34,72] Participation of type-II IFN-producing cells other than stromal cells in HTLV-1 suppression *in vivo* is also conceivable.

Potential involvement of type-I IFNs in HTLV-1 suppression *in vivo*. In *in vitro* experiments, co-cultured stromal cells suppressed viral expression in HTLV-1-infected cells. Interestingly, when infected cells were re-isolated from the co-cultures, viral expression was restored to the original level over the following 48 h (Fig. 3).[34] This observation shows a striking similarity to the rapid induction of HTLV-1 expression in freshly isolated ATL cells after culture *in vitro*.

Involvement of type-I IFN responses in the suppression of HTLV-1 expression *in vivo* was confirmed using interferon regulatory factor-7-KO mice, which are deficient in most type-I IFN responses. Viral expression in HTLV-1-infected cells was significantly suppressed when the infected cells were intraperitoneally injected into WT mice but not into interferon regulatory factor-7-KO mice.[34]

It is speculated that the levels of viral expression in HTLV-1-infected lymphocytes may differ among various tissues depending upon the strength of IFN responses. Thus far, there is little information regarding HTLV-1 expression in various tissues. In transgenic mice with an HTLV-1 LTR-driven construct of the pX gene, expression of the transgene was only observed in limited organs including the central nervous system, eyes, salivary glands and joints.[73] It is intriguing that all of these tissues are involved in human inflammatory diseases related to HTLV-1 infection. Such coincidences suggest the involvement of HTLV-1 gene expression in the pathogenesis of these inflammatory diseases.

Double control of HTLV-1 by innate and acquire immunity

Relationship between acquired and innate immune control in HTLV-1 infection. At the primary infection, type-I IFNs generally play a critical role in limiting viral replication, and have positive effects on antigen presentation by activating DCs, inducing type-II IFN, and upregulating MHC-I, which subsequently augments T-cell responses.[74] However, the role of type-I IFNs in the chronic phase of viral infection may not always be positive. In HIV-1 infection, type-I IFNs may be a progressive factor for the disease by accelerating T-cell exhaustion.[75]

Suppression of HTLV-1 expression by type-I IFNs may reduce the efficacy of T-cell-mediated surveillance against HTLV-1-infected cells, because T-cells require viral proteins for recognition. On the contrary, if the IFN-mediated suppressive system is insufficient, HTLV-1-specific T-cell responses will be activated in response to viral antigens.

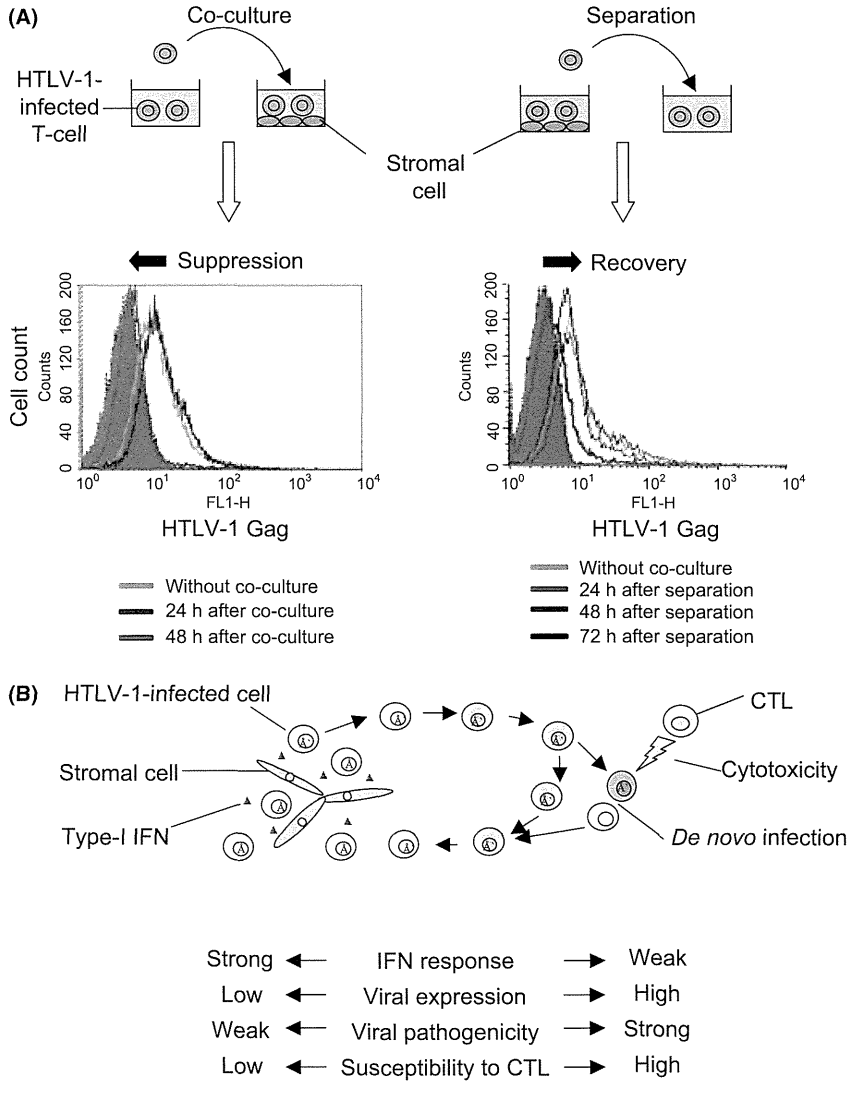

Fig. 3. Reversible suppression of human T-cell leukemia virus type 1 (HTLV-1) expression by innate immunity. (A) When IL-2-dependent HTLV-1-infected cells are co-cultured with 293T cells, intracellular HTLV-1 Gag proteins in the infected cells are decreased within 48 h (left panel). When the infected cells are re-isolated and further cultured on their own, Gag expression is recovered within 48 h (right panel).[34] (B) Scheme of the presumed status of HTLV-1-infected cells *in vivo*. Viral expression (indicated as pink) would be suppressed in tissues with strong IFN responses (left) and increased in tissues with weak IFN responses (right). CTL function, if any, is only effective upon viral expression, resulting in an infected cell reservoir without viral expression (left) and a T-cell surveillance system with low efficiency (right).

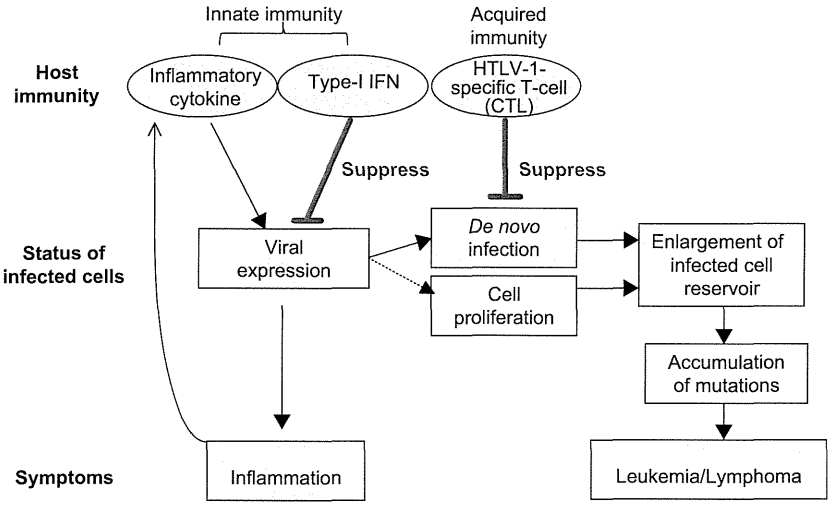

Fig. 4. Hypothetical relationships among the host immunity, status of human T-cell leukemia virus type 1 (HTLV-1)-infected cells and symptoms. HTLV-1-infected cells are controlled by at least two systems: type-I IFNs (innate immunity) and HTLV-1-specific T-cells (acquired immunity). The former suppress viral expression and the latter kill infected cells. An increase in viral expression would accelerate inflammation, increase the number of infected cells through *de novo* infection and activate HTLV-1-specific T-cells that determine an equilibrium level of proviral load within an individual. Viral expression may be a positive, but not absolute, factor for cell proliferation. When the viral expression is well controlled, the viral pathogenesis will proceed slowly, and may not be apparent until infected cell clones with a malignant phenotype finally emerge from the enlarged infected cell reservoir. Without proper T-cell responses, the emergence of such clones may occur earlier, because they would have more chance to survive.

The relationship between innate and acquired immunity may also differ among tissues. In tissues with strong IFN responses, viral expression in the infected cells would be suppressed and CTLs would ignore these cells. However, in tissues with weak IFN responses, infected cells would express viral antigens to be recognized by CTLs (Fig. 3). These presumptions can explain the status of HTLV-1-infected cells *in vivo*, which comprises a large reservoir of infected cells without viral expression and a low-efficiency surveillance system by CTLs that can only work on limited occasions.

Potential relationship between disease manifestation and innate and acquired host immunity in HTLV-1 infection. Although suppression of HTLV-1 expression may partly interfere with the efficacy of T-cell immunity, it may contribute to a slowing down of the Tax-mediated pathogenesis, tumorigenesis and inflammation (Fig. 4). In a rat model, shRNA-mediated suppression of Tax in HTLV-1-transformed cells rendered these cells resistant to Tax-specific CTLs but also reduced their ability for tumorigenesis *in vivo*.[76] Continuous suppression of HTLV-1 expression in humans may have a similar decelerating effect against Tax-mediated tumorigenesis. This might be a reason why it takes so long for ATL to develop. So long as the viral expression is well controlled, the viral pathogenesis may not be apparent until malignant cell clones finally come through the process of clonal evolution in the infected cell reservoir. Without proper T-cell responses, the emergence of such clones may occur earlier, because they would have more chance to survive.

HAM/TSP patients show elevated levels of viral expression for an unknown reason. Increased levels of inflammatory cytokines could be either a cause or a result of this phenomenon. The involvement of HTLV-1 proviral integration sites in transcription units in elevated viral expression has also been suggested.[77] An experimental rat model of HAM/TSP using a certain WKAH strain exhibits increased Tax mRNA expression in the spinal cord without T-cell infiltration,[78] suggesting that viral expression is a primary event while T-cell responses are not. Further studies revealed that this particular rat strain contains mutations in the promoter region of the IL-12 receptor, which potentially lead to reduced IFN-γ production in the spinal cord.[72] The associations of genetic factors related to the IFN system with HAM/TSP patients have remained obscure. Very recently, a gene expression profiling study indicated that expression of suppressor of cytokine signaling 1 (SOCS1) is upregulated in HAM/TSP patients and ACs, and is positively correlated with high HTLV-1 mRNA loads.[79]

Conclusions

HTLV-1 is controlled by both acquired and innate immunity. HTLV-1-specific T-cells contribute to antitumor surveillance, and type-I IFNs contribute to silencing viral expression. The presence of the double control systems with partial conflicts would explain some of the puzzles in HTLV-1 infection, such as the transient suppression of viral expression *in vivo*, apparently reciprocal occurrence of ATL and HAM/TSP, inconsistent correlations of proviral loads with T-cell responses, and a long incubation period.

Insufficient T-cell responses are regarded as a risk factor for ATL, and vaccines that augment HTLV-1-specific T-cell responses would be beneficial in reducing the risk in a subpopulation of HTLV-1 carriers exhibiting insufficient T-cell responses and elevated proviral loads.

Innate immune responses in HTLV-1 infection should be further investigated, because they could be another important determinant of disease manifestation and represent therapeutic targets in HTLV-1-related diseases.

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Functional impairment of Tax-specific but not cytomegalovirus-specific CD8$^+$ T lymphocytes in a minor population of asymptomatic human T-cell leukemia virus type 1-carriers

Ayako Takamori[1], Atsuhiko Hasegawa[1*], Atae Utsunomiya[2], Yasuhiro Maeda[3,9], Yoshihisa Yamano[4], Masato Masuda[5], Yukiko Shimizu[4], Yotaro Tamai[1], Amane Sasada[1], Na Zeng[1], Ilseung Choi[6], Naokuni Uike[6], Jun Okamura[7], Toshiki Watanabe[8], Takao Masuda[1] and Mari Kannagi[1]

Abstract

Background: Human T-cell leukemia virus type 1 (HTLV-1) causes adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in a small percentage of infected individuals. ATL is often associated with general immune suppression and an impaired HTLV-1-specific T-cell response, an important host defense system. We previously found that a small fraction of asymptomatic HTLV-1-carriers (AC) already showed impaired T-cell responses against the major target antigen, Tax. However, it is unclear whether the impaired HTLV-1 Tax-specific T-cell response in these individuals is an HTLV-1-specific phenomenon, or merely reflects general immune suppression. In this study, in order to characterize the impaired HTLV-1-specific T-cell response, we investigated the function of Tax-specific CD8$^+$ T-cells in various clinical status of HTLV-1 infection.

Results: By using tetramers consisting of HLA-A*0201, -A*2402, or -A*1101, and corresponding Tax epitope peptides, we detected Tax-specific CD8$^+$ T-cells in the peripheral blood from 87.0% of ACs (n = 20/23) and 100% of HAM/TSP patients (n = 18/18) tested. We also detected Tax-specific CD8$^+$ T-cells in 38.1% of chronic type ATL (cATL) patients (n = 8/21), although its frequencies in peripheral blood CD8$^+$ T cells were significantly lower than those of ACs or HAM/TSP patients. Tax-specific CD8$^+$ T-cells detected in HAM/TSP patients proliferated well in culture and produced IFN-γ when stimulated with Tax peptides. However, such functions were severely impaired in the Tax-specific CD8$^+$ T-cells detected in cATL patients. In ACs, the responses of Tax-specific CD8$^+$ T-cells were retained in most cases. However, we found one AC sample whose Tax-specific CD8$^+$ T-cells hardly produced IFN-γ, and failed to proliferate and express activation (CD69) and degranulation (CD107a) markers in response to Tax peptide. Importantly, the same AC sample contained cytomegalovirus (CMV) pp65-specific CD8$^+$ T-cells that possessed functions upon CMV pp65 peptide stimulation. We further examined additional samples of two smoldering type ATL patients and found that they also showed dysfunctions of Tax-specific but not CMV-specific CD8$^+$ T-cells.

Conclusions: These findings indicated that Tax-specific CD8$^+$ T-cells were scarce and dysfunctional not only in ATL patients but also in a limited AC population, and that the dysfunction was selective for HTLV-1-specifc CD8$^+$ T-cells in early stages.

* Correspondence: hase.impt@tmd.ac.jp
[1]Department of Immunotherapeutics, Tokyo Medical and Dental University, Tokyo, Japan
Full list of author information is available at the end of the article

Background

Human T-cells leukemia virus type 1 (HTLV-1) is the causative agent of a highly aggressive $CD4^+$ T-cell malignancy, adult T-cell leukemia (ATL)[1,2]. As many as 10 million individuals are thought to be infected worldwide, in southern Japan, the Caribbean basin, South America, Melanesia, and equatorial Africa[3]. Unlike human immunodeficiency virus (HIV), the majority of HTLV-1-infected individuals are clinically asymptomatic during their lifetime. However, approximately 5% develop ATL, and another 2-3% develop a variety of chronic inflammatory diseases such as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[4-8].

HTLV-1-specific cytotoxic T-lymphocytes (CTLs) are thought to play a pivotal role in containing the proliferation of HTLV-1-infected T-cells[9,10]. Tax is known to be the dominant target antigen for HTLV-1-specific CTLs[10-13], and a high frequency of Tax-specific CTLs can be detected in HAM/TSP patients and some asymptomatic HTLV-1 carriers (ACs)[10-14]. However, ATL patients show general immune suppression[15], reduced frequency and dysfunction of Tax-specific CTLs[16,17]. Regulatory T cell (Treg)-like function of $FoxP3^+$ ATL cells and diminished function of dendritic cells may be involved in the immune suppression in ATL patients [18,19], but the precise mechanism is not yet clarified. We previously demonstrated that a fraction of ACs also exhibit reduced T-cell responses against Tax protein [20]. These observations suggest that the reduced HTLV-1-specific T-cell response might be an underlying risk of ATL development, but not the result of ATL. However, it is unknown how the function of HTLV-1-specific $CD8^+$ T-cells becomes impaired in a small percentage of ACs and whether its dysfunction is specific for HTLV-1 antigen or due to general immune suppression.

During chronic stage of infection with several viruses, such as HIV and hepatitis C virus (HCV), virus-specific CTLs gradually lose their cytotoxic activity, the ability to proliferate and secrete a diverse profile of cytokines, ultimately leading to exhaustion, anergy or even deletion of these cells[21-26]. Programmed death-1 (PD-1), a negative regulator in the CD28 superfamily, has recently been shown to be highly expressed on virus-specific T-cells during many chronic viral infections[27-29]. It has also been reported that the interaction of PD-1 with PD-ligand 1 (PD-L1) negatively regulates cytokine production and proliferation of T-cells[30,31]. A previous report indicates that PD-1 is up-regulated on the dominant Tax-specific CTLs in ATL patients and ACs and that immune regulation through the PD-1/PD-L1 pathway may be involved in the dysfunction of HTLV-1-specific CTLs in ATL patients[32].

Studies on memory T-cell differentiation have shown that phenotype, function, and homeostasis of memory T-cells vary for different persistent virus infections[33]. Central memory T-cells (T_{CM}; $CD45RA^-CCR7^+$) are elicited by non-persisting virus that provide transient antigen stimulation, such as in Influenza virus infection. In contrast, effector memory T-cells (T_{EM}; $CD45RA^-CCR7^-$) predominate when relatively high levels of antigen persist, such as in HIV infection. Terminally differentiated memory (T_{Diff}; $CD45RA^+CCR7^-$) can be seen when antigen persists at a low level, such as in cytomegalovirus (CMV) infection. In HTLV-1 infection, it has been reported that dominant Tax-specific CTLs in HAM/TSP patients consist of T_{EM} and T_{Diff} compartments[34].

We previously identified some major epitopes recognized by HTLV-1-specific CTLs in infected individuals carrying HLA-A2, -A11, or -A24[12,35,36]. These allowed us to monitor HTLV-1-specific CTLs and analyze their functions *ex vivo*, by using antigen/HLA tetrameric complexes. In this study, we demonstrate that IFN-γ production and proliferative capacity of tetramer-binding Tax-specific $CD8^+$ T-cells were severely impaired not only in ATL patients but also in a minor population of asymptomatic HTLV-1 carriers (ACs). Importantly, the T-cell dysfunction at the asymptomatic stage was selective for HTLV-1 but not for CMV antigen. In addition, severely impaired HTLV-1-specific but not CMV-specific $CD8^+$ T-cells responses were also observed in patients diagnosed as smoldering ATL, the clinical condition of which is close to that of AC. The dysfunction of HTLV-1-specific $CD8^+$ T-cells in an early clinical stage implies HTLV-1-specific immune suppressive mechanism might be an underlying risk for ATL.

Results

Incidence and frequency of Tax-specific $CD8^+$ T-cells in ACs, and HAM/TSP and cATL patients

In 23 ACs and 18 HAM/TSP and 21 cATL patients carrying HLA-A2, -A11 and/or -A24 alleles, we evaluated the frequencies of Tax-specific $CD8^+$ T-cells by using cognate Tax/HLA tetramers (Figure 1 and Table 1). Tax-specific $CD8^+$ T-cells were detected in 87.0% of ACs and all HAM/TSP patients tested. In contrast, only 38.1% of cATL patients have detectable frequencies of Tax-specific $CD8^+$ T-cells (Table 1). Figure 1B shows that the average frequency of Tax-specific $CD8^+$ T-cells in the $CD8^+$ T-cells of cATL patients (n = 21, 0.90% range: 0%-9.45%) was significantly lower than that in ACs (n = 23, 2.37%, range: 0%-8.23%, $P = 0.0023$). HAM/TSP patients had the highest average frequency of Tax-specific $CD8^+$ T-cells among the three groups (n = 18, 8.88%, range: 1.86%-29.9%, $P = 0.0001$; vs. AC, $P < 0.0001$; vs. cATL patients), which is consistent with

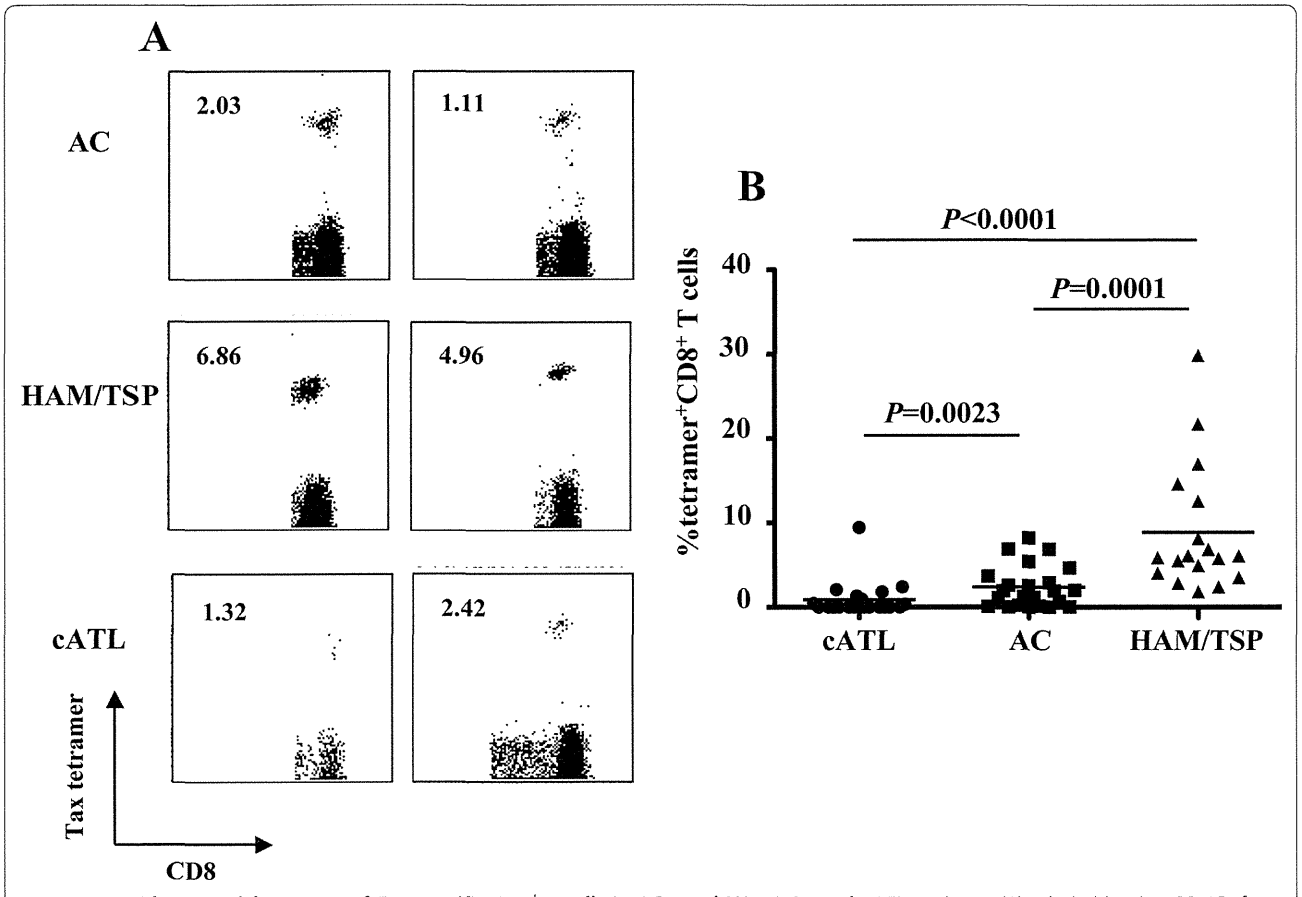

Figure 1 Incidence and frequency of Tax-specific CD8$^+$ T-cells in ACs, and HAM/TSP and cATL patients. (A) Whole blood or PBMCs from AC (top), and HAM/TSP (middle) and cATL (bottom) patients were stained with Tax/HLA tetramer. Number indicates the percentage of tetramer$^+$ cells in CD8$^+$ T-cells. (B) The percentage of Tax-tetramer$^+$ CD8$^+$ T-cells in AC (n = 23), and HAM/TSP (n = 18) and cATL (n = 21) patients. *P* value was determined by the Mann-Whitney *U* test. Horizontal bars indicate the average percentage of Tax-tetramer$^+$ CD8$^+$ T-cells for the group.

previous reports [10,17,37]. It is of note that Tax-specific CD8$^+$ T-cells are detectable even in cATL patients, although the frequency is very low.

Impaired cell proliferation and IFN-γ production of Tax-specific CD8$^+$ T-cells in cATL but not HAM/TSP patients

We next examined IFN-γ production and cell proliferation of Tax-specific CD8$^+$ T-cells in HAM/TSP and cATL patients (Figure 2A). Intracellular IFN-γ staining showed that Tax-specific CD8$^+$ T-cells in all HAM/TSP patients tested produced IFN-γ when stimulated with Tax peptide (Figure 2A). Tax-specific CD8$^+$ T-cells in those HAM/TSP patients proliferated regardless of stimulation with Tax peptide (Figure 2B). In contrast to HAM/TSP patients, IFN-γ production from Tax-specific CD8$^+$ T-cells in a cATL patient was hardly detectable even when stimulated with Tax peptide (4.8%, Figure 2A). In the same donor, Tax-specific CD8$^+$ T-cells

Table 1 The number of blood samples with detectable Tax-specific CD8$^+$ T-cells in all samples tested in this study

Tax/HLA tetramers used in this study	Disease Status		
	AC	HAM/TSP	cATL
HLA-A*0201/Tax11-19	12/14[1]	7/7	2/11
HLA-A*1101/Tax88-96	4/4	4/4	3/5
HLA-A*2402/Tax301-309	13/15	13/13	5/16
No. of tetramer$^+$ samples/total no. of blood samples[2]	20/23 (87.0%)	18/18 (100%)	8/21 (38.1%)

[1] No. of samples with detectable Tax-specific CD8$^+$ T-cells/total no. of samples carrying each HLA allele. When the frequency of tetramer$^+$ cells was more than 0.04% of CD8$^+$ T-cells, the sample was regarded as detectable.

[2] In case Tax-specific CD8$^+$ T-cells was detectable by either tetramer in a sample carrying two of three HLA-A alleles above, the sample was regarded as positive.

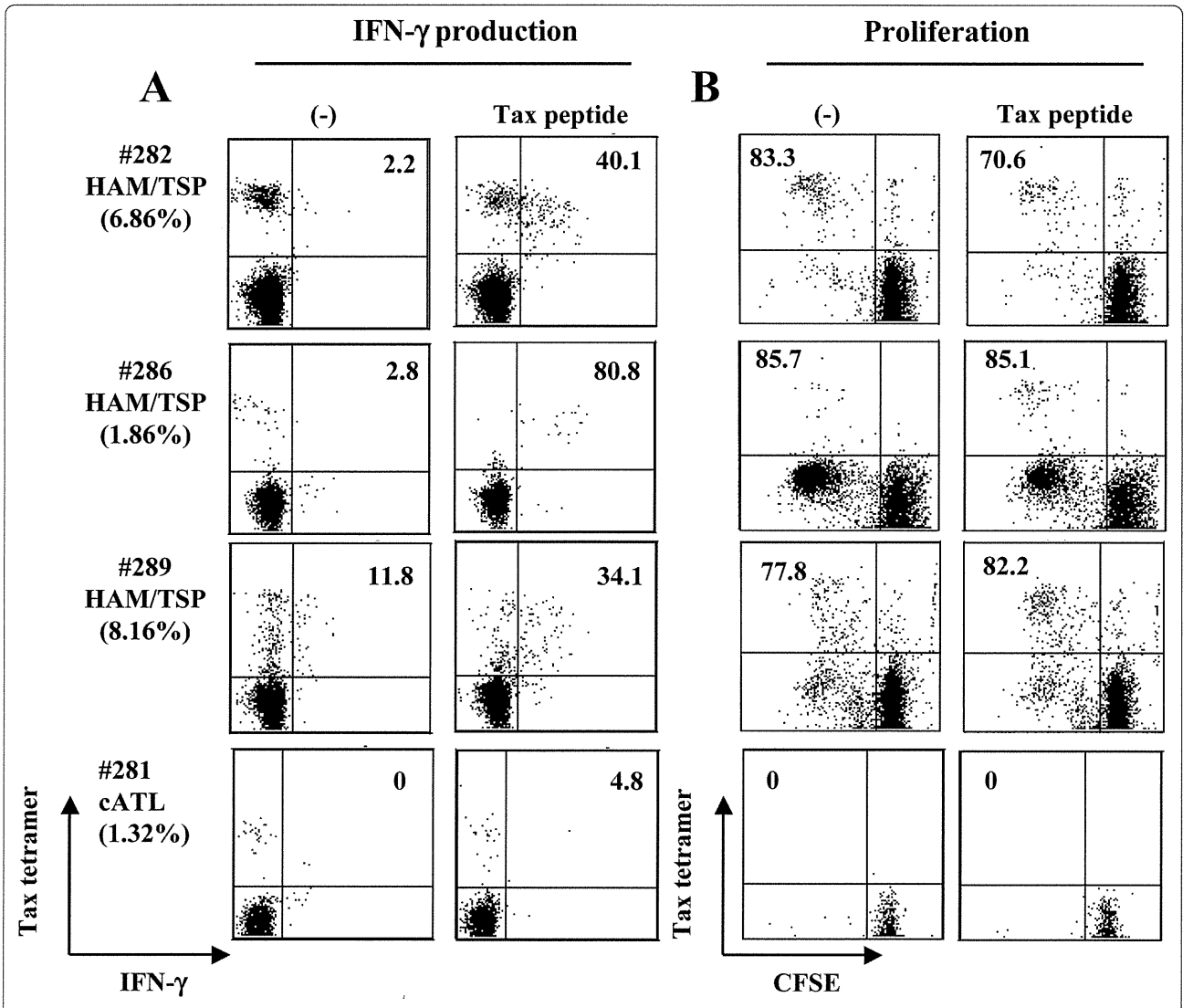

Figure 2 IFN-γ production and proliferation of Tax-specific CD8$^+$ T-cells in HAM/TSP and cATL patients. (A) PBMCs from HAM/TSP and cATL patients were stimulated with or without 10 μM Tax peptide for 6 hrs. The number indicates the percentage of IFN-γ-producing cells in tetramer$^+$ cells. (B) For CFSE-based T-cell proliferation, CFSE-labeled PBMCs from HAM/TSP and cATL patients were cultured in the presence or absence of 100 nM Tax peptide for 6 days. The number indicates the percentage of dividing (CFSE^{low}) cells in tetramer$^+$ cells. The percentage of tetramer$^+$ cells among CD8$^+$ T cells in fresh blood is indicated in parenthesis under the patient ID.

could be detected in fresh blood (1.32%) and after 6 hrs incubation as shown in Figure 2A, but not after 6 day-culture, suggesting that Tax-specific CD8$^+$ T-cells in this cATL patient had no proliferative capacity (Figure 2B). We tested PBMC from four other cATL patients who had detectable Tax-specific CD8$^+$ T-cells, but none of them showed proliferation of Tax-specific CD8$^+$ T-cells by either the CFSE-based proliferation assay or 13-day culture (Additional file 1). Collectively, these results indicate that Tax-specific CD8$^+$ T-cells from most cATL patients are impaired in their capacities to proliferate and produce IFN-γ.

Diversity in the IFN-γ production and cell proliferation of Tax-specific CD8$^+$ T-cells in ACs

Our recent studies using the GST-Tax protein-based assay demonstrated that the extent of Tax-specific T-cell responses varied widely in ACs[20]. We then evaluated proliferation and/or IFN-γ production of tetramer-binding Tax-specific CD8$^+$ T-cells in 14 ACs (Table 2). Representative data on 4 of 14 ACs are shown in Figures 3A and 3B. In 3 ACs (#251, #313, and #360), Tax-specific CD8$^+$ T-cells produced IFN-γ and proliferated in response to Tax peptide (Figures 3A and 3B). Similarly to HAM/TSP samples, a large proportion of Tax-

Table 2 Clinical information and summary for Tax-specific CD8$^+$ T cells in 14ACs

ID	Age	Sex	WBC (/μl)	CD4 (%)[1]	CD8 (%)[1]	HLA	Tetramer (%)[2]	Functions and phenotype of Tax-specific CD8$^+$ T-cells[3]			Ably (%)[7]	PVL[8]
								IFN-γ$^+$ (%)[4]	CFSE^{low} (%)[5]	PD-1$^+$ (%)[6]		
#217	70s	F	6800	ND[9]	5.72	A24	1.94	27.7	78.9	78.7	0	14
#236	30 s	F	6500	ND	11.9	A24	2.54	31.1	0	54.1	0	22
#238	60 s	F	5700	ND	12.7	A11	1.29	36.4	100	0	0	2
#243	50 s	F	4100	ND	24.6	A2/24	0.39/3.67	11.3	27.6	93.8	0	3
#245	40 s	F	5000	ND	22.6	A2	0.73	62.5	75	ND	1	58
#251	60 s	M	4800	ND	11.9	A2/11	0.70/8.23	35.8	84.4	36.7	0	2
#279	40 s	M	6200	34.1	11.6	A2/24	4.70/0.18	12.9	30.8	70.2	1	48
#287	70 s	M	4800	72.5	10.0	A2/24	1.17/0.23	11.1	0	55.6	2	81
#309	60 s	F	4600	37.5	24.8	A11/24	6.88/4.26	51.7	76.2	85.3	1.5	29
#311	60 s	F	3200	30.6	14.8	A2/24	1.02/1.94	51.3	ND	ND	0	6
#312	50 s	F	2700	27.3	36.4	A24	2.03	77.8	ND	ND	ND	UN[10]
#313	60 s	M	7300	25.4	31.0	A24	1.11	55.7	60	90.6	ND	4
#315	50 s	F	7500	26.5	7.9	A2/24	6.88/0	24.5	84.7	20	0.6	17
#360	50 s	M	6200	37.7	29.9	A2	2.6	63.1	68.4	10.2	0	UN

[1]The number indicates percentage of CD4$^+$ or CD8$^+$ T cells in lymphocytes.

[2]The number indicates percentages of tetramer$^+$ cells in CD8$^+$ T-cells. Two numbers divided by a slash represent those detected by two different tetramers corresponding to two HLA alleles shown in the HLA column.

[3]In case of a sample carrying two of three HLA-A alleles (A2, A11, or A24), Tax-specific CTLs predominantly detected by a tetramer were used. The number represents percentage of indicated cells in the tetramer-binding CD8$^+$ T cells.

[4]Evaluated by intracellular IFN-γ staining following 6 hours stimulation with corresponding Tax peptide.

[5]Evaluated by CFSE intensities in labeled PBMC after 6 days incubation with corresponding Tax peptide stimulation.

[6]The number represents percentage of indicated PD-1$^+$Tax-specific CD8$^+$ T cells without culture.

[7]Ably; abnormal lymphocytes

[8]PVL; proviral load. The number represents copy number per 1000 PBMCs.

[9]ND; not determined

[10]UN; undetectable

specific CD8$^+$ T-cells in these ACs spontaneously proliferated without stimulation with Tax peptide, probably due to viral reactivation in HTLV-1-infected cells *in vitro*[38,39]. IFN-γ production was specifically detected for peptide stimulation, and 35.8-55.7% of Tax-specific CD8$^+$ T-cells produced a good amount of IFN-γ (mean fluorescence intensity, MFI: 63.7-195.3) upon stimulation in the samples of #251, #313, and #360. In contrast, Tax-specific CD8$^+$ T-cells in one AC (#287) did not proliferate in response to Tax peptide and showed a very weak IFN-γ response with low amounts of IFN-γ (MFI: 37.5) in a low percentage (11.1%) of Tax-specific CD8$^+$ T-cells (Figures 3A and 3B). In other ACs (#243 and #279), low frequency of IFN-γ$^+$ Tax-specific CD8$^+$ T-cells was observed, but the levels of IFN-γ production (MFI: #243; 58.8, #279; 77.6) and the proliferative responses were comparable to other ACs (Table 2). Tax-specific CD8$^+$ T-cells in #236 failed to proliferate but showed favorable IFN-γ production (MFI: 80.1) in 31.1% of the cells.

Among AC samples tested, AC#287 carried higher proviral load (81 copies in 1000 PBMCs) than any other ACs (Table 2). Since Tax-specific CD8$^+$ T-cells in #287 had severely impaired IFN-γ production and proliferative potential, we examined the relationship of the function of these T-cells with proviral loads. Both percentages of IFN-γ$^+$ and dividing Tax-specific CD8$^+$ T-cells among CD8$^+$ T-cells were likely to be inversely correlated with proviral loads although they were not statistically significant (Figure 3C and 3D). Because of the limited availability of the samples, we focused mainly on two ACs (#287 and #313) in the studies hereafter.

Dysfunction of Tax-specific CD8$^+$ T-cells and inefficient CD8$^+$ cell-mediated HTLV-1 control in AC #287

To examine whether Tax-specific CD8$^+$ T-cell responses were influenced by activation of antigen-presenting cells (APCs), PBMC from #313 (responder) and #287 (low responder) were stimulated with Tax peptide in the presence or absence of LPS, a potent activator of APCs such as dendritic cells (DCs) and monocytes/macrophages. In #313, the frequency of Tax-specific CD8$^+$ T-cells increased from 1.11% to 6.47% or 4.07% at day 13, after stimulation with or without Tax peptide, respectively. The frequency of Tax-specific CD8$^+$ T-cells in #313 further increased in the presence of Tax peptide and LPS (15.81%). In contrast to #313, the frequency of Tax-specific CD8$^+$ T-cells in #287 decreased from 1.17%