Table 3 | Summary of reports on treatment for HAM/TSP.

Authors	Country	Study design	Reagents	Treatment regimen	Study period	No. of patients	Rate of Efficacy	Note
Osame et al. (1990b)	Japan	Open-label	Prednisolone	60–80 mg qod for 2 month → 10 mg off/month for 6 month → 5 mg qod for 3 month	11 Month	65	90.8% (59/65) 56.9% (>1)	Incidence of side effects: 20% (13/65)
Croda et al. (2008)	Brazil	Case series	Methylprednisolone	1 g × 3 days/month for 3–4 month	2.2 Years	39	24.5%	Transient effect
Nakagawa et al. (1996)	Japan	Open-label	Prednisolone	1–2 mg/kg qd or qod for 1–2 month → tapering	6–12 Month	131	81.7% 69.5% (>1)	Decrease of CSF neopterin
			Methylprednisolone	500 mg–1g × 3 days		10	30.0%	For rapid progression
			Interferon-α	3 MU/day × 30 days	1–3 Month	32	62.5% 21.9% (>1)	Transient effect Incidence of side effects: 65.6% (21/32)
Martin et al. (2012)	UK	Open-label	Cyclosporine A	2.5–5 mg/kg/day bd for 48 week	72 Week	7	71.4% (5/7) after 3 Month	Clinical failure: two patients
Izumo et al. (1996)	Japan	Multicenter double-blind RCT	Interferon-α	0.3 MU/day × 28 days	8 Week	15	7.1%	Incidence of side effects: 26.7% (4/15)
				1 MU/day × 28 days		17	23.5%	29.4% (5/17)
				3 MU/day × 28 days		16	66.7%	50.0% (8/16)
Yamasaki et al. (1997)	Japan	Case series	Interferon-α	6 MU/day × 14 days → 6 MU/3 times/week × 22 week	6 Month	7	71.4% (5/7)	Clinical failure: two patients
Arimura et al. (2007)	Japan	Phase IV	Interferon-α	3 MU/day × 4–793 days (median 30 days)	6 Month	167	66.2% 29.2% (>1)	Side effects: 87.4% Serious side effects: 7.0%
Taylor et al. (2006)	UK and Japan	Double-blind RCT	Zidovudine + lamivudine	AZT 300 mg + 3TC 150 mg bd	48 Week	16	No clinical improvement	No change in proviral load
Macchi et al. (2011)	UK	Case series	Tenofovir	245 mg/day	2–16 Month	6	No clinical improvement	No change in proviral load

>1, improvement of more than one grade in the Osame's motor disability score.

No., number; qod, every other day; mo: month(s); yr, year(s); qd, every day; MU, million unit; wk, week(s)' bd, twice daily; RCT, randomized controlled trial; AZT, zidovudine; 3TC, lamivudine.

anti-inflammatory and/or antiviral in nature. In fact, there is a recent report on the high efficacy of cyclosporine A therapy targeted at early phase or progressive HAM/TSP patients. In this study, clinical improvement was observed in five of seven patients, with reduction of provirus DNA load observed in the CSF (Martin et al., 2012).

Type I IFNs (α and β), which have immunomodulatory and antiviral properties (Borden et al., 2007), have been tested as anti-HAM/TSP drugs. IFN-α demonstrated clinical benefits in a multicenter, randomized, double-blind, controlled trial of HAM/TSP patients in Japan (Izumo et al., 1996). In this study, 3 million units (MU) of human lymphoblastoid natural IFN-α given daily by intramuscular injection for 28 days showed better clinical benefit than 0.3 or 1 MU of IFN-α. The reduction of proviral DNA load and memory $CD8^+$ cells in PBMCs (Saito et al., 2004) and the reduction of CD4/CD8 ratio and $CD4^+CCR5^+$ cells in CSF (Kambara et al., 2002) after short-term IFN-α therapy was demonstrated. However, the benefit of long-term IFN-α therapy has not been well demonstrated. A small study extending IFN-α treatment for 24 weeks reported sustained clinical response (Yamasaki et al., 1997). In a post-marketing surveillance of IFN-α in Japan, sustained improvements in motor disability for 5 months after cessation of IFN-α administration were observed in 11 of 30 patients, and a high adverse event rate (536 events reported in 146 patients; 46 classified as serious) was indicated (Arimura et al., 2007). In this surveillance study, it is notable that IFN-α treatment was more effective in patients with lower motor disability and shorter duration of illness and progression phase, suggesting the existence of therapeutic windows of opportunity in the treatment of HAM/TSP. It is also notable that rapidly progressing HAM/TSP patients showed no response and dropped out from the IFN-α therapy (Yamasaki et al., 1997; Arimura et al., 2007). Therefore, well-designed controlled clinical trials to guide the clinician with regard to the appropriate target, time of initiation, and the dose or duration of IFN-α therapy in HAM/TSP will be important for future studies.

Thus, corticosteroids and IFN-α may have therapeutic efficacy for HAM/TSP to some extent; however, the effect may not be sufficient for avoiding long-term disability. Moreover, in some cases, it might be difficult to continue therapy because of the side effects of these drugs and their insufficient benefit. Therefore, it is essential that revolutionary drugs that can lead to a paradigm shift in the therapeutic strategies for HAM/TSP be developed. Considering the pathogenesis of HAM/TSP, therapies to eliminate HTLV-1-infected cells from the peripheral blood and CNS should be developed. However, antiviral therapy has not been successful in the clinical trial for HAM/TSP. A randomized, double-blind, placebo-controlled, 6-month study of zidovudine and lamivudine combination therapy, which demonstrated activity against HTLV-1 reverse transcriptase *in vitro*, was conducted in 16 patients, and no significant changes were observed in the clinical symptoms and HTLV-1 proviral load (Taylor et al., 2006). A pilot trial of tenofovir, which also demonstrated activity against HTLV-1 reverse transcriptase *in vitro*, was conducted in six patients, and no significant change in HTLV-1 proviral load was observed (Macchi et al., 2011). Thus, the impact of therapy with viral reverse transcriptase inhibitors with the aim of reducing the HTLV-1 proviral load *in vivo* has been minimal. These results support the hypothesis that HTLV-1 proviral load in HTLV-1-infected patients is mainly maintained through cell division of infected cells and not by viral replication and new infection (Wattel et al., 1995; Cavrois et al., 1998). Therefore, development of therapies directly targeting HTLV-1-infected cells could be more promising to reduce the viral load. Recently, we have demonstrated that CC chemokine receptor 4 (CCR4), expressed on the surface of ATLL cells (Ishida et al., 2004), is also expressed on HTLV-1-infected cells in HAM/TSP patients (Yamano et al., 2009). More recently, a humanized anti-CCR4 monoclonal antibody has been developed; the safety and efficacy of this antibody has been proven in phase I and II studies (Yamamoto et al., 2010; Ishida et al., 2012) and subsequently approved by the Ministry of Health, Labour and Welfare as a therapeutic agent for relapsed patients with ATLL in Japan. Further clinical trials on the safety and efficacy of anti-CCR4 therapy for HAM/TSP patients should be conducted in future studies.

CONCLUSION

Advances in study of the epidemiology and pathogenesis of HAM/TSP have led to the identification of several biomarkers and therapeutic targets. However, these findings have not yet translated into an optimal therapeutic strategy for this hitherto intractable neurological disease. Well-designed clinical trials in HAM/TSP will provide opportunities for further quantification of biomarkers and refinement of therapeutic drugs. The development of an effective therapy to improve long-term prognosis in HAM/TSP is of paramount importance, and clinical trials for the validation of HAM/TSP relevant biomarkers and new therapeutic targets will be key challenges in this therapy.

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7 HAM(HTLV-1関連脊髄症)

山野嘉久

1 疫学

ヒトTリンパ球向性ウイルス1型（HTLV-1）関連脊髄症（HAM）は，脊髄の慢性炎症による進行性の錐体路障害を特徴とする．HTLV-1感染者は全国で約110万人存在するが，その約0.3％がHAMを発症し，日本での患者数は約3,000名．現在，HAM患者登録サイト（http://hamtsp-net.com）がある．

HTLV-1は成人T細胞白血病も起こすことがあるがその合併は稀である．

2 診断

HAMは，早期の診断と治療介入がきわめて重要で，病気を見逃さない注意が必要である．表のような症状の患者を診たら，ぜひHAMを思い浮かべてほしい．症状の特徴から整形外科や泌尿器科を受診するケースも多いが，**HAMを疑ったらすぐに神経内科医に紹介**してほしい．

痙性対麻痺を呈しHAMの可能性が考えられる場合，血清中の抗HTLV-1抗体の有無をEIA法またはPA法でスクリーニングし，陽性の場合はウエスタンブロット法で確認，感染を確定する．感染が確認されたら髄液検査を施行し，髄液の抗HTLV-1抗体が陽性の場合，他のミエロパチーをきたす脊髄圧迫病変，脊髄腫瘍，多発性硬化症，視神経脊髄炎などを鑑別したうえで，HAMと確定診断する．

3 検査

髄液検査では細胞数増加（単核球優位）を約3～4割に認め，ネオプテリン（保険未承認，外注測定可）の増加を約8割に認める．細胞数よりネオプテリンの方が脊髄炎症の程度を把握するうえで感度に優れており，また細胞数やネオプテリンの値は病勢と相関が高く，治療方針決定や治療効果判定に有用である．MRIでは胸髄萎縮がしばしば認められ，発症早期にT2強調での髄内強信号が認められる場合がある．

表　HAMの初期症状

何となく歩きにくい，両下肢のつっぱり感，足がもつれる，つまずく，走ると転びやすい，などの歩行障害に関する症状
排尿障害や便秘も早期から自覚されることが多く，尿閉や頻尿，くり返す膀胱炎で泌尿器科を受診しHAMと診断されることもある
稀に，持続する両下肢のしびれ感，痛みなどを早期から認めることがある

4 経過

HAMは10～20年の経過で歩行不能になる場合が多いが（緩徐進行例），時に数カ月～2年以内で歩行不能になる例（急速進行例）もあり，重症例では両下肢の完全麻痺，躯幹の筋力低下による座位保持不能で寝たきりとなる例もある．一方，歩行障害が軽度のまま数十年以上症状の進行が乏しい例（軽症例）もある（図）．このように，HAMの経過には個人差が大きいが，その経過は脊髄炎症の程度を反映している場合が多く，病勢の把握は治療方針を決定する指標となる．

5 治療

HAMは病勢に応じた治療が必要である．発症後数カ月単位で階段昇降や歩行に補助が必要となるような急速進行例は，髄液の細胞数やネオプテリンも高く，ステロイドパルス療法とその後の内服療法が有効の場合がある．また症状が緩徐に進行し，髄液所見が炎症活動期と判断される慢性進行例では，ステロイド少量内服やインターフェロンα療法が有効の場合がある．ほとんど進行が認められず髄液所見もおとなしい軽症例は，これら治療薬の必要性に乏しく，排尿・排便障害や痙性に対する対症療法や継続的なリハビリをしながら経過観察が推奨される．また，常に副作用を念頭におき，症状の進行具合や髄液所見を参考に，できるだけ減量や中止の可能性を検討する．特にステロイド性骨粗鬆症には注意が必要で，ガイドライン[1]に基づいた対応が求められる．

6 合併症の治療

HAM患者は多彩な合併症を伴う．排尿障害に関しては，適切な治療薬の選択や間欠的自己導尿を行うことによりADLが大きく改善するので，泌尿器科医と連携した対応が望まれる．痙性に対しては，その程度に応じて抗痙縮薬の量を調整する．下肢の激しい疼痛を伴う場合は，神経障害性疼痛治療ガイドライン[2]に基づいた対応が推奨される．その他，便秘，褥瘡，

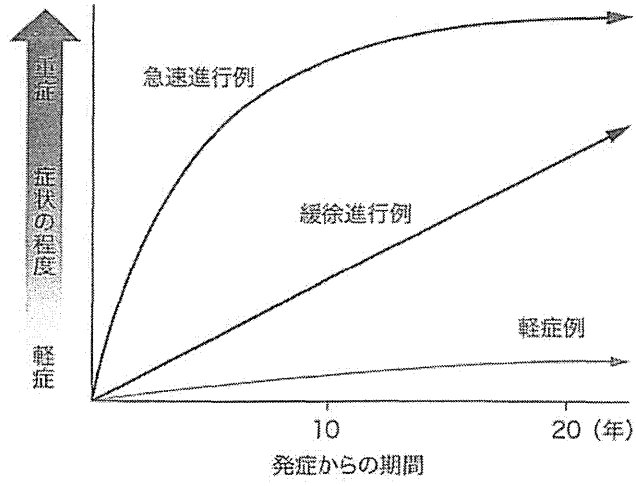

図 HAMの臨床経過の特徴

ブドウ膜炎や肺胞炎などを伴うこともあり，全身検索も忘れてはならない．これら合併症のコントロールや継続的なリハビリは，患者の日常生活を維持するうえできわめて重要であり，他科と連携しながらきめ細かな治療を行う必要がある．

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3）山野嘉久ら：HTLV-1関連脊髄症（HAM）の治療法を確立していくために―その現状と展望―．日本臨床，70（4）：705-713，2012

a0005

HTLV-1

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Glossary

d0005 **Cytotoxic T cell** A cytotoxic T cell belongs to a subgroup of T lymphocytes with CD8 receptor that are antigen- pecific and capable of inducing the death of virus-infected somatic or tumor cells.

d0010 **Gliosis** Gliosis is the process of scarring in the central nervous system, caused by a proliferation of astrocytes.

d0015 **Oligoclonal band** Oligoclonal bands are bands of immunoglobulins that are seen when a blood serum (or plasma) or cerebrospinal fluid (CSF) is analyzed by protein electrophoresis. The presence of oligoclonal bands in CSF but not in blood serum (or plasma) means the production of immunoglobulins in central nervous system, that is, inflammation in the central nervous system.

Provirus A provirus is the form of the virus which is capable of being integrated into the chromosome of the host cell. d0020

Spastic paraparesis Mild or moderate loss of motor function accompanied by spasticity in the extremities mainly caused by central nervous system (brain and spinal cord) diseases. d0025

p0030 Human T-lymphotropic virus type-1 (HTLV-1) belongs to the *Deltaretrovirus* genus of the Orthoretrovirinae subfamily and infects 10–20 million people worldwide. HTLV-1 can be transmitted through sexual contact, intravenous drug use, and breastfeeding from mother to child. The infection is endemic in south-west Japan, the Caribbean, sub-Saharan Africa, South America, with smaller foci in Southeast Asia, South Africa, and northeastern Iran. HTLV-1 was initially isolated in 1980 from two T-cell lymphoblastoid cell lines and the blood of a patient originally thought to have a cutaneous T-cell lymphoma. It was the first human retrovirus ever associated with a human cancer. Three years before the isolation of HTLV-1, a Japanese group reported adult T-cell leukemia (ATL), a rare form of leukemia endemic to southwest Japan, as a distinct clinical entity. In 1981, the same group demonstrated that ATL was caused by a new human retrovirus originally termed 'ATLV'. Later, ATLV and HTLV have been shown to be identical, and a single name HTLV-1 has been adopted. In the mid-1980s, epidemiological data linked HTLV-1 infection with a chronic progressive neurological disease, which was termed 'tropical spastic paraparesis (TSP)' in the Caribbean and 'HTLV-1 associated myelopathy (HAM)' in Japan. HTLV-1-positive TSP and HAM were subsequently found to be clinically and pathologically identical and the disease was given a single designation as HAM/TSP. HTLV-1 can cause other chronic inflammatory diseases such as uveitis, arthropathy, pulmonary lymphocytic alveolitis, polymyositis, Sjögren syndrome, and infective dermatitis. Only approximately 2–3% of infected persons develop ATL and another 0.25–4% develop chronic inflammatory diseases, while the majority of infected individuals remain lifelong asymptomatic carriers (ACs). Thus, the viral, host, and environmental risk factors, as well as the host immune response against HTLV-1 infection, appear to regulate in the development of HTLV-1-associated diseases. For over two decades, the investigation of HTLV-1-mediated pathogenesis has focused on Tax, an HTLV-1-encoded viral oncoprotein. Tax activates many cellular genes by binding to groups of transcription factors and coactivators and is necessary and sufficient for cellular transformation. However, recent reports have identified another regulatory protein, HTLV-1 basic leucine zipper factor (HBZ), that plays a critical role in the development of ATL and HAM/TSP.

HTLV-1-Associated Diseases s0005

Adult T-cell leukemia s0010

p0035 ATL is a fatal malignancy of mature CD4+ T cells. It arises in only a small proportion of HTLV-1-infected people (1–5% of infected individuals) after long latency periods following primary infection. ATL shows diverse clinical features, but can be divided into four clinical subtypes: smoldering, chronic, lymphoma, and acute. Each subtype is directly correlated with the prognosis of patients: the smoldering and chronic types are indolent, while the acute and lymphoma types are aggressive and characterized by resistance to chemotherapy and poor prognosis. Development of ATL is characterized by infiltration of various tissues with circulating ATL cells, called 'flower cells', which have conspicuous lobulated nuclei. These cells cause further symptoms including lymphadenopathy, lytic bone lesions, skin involvement, hepatosplenomegaly, and hypercalcemia. Laboratory findings of ATL patients typically reveal a marked leukocytosis, hypercalcemia, high serum levels of lactate dehydrogenase (LDH), and a soluble form of interleukin-2 receptor (IL-2R). In cohort studies of HTLV-1 carriers, the risk factors for ATL appeared to include vertical infection (mother to child transmission), male gender, older age, and increasing numbers of abnormal lymphocytes. Since ATL occurs mainly in vertically infected individuals, but not in those who become infected later in life, the impairment of HTLV-1-specific T-cell responses caused by vertical HTLV-1 infection has been suggested as a possible cause of disease development. The HTLV-1-specific cytotoxic T-cell (CTL) responses from ATL patients are significantly lower than that of HAM/TSP patients. However, insufficient HTLV-1-specific T-cell responses might also occur during and after the onset of

ATL. Although ATL has a poor prognosis, recent advances in its treatment have led to significant gains in response rates and survival. Accumulating evidence suggests that allogeneic bone marrow transplantation and allogeneic peripheral blood stem cell transplantation are potent therapies for aggressive ATL (i.e., the acute and lymphoma type). The combination of the antiretroviral agent zidovudine (AZT) and interferon-alfa (IFN-α) is also beneficial for overall survival in smoldering and chronic (i.e., indolent) ATL, although its efficacy has not yet been confirmed in well-designed prospective studies.

p0040 Since the discovery of HTLV-1, the viral transactivator Tax has been viewed as critical for leukemogenesis, due to its pleiotropic effects on both viral and many cellular genes responsible for cell proliferation, genetic instability, dysregulation of the cell cycle, and apoptosis. However, Tax expression is not detected in about 60% of freshly isolated samples from ATL cases. Recently, the expression of another regulatory protein, HBZ, has been reported in association with all ATL cases. This protein, which is encoded in the minus or antisense strand of the virus genome, promotes proliferation of ATL cells and induces T-cell lymphomas in CD4+ T cells by transgenic expression, indicating involvement of HBZ expression in the development of ATL. In addition, among the HTLV-1-encoded viral genes, only the HBZ gene sequence remains intact, unaffected by nonsense mutations and deletion. Thus, HBZ expression is indispensable for proliferation and survival of ATL cells and HTLV-1-infected cells, and Tax expression is not always necessary for the development of ATL.

s0015 HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis

p0045 HAM/TSP is a chronic progressive myelopathy characterized by spastic paraparesis, sphincter dysfunction, and mild sensory disturbance in the lower extremities. In addition to neurological symptoms, some HAM/TSP cases also exhibit autoimmune-like disorders, such as uveitis, arthritis, T-lymphocyte alveolitis, polymyositis, and Sjögren syndrome. To date, more than 3000 cases of HAM/TSP have been reported in HTLV-1-endemic areas. Sporadic cases have also been described in nonendemic areas such as the United States and Europe, mainly in immigrants from an HTLV-1-endemic area. The lifetime risk of developing HAM/TSP is different among ethnic groups, ranging between 0.25% and 4%. The annual incidence of HAM/TSP is higher among Jamaican subjects than among Japanese subjects (20 vs. three cases/100 000 population), with a 2 to 3 times higher risk for women in both populations. The period from initial HTLV-1 infection to the onset of HAM/TSP is assumed to range from months to decades, a shorter time than for ATL onset. HAM/TSP occurs both in vertically infected individuals and in those who become infected later in life (i.e., through sexual contact (almost exclusively from male to female), intravenous drug use, contaminated blood transfusions, etc.). The mean age at onset is 43.8 years and, like other autoimmune diseases, the frequency of HAM/TSP is higher in women than in men (the male to female ratio of occurrence is 1:2.3).

p0050 The essential histopathological feature of HAM/TSP is a chronic progressive inflammation in the spinal cord, predominantly at the thoracic level. The loss of myelin sheaths and axons in the lateral, anterior, and posterior columns is associated with perivascular and parenchymal lymphocytic infiltration, reactive astrocytosis, and fibrillary gliosis. In addition to HTLV-1 antibody positivity, other laboratory findings of HAM/TSP include the presence of atypical lymphocytes (the so-called flower cells) in peripheral blood and cerebrospinal fluid (CSF), a moderate pleocytosis, and raised protein content in CSF. Oligoclonal bands, raised concentrations of inflammatory markers such as neopterin, tumor necrosis factor (TNF)-α, IL-6 and IFN-γ, and an increased intrathecal antibody synthesis specific for HTLV-1 antigens have also been described in CSF of HAM/TSP patients.

p0055 A previous population association study in HTLV-1 endemic in southwest Japan revealed that one of the major risk factors is the HTLV-1 proviral load (PVL), as the PVL is significantly higher in HAM/TSP patients than in ACs. A high PVL was also associated with an increased risk of progression to disease. Higher PVL in HAM/TSP patients than in ACs was also observed in other endemic areas such as the Caribbean, South America, and the Middle East. In southwest Japan, an association was suggested between possession of the HLA-class I genes HLA-A*02 and Cw*08 and a statistically significant reduction in both PVL and the risk of HAM/TSP. By contrast, possession of HLA-class I HLA-B*5401 and class II HLA-DRB1*0101 predisposed patients in the same population to HAM/TSP. Since the function of class I HLA proteins is to present antigenic peptides to CTL, these results imply that individuals with HLA-A*02 or HLA-Cw*08 mount a particularly efficient CTL response against HTLV-1, which may be an important determinant of HTLV-1 PVL and the risk of HAM/TSP.

p0060 To date, no generally agreed standard treatment regimen has been established for HAM/TSP, as no treatment for HAM/TSP has proven to be consistently effective and long term. Therefore, current clinical practice for treatment of HAM/TSP is based on case series and open, nonrandomized uncontrolled studies. Although mild to moderate beneficial effects have been reported with corticosteroids, immunosuppressants, high-dose intravenous gammaglobulin, antibiotics (erythromycin and fosfomycin), and vitamin C, the clinical benefits are only transient and limited. The complications of steroid use limit their use particularly in postmenopausal females, who are at higher risk of developing HAM/TSP. Only three randomized placebo-controlled trials have been conducted for HAM/TSP treatment. These studies indicate that IFN-α is an effective therapy, with an acceptable side-effects profile. By contrast, no evidence yet exists of any benefit of zidovudine plus lamivudine for treating HAM/TSP. More clinical trials with adequate power are needed in the future.

Other HTLV-1-Associated Diseases s0020

p0065 HTLV-1 has been implicated in the pathogenesis of other inflammatory disorders such as uveitis, arthropathy, infective dermatitis, pulmonary lymphocytic alveolitis, polymyositis, Sjögren syndrome, and autoimmune thyroid diseases, based on the higher HTLV-1 PVL and the higher seroprevalence in patients than in ACs. However, direct evidence for an association between these disorders and HTLV-1 infection is still lacking. Nonetheless, HTLV-1 may be a significant trigger for the development of these autoimmune disorders. AU2

See also: Retroviruses (01323)

Further Reading

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11 ヒトTリンパ球向性ウイルス脊髄症（HAM）

辻野　彰，中村龍文

HTLV-1関連脊髄症［human T-lymphotropic virus type 1（HTLV-1）-associated myelopathy：HAM］は，1986年に納らによって提唱された疾患単位で，HTLV-1感染者（キャリア）に見出される慢性進行性の痙性脊髄麻痺を示す一群である．HTLV-1は，1977年に高月らによって発見された成人T細胞白血病・リンパ腫（adult T-cell leukemia：ATL）の原因ウイルスであるヒトレトロウイルスである．カリブ海諸国で熱帯性痙性麻痺（tropical spastic paraparesis：TSP）患者の一部にもHTLV-1キャリアがいることが明らかとなり，HAM/TSPとして疾患概念が確立した．HAMはHTLV-1キャリアの極一部で発症し，HTLV-1キャリア1,000人に1人位の割合でHAM患者が存在すると報告されている．発症は中年以降の成人が多いが，10歳代，あるいはそれ以前の発症と考えられる例もある．男女比は1：2.3と女性に多い．HTLV-1の感染経路として母乳を介する母子間垂直感染と，輸血，性交渉による水平感染が知られているが，そのいずれでもHAMは発症し，輸血後数週間で発症した例もある．輸血後発症するHAMの存在の指摘を受け

Topics

HAM臨床試験

a) Nishiura Y et al：Disulfide-mediated apoptosis of human T-lymphotropic virus type-I（HTLV-I）-infected cells in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis. Antivir Ther 14：533-542, 2009

b) Araya N et al：Fucoidan therapy decreases the proviral load in patients with human T-lymphotropic virus type-1-associated neurological disease. Antivir Ther 16：89-98, 2011

HAMはあくまでもHTLV-1感染症であり，その原因療法を考える場合，HTLV-1感染細胞を標的とした治療法の開発が望まれる．その場合，長期にかつ安全に投与できる薬剤でなければならない．このような観点からの新規治療法の開発をめざした日本における最近の臨床試験の成績について紹介したい．

1．prosultiamine（アリナミン®）療法

prosultiamineはアリシンとチオール型ビタミンB_1で合成されたアリチアミン誘導体の1つである．著者らは本薬剤の中に存在するSS結合に着目し検討を行った結果，prosultiamineで処理されたHAM患者末梢血CD4陽性細胞では，その中のHTLV-1感染細胞がアポトーシスによってHTLV-1プロウイルス量が有意に減少することを明らかにした．この事実を踏まえ，6例のHAM患者に対してprosultiamine 40 mg，14日間連日点滴静注を行い臨床試験を施行した．その結果，短い投与期間にもかかわらず6例全例で末梢血HTLV-1プロウイルス量は30～50%にまで減少した．臨床的には著効は1例であったが，その他の症例でも痙縮を中心に改善がみられた[a]．現在，本薬剤による長期療法をめざし，経口prosultiamineによる臨床試験が進行中である．

2．フコイダン療法

HTLV-1はフリーのウイルスの形では感染は成立せず，感染はcell to cellで伝播していく．Arayaらは，HTLV-1のこのような感染様式での生体内拡大阻止を目的として，13例のHAM患者に対して6gのフコイダンを6～13ヵ月の間，連日経口投与を行った．その結果，末梢血HTLV-1プロウイルス量を約40%減少させ，臨床的にもその間の症状の悪化はなかったと報告している[b]．

これらの報告はあくまでも臨床試験の成績であるが，このように現在いくつかの施設でHTLV-1感染細胞を標的としたHAM治療法の開発へ向けた研究がスタートしている．

て，1986年11月より日本赤十字社の献血に抗HTLV-1抗体のスクリーニングが開始され，以後，輸血後発症はなくなっている．

HTLV-1キャリアは，以前から九州・沖縄地方に多いとされてきた．しかしながら，平成21年度厚生労働科学研究班「本邦におけるHTLV-1感染及び関連疾患の実態調査と総合対策」(研究代表者：国立感染症研究所 血液・安全性研究部 山口一成)の報告[1]では，全国のキャリア数が約108万人と推定され，九州・沖縄地方のキャリアの割合が減少している一方，関東地方と近畿地方の大都市圏での増加が示され，日本のHTLV-1キャリアは依然として多数存在し，全国に拡散する傾向があることが指摘された．HAM患者はHTLV-1キャリアの全国へ拡散とともに，東京や大阪など，人口の集中する大都市では九州に匹敵する数の患者が見出されるようになった．1998年の全国調査では1,422人の患者が確認されていたが，平成21年度の前述の全国調査では人口10万人あたり3人程度(3,000人)の患者数と推定されている．この10年を通して，年間，少なくとも30人以上が新たに発症していることになる．HAMに関しては，その稀少性ゆえに病態解明や治療薬開発のための研究が進展しにくい面があったが，HAM患者およびHTLV-1キャリアの患者会などの多大な尽力により，2008年にHAMが特定疾患(難病)に指定され，難治性疾患克服研究事業の対象疾患として組織的な研究が開始されている．

A 発症機序

HAM患者ではHTLV-1キャリアに比較して末梢血におけるHTLV-1プロウイルス量が有意に多く，このことはHTLV-1感染細胞数が増加していることを意味している．病理学的には，HAM患者脊髄は胸髄全長にわたって萎縮しており，リンパ球・マクロファージの浸潤による慢性炎症が胸髄中・下部に強く認められ，その周囲に脊髄実質の軸索，髄鞘の崩壊変性がみられる，いわゆる慢性脊髄炎である[2]．HTLV-1は脊髄に浸潤しているTリンパ球のみに感染しており，そのプロウイルス量に比例して炎症が強い．以上のことから，脊髄で起こっている病態機序としては，バイスタンダーメカニズム—脊髄に浸潤してきたHTLV-1感染CD4陽性T細胞とHTLV-1特異的細胞傷害性T細胞の相互作用の結果生ずる周囲組織の破壊—が考えられている．つまり，HAMはHTLV-1感染によって惹起されるリンパ球(Th1細胞)の活性化をトリガーとして，脊髄に浸潤するHTLV-1感染CD4陽性T細胞とそれを排除させようとする細胞との凌ぎ合いが脊髄という場所で起こる結果，惹起される疾患と言える．したがって，活性化されて高い組織浸潤能をもったHTLV-1感染Th1細胞の増加がHAMの発症・病態を形成するうえで重要な役割を果たしている可能性がある[3]．

B 臨床像

基本的な臨床症状は緩徐進行性の両下肢痙性不全麻痺で，下肢筋力低下と痙性による歩行障害を示す．膝蓋腱反射，アキレス腱反射は亢進し，病的反射がみられる．通常，上肢は筋力低下などの自覚症状を欠いているが，深部腱反射は亢進していることが多い．感覚障害は運動障害に比して軽度にとどまる例が多く，しびれ感や痛みなど，自覚的なものが多い．一方，自律神経症状は高率にみられ，特に排尿困難，頻尿，便秘などの膀胱直腸障害は病初期よりみられ，主訴となることも多い．その他，進行例では下半身の発汗障害，起立性低血圧，インポテンツなども認められる．これらの症状はいずれも脊髄の傷害を示唆するものであり，HAMの中核症状となっている．それに加え，手指振戦，運動失調，眼球運動障害，あるいは軽度の痴呆を示し，病巣の広がりが想定される例もある．しかし，そのような症例でも中核症状としての両下肢痙性不全麻痺は共通に認められる．

通常は緩徐進行性で慢性に経過するが進行が早い例もみられる．高齢での発症で進行度が早い傾向があり，重症例では両下肢の完全麻痺，体躯の筋力低下による座位障害で寝たきりとなる．一方で，運動障害が軽度のまま長期にわたり症状の進行がほとんどみられない患者も多い．基本的に生命予後は良好であるが，転倒による大腿骨頸部骨折，尿路感染の繰り返しや褥瘡は予後不良の因子として重要である．

C 診断と検査

診断は，緩徐進行性で膀胱直腸障害を伴う痙性対麻痺と，抗HTLV-1抗体が血清，髄液ともに陽性であることによってなされる．髄液では軽度の異常が認められることがあるが，髄液ネオプテリンは高く，活動性炎症を反映しているとされている．末梢血のプロウイルス量は，HTLV-1キャリアに比し高値で，その変動は病勢と連動している．画像診断では，脊髄MRIで通常は正常，重症例では胸髄を中心にびまん性に萎縮していることがあり，局所性病変はみられないことが多い．しかしながら，発症後間もない症例で，びまん性の腫大やT2強調画像での髄内の強信号像が報告されている．また，大脳MRIのT2強調画像で深部白質の異常信号像がみられる例がある．

D 治療の一般方針

1 治療方針の立て方

HAMに対する根本的な治療法は確立されていない．現時点では免疫調節療法が一般的である．すなわち，明らかな症状の進行がみられ，末梢血中プロウイルス高値，髄液ネオプテリン高値などの指標より炎症の活動期と判断される症例ではこの療法が必要とされている．免疫調節療法は，HAM患者において活性化HTLV-1感染リンパ球によって引き起こされた脊髄の慢性炎症を抑制することを目的としている．その治療戦略として，①活性化HTLV-1感染リンパ球の抑制，②脊髄へのリンパ球浸潤の阻止，③種々の炎症性サイトカインやリンパ球の接着因子の制御などがターゲットとして考えられている．HAMに対する治療の中で主体をなしているのが，ステロイドホルモン療法と抗ウイルス効果も併せ持つインターフェロンα療法である．一方，炎症の活動性がほとんどないと考えられる例では，痙性対麻痺や排尿障害に対する対症療法が行われ，継続的なリハビリテーションが推奨される．

2 薬物療法の実際

a) ステロイドホルモン療法

現在でも最も使用されている薬剤である．経口prednisolone（プレドニン®）はHAM患者の7割に有効とされている．活動期のHAM患者ではprednisolone 20～30 mgの隔日投与で改善がみられることが多い．しかしながら，骨粗鬆症，糖尿病，感染症などの副作用のため長期連用が難しく，中止によりしばしば明らかな再燃がみられている．進行性のHAM患者に副腎皮質ステロイドホルモン大量投与（ソル・メドロール®）を500～1,000 mg/日，3～5日連日投与が有効な場合もある．

b) インターフェロンα療法

HAMに対して唯一医療保険適用となっている薬剤である．インターフェロンα（スミフェロン®）は免疫調整作用に加えて抗ウイルス効果を期待して，スミフェロン®の多施設無作為抽出二重盲検法による治験が行われ，HAMに対する効能が2001年1月より保険適用となっている．HAM患者の6～7割に有効とされているが，ステロイドホルモンより効果は劣る．スミフェロン® 300万単位を筋注または皮下注で28日間連日投与し，引き続いて週2～3回投与に移行するか，当初より週2～3回投与を継続する方法が試みられている．この治療でもやはり，副作用に十分注意する必要がある．発熱やうつ状態による長期間の活動性低下は運動機能の低下につながる．

3 その他の治療法

非活動期の治療は痙縮や排尿障害，便秘に対する対症的な薬物療法やリハビリテーションが重要で，腰帯筋・傍脊柱筋の筋力増強やアキレス腱の伸張により，歩行の改善が得られる．間欠自己導尿の導入により外出への不安解消や夜間頻尿による不眠の改善など，ADLの改善が期待される．

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Potential Contribution of a Novel Tax Epitope–Specific CD4$^+$ T Cells to Graft-versus-Tax Effect in Adult T Cell Leukemia Patients after Allogeneic Hematopoietic Stem Cell Transplantation

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for adult T cell leukemia/lymphoma (ATL) caused by human T cell leukemia virus type 1 (HTLV-1). We previously reported that Tax-specific CD8$^+$ cytotoxic T lymphocyte (CTL) contributed to graft-versus-ATL effects in ATL patients after allo-HSCT. However, the role of HTLV-1–specific CD4$^+$ T cells in the effects remains unclear. In this study, we showed that Tax-specific CD4$^+$ as well as CD8$^+$ T cell responses were induced in some ATL patients following allo-HSCT. To further analyze HTLV-1–specific CD4$^+$ T cell responses, we identified a novel HLA-DRB1*0101–restricted epitope, Tax155–167, recognized by HTLV-1–specific CD4$^+$ Th1-like cells, a major population of HTLV-1–specific CD4$^+$ T cell line, which was established from an ATL patient at 180 d after allo-HSCT from an unrelated seronegative donor by in vitro stimulation with HTLV-1–infected cells from the same patient. Costimulation of PBMCs with both the identified epitope (Tax155–167) and known CTL epitope peptides markedly enhanced the expansion of Tax-specific CD8$^+$ T cells in PBMCs compared with stimulation with CTL epitope peptide alone in all three HLA-DRB1*0101$^+$ patients post–allo-HSCT tested. In addition, direct detection using newly generated HLA-DRB1*0101/Tax155–167 tetramers revealed that Tax155–167-specific CD4$^+$ T cells were present in all HTLV-1–infected individuals tested, regardless of HSCT. These results suggest that Tax155–167 may be the dominant epitope recognized by HTLV-1–specific CD4$^+$ T cells in HLA-DRB1*0101$^+$–infected individuals and that Tax-specific CD4$^+$ T cells may augment the graft-versus-Tax effects via efficient induction of Tax-specific CD8$^+$ T cell responses. *The Journal of Immunology*, 2013, 190: 000–000.

Human T cell leukemia virus type 1 (HTLV-1) is the causative agent of a highly aggressive CD4$^+$ T cell malignancy, adult T cell leukemia/lymphoma (ATL) (1, 2). This virus has infected 10–20 million people worldwide, especially in southern Japan, the Caribbean basin, South America, Melanesia, and equatorial Africa (3). Approximately 5% of HTLV-1–seropositive individuals develop ATL, and another 2–3% develop a slow progressive neurologic disorder known as HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) or various chronic inflammatory diseases (4). The majority of HTLV-1–infected individuals remain asymptomatic throughout their lives.

ATL is characterized by extremely poor prognosis, mainly because of intrinsic drug resistance to cytotoxic agents. It has been reported that allogeneic hematopoietic stem cell transplantation (allo-HSCT), but not autologous HSCT, improved the outcome of ATL (5, 6). In previous clinical studies carried out by the ATL allo-HSCT Study Group, the overall survival rate within 3 y after allo-HSCT with reduced intensity conditioning (RIC) was 36% (7). HTLV-1 proviral load became and remained undetectable in some ATL patients with complete remission after allo-HSCT, suggesting that it is an effective treatment for ATL (7–9). In these studies, we reported that donor-derived HTLV-1 Tax-specific CD8$^+$ CTLs were induced in some ATL patients who achieved complete remission after allo-HSCT (10). These CTLs were able to lyse recipient-derived HTLV-1–infected T cells in vitro, suggesting potential contributions to graft-versus-leukemia effects. CD8$^+$ T cells, especially CTLs, generally play an important role in controlling viral replication in various infections, such as those

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Abbreviations used in this article: AC, asymptomatic carrier; allo-HSCT, allogeneic stem cell transplantation; ATL, adult T cell leukemia/lymphoma; HAM/TSP, HTLV-1–associated myelopathy/tropical spastic paraparesis; HTLV-1, human T cell leukemia virus type 1; ILT, IL-2–dependent T cell line; LCL, lymphoblastoid B cell line; rhIL-2, recombinant human IL-2; RIC, reduced intensity conditioning; Treg, regulatory T.

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involving HIV, hepatitis B virus, and hepatitis C virus. In HTLV-1 infection, HTLV-1–specific $CD8^+$ T cells predominantly recognize the Tax Ag and are believed to contribute to controlling infected cells (11, 12). A high frequency of functional Tax-specific $CD8^+$ T cells can be detected in HAM/TSP patients and some asymptomatic carriers (ACs), whereas most ATL patients and a small population of ACs show severely reduced Tax-specific $CD8^+$ T cell responses (13, 14). The mechanism underlying the suppression of HTLV-1–specific $CD8^+$ T cell responses in these patients has not yet been fully elucidated.

For induction and maintenance of virus-specific CTLs, virus-specific $CD4^+$ Th cell responses are required in many virus infections (15–19). However, there are only a few reports of HTLV-1–specific Th cell responses (20–23), presumably because of their susceptibility to HTLV-1 infection in vivo and in vitro (24). Preferential HTLV-1 infection in HTLV-1–specific $CD4^+$ T cells could be one of the reasons for immune suppression in ATL patients. In addition, it has been reported that a higher frequency of $CD4^+FOXP3^+$ regulatory T (Treg) cells is observed in infected individuals compared with uninfected healthy donors. The frequency of Tax^- Treg cells, which are a major population of Treg cells in infected individuals, is negatively correlated with HTLV-1–specific CTL responses (25). HTLV-1 basic leucine zipper factor might also be involved in immune suppression, because HTLV-1 basic leucine zipper was constitutively expressed in infected cells (26) and inhibited the activity of IFN-γ promoters by suppressing NFAT and AP-1 signaling pathways, resulting in the impaired secretion of Th1 cytokines from $CD4^+$ Th cells in a transgenic mouse model (27) These reports suggest that both the dysfunction of HTLV-1–specific $CD4^+$ Th cells and the increased number of uninfected Treg cells might be implicated in the immunosuppression observed in ATL patients. Conversely, in HAM/TSP patients, $CD4^+$ T cells are predominantly found in early active inflammatory spinal cord lesions (28, 29) with spontaneous production of proinflammatory, neurotoxic cytokines, such as IFN-γ and TNF-α (30), suggesting their contributions to the pathogenesis of HAM/TSP. However, the precise roles of HTLV-1–specific $CD4^+$ T cells in HTLV-1 infection remain unclear.

In some ATL patients who achieved complete remission after allo-HSCT, it has been suggested that donor-derived HTLV-1 Tax-specific CTLs may contribute to elimination of ATL cells (graft-versus-Tax effects) (10). We believe that $CD4^+$ T cells also play a critical role in the graft-versus-ATL effects because $CD4^+$ T cells are required for induction and maintenance of optimal CTL responses (15–19). It therefore is important to clarify the role of HTLV-1–specific $CD4^+$ T cells in the effects for understanding HTLV-1–specific T cell immunity in ATL patients after allo-HSCT and for developing new vaccine strategies to prevent recurrence of ATL.

Several studies have reported some HTLV-1–specific $CD4^+$ T cell epitopes restricted by different HLA haplotypes (20–23). The helper functions of these epitopes in HTLV-1–specific CTL responses in HTLV-1–infected individuals have not been well understood. However, Jacobson et al. (20) showed that $CD4^+$ T cells specific for Env gp46 196–209, an epitope restricted by HLA-DQ5 or -DRw16, exhibited a cytotoxic function by directly recognizing HTLV-1–infected cells. This observation raises the possibility that some HTLV-1–specific $CD4^+$ T cells may contribute to the graft-versus-ATL effects through their cytotoxic function in ATL patients after allo-HSCT.

In the current study, we demonstrated that both $CD4^+$ and $CD8^+$ Tax-specific T cell responses were induced in patients after allo-HSCT with RIC for ATL. To further analyze HTLV-1–specific $CD4^+$ T cell responses in ATL patients after allo-HSCT, we determined a novel HLA-DRB1*0101–resricted epitope, Tax155–167, recognized by HTLV-1–specific $CD4^+$ Th1-like cells, a major population of HTLV-1–specific $CD4^+$ T (T4) cell line, which was established from a patient in complete remission following allo-HSCT with RIC. Costimulation with oligopeptides corresponding to the Th1 epitope, Tax155–167, together with a known CTL epitope led to robust expansion of Tax-specific $CD8^+$ T cells in PBMCs from three HLA-DRB1*0101^+ patients after allo-HSCT tested. Furthermore, Tax155–167-specific $CD4^+$ T cells were found to be maintained in all HTLV-1–infected HLA-DRB1*0101^+ individuals tested, regardless of HSCT, by direct detection with newly generated HLA-DRB1*0101/Tax155–167 tetramers. Our results suggest that Tax155–167 may be a dominant epitope recognized by HTLV-1–specific $CD4^+$ T cells in HTLV-1–infected individuals carrying HLA-DRB1*0101 and that Tax-specific $CD4^+$ T cells may strengthen the graft-versus-ATL effects through efficient induction of Tax-specific CTL responses.

Materials and Methods

Subjects

A total of 18 ATL patients who underwent allo-HSCT with RIC regimen, and one HTLV-1–seronegative (#365) and two seropositive donors (one AC #310 and one HAM/TSP patient #294) carrying HLA-DRB1*0101 donated peripheral blood samples after providing written informed consent. Approximately one-half of these patients received allogeneic peripheral blood stem cell transplantation from HLA-A-, B-, and -DR–identical sibling donors. The other half received allogeneic bone marrow cells from HLA-A–, B–, and DR–identical seronegative unrelated donors (Table I). These patients were the participants of clinical studies organized by the ATL allo-HSCT Study Group, supported by the Ministry of Health, Welfare, and Labor of Japan. This study was also reviewed and approved by the Institutional Ethical Committee Review Board of the Tokyo Medical and Dental University.

Generation of cell lines derived from patients and donors

PBMCs were isolated using Ficoll-Paque PLUS (GE Healthcare, Buckinghamshire, U.K.) density gradient centrifugation and stored in liquid nitrogen in Bambanker stock solution (NIPPON Genetics, Tokyo, Japan) until required. These were used in part to obtain HTLV-1–infected IL-2–dependent T cell lines (ILT) and EBV-transformed lymphoblastoid B cell lines (LCL). ILT-#350 was spontaneously immortalized during long-term culture of PBMCs from patient #350 before allo-HSCT and maintained in RPMI 1640 medium (Life Technologies, Grand Island, NY) containing 20% FCS (Sigma Aldrich, St. Louis, MO) and 30 U/ml recombinant human IL-2 (rhIL-2; Shionogi, Osaka, Japan). LCL-#307, -#341, and -#350 were established by maintaining PBMCs from ATL patients #307, #341, and #350, respectively, after allo-HSCT. These PBMCs were maintained in RPMI 1640 medium containing 20% FCS, following infection with the EBV-containing culture supernatant of the B95-8 cell line, LCL-Kan, derived from a healthy individual was also used.

Synthetic peptides

A total of 18 overlapping peptides, 12- to 25-mer in length, spanning the central region of Tax (residues 103–246) were purchased and used for epitope mapping (Scrum Tokyo, Japan) (Table II). HLA-A*2402–restricted CTL epitopes (Tax301–309, SFHSLHLLF) (10) were used for in vitro stimulation of Tax-specific CTLs (Hokudo, Sapporo, Japan).

GST–Tax fusion protein–based immunoassay

HTLV-1 Tax-specific T cell responses were evaluated using GST–fusion proteins of the N-terminal (residues 1–127), central (residues 113–237), and C-terminal (residues 224–353) regions of HTLV-1 Tax (GST–Tax-A, -B, and -C, respectively) as described previously (13, 31). PBMCs (1×10^6 cells/ml) were incubated with or without a mixture of GST–Tax-A, -B, and -C proteins (GST–TaxABC) in 200 μl RPMI 1640 medium supplemented with 10% FCS. After 4 d, the supernatant was collected, and the concentration of IFN-γ in the supernatant was determined using an OptiEIA Human IFN-γ ELISA Kit (BD Biosciences, San Jose, CA). The minimum detectable dose for this assay was determined to be 23.5 pg/ml IFN-γ. $CD8^+$ cells were depleted from PBMCs by negative selection using Dynabeads M-450 CD8 (Invitrogen, Carlsbad, CA), according to the

manufacturer's instructions. For cytokine profiling of a HTLV-1–specific $CD4^+$ T cell line, cells were stimulated with formaldehyde-fixed ILT-#350 for 48 h. Culture supernatant was collected, and various cytokines were measured using a Human Th1/Th2/Th17 Cytokine Kit for a Cytokine Beads Array (BD Biosciences).

Induction of HTLV-1–specific $CD4^+$ T cell line (T4 cells)

PBMCs (1×10^6 cells/ml) from patient #350, in complete remission at 180 d after allo-HSCT, were cultured for 2 wk with 100 nM Tax301–309 peptide in 96-well round-bottom tissue culture plate (BD Biosciences) in a final volume of 200 μl RPMI 1640 medium with 20% FCS and 10 U/ml rhIL-2. $CD4^+$ cells were then isolated by negative selection using a Human CD4 T lymphocyte Enrichment Set-DM (BD Biosciences) and maintained in RPMI 1640 medium with 20% FCS and 100 U/ml rhIL-2. Cells (1×10^6 cells/ml) were stimulated with formaldehyde-fixed ILT-#350 (2.5×10^5 cells/ml) every 2–3 wk. After multiple rounds of stimulation, the resulting $CD4^+$ T cell line was assessed for HTLV-1 specificity by comparing IFN-γ production against ILT-#350 to that against an HTLV-1–negative cell line, LCL-#350.

RT-PCR

Total RNA from cells was isolated using Isogen (Nippon Gene, Tokyo, Japan) and Turbo DNA-free (Life Technologies). First-strand cDNA was prepared from 0.5 μg RNA using ReverTra Ace and $Oligo(dT)_{20}$ primers provided in a ReverTra Ace-α-kit (Toyobo, Osaka, Japan). PCRs were performed in 50 μl reaction mixture containing ReverTra Dash (Toyobo), 0.5 μM of each HTLV-1 pX-specific primer (pX1, 5′-CCA CTT CCC AGG GTT TAG ACA GAT CTT C-3′ and pX4, 5′-TTC CTT ATC CCT CGA CTC CCC TCC TTC CCC-3′), and 2 μl cDNA. GAPDH-specific primers (GAPDH5′, 5′-ACC ACA GTC CAT GCC ATC AC-3′; GAPDH3′, 5′-TCC ACC ACC CTG TTG CTG TA-3′) were used as an internal control. The thermal cycling conditions comprised an initial activation step at 94°C for 1 min, followed by 30 cycles of denaturation (98°C, 10 s), annealing (60°C, 2 s), and extension (74°C, 30 s). The PCR amplicons were visualized by ethidium bromide staining following 2% (w/v) agarose gel electrophoresis.

Flow cytometry

For cell surface staining, the following fluorochrome-conjugated mouse anti-human mAbs were used: CD3-FITC (UCHT1; BioLegend, San Diego, CA), CD4-FITC (RPA-T4; BioLegend), CD8-FITC (RPA-T8; BioLegend), and CD8-PE-Cy5 (HIT8a; BD Biosciences, San Jose, CA). For tetramer staining, PE-conjugated HLA-A*0201/Tax11–19, HLA-A*1101/Tax88–96, HLA-A*1101/Tax272–280, and HLA-A*2402/Tax301–309 tetramers were purchased from Medical & Biological Laboratories (Nagoya, Japan). PE-conjugated HLA-DRB1*0101/Tax155–167 tetramer were newly generated through the custom service of Medical & Biological Laboratories. Whole-blood or cultured cells were stained with PE-conjugated Tax/HLA tetramer in conjunction with CD3-FITC and CD8-PE-Cy5 or CD4-PE-Cy5. For whole-blood samples, RBCs were lysed and fixed in BD FACS lysing solution (BD Biosciences) before washing. Samples were analyzed on a FACSCalibur (BD Biosciences), and data analyses were performed using FlowJo software (Tree Star, Ashland, OR).

Epitope mapping

T4 cells (3×10^5 cells/ml) were stimulated with LCL-#350, pulsed with various concentrations of synthetic peptides for 1 h at 37°C, at a responder/stimulator (R/S) ratio of 3. The culture supernatant was collected at 6 h poststimulation, and peptide-specific IFN-γ production from T4 cells was determined by ELISA.

HLA class II restriction assay

T4 cells (5×10^5 cells/ml) were cocultured for 6 h with ILT-#350 (1×10^5 cells/ml) in the presence or absence of anti-human HLA-DR (10 μg/ml; L243; BioLegend), anti-human HLA-DQ (10 μg/ml; SPVL3; Beckman Coulter, Fullerton, CA), or anti–HLA-ABC (10 μg/ml; W6/32; BioLegend). The IFN-γ in the supernatant was measured by ELISA.

To identify a HLA class II molecule responsible for Ag presentation to T4 cells, Tax155–167 peptide-specific IFN-γ responses were evaluated using various HLA-typed LCLs (LCL-#350, LCL-#341, LCL-#307, and LCL-Kan). These LCLs (1×10^5 cells/ml) were pulsed with 100 ng/ml Tax155–167 peptide for 1 h, fixed with 2% formaldehyde, and then cultured with T4 cells (3×10^5 cells/ml) for 6 h. The culture supernatant was collected, and IFN-γ in the supernatant was measured by ELISA.

Tetramer-based proliferation assay

PBMCs (1.0×10^6 cells/ml) were cultured for 13 or 14 d with or without 100 nM antigenic peptides in the presence of 10 U/ml rhIL-2. Cells were stained with HLA/Tax tetramer-PE, CD3-FITC, and CD8-PE-Cy5 or CD4-PE-Cy5 and then analyzed by flow cytometry.

Statistic analysis

Statistical significance was evaluated with the unpaired t test using Graphpad Prism 5 (Graphpad Software, La Jolla, CA). In all cases, two-tailed p values <0.05 were considered significant.

Results

Tax-specific T cell responses in ATL patients who received allo-HSCT with RIC

We previously reported that Tax-specific $CD8^+$ T cells were induced in some ATL patients after allo-HSCT with RIC from HLA-identical sibling donors (10). In this study, we examined the Tax-specific T cell response in a larger number of ATL patients who received allo-HSCT with RIC. Table I provides a summary of the

Table I. Clinical information and summary for Tax-specific $CD8^+$ T cells in 18 ATL patients at 180 d post–allo-HSCT with RIC

ATL Subtype	Type of Donor	Donor-HLA	Donor HTLV-1 Sero Status	Chimerism (%)[a]	Tetramer (%)[b]	Proviral Load[c]
Lymphoma	r-PB	A 26/33, DR 4/13	(−)	<5	NT	0.1
Acute	r-PB	A 2/26, DR 10/18	(−)	<5	0.00	0.1
Lymphoma	r-PB	A 24/−, DR 9/15	(−)	<5	0.07	0.1
Lymphoma	r-PB	A 24/33, DR 13/15	(−)	<5	0.00	0.0
Lymphoma	r-PB	A 24/26, DR 4/15	(+)	<5	1.34	4.8
Acute	ur-BM	A 24/33, DR 13/15	(−)	<5	0.72	0.0
Acute	r-PB	A 2/11, DR 14/15	(+)	<5	0.10	5.4
Acute	ur-BM	A 2/24, DR 14/15	(−)	<5	0.92	0.0
Acute	ur-BM	A 11/24, DR 8/9	(−)	<5	0.75	NT
Acute	r-PB	A 2/24, DR 4/8	(−)	<5	1.40	0.7
Acute	ur-BM	A 24/33, DR 1/15	(−)	<5	0.45	0.1
Lymphoma	ur-BM	A 2/24, DR 4/−	(−)	<5	0.44	0.0
Acute	r-PB	A 24/−, DR 8/15	(+)	<5	0.00	0.0
Acute	ur-BM	A 24/26, DR 1/14	(−)	<5	0.59	0.6
Acute	ur-BM	A 24/26, DR 9/12	(−)	<5	0.45	0.0
Lymphoma	r-PB	A 2/11, DR 4/14	(−)	<5	0.42	0.0
Acute	ur-BM	A 11/26, DR 8/15	(−)	<5	0.14	0.0
Acute	r-PB	A 24/26, DR 1/−	(−)	<5	0.11	0.0

[a]Indicates percentage of recipient-derived T cell chimerism.
[b]Indicates percentage of $tetramer^+$ cells among $CD8^+$ T cells in PBMCs.
[c]Indicates copy number per 1000 PBMCs.
F, Female; M, male; NT, not tested; r-PB, related donor-derived peripheral blood stem cell; ur-BM, unrelated donor-derived bone marrow cell.

results of Tax-specific $CD8^+$ T cell detection by flow cytometry, using the Tax/HLA tetramers, in the peripheral blood of 18 ATL patients at 180 d after allo-HSCT, together with clinical information. During this period, all patients achieved a complete chimera state consisting of >95% of donor-derived hematopoietic cells. By using four available tetramers (HLA-A*0201/Tax11–19, HLA-A*2402/Tax301–309, HLA-A*1101/Tax88–96, and HLA-A*1101/Tax272–280), Tax-specific $CD8^+$ T cells were found in 14 patients. Because the donors were uninfected individuals in the majority of cases (Table I), induction of the Tax-specific donor-derived $CD8^+$ T cells in recipients indicated the presence of newly occurring immune responses against HTLV-1 in the recipients. This evidence strengthens our previous observation (10, 32).

We also used a GST–Tax fusion protein-based assay to evaluate Tax-specific T cell responses. The tetramer-based assay was limited to four kinds of epitopes and restricted by three HLA alleles but did not detect T cells directed to other epitopes or HLAs. The GST–Tax fusion protein-based assay can detect both $CD4^+$ and $CD8^+$ T cell responses, irrespective of HLA types. However, this sensitivity is not as good as single-cell analysis by flow cytometry (31). As shown in Fig. 1A, there was a wide variation in the IFN-γ responses to the Tax protein in the PBMCs among the 16 patients tested. In five patients (#247, #270, #328, #340, and #349), IFN-γ production of PBMCs against GST–TaxABC proteins was very low or not specific for the Tax protein. PBMCs from the other 11 patients (#239, #241, #301, #317, #341, #344, #350, #351, #352, #358, and #364) produced higher amounts of IFN-γ in response to GST–TaxABC proteins compared with GST. However, the levels of IFN-γ production varied among the patients.

We also evaluated the extent to which Tax-specific $CD4^+$ T cells were responsible for IFN-γ in the GST–Tax-based immunoassay system. We used PBMCs from patients #350 and #341, who showed high Tax-specific T cell responses. $CD8^+$ cell–depleted PBMCs from patient #350 and #341 showed a reduced but still significant level of Tax-specific IFN-γ–producing response compared with whole PBMCs (Fig. 1B). These results indicate that not only $CD8^+$ but also $CD4^+$ T cells against Tax are present in the peripheral blood from patient #350 and #341 after allo-HSCT with RIC.

Induction of an HTLV-1–specific $CD4^+$ T cell line from patient #350

We next attempted to induce HTLV-1–specific $CD4^+$ T cells from the PBMCs of patient #350 at 180 d after allo-HSCT, using an HTLV-1–infected T cell line (ILT-#350) as APCs. Freshly isolated PBMCs were stimulated for 2 wk with Tax301–309, a dominant CTL epitope presented by HLA-A*2402, to eliminate HTLV-1–infected cells, which potentially existed in PBMCs. The $CD4^+$ cells were then isolated from the cultured cells and stimulated with formaldehyde-fixed ILT-#350 every 2–3 wk. The established cell line was found to be a $CD4^+$ T cell line (designated as T4 cells thereafter) because cells expressed CD3 and CD4 but not CD8

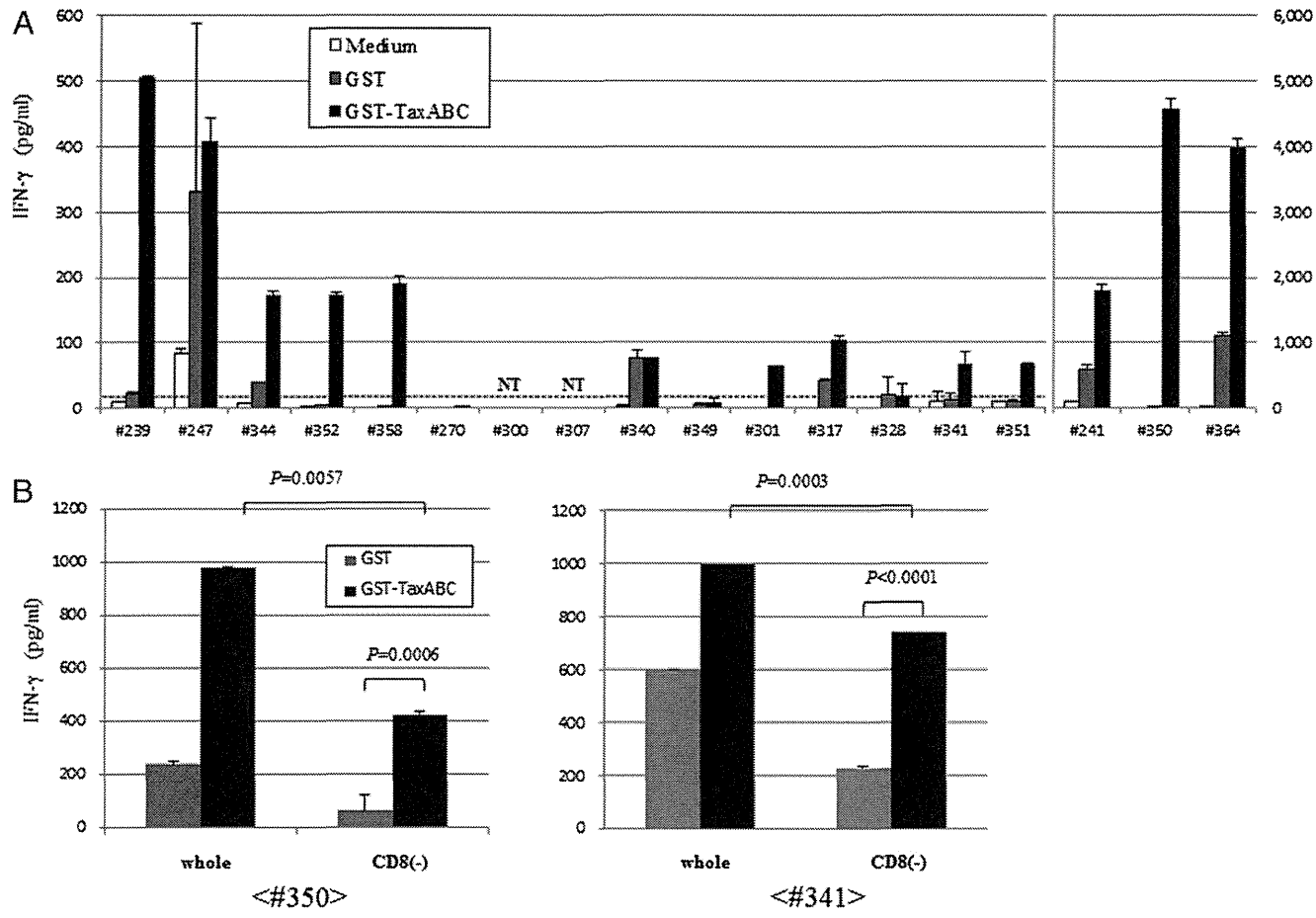

FIGURE 1. Diversity of Tax-specific T cell responses in ATL patients who received allo-HSCT with RIC. (**A** and **B**) PBMCs from 18 ATL patients at 180 d after allo-HSCT (A) or whole and $CD8^+$ cell–depleted PBMCs from two patients at 540 d after allo-HSCT (#350 and #341) (B) were cultured for 4 d in the absence (open square) or presence of GST (gray square), or GST–Tax (black square) proteins. The concentration of IFN-γ in the supernatant was determined by ELISA. The *y*-axis on the right side indicates the results from three patients (#241, #350, and #364). The dotted horizontal line indicates the detection limit (23.5 pg/ml). The error bars represent SD of duplicated wells. The representative result of two independent experiments is shown in (B).

(Fig. 2A). Because HTLV-1 has been shown to preferentially infect $CD4^+$ T cells in vivo and in vitro (24), we examined HTLV-1 expression in T4 cells by RT-PCR (Fig. 2B). As expected, the T4 cells did not express HTLV-1 Tax, indicating that the cells were not infected with HTLV-1. We assessed expression of various cytokines in T4 cells (Fig. 2C). The T4 cells were stimulated with formaldehyde-fixed ILT-#350 or LCL-#350. The cells produced large amounts of IFN-γ and TNF-α and small amounts of IL-2, IL-4, and IL-10 in response to ILT-#350 but not against LCL-#350. IL-6 and IL-17A were not detected in the culture supernatant. These data indicate that T4 cells are mainly HTLV-1–specific $CD4^+$ Th1-like cells but contain minor populations to produce Th2 cytokines.

Determination of the minimum epitope recognized by T4 cells

Freshly isolated PBMCs in the patient #350 produced IFN-γ in response to GST–Tax (Fig. 1A). We expected that the epitope recognized by the T4 cells should be present in the Tax protein. We therefore examined whether the T4 line responded to Tax using LCL-#350 pulsed with GST–Tax proteins as APCs. As shown in Fig. 3A, the T4 cells produced significantly higher amounts of IFN-γ in response to GST–TaxABC and GST–Tax-B (residues 113–237) (31) but not GST–Tax-A (residues 1–127) (31) and -C (residues 224–353 (31), when compared with the GST control protein, indicating that the T4 cells recognized the central region (residues 113–237) of the Tax Ag. We next synthesized eight overlapping 25-mer peptides spanning the central region of Tax (residues 103–246) and analyzed their abilities to stimulate T4 cells (Table II). The cell line produced high amounts of IFN-γ only when stimulated with Tax154–178 (Fig. 3B). We then prepared four overlapping 15-mer peptides, covering residues 154–178 of Tax, to examine the IFN-γ responses of the T4 cells (Table II). Both Tax151–165 and Tax156–170-stimulated cells to induce IFN-γ responses but not at a comparable level to Tax154–178 (Fig. 3C). These results suggest that the epitope recognized by T4 cells might be present in the N-terminal half of Tax154–178. We therefore stimulated the cells with Tax154–168, Tax155–169, or Tax156–170. The cells showed higher IFN-γ responses against Tax154–168 and Tax155–169 than Tax156–170, indicating that the minimum epitope might be within residues 155–168 of Tax (Fig. 3D). To identify the minimum epitope recognized by T4 cells, we next synthesized three overlapping peptides of 12- to 14-mer lengths beginning at residue 155 of Tax (Table II). Tax155–167 induced IFN-γ responses in cells at a similar level to Tax155–169 and Tax155–168, although Tax155–166 did not (Fig. 3E). Moreover, IFN-γ production of cells in response to various concentrations of Tax155–167 was comparable to that against Tax155–169 and Tax155–168 (Fig. 3F). These data clearly show that the minimum epitope recognized by the T4 cells is Tax155–167.

*HLA-DRB1*0101 restriction of Tax-specific T4 cells*

To analyze HLA class II molecules involved in the presentation of the minimum epitope, T4 cells were stimulated with ILT-#350 in the presence or absence of anti–HLA-DR, -DQ, and anti-HLA class I blocking Abs. As shown in Fig. 4A, the addition of an anti–HLA-DR blocking Ab abrogated IFN-γ responses of the T4 cells against ILT#-350, indicating that the epitope was HLA-DR restricted.

We further investigated the HLA-DR alleles responsible for the presentation of the minimum epitope by using four HLA-typed LCLs displaying different HLA-DRs. As shown in Fig. 4B, the T4 cells responded by producing IFN-γ when Tax155–167 was presented by autologous LCL-#350 (DR1/14) and allogeneic LCL-#341 (DR1/15). These results clearly indicate that this epitope is presented by HLA-DRB1*0101 on APCs. We searched for a known HLA-DRB1*0101 motif in the identified epitope Tax155–167 and found that this epitope contained the HLA-DRB1*0101 motif (Fig. 4C) (33).

Enhancement of Tax-specific $CD8^+$ T cell expansion by Tax155–167-specific $CD4^+$ T cell help

As T4 cells were established from PBMCs of an HTLV-1–infected patient #350, it is suggested that Tax155–167-specific $CD4^+$ T cells may be maintained in the HLA-DRB1*0101$^+$ patient #350.

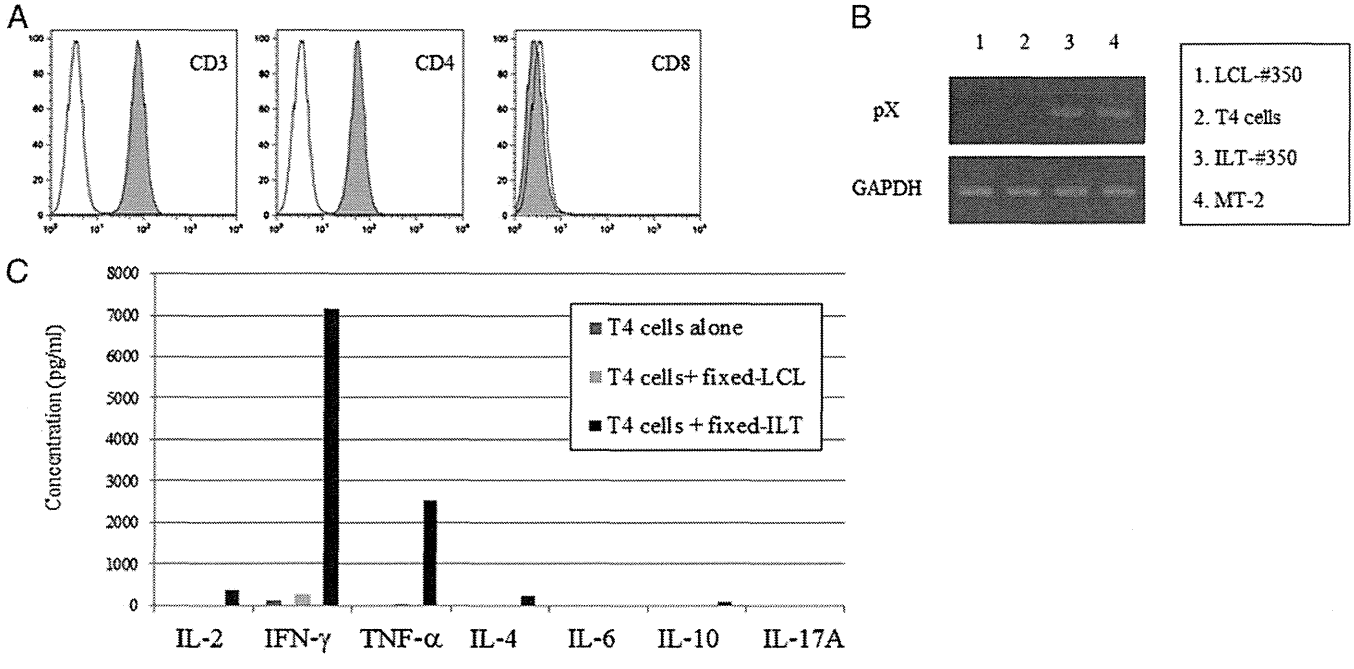

FIGURE 2. Phenotype and function of $CD4^+$ T cell line (T4) generated from patient #350. (**A**) Cell surface phenotype of T4 cells was analyzed by flow cytometry. (**B**) Total RNA was extracted from LCL-#350 (*lane 1*), T4 cells (*lane 2*), ILT-#350 (*lane 3*), and MT-2 (*lane 4*). Tax mRNA expression for each cell type was analyzed by RT-PCR. GAPDH was used as an internal control. (**C**) T4 cells were stimulated for 24 h with or without formaldehyde-fixed ILT-#350 or LCL-#350 cells. The concentration of indicated cytokines in the supernatants was measured using a cytometric bead array system.

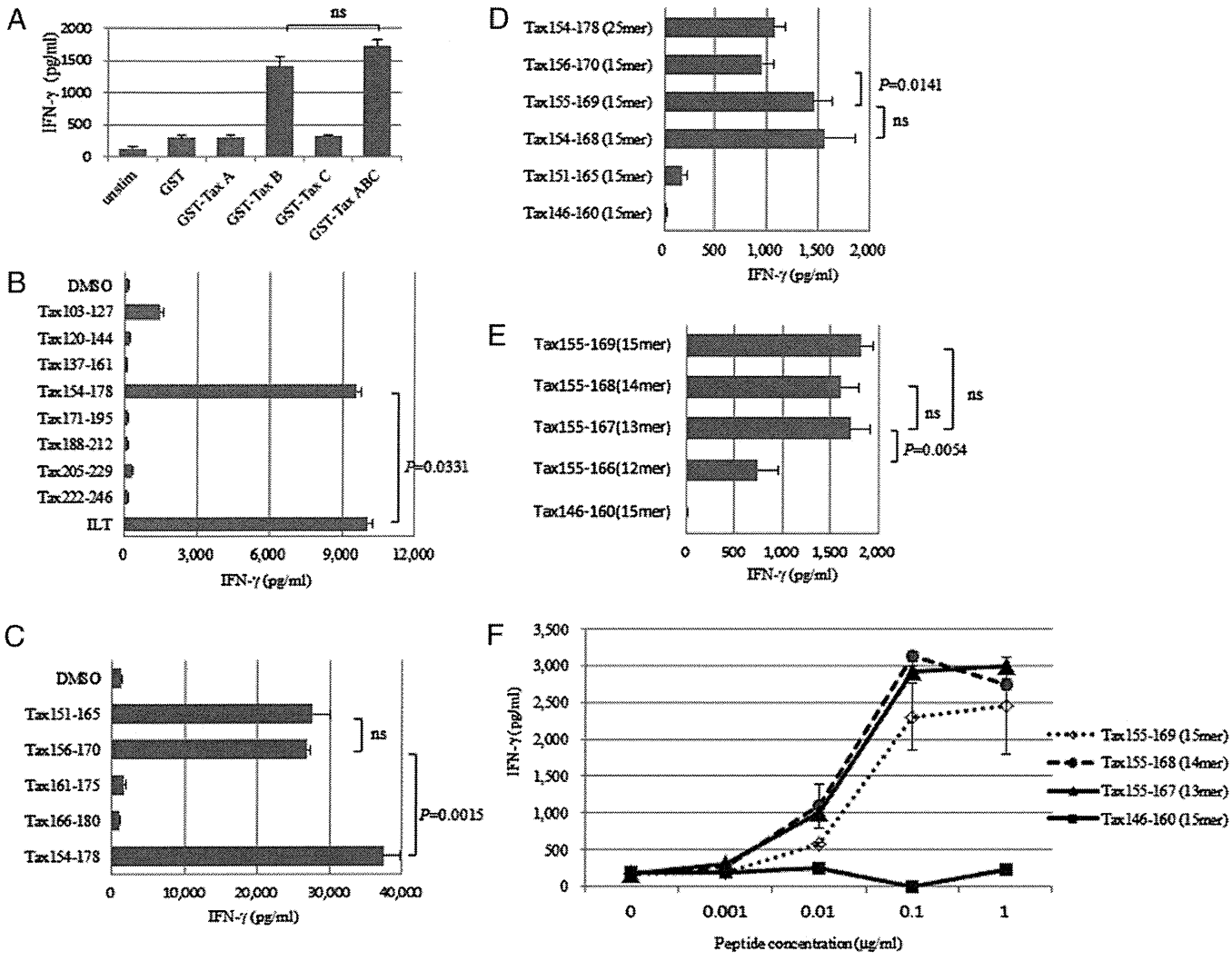

FIGURE 3. Identification of the dominant Tax-derived epitope recognized by established T4 cells. (**A**) Donor-derived LCL-#350 was pulsed with GST, GST–Tax-A, GST–Tax-B, GST–Tax-C, or a mixture of GST–Tax-A, -B, and -C (GST–TaxABC) for 24 h and then cocultured for 24 h with the T4 cells at a responder/stimulator (R/S) ratio of 3. IFN-γ production from T4 cells was analyzed by ELISA. (**B** and **C**) LCL-#350 was pulsed with the indicated overlapping 25-mer-long (B) or 15-mer-long (C) synthetic peptides (10 μg/ml) within the Tax-B region for 1 h. Formaldehyde-fixed ILT-#350 cells were cocultured with T4 cells for 6 h. IFN-γ in the supernatant was measured by ELISA. (**D** and **E**) IFN-γ responses of T4 cells were assessed using the indicated overlapping 12- to 25-mer-long synthetic peptides (100 ng/ml). (**F**) IFN-γ responses of T4 cells against indicated concentrations of 13- to 15-mer-long peptides were assessed as in (B) and (C). (A–F) Results are representative of two or three independent experiments. The error bars represent SD of triplicate wells. Statistical significance was analyzed by the unpaired *t* test.

We therefore evaluated the helper function of Tax155–167-specific $CD4^+$ T cells on the expansion of dominant Tax-specific CTLs in fresh PBMCs of the patient #350. Freshly isolated PBMCs from patient #350 (A24/26, DR1/14) at 540 d after allo-HSCT were stimulated for 13 d with the HLA-A24–restricted CTL epitope peptide (Tax301–309) in the presence or absence of the HLA-DRB1*0101–restricted $CD4^+$ Th epitope peptide (Tax155–167), and Tax-specific $CD8^+$ T cell expansion was evaluated using the HLA-A*2402/Tax301–309 tetramer. As shown in Fig. 5, Tax301–309-specific $CD8^+$ T cells proliferated to 9.26% of $CD8^+$ T cells when stimulated with Tax301–309 alone. Surprisingly, a highly elevated frequency (62.3%) of tetramer-binding $CD8^+$ T cells was detected by in vitro costimulation with Tax301–309 and Tax155–167, suggesting the presence of Tax155–167-specific $CD4^+$ Th cells in patient #350.

We examined whether Tax155–167-specific $CD4^+$ T cells existed and functioned as helper cells in the other two HTLV-1–infected HLA-DRB1*0101^+ patients after allo-HSCT (day 360 for patient #341 and day180 for #364). These patients had detectable levels of HLA-A*2402/Tax301–309 tetramer-binding $CD8^+$ T cells in the peripheral blood (Fig. 5). In patients #341 and #364, the tetramer-binding cells expanded to 7.7 and 0.849% of $CD8^+$ T cells at 13 d of culture when stimulated with the CTL epitope peptide, Tax301–309, alone. Costimulation of PBMCs with both peptides Tax155–167 and Tax301–309 led to a vigorous proliferation of tetramer-binding $CD8^+$ T cells (59.6% for patient #341 and 15.5% for patient #364) as observed in patient #350 (Fig. 5). These results indicate that Tax155–167-specific $CD4^+$ T cells may be present and contribute to enhancing $CD8^+$ T cell responses in HTLV-1–infected HLA-DRB1*0101^+ individuals after allo-HSCT.

*Tax155–167-specific $CD4^+$ T cells were maintained in HTLV-1–infected HLA-DRB1*0101^+ individuals*

We next generated the HLA-DRB1*0101/Tax155–167 tetramer to directly detect Tax155–167-specific $CD4^+$ T cells and examined the presence of Tax155–167-specific $CD4^+$ T cells in the PBMCs freshly isolated from two HLA-DRB1*0101^+ patients after allo-HSCT (day 180 for patient #350 and day 360 for patient #364).