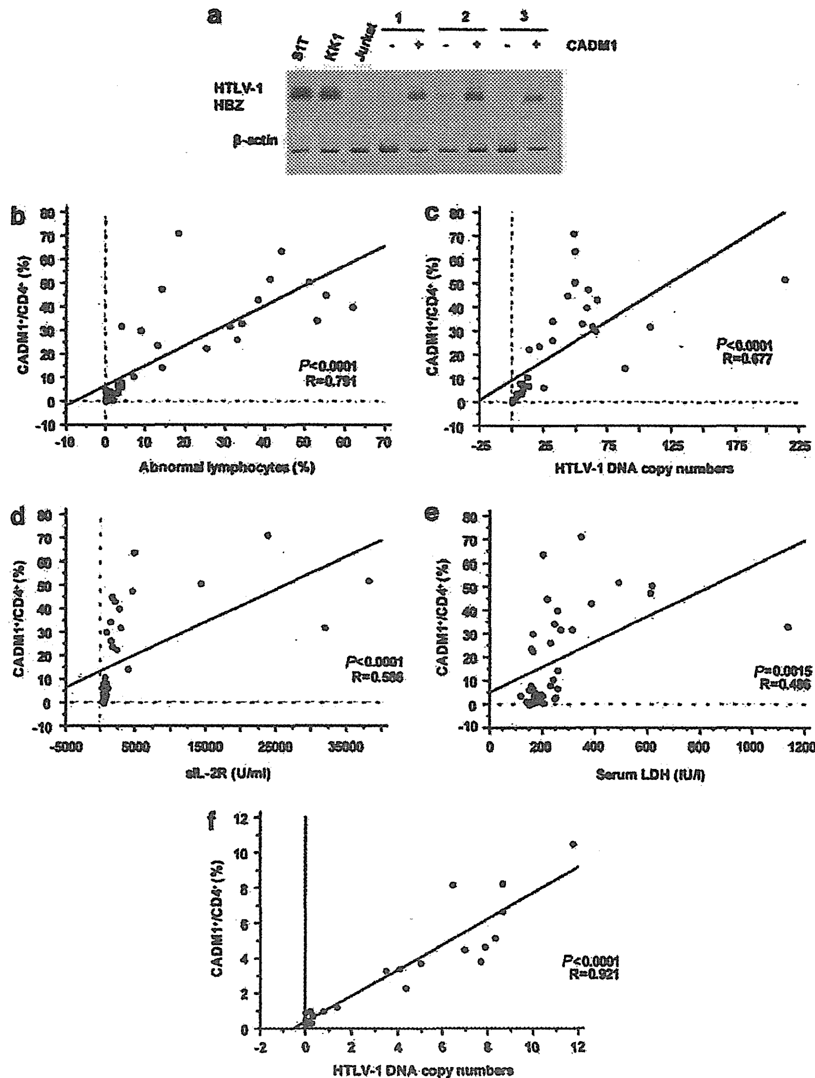

Figure 3. Correlation of the percentages of the CD4$^+$CADM1$^+$ fraction with the percentages of abnormal lymphocytes, HTLV-1 DNA copy number and the levels of soluble IL-2Rα and serum LDH in both various types of patients with ATLL and HTLV-1 carriers. (**a**) Identification of the HTLV-1 genome by PCR amplification after separation by CADM1-magnetic beads. After separation of the peripheral blood of three ATLL patients by magnetic beads, genomic DNA was extracted from both the CADM1 and non-CADM1 fractions and amplified by specific PCR primers for HTLV-1 *HBZ*. Two ATLL cell lines (S1T and KK1) were used as positive controls, and a T-ALL cell line (Jurkat) was used as a negative control for the HTLV-1 *HBZ*. Lane 1, smoldering ATLL; lane 2, chronic ATLL; lane 3, HTLV-1 carrier. (**b**-**e**) The percentage of the CD4$^+$CADM1$^+$ fraction was compared with the percentage of abnormal lymphocytes, the HTLV-1 DNA copy number and the levels of soluble IL-2Rα and serum LDH in both various types of patients with ATLL and HTLV-1 carriers. In (**d**), data from one acute-type patient were not included in the analysis because of the extremely high levels of soluble IL-2Ra (CD4$^+$CADM1$^+$, 32.9%; IL-2Ra, 96 900 U/ml). (**f**) The percentage of the CD4$^+$CADM1$^+$ fraction was compared with the HTLV-1 DNA copy number in HTLV-1 carriers.

of ATLL patients, we compared the serum levels of soluble CADM1 and soluble IL-2Rα in individual cases. As shown in Figure 4b, significantly higher levels of soluble CADM1 were detected in the serum of ATLL patients who had increased levels of soluble IL-2Rα; thus, serum CADM1 levels may be a diagnostic tool for the prediction of disease progression in ATLL.

High expression of CADM1 in ATLL-derived lymphomas

To examine the expression of CADM1 in tissue sections from lymphoma-type ATLL, formalin-fixed lymphoma samples from different types of malignant lymphomas were immunostained with the anti-CADM1 antibody. For these studies, we used a monoclonal antibody (1-10C) raised against the recombinant extracellular domain of the CADM1 protein. To confirm the reactivity of the anti-CADM1 antibody in formalin-fixed ATLL cells, cell pellets from various leukemia cell lines were fixed in 10% formalin, embedded in paraffin and stained for CADM1. The anti-CADM1 antibody specifically stained the surface of the CADM1-positive S1T-ATLL cell line but did not react with the CADM1-negative ED-ATLL and all non-ATLL cell lines (Figure 5a, panels 1 and 2, and Supplementary Figure 5a). Western blot analysis confirmed the lack of CADM1 expression in these cell lines (Figure 1a and Supplementary Figure 5b). We next performed immunostaining of lymph node biopsies from ATLL patients with malignant lymphoma using the anti-CADM1 antibody. As positive controls, we used erythrocytes and peripheral nerve tissue (Figure 5a, panels 3 and 4).[17,18] In addition, we examined CADM1

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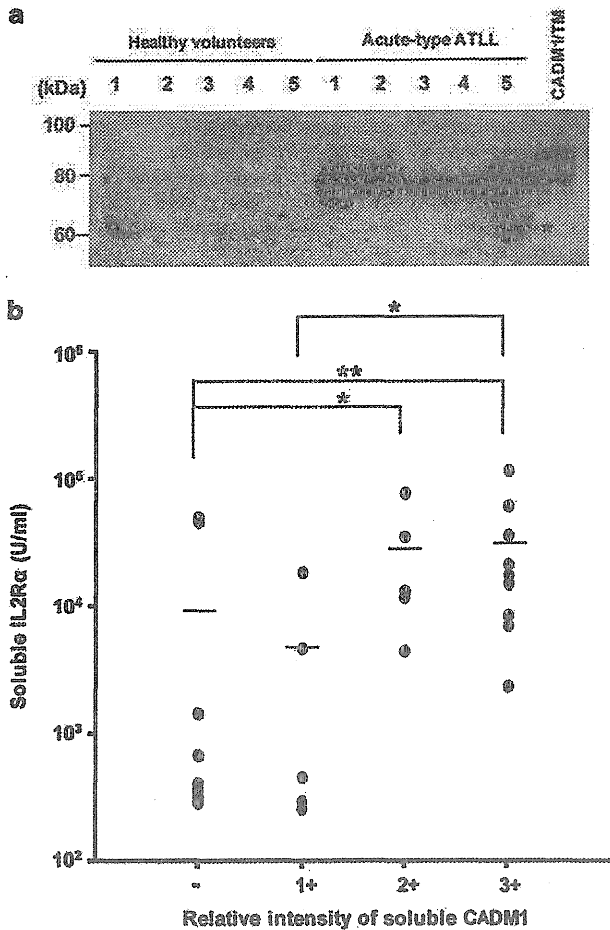

Figure 4. Identification of a soluble form of CADM1 in ATLL patients. (**a**) The soluble form of CADM1 in the peripheral blood from five healthy volunteers and five patients with acute-type ATLL was identified by immunoblot analysis using an anti-CADM1 antibody. The asterisk indicates an albumin band. Truncated CADM1 with an extracellular domain was purified from the culture supernatant of 293 cells after transfection of the CADM1 expression plasmid as a positive control. (**b**) The relative band intensity of CADM1 by immunoblot was compared with the level of sIL-2Rα in various serum samples from healthy volunteers, HTLV-1 carriers and ATLL patients. The band intensity was measured by the Image Gauge software (Fujifilm, Tokyo, Japan). The signal intensities were classified as either high (3+), medium (2+), low (1+) or undetectable (-). Asterisks indicate a significant difference between the band intensities of the groups (*$P<0.001$, **$P<0.0001$).

expression in three cases of lymph nodes with reactive follicular and/or paracortical hyperplasia (reactive lymph nodes) and found that most of the lymphocytes in the reactive lymph nodes were negatively stained and <1% of the cells were positively stained (Figure 5a, panel 5). The staining pattern of the CADM1-positive cells in the reactive lymph nodes mainly shows a uniform cytoplasmic pattern rather than the specific membranous staining that was seen in ATLL cells (as shown below and in Figure 1b). The CADM1-positive cells in reactive lymph node possibly correspond to histiocytes, including dendritic cells because a subset of T-cell zone dendritic cells was reported to express CADM1 (Necl-2) within the lymph node.[24,25] We examined 90 tissue samples from patients with various types of lymphoma, including 36 patients with ATLL and 54 with non-ATLL lymphomas, using erythrocytes and nerve fascicles as positive controls. Of the non-ATLL samples, 29 cases were T- or NK-cell lymphomas, 37 cases were B-cell lymphomas and 2 cases were null-cell lymphomas. Using a four-grade scale to score CADM1 immunohistochemical staining (0 to 3+, Figure 5b), we found that 92% of ATLL lymphomas were positive for CADM1, and 50% of them were heavily stained and were scored 2+ or higher (Table 1). Of note, a few lymphoma cells showed diffuse cytoplasmic staining in addition to membrane staining with CADM1. Among the non-ATLL lymphomas, a few CADM1-positive cells were observed, the morphology of which was small to medium in size with normochromatic round to ovoid nuclei and lacking nuclear atypia (Figure 5c). Based on the morphology and the CADM1-staining patterns, the CADM1-positive cells in the non-ATLL lymphomas were not considered as lymphoma cells but may correspond to histiocytes, including dendritic cells, because these cells were similar to the CADM1-positive cells found in reactive lymph nodes (Figure 5a, panel 5 and Figure 5c). Based on these results, a high degree of cell membrane staining for CADM1 with a score of 2+ may provide high specificity in the diagnosis of ATLL, and combined staining with CADM1 and other T-cell-specific markers may be necessary for a more accurate diagnosis of lymphoma-type ATLL.

DISCUSSION

In this study, we made a series of antibodies against CADM1 to be used as diagnostic tools for ATLL, such as for the identification and separation of ATLL and HTLV-1-infected cells, the detection of the soluble form of CADM1 in peripheral blood and the pathological identification of lymphoma-type ATLL after formalin fixation. Expression of CADM1 by flow cytometry was clearly detected on the surface of ATLL cells and HTLV-1-infected T lymphocytes, which was confirmed by detection of the HTLV-1 genome after separation by magnetic beads with a CADM1 antibody. The percentage of $CD4^+CADM1^+$ cells in the peripheral blood correlated highly with the DNA copy number of HTLV-1 in lymphocytes from HTLV-1 carriers and ATLL patients. In particular, we identified the soluble form of the CADM1 protein in the peripheral blood of HTLV-1 carriers and ATLL patients. The definitive diagnosis of ATLL is based on the confirmation of ATLL cells in the peripheral blood or in lymphoma tumors by detection of HTLV-1 genomic integration; therefore, measurement of serum levels of soluble CADM1 protein as well as detection of $CD4^+CADM1^+$ cells in the blood, when used in conjunction with other standard diagnostic methods, would be useful for identifying and monitoring disease progression in HTLV-1 carriers with increased accuracy and may aid in the early diagnosis and measurement of treatment effects for ATLL.

It has been proposed that HTLV-1 infects various types of cells, including T-reg cells and subsets of T helper cells (Th2 and Th17), in a cell-to-cell manner.[26-29] There is also evidence that ATLL cells act as T-reg cells that express CD4, CD25 and FoxP3 and are thought to contribute to the immune suppression of ATLL patients;[6] however, it was reported that CADM1 is expressed at low levels on resting naive T cells, and its expression is further downregulated 14 h following TCR activation.[30] Therefore, we determined the expression of CADM1 in the T-reg cell fraction of the peripheral blood of healthy volunteers. The results showed that a subset of the T-reg fraction weakly expressed CADM1, suggesting that CADM1 is not a major marker for the T-reg fraction and that CADM1 expression on ATL cells may reflect the fact that ATL cells originate from T-reg cells. As ATLL cells that constitutively express CD25 exhibited heterogeneous Foxp3 expression patterns,[5] a part of ATLL is likely derived from $FoxP3^+$ T-reg cells. In another report, a population of $FoxP3^+$ cells distinct from ATLL cells was shown to have a regulatory function and was found to impair the cell-mediated immune response to HTLV-1 in patients with ATLL.[31] Although we do not know whether the population of T-reg cells with weak expression of CADM1 in the

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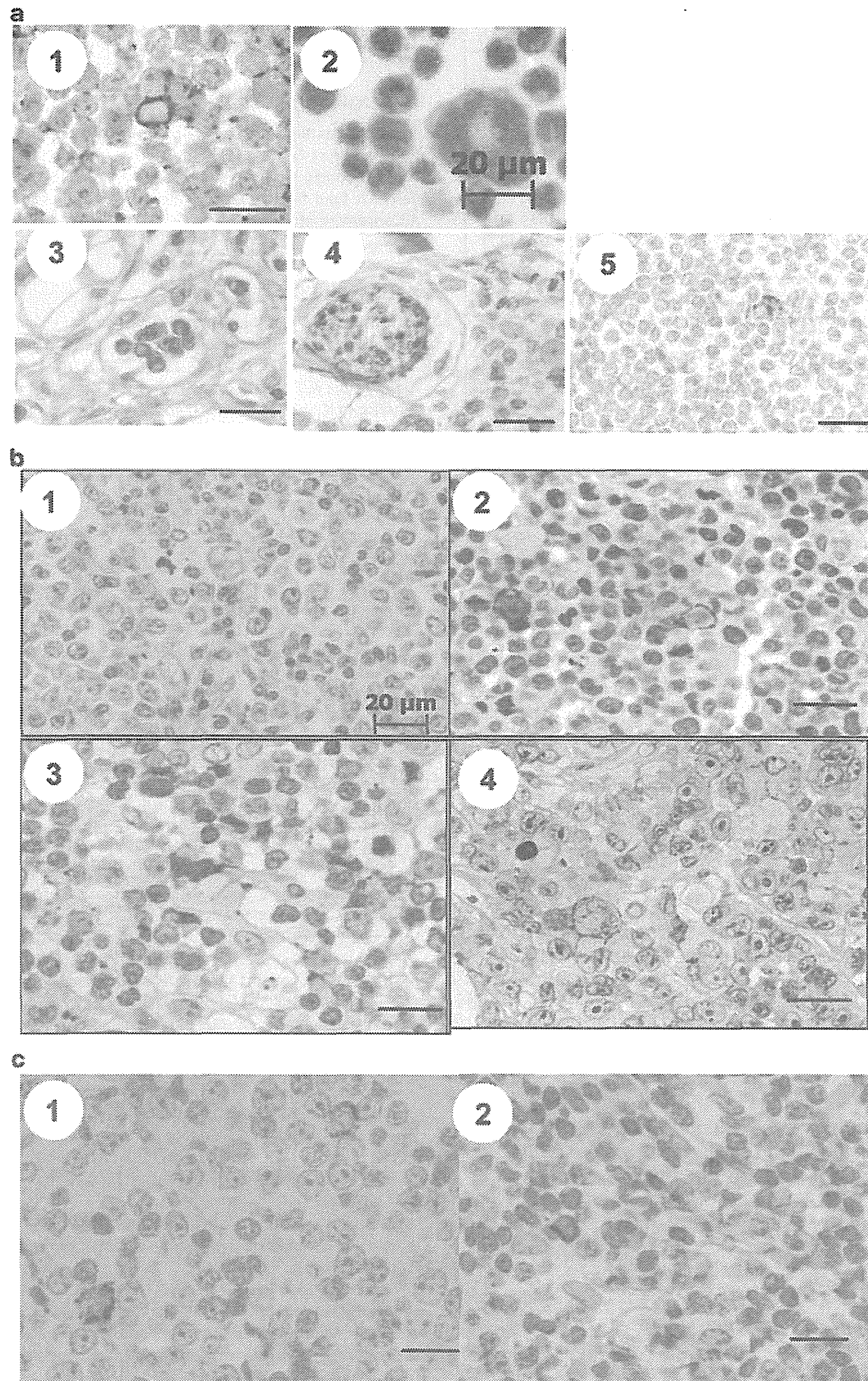

Figure 5. Expression of CADM1 in lymphoma-type ATLL. (**a**) Immunostaining of CADM1 in the S1T-ATLL cell line was used as a positive control (a1), and the ED-ATLL cell line was used as a negative control (a2) for CADM1 expression using an anti-CADM1 antibody (1-10C). Immunostaining of erythrocytes in the blood vessels (**c**), peripheral nerve cells (a3) and reactive lymph nodes (a4) using the same antibody. Scale bar, 20 µm. (**b**) The immunoreactivity for CADM1 was scored using a standardized four-tiered scoring scale as follows: staining in >30% of cells was scored as 3+ (b4); staining in >5% but <30% of cells was scored as 2+ (b3); staining in <5% of cells was scored as 1+ (b2); and a lack of staining was scored as 0 (b1). These images were taken from immunostained ATLL lymphoma sections. Scale bar, 20 µm. (**c**) Representative CADM1 immunostaining in B-cell (c1) and NK-cell (c2) lymphomas. Scale bar, 20 µm.

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Table 1. Immunohistochemical staining of CADM1 in various types of lymphomas, including ATLL

	Case numbers	*Staining scores*				*Positive rates (%)*	
		Negative	*1+*	*2+*	*3+*	*⩾1+*	*⩾2+*
ATLL	36	3	15	14	4	33/36 (92)	18/36 (50)
Non-ATLL	54	37	16	1	0	17/54 (31)	1/54 (1.8)
T/NK	15	12	3	0	0	3/15 (20)	0/15 (0)
B	37	23	13	1	0	14/37 (38)	1/37 (2.7)
Null	2	2	0	0	0	0/2 (0)	0/2 (0)

Abbreviations: ATLL, adult T-cell leukemia/lymphoma; CADM1, cell adhesion molecule 1. The immunoreactivity for CADM1 was scored using a standardized four-tiered scoring scale as follows: staining in >30% of cells was scored as 3+; staining in >5% but <30% of cells was scored as 2+; staining in <5% of cells was scored as 1+; lack of staining was scored as 0.

PBMCs of healthy volunteers is the cellular origin for ATLL cells, CADM1 is thought to be one of the major markers for the various types of ATLL cells. In fact, we observed strong expression of CADM1 in rare cases of ATLL characterized by the $CD4^+CD8^+$, $CD4^-CD8^+$ or $CD4^-CD8^-$ phenotypes (data not shown); therefore, the $CADM1^{high}$ population of T-lymphocytes in peripheral blood can be considered ATLL cells.

The question of why CADM1 is strongly expressed on the surface of various types of ATLL remains unclear. Previously, we investigated whether the expression of CADM1 was induced by HTLV-1/Tax expression and found that Tax protein expression did not activate the expression of CADM1 in JPX-9 cells (data not shown). We also introduced a Tax expression vector into MOLT4 and 293T cells and determined the expression level of CADM1. We found that Tax could not induce CADM1 expression in these cells, suggesting that Tax expression is not related to the high expression of CADM1. As HBZ is known to be constitutively expressed in both HTLV-1-infected cells and ATLL cells and can modulate transcription of cellular genes,[16] it is possible that HBZ activates CADM1 expression. We also speculate that $CADM1^{high}$ expression in ATLL cells may be associated with transcriptional abnormalities in ATLL cells through the accumulation of genomic or epigenomic alterations. In this study, we found a good correlation between HTLV-1 copy numbers and the percentages of $CD4^+CADM1^+$ cells in the peripheral blood of HTLV-1 carriers, suggesting that HTLV-1 carriers with high percentages of $CD4^+CADM1^+$ cells could be associated with progressive genetic alterations and might be at high risk for developing ATLL.

Recent studies have shown that a few markers, such as CCR4 and CD70, are unique ATLL surface markers.[32,33] Although the proportion of $CD4^+CCR4^+$ cells and $CD4^+CD70^+$ cells in the PBMCs from healthy individuals were found to be approximately 5%,[27,33] the proportion of $CD4^+CADM1^+$ cells was <1% (Figure 2); therefore, measurement of $CADM1^+$ T cells is particularly efficient in the diagnosis of HTLV-1 infection in individuals who carry a small number of HTLV-1-infected cells. We have demonstrated previously that CADM1 has important functions in increasing cell adhesion and mediating progression to organ invasion.[19] In this study, we succeeded in isolating a low percentage of both HTLV-1-infected cells from the PBMCs of HTLV-1 carriers with high HTLV-1 copy numbers and ATLL cells from patients with ATLL. The sorted HTLV-1-infected cells and ATLL cells could become useful tools for transcriptional and/or genomic analysis that may be used to compare their results with those of PBMCs from either healthy volunteers or peripheral leukemia cells from patients with ATLL. The results may provide important information on the expression patterns and/or genomic abnormalities that occur at the early stages of HTLV-1 infection and/or ATLL development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by Grants-in-Aid for Scientific Research of Priority Area and for twenty-first Century COE program (Life science) from the Ministry of Education, Culture, Sports, Science and Technology, Japan Leukemia Research fund, and Research fund from Miyazaki Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence, JST.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

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Journal of Medical Virology 83:501–509 (2011)

Target Epitopes of HTLV-1 Recognized by Class I MHC-Restricted Cytotoxic T Lymphocytes in Patients With Myelopathy and Spastic Paraparesis and Infected Patients With Autoimmune Disorders

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Human T-cell lymphotropic virus type I (HTLV-1) causes adult T-cell leukemia/lymphoma and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). The different patterns of clinical diseases are thought to be linked to immunogenetic host factors. A variety of autoimmune diseases, such as Sjögren's syndrome, have been reported in persons infected with HTLV-1, although the precise relationship between these disorders and HTLV-1 infection remains unknown. There is no report on the repertoire of HTLV-1-specific $CD8^+$ T-cells in HAM/TSP patients or carriers with autoimmune diseases, both characterized by an abnormal immune state. In this study, to characterize HTLV-1-specific $CD8^+$ T-cells in asymptomatic HTLV-1 carriers, HAM/TSP patients and carriers with autoimmune diseases, we examined the frequency and diversity of HTLV-1-specific $CD8^+$ T-cells using HTLV-1 tetramers. HTLV-1 Env-specific $CD8^+$ T-cells were significantly more frequent in HAM/TSP and carriers with autoimmune diseases compared with asymptomatic HTLV-1 carriers, while the frequency of HTLV-1 Tax-specific $CD8^+$ T-cells was not significantly different among them. $CD8^+$ cells binding to HTLV-1 Tax tetramers in carriers with autoimmune diseases were significantly reduced compared with HAM/TSP patients. This study demonstrates the importance of $CD8^+$ T-cells recognizing HTLV-1 Env-tetramers in HAM/TSP patients and carriers with autoimmune diseases, thereby suggesting that the diversity, frequency and repertoire of HTLV-1 Env-specific $CD8^+$ T-cell clones may be related to the hyperimmune response in HAM/TSP and carriers with autoimmune diseases, although different immunological mechanisms may mediate the hyperimmunity in these conditions. ***J. Med. Virol. 83:501–509, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: HTLV-1; HAM/TSP; autoimmune diseases; MHC; CD8+ T cells

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INTRODUCTION

Adult T-cell leukemia/lymphoma (ATL) [Poiesz et al., 1980; Hinuma et al., 1981; Tsukasaki et al., 2009] and human T-cell lymphotropic virus type I (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [Gessain et al., 1985; Osame et al., 1986; Casseb, 2009] are two of the most important diseases associated with long-term infection with HTLV-1, which has infected approximately 10–20 million people world-

The authors declare no financial or commercial conflict of interest.

Grant sponsor: Japanese Ministry of Health, Labour, and Welfare (to T.K. and N.A.); Grant sponsor: Kagoshima University for Frontier Science Research Center Program (to N.A.).

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Accepted 30 September 2010

DOI 10.1002/jmv.21985

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wide, particularly in Equatorial Africa, the Caribbean basin, South America, Melanesia, and southern Japan [Proietti et al., 2005]. The different patterns of clinical disease are thought to be linked to host immunogenetic factors. HTLV-1 is also associated with a variety of autoimmune disorders including T cell alveolitis, myopathy, uveitis, arthritis, and Sjögren's syndrome [Sugimoto et al., 1987; Vernant et al., 1988; Nishioka et al., 1989; Terada et al., 1994], although the precise relationship between these disorders and HTLV-1 infection remains unclear. Patients with systemic lupus erythematosus (SLE) and concomitant HTLV-1 infection, for example, seem to have a more indolent clinical course compared with patients with SLE who are not infected with HTLV-1 [Akimoto et al., 2007b].

HTLV-1 Tax-specific cytotoxic T lymphocytes (CTLs) play an important role in suppressing proliferation of HTLV-1-infected or transformed T cells in vitro [Jacobson et al., 1990; Bangham, 2008, 2009]. HTLV-1 Tax and envelope (Env) epitopes recognized by HLA class I molecules [Yashiki et al., 2001], and the association between HTLV-1 Tax-specific CTL frequency, as well as anti-HTLV-1 Tax antibody titers, with reduced HTLV-1 proviral load in asymptomatic HTLV-1 carriers have been described previously [Kozako et al., 2006, 2009c; Akimoto et al., 2007a]. In HAM/TSP, HTLV-1 specific $CD8^+$ CTLs target infected $CD4^+$ cells that have entered the central nervous system, resulting in direct and/or bystander injury [Ijichi et al., 1993]. These CTLs primarily target p40 Tax epitopes, although the less well-characterized *env* and *pol* encoded epitope targets have also been detected [Jacobson et al., 1991; Kannagi et al., 1991; Parker et al., 1992; Furukawa et al., 1994]. Like HAM/TSP, it has been demonstrated using vaccinia virus-HTLV-1 recombinants that $CD8^+$ CTLs from patients with Sjögren's syndrome show major histocompatibility (MHC) class I restricted cytotoxicity to target cells expressing various HTLV-1 proteins, primarily HTLV-1 Tax [Kannagi et al., 1991].

There appears to be no difference in the frequency of amino acid residues favoring MHC class I restricted binding when Env and Tax epitopes are compared, but among non-binding peptides, Env peptides seem more likely to have detrimental amino acid residues at the anchor sites [Pique et al., 1996]. Furthermore, because there seems to be no difference in the functional capacity of HTLV-1 virus-encoded envelope proteins originating from HAM/TSP patients compared with virus from ATL patients, host factors seem to play an important role in mediating immunopathogenesis [Pique et al., 1994]. Our current understanding of the immunological response to HTLV-1 envelope epitopes includes the broad immunogenicity of the surface protein Env175–199 that elicits helper T cell, cytotoxic T cell, as well as humoral responses [Baba et al., 1993]. Despite the apparent association between some autoimmune diseases and HTLV-1 infection, CTL responses targeting HTVL-1 Env epitopes have been less well characterized in individuals infected with HTLV-1 with autoimmune diseases.

MATERIALS AND METHODS

Subjects and PBMCs

The study sample consisted of 26 asymptomatic HTLV-1 carriers (range, 22- to 82-year-old; median, 60-year-old), 18 HAM/TSP patients (range, 34- to 73-year-old; median, 54-year-old), and 25 individuals with autoimmune disorders and HTLV-1 infection (range, 32- to 80-year-old; median, 60-year-old) from the Kagoshima University Hospital. The individuals with autoimmune disorders included eight with Sjögren's syndrome, seven with SLE, five with rheumatoid arthritis (RA), three with systemic sclerosis (SSc), and two with polymyositis (PM). Diagnoses of Sjögren's syndrome, SLE, and RA were made according to the revised Japan criteria for Sjögren's syndrome [Miyawaki, 2000], the revised criteria of the American College of Rheumatology (ACR) [Tan et al., 1982], and the 1987 ACR criteria [Silman, 1988], respectively. Anti-HTLV-1 antibody was measured by electrochemiluminescence immunoassay (ECLIA) (Picolumi® HTLV-I; Eisai, Tokyo, Japan), using beads coated with purified HTLV-1 antigen and synthetic Env peptides. This study protocol was in compliance with the Helsinki Declaration, and approved by the Medical Ethical Committee of Kagoshima University, and participants provided informed consent. Peripheral blood mononuclear cells (PBMCs) were separated from heparinized whole blood by centrifugation on Ficoll Hypaque (Amersham Biosciences, Uppsala, Sweden). For some experiments, cells were cryopreserved in liquid nitrogen until assayed [Kozako et al., 2006, 2009b,c].

HLA Typing of PBMCs

Based on prior reports, HLA type analysis revealed that 88% of people infected with HTLV-1 were HLA-A*02 or HLA-A*24 [Kozako et al., 2006], consistent with other studies of HLA allele types in the population of Southern Kyushu, Japan [Sonoda et al., 2000]. PBMC samples were screened initially by serological staining with monoclonal antibodies (mAbs) for HLA-A*02 supertype (clone BB7.2) and HLA-A*24 supertype (clone 17A10; Medical and Biological Laboratories, Nagoya, Japan), followed by secondary staining with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG (Immunotech, Praka, Czech) and subjected to flow cytometry on a FACScan (BD Biosciences, San Jose, CA). Individuals with neither HLA-A*02 nor HLA-A*24 were excluded from this study. HLA allele types of asymptomatic HTLV-1 carriers were also confirmed by the Luminex method using DNA isolated from cryopreserved PBMCs, as has been previously described (G&G Science, Fukushima, Japan) [Itoh et al., 2005].

Tetramer Assay for HTLV-1 Tax/Env-Specific CD8+ T Cells

Sixteen distinct phycoerythrin (PE)-conjugated HLA-A*0201 and HLA-A*2402 tetramers for HTLV-1 Tax and Env peptides (Medical and Biological Laboratories)

based on known HTLV-1 Tax and Env CTL epitope mapping data were used in this study [Yashiki et al., 2001] (Table II). HLA tetramers were produced as previously described [Baenziger et al., 1986; Altman et al., 1996; Kozako et al., 2006, 2009c]. Aliquots of 1×10^6 PBMCs were incubated with each of 16 distinct HTLV-1 Tax or Env peptides, followed by staining with FITC-conjugated mouse anti-human CD8 mAbs (Beckman Coulter, Fullerton, CA) and peridin chlorophyll a protein (PerCP)-conjugated anti-CD45 (BD Biosciences) according to the manufacturer's instructions. $CD45^+$ lymphocytes were applied to a FACScan [Kuzushima et al., 2001] and 1×10^5 events analyzed with FlowJo software (Tree Star, San Carlos, CA) [Betts et al., 2004]. Human immunodeficiency virus (HIV)/HLA tetramers (Medical and Biological Laboratories) were also stained as negative controls. Based on the negative controls, a cut-off point of 0.1% for HTLV-1/HLA tetramer positivity in $CD8^+CD45^+$ T lymphocytes was used, as previously described [Akimoto et al., 2007a; Kozako et al., 2006].

Statistical Analysis

The proportion of people with HTLV-1-specific $CD8^+$ T cell positivity for respective subject groups was compared with a χ^2 test or Fisher's exact test as appropriate. The percentage of tetramer positive cells was compared using the Mann–Whitney U-test. SPSS for Windows (version 14.0J; SPSS, Inc., Chicago, IL) was used for statistical analyses, and $P < 0.05$ was considered statistically significant.

RESULTS

Adopting HLA-A*0201 Tetramers for Carriers Possessing HLA-A*0201 and HLA-A*0206

Consistent with prior studies in the Southern Kyushu population [Sonoda et al., 2000], most of the asymptomatic HTLV-1 carriers with the HLA-A*02 haplotype were either HLA-A*0201 or HLA-A*0206, and nearly all HLA-A*24 subjects were HLA-A*2402. Of 26 asymptomatic HTLV-1 carriers, six were HLA-A*0201, five were HLA-A*0206, 18 were HLA-A*2402, and three were heterozygous for HLA-A*0201 and HLA-A*2402. Because existing HTLV-1/HLA tetramers were developed for carriers possessing HLA-A*0201 or HLA-A*2402, but not for HLA-A*0206, HLA-A*0201 tetramers were adopted for subjects of the HLA-A*0206 haplotype. In comparing asymptomatic HTLV-1 carriers with HLA-A*0201 and HLA-A*0206 haplotypes, there was no significant difference in frequency of Tax-specific $CD8^+$ T cells (27% and 16%, respectively; $P = 0.52$), or the proportion of individuals with detectable $CD8^+$ T cells for all HTLV-1 tetramers tested (Table I). Furthermore, the CMV p65/HLA-A*0201 tetramer was recognized by $CD8^+$ T cells from individuals with HLA-A*0201 (n = 6) and HLA-A*0206 (n = 4) haplotypes. These normally HLA-A*0201-restricted tetramers were thus also considered to be reliable in selecting $CD8^+$ T cells in HLA-A*0206 individuals. The lower limit of this assay for detecting tetramer specific $CD8^+$ T cells was 0.1%, defined using HIV-1 tetramers as negative control, and consistent with previous reports [Kozako et al., 2006, 2009a,b,c]. The percentage of HTLV-1 tetramer positive cells in the $CD8^+CD45^+$ T cell subset ranged from 0% to 1.28% (Fig. 1).

Env-Derived Epitopes are Recognized More Frequently by Patients With Autoimmune Diseases

The proportion of individuals with detectable $CD8^+$ T cells binding the various envelope epitope tetramers tested was significantly higher in HAM/TSP patients and carriers with autoimmune diseases (15% and 25%, respectively), compared with asymptomatic HTLV-1 carriers (1%, $P < 0.001$, Table II). There was no statistically significant difference in the proportion of individuals with detectable $CD8^+$ T cells specific for the different HTLV-1 Tax epitope tetramers. Individuals with autoimmune diseases had detectable $CD8^+$ T cells that primarily recognized envelope-derived epitopes to a greater extent than Tax-derived epitope tetramers. A

TABLE I. Frequency of HTLV-1-Specific CTL Positivity in Asymptomatic HTLV-1 Carriers Possessing HLA-A*0201 and HLA-A*0206

Tetramers	HLA-A*0201	HLA-A*0206
T11	100% (6/6)[a]	60% (3/5)
T123	17% (1/6)	0% (0/5)
T155	0% (0/6)	0% (0/5)
T178	0% (0/6)	20% (1/5)
T307	17% (1/6)	0% (0/5)
E175	0% (0/6)	0% (0/5)
E239	0% (0/6)	0% (0/5)
E442	0% (0/6)	0% (0/5)
HTLV-1 Tax CTL positives	27% (8/30)	16% (4/25)*
HTLV-1 Env CTL positives	0% (0/18)	0% (0/15)
Total HTLV-1-specific CTL positives	17% (8/48)	10% (4/40)**
CMV pp65-specific CTL positives	100% (6/6)	100% (4/4)

[a]Epitopes detected by HTLV-1/HLA tetramers/number of tetramers tested.
*$P = 0.52$.
**$P = 0.55$.

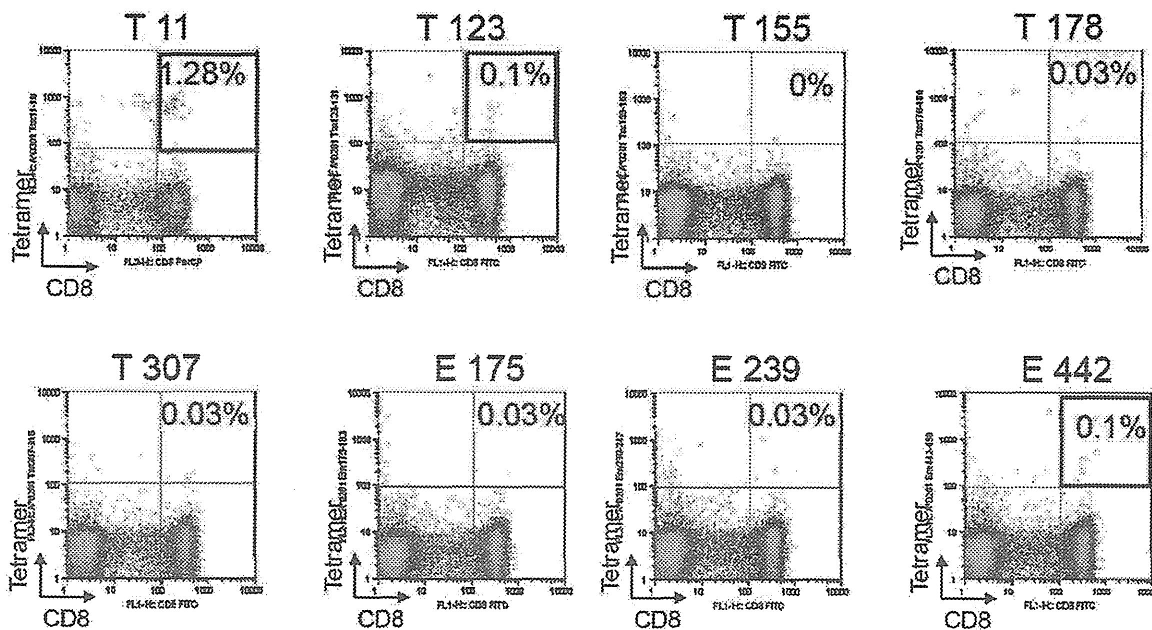

Fig. 1. Variety of HTLV-1-specific CD8$^+$ T cells in fresh PBMCs. Tetramer$^+$CD8$^+$ T cells were estimated among the CD45$^+$ T lymphocyte population. Fresh PBMCs isolated from carriers with autoimmune diseases were stained with eight distinct HTLV-1/HLA-A*0201-tetramers (T11, T123, T155, T178, T307, E175, E239, and E442). Numbers in the upper right quadrants represent the percentages of tetramer$^+$CD8$^+$ T cells among CD8$^+$CD45$^+$ T lymphocytes.

higher proportion of HTLV-1 infected individuals with Sjögren's syndrome, SLE, or SSc had detectable levels of envelope tetramer specific CD8$^+$ T cells (30%, 29%, and 33%, respectively), compared with asymptomatic carriers (1.1%, $P < 0.001$). In contrast, the proportion of individuals with detectable Tax-specific CD8$^+$ T cells was not significantly different among the subject groups, except for individuals with Sjörgren's syndrome, who were more likely than asymptomatic carriers to have detectable Tax-specific CD8$^+$ T cells (34% vs. 22%, respectively). In fact, individuals with Sjörgren's syndrome were more likely to have detectable overall tetramer (Tax- and Env-) specific CD8$^+$ T cells compared with asymptomatic carriers (34% vs. 14%, respectively; $P < 0.001$). There were no significant differences observed in the proportion of individuals with detectable tetramer specific CD8$^+$ T cells when individuals with rheumatoid arthritis or polymyositis were compared with asymptomatic carriers for Tax-specific (24%, 40%, and 22%, respectively) or Env-specific CD8$^+$ T cells (6.7%, 17%, and 1.1%, respectively). Table III summarizes the proportion of individuals with detectable CD8$^+$ T cells binding the various epitope tetramers tested. There was no significant difference in the proportion of individuals with CD8$^+$ T cells binding Tax-derived epitope tetramers, but a higher proportion of HAM/TSP patients (50%) and carriers with autoimmune diseases (64%) had Env-tetramer specific CD8$^+$ T cells overall, when compared with asymptomatic HTLV-1 carriers (3.8%, $P < 0.001$).

CD8$^+$ T Cells From Asymptomatic HTLV-1 Carriers Exhibit a Narrower HTLV-1 Epitope Repertoire Than HAM/TSP Patients and Carriers With Autoimmune Diseases

Fifty-nine individuals were assessed using eight distinct tetramers corresponding to the immunodominant HLA-A*02 or HLA-A*24 restricted epitopes. In the case of individuals with both HLA alleles (three asymptomatic HTLV-1 carriers, seven HAM/TSP patients, and three HTLV-1 carriers with autoimmune diseases), epitope-specific CD8$^+$ T-cells were analyzed using eight tetramers for each HLA type. The array of HTLV-1 epitopes recognized by CD8$^+$ T cells varied, not only by HLA/HTLV-1 peptide, but also according to clinical diagnosis (Table II). Among asymptomatic HTLV-1 carriers, CD8$^+$ T cells predominantly recognized the HLA-A*0201-restricted Tax11–19 and the HLA-A*2402-restricted Tax301–309 tetramers. None of the asymptomatic HTLV-1 carriers in this study had detectable CD8$^+$ T cells binding T155, T289, or T311 tetramers (Table II), whereas these tetramers were recognized by HAM/TSP patients and HTLV-1 infected carriers with autoimmune diseases. Envelope epitopes were rarely recognized by CD8$^+$ T cells from asymptomatic carriers, while HAM/TSP patients and carriers with autoimmune diseases consistently showed detectable levels of Env-specific CD8$^+$ T cells binding HLA-A*0201-restricted Env175–183, Env239–246, Env442–450; and HLA-A*2402-restricted Env11–19,

TABLE II. Diversity of HTLV-1 Epitopes Recognized by CTL m Asympiomatic HTLV-1 Carriers, HAM/TSP Patients, and Earners With Autoimmune Diseases

Tetramers	HLA allele	HTLV-l peptide	Asymptomatic HTLV-1 carriers	HAM TSP	Carriers with autoimmune diseases
T11	A*0201	Taxll–19	73% (8/11)[a]	90% (9/10)	55% (6/11)
T123	A*0201	Taxl23–131	9% (1/11)	20% (2/10)	45% (5/11)
T155	A*0201	Taxl55–163	0% (0/11)	10% (1/10)	0% (0/11)
T178	A*0201	Taxl78–186	9% (1/11)	20% (2/10)	18% (2/11)
T3O7	A*0201	Tax307–315	9% (1/11)	0% (0/10)	0% (0/11)
E175	A*0201	Envl75–183	0% (0/11)	0% (0/10)	9% (1/11)
E239	A*0201	Env239–247	0% (0/11)	10% (1/11)	18% (2/11)
E442	A*0201	Env442–450	0% (0/12)	0% (0/12)	18% (2/12)
T12	A*2402	Taxl2–20	11% (2/13)	13% (2/13)	24% (4/13)
T187	A*2402	Taxl87–195	11% (2/18)	7% (1/15)	18% (3/17)
T289	A*2402	Tax289–297	0% (0/18)	13% (2/15)	18% (3/17)
T301	A*2402	Tax301–309	94% (17/18)	87% (13/15)	76% (13/17)
T311	A*2402	Tax311–319	0% (0/16)	7% (1/16)	6% (1/16)
E11	A*2402	Euvll–19	6% (1/17)	40% (6/17)***	47% (8/17)***
E21	A*2402	Env21–29	0% (0/18)	13% (2/18)	35% (6/18)***
B153	A*2402	Envl 53–161	0% (0/18)	13% (2/I?)	24% (4/17)
Tax CTL positives			22% (32/145)	26% (33/125)	26% (37/140)
Env CTL positives			1% (1/87)	15% (11/75)*	27% (23/84)*
Total CTL positives			14% (33/232)	22% (44/200)***	27% (60/224)**

Tetramers	Sioaren's syndrome	SLE	RA	Sarcoidosis	PM	SSc
T11	100% (3/3)	25% (1/4)	33% (1/3)	100% (1/1)	100% (1/1)	NT
T123	67% (2/3)	50% (2/4)	33% (1/3)	0% (0/1)	0% (0/1)	NT
T155	0% (0/3)	0% (0/4)	0% (0/3)	0% (0/1)	0% (0/1)	NT
T178	0% (0/3)	25% (1/4)	33% (1/3)	0% (0/1)	0% (0/1)	NT
T307	0% (0/3)	0% (0/4)	0% (0/3)	0% (0/1)	0% (0/1)	NT
E175	33% (1/3)	0% (0/4)	0% (0/3)	0% (0/1)	0% (0/1)	NT
E239	33% (1/3)	25% (1/4)	0% (0/3)	0% (0/1)	0% (0/1)	NT
E442	33% (1/3)	25% (1/4)	0% (0/3)	0% (0/1)	0% (0/)	NT
TI2	29% (2/7)	0% (0/4)	0% (0/2)	50% (1/2)	[00% (1/)	33% (1/3)
T187	43% (3/7)	0% (0/4)	0% (0/2)	0% (0/2)	0% (0/)	0% (0/3)
T2S9	0% (0/7)	0% (0/4)	50% (1/2)	0% (0/2)	100% (1/)	33% (1/3)
T301	86% (6/7)	50% (2/4)	100% (2/2)	100% (2/)	100% (1/)	67% (2/3)
T311	14% (1/7)	0% (0/4)	0% (0/2)	0% (0/)	0% (0/)	0% (0/3)
Ell	43% (3/7)	50% (2/4)	0% (0/2)	100% (2/2)***	0% (0/1)	33% (1/3)
E21	14% (1/7)	50% (2/4)	50% (1/2)	0% (0/2)	100% (1/1)	33% (1/3)
El 53	29% (2/7)	25% (1/4)	0% (0/2)	0% (0/2)	0% (0/1)	33% (1/3)
Tax CTT, positives	34% (17/50)	15% (6/40)	24% (6/25)	27% (4/15)	40% (4/10)	27% (4/15)
Env CTL positives	30% (9/40)*	29% (7/24)*	7% (1/15)	22% (2/9)***	17% (1/6)	33% (3/9)**
Total CTL positives	33% (26/80)*	20% (13/64)	18% (7/40)	25% (6/24)	31% (5/16)	29% (7/24)

NT, not tested.
[a]Epitopes detected by HTLV-1/HLA tetramers/number of tetramers tested.
*$P < 0.001$.
**$P < 0.01$.
***$P < 0.05$, versus ACs.

Env21–29, and Env153–161 epitope tetramers. The epitope repertoire of HTLV-1 Env-specific CD8$^+$ cells in asymptomatic carriers showed considerably less breadth than that of HAM/TSP patients and carriers with autoimmune diseases.

Differences in Frequency of HTLV-1-Specific Tetramer Binding CD8$^+$ T Cells Among Asymptomatic HTLV-1 Carriers, HAM/TSP Patients, and Carriers With Autoimmune Diseases

There were significant differences related to clinical status with respect to the percentages of Tax-specific CD8$^+$ T cells among individuals. Among HLA-A*0201 subjects, the percentage of CD8$^+$ T cells binding Tax11–19/HLA-A*0201 tetramer in CD8$^+$/CD45$^+$ T lymphocytes ranged from 0.03% to 3.77% in asymptomatic HTLV-1 carriers, 0–17.1% in HAM/TSP patients, and 0–1.21% in carriers with autoimmune diseases. A similar trend was observed among HLA-A*2402 subjects, for whom the percentages of CD8$^+$ T cells binding Tax301–309/HLA-A*2402 tetramer in CD8$^+$/CD45$^+$ T lymphocytes ranged from 0.09% to 15.6% in asymptomatic HTLV-1 carriers, 0–26.0% in HAM/TSP patients, and 0–3.83% in carriers with autoimmune diseases. For both immunodominant HTLV-1 Tax epitopes, the mean percentage of tetramer-specific T cells within the CD8$^+$ T cell subset in HAM/TSP patients was significantly greater than in asymptomatic HTLV-1 carriers

TABLE III. The Number of Subjects Positive for HTLV-1-Specific CD8+ T Cells in Asymptomatic HTLV-1 Carriers, HAM/TSP Patients, and Carriers With Autoimmune Diseases

HLA allele	Tetramers	Asymptomatic HTLV-1 carriers	HAM/TSP	Carriers with autoimmune diseases
A*02	Tax	82% (9/11)	90% (9/10)	64% (7/11)
A*02	Env	0% (0/11)	10% (1/10)	27% (3/11)
A*24	Tax	94% (17/18)	100% (15/15)	88% (15/17)
A*24	Env	6% (1/18)	40% (6/15)*	82% (14/17)*
Tax CTL positivities		96% (25/26)	100% (18/18)	80% (20/25)
Env CTL positivities		4% (1/26)	50% (9/10)*	64% (16/25)*

*$P < 0.001$, versus ACs.

($P < 0.05$%; Fig. 2), and carriers with autoimmune diseases ($P < 0.01$; Fig. 2), who in turn consistently had the lowest percentages of tetramer-specific $CD8^+$ T cells. HTLV-1 proviral load in HTLV-1-infected persons with autoimmune diseases was significantly lower than in asymptomatic HTLV-I carriers (28.8 and 62.2 copies/ 1,000 PBMCs, respectively; $P < 0.05$). With respect to Env11-19/HLA-A*2402 tetramer-binding, asymptomatic carriers consistently had the lowest percentages of Env-tetramer specific $CD8^+$ T cells (mean ± SD, 0.036% ± 0.026), which was significantly lower than in carriers with autoimmune diseases (0.067% ± 0.043, $P < 0.05$), and marginally lower than in HAM/TSP patients (0.066% ± 0.042, $P = 0.064$).

DISCUSSION

Myriad autoimmune diseases, including T cell alveolitis, myopathy, uveitis, certain types of arthritis, Sjögren's syndrome, and SLE, are seen in persons infected with HTLV-1 [Sugimoto et al., 1987; Vernant et al., 1988; Nishioka et al., 1989; Terada et al., 1994; Akimoto et al., 2007b]. The precise nature of the association between HTLV-1 and these diseases, however, remains unclear. Previous studies have focused on the rather robust cytotoxic T cell response to the HTLV-1 Tax protein in patients with HAM/TSP [Jacobson et al., 1990; Kubota et al., 2003; Bangham, 2008], but there has been little attention given to other HTLV-1

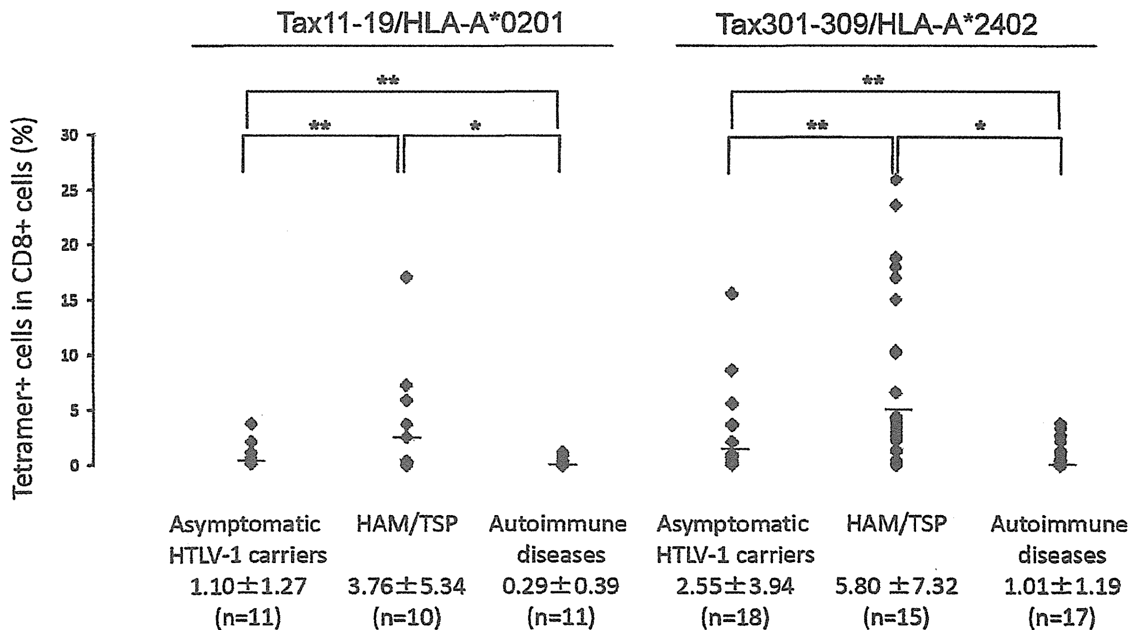

Fig. 2. Frequency of Tax11-19/HLA-A*0201 or Tax301-309/HLA-A*0201 tetramer binding $CD8^+$ T cells in asymptomatic HTLV-1 carriers, HAM/TSP patients, and carriers with autoimmune diseases. The percentage of $tetramer^+$ cells in $CD8^+$ lymphocytes in asymptomatic HTLV-1 carriers, HAM/TSP patients, and carriers with autoimmune diseases. Horizontal bars indicate the mean percentages of tetramer . The numbers below each subject group are the means ± SD. *$P < 0.01$; **$P < 0.05$ (significantly different as determined by Mann–Whitney U-test).

preferential CTL targets, particularly the HTLV-1 envelope [Pique et al., 1994, 1996]. In this study, HLA class I tetramers were used to measure the CTL response to HTLV-1 Tax and Env in a group of asymptomatic people infected with HTLV-1, patients with HAM/TSP, and persons with autoimmune diseases and concomitant HTLV-1 infection. The proportion of individuals with detectable Env tetramer-specific CTL responses was significantly higher in patients with HAM/TSP or autoimmune diseases than in asymptomatic carriers (Table II). Furthermore, patients with HAM/TSP or autoimmune diseases recognized a significantly broader repertoire of Env epitopes (four and six out of a total of six Env epitope tetramers tested, respectively), compared with asymptomatic carriers (one out of six Env epitope tetramers tested). Consistently, patients with HAM/TSP or autoimmune disease had a higher percentage of Env11-19/HLA-A*2402 specific CTLs. These findings were more pronounced for people with HLA-A*2402 than HLA-A*0201 alleles.

Antigen-mediated activation of $CD4^+$ helper T cells (Th) is essential for $CD8^+$ CTL activation. Antigen specific Th cells can be activated by Env-specific B cells, and $CD4^+$ T cells specific for intracellular viral antigens, giving cognate assistance to B cells [Scherle and Gerhard, 1986]. HTLV-1 Env epitopes eliciting both Th and B cell responses have been previously described [Jacobson et al., 1991]. Kitze et al. [1998] have also demonstrated a $CD4^+$ Th response to HTLV-1 Env glycoprotein gp21, an important target antigen in patients with HAM/TSP. The enhanced $CD4^+$ T cell responsiveness known to be characteristic of autoimmune conditions, and similarly described in HAM/TSP [Yamano et al., 1997], may therefore explain the broader Env epitope repertoire and increased Env specific CTL frequency in people with HAM/TSP or autoimmune diseases observed in this study. Previous studies examining the breadth of the T cell receptor repertoire in $CD8^+$ T cells in people infected with HTLV-1 have reported conflicting results. One study found no significant difference in the number of expanded $CD8^+$ T cell clones when asymptomatic carriers were compared with HAM/TSP patients [Eiraku et al., 1998]. Another study, using Immunoscope methods to examine the breadth of T cell clonal expansion, reported significantly greater breadth of the CTL repertoire in HAM/TSP patients compared with asymptomatic carriers [Ureta-Vidal et al., 2001]. Peripheral blood derived T lymphocytes, examined using reverse transcription-polymerase chain reaction/single-stranded conformational polymorphism methods, were also found to have a wider variety of HTLV-1 specific T-cell clonotypes in HAM/TSP patients compared with carriers [Hoger et al., 1997]. This study also demonstrates clearly, that a greater proportion of HAM/TSP patients had detectable Env specific CTLs, and that HAM/TSP patients had a broader repertoire of CTLs recognizing Env epitopes, as well as higher mean percentage of Env-specific CTLs within the $CD8^+CD45^+$ T cell subset, compared with asymptomatic carriers. This study goes further to demonstrate that like HAM/TSP patients, people with autoimmune diseases and HTLV-I infection, also have a broader CTL repertoire and higher frequency of Env-specific CTLs compared with asymptomatic carriers.

Increased proviral load in asymptomatic carriers is associated with an increased risk for progression to HAM/TSP. The Tax-specific CTL response is critical in controlling proviral load. Although increased in HAM/TSP compared with asymptomatic carriers [Nagai et al., 1998], the CTLs in HAM/TSP have been demonstrated to have lower cytolytic efficiency [Bangham, 2008; Kattan et al., 2009]. The higher Tax-specific CTL frequency, but with higher proviral load, observed in HAM/TSP patients compared with asymptomatic persons infected with HTLV-1 is consistent with low CTL efficiency. Conversely, higher CTL efficiency could explain the lower $CD8^+$ Tax-specific CTL frequency seen in people with autoimmune diseases, who have a significantly lower proviral load than asymptomatic carriers in this study. This difference in CTL efficiency remains to be confirmed.

In this study, it was demonstrated that the difference in frequency of HTLV-1 Tax-specific $CD8^+$ T cells was not statistically significant between asymptomatic HTLV-1 carriers, HAM/TSP or carriers with autoimmune diseases, while HTLV-1 Env-specific $CD8^+$ T cells were significantly more frequent in HAM/TSP and carriers with autoimmune diseases than those in asymptomatic HTLV-1 carriers. These results suggest that the diversity, frequency, and repertoire of HTLV-1-specific $CD8^+$ T cell clones, especially HTLV-1 Env $CD8^+$ T cells may be related to the hyperimmune response in HAM/TSP and carriers with autoimmune diseases, although different immunological mechanisms may mediate the hyperimmune responses in HAM/TSP and autoimmune diseases.

ACKNOWLEDGMENTS

We thank Mrs. Hokkoku and Mrs. Higashi for technical assistance.

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REVIEW

HTLV infection and the eye

Koju Kamoi and Manabu Mochizuki

Purpose of review
Human T-cell lymphotropic virus (HTLV) is the first discovered retrovirus causing malignancy in human. HTLV infection affects host's ocular tolerance and causes various diseases in the eye. Here we discuss the manifestations, mechanisms, treatments, and future directions of HTLV-related ocular diseases.

Recent finings
Recent serological researches showed that the number of HTLV-1 carriers in metropolitan area was increasing, although seroprevalence of HTLV-1 in general population was decreased after screening serological tests in blood donors started. The most common clinical entity of uveitis was still HTLV-1 uveitis in HTLV-1 highly endemic area, but prevalence of HTLV-1 uveitis varies in different parts of the world. As for treatment of inflammation, tacrolimus and 5-azacytidine were reported to be effective for autoimmune manifestations in HTLV-1-related overlap syndrome (deratomyositis/Sjogren's syndrome) and HTLV-1-related myelodysplastic syndrome. Interleukin-2 receptor targeted therapies improved scleritis in patients with adult T-cell leukemia/lymphoma caused by HTLV-1. Basic researches identified that HTLV-1 tax and HTLV-1 basic leucine zipper factor play critical roles in the HTLV-1-related disease and are now being investigated as targeted therapies.

Summary
Development of modern molecular biology makes it possible to reveal deep insights of HTLV-1-related ocular diseases. Although effective therapies based on basic researches have been reported, further endeavor is necessary to establish much more specific treatments of the ocular diseases.

Keywords
adult T-cell leukemia/lymphoma, Keratoconjunctivitis sicca, HTLV-1 basic leucine zipper factor, HTLV-1 tax, HTLV-1 uveitis

INTRODUCTIONS

Retrovirus is a RNA virus encoding for a reverse transcriptase, which translate the viral RNA into a DNA provirus, which in turn is rapidly incorporated into the host's genome [1]. Retroviruses are currently classified into oncoviruses and lentiviruses. Oncoviruses are associated with haematological proliferations and tumours of connective tissues in animal species. Human T-cell lymphotropic viruses (HTLVs) are the representative viruses and HTLV-1 was the first retrovirus described as a causative agent of human disease [2,3]. Lentivirus induces chronic and progressive pulmonary and/or neurological diseases in animal species. The representative virus in human is HIV, which was previously named HTLV-3 and now reclassified into HIV and is a causative agent of AIDS [1].

Human retrovirus (HTLV/HIV) infection affects on host's ocular tolerance, which results in various diseases, particularly uveitis [4–6]. Although HIV-related ocular manifestations are well known among ophthalmologists, their knowledge of HTLV-related ocular manifestation seems to be insufficient. The information of HTLV infection is now important because a recent survey indicates that HTLV-1 carriers are estimated to spread from local endemic areas to nonendemic metropolitan areas [7]. Therefore, we believe that this review of HTLV might be enough for ophthalmic practice and hope that this might highlight scientific attention to HTLV-1 infection among ophthalmologists.

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Curr Opin Ophthalmol 2012, 23:557–561

DOI:10.1097/ICU.0b013e328358b9ec

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KEY POINTS

- HTLV-1 carriers are estimated to spread from local endemic areas to nonendemic metropolitan areas.
- HTLV-1 ocular manifestations can be mainly classified into three groups, uveitis, opportunistic infection/malignant infiltration of the eye in ATL patients and keratoconjunctivitis sicca.
- The most frequent ocular manifestation of HTLV-1 infection that ophthalmologists should keep in mind is HTLV-1 uveitis.
- HTLV-1 tax and HTLV-1 basic leucine zipper factor (HBZ) are thought to be responsible for immune dysregulation and may contribute to ocular manifestations.

HUMAN T-CELL LYMPHOTROPIC VIRUS INFECTION AND SEROLOGY

Retrovirus was first described in 1970s [8], but the causal relationship with human diseases was identified in the early 1980s when HTLV-1 was identified as a causative agent of adult T-cell leukemia/lymphoma (ATL) [2,3]. After the discovery of HTLV-1, related viruses have been isolated and HTLV is now composed of four related HTLVs, that is HTLV-1 to HTLV-4 [9]. However, only HTLV-1 has been obviously linked to human diseases at present.

HTLV-1 infection is known to have unique geographic distribution, and is prevalent in southern part of Japan, Melanesia, the Caribbean Islands, Central and South America, as well as central Africa. It is estimated that 20 million people carry the virus worldwide [10]. The current survey indicated that the seroprevalence of HTLV-1 in general population (in Japan) is known to be decreased after screening serological tests of HTLV-1 in blood donors started in 1987 because blood transfusion and breastfeeding from mother to child are major routes of the viral transmission [11]. However, another survey indicated that the number of HTLV-1 carriers in the metropolitan area is significantly increasing because of migration from the endemic areas to the metropolitan areas [7]. Therefore, ophthalmologists, especially in the metropolitan area, are required to know HTLV-related ocular manifestation to avoid misdiagnosis.

OCULAR MANIFESTATIONS IN HUMAN T-CELL LYMPHOTROPIC VIRUS-1 INFECTION

A recent report indicated that ocular complains were the first manifestation of HTLV-1 infection to come to clinical attention, in addition to neurologic and rheumatologic complains [12]. The most frequent ocular manifestation of HTLV-1 infection that ophthalmologists should keep in mind is HTLV-1 uveitis [13]. HTLV-1 uveitis is now recognized as the third clinical entity of HTLV-1 infection following ATL and HTLV-1-associated myelopathy/tropical spastic paraparesis [14–16]. Clinical entity of HTLV-1 uveitis was established by a series of research conducted by Mochizuki *et al.* in 1990s. They showed clinical and laboratory data consisting of seroepidemiology, clinical features, detection of proviral DNA and mRNA of HTLV-1 from ocular tissues, and detection of viral particles from T-cell clones (TCCs) derived from the aqueous humor of the patient [4,5]. Since then, it has been well established that uveitis is significantly related to HTLV-1. In addition to HTLV-1 uveitis, many other ocular manifestations, which are proved linkage to HTLV-1 infection, have been reported all over the world. Taken together, HTLV-1 ocular manifestations can be mainly classified into three groups, uveitis, opportunistic infection/malignant infiltration of the eye in ATL patients and keratoconjunctivitis sicca (KCS).

HUMAN T-CELL LYMPHOTROPIC VIRUS-1 UVEITIS

Seroepidemiological comparison study [4,5,17] in endemic/nonendemic area revealed that the HTLV-1 seroprevalence in patients with idiopathic uveitis was significantly higher than that in following two control groups: patients with cause defined as uveitis and patients with nonuveitic ocular diseases. This was the first clue suggesting that HTLV-1 infection is significantly related to uveitis. The uveitis is now recognized as a distinct clinical entity related to HTLV-1 and designated as HTLV-1 uveitis.

Recent survey [18] in the endemic area revealed that the most common clinical entity of uveitis was still HTLV-1 uveitis, followed by Vogt–Koyanagi–Harada (VKH) disease, sarcoidosis and others. However, the new patients of HTLV-1 uveitis clearly decreased with time, although VKH disease, sarcoidosis and others do not have changed much in the last two decade. As for the prevalence of HTLV-1 uveitis in different parts of the world, the prevalence of HTLV-1 uveitis in Martinique [19], and that of HTLV-1 uveitis in Brazil are lower than that reported in Japan [20,21]. HTLV-1 uveitis's major symptoms at initial presentation were sudden onset of floater, foggy vision and blurred vision. Other complains are pain/burning, itching and foreign body sensation. Most patients had an intermediate uveitis with moderate or heavy vitreous opacities (fine cells and lacework-like membranous opacities). The vitreous opacities were the most impressive findings and were accompanied by mild iritis and mild

retinal vasculitis but no uveoretinal lesions [22]. The ocular inflammation of HTLV-1 uveitis was unilateral or bilateral [19,20,22]. Interesting observation was reported in HTLV-1 uveitis patients that was an association with Graves' disease. HTLV-1 uveitis occurred after the onset of Graves' disease in all cases [23]. The incidence of HTLV-1 uveitis after Graves' disease in the first report was very similar to that of the latest [18].

As for the mechanism of HTLV-1 uveitis, analysis of infiltrating cells in the eye with HTLV-1 uveitis revealed that the majority of infiltrating cells were $CD3^+$ T cells, but not malignant cells or leukemic cells based on their T-cell receptor usage [24]. HTLV-1 proviral DNA, HTLV-1 protein and viral particle were detected from ocular infiltrating cells in the eye of HTLV-1 uveitis patients [4,5]. A series of researches showed that HTLV-1 uveitis was caused by inflammatory cytokines produced by HTLV-1-infected $CD4^+$ T cells that are significantly accumulated in the eye of the patients. HTLV-1-infected $CD4^+$ TCCs established from infiltrating cells in eyes of HTLV-1 uveitis patients produced a large amount of various inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, tumour necrosis factor (TNF-α) and interferon (IFN)-γ [25]. Furthermore, addition of corticosteroids in the culture medium suppressed the cytokine production [26].

ADULT T-CELL LEUKEMIA/LYMPHOMA-RELATED OCULAR SYMPTOM

Opportunistic infection and malignant infiltration of the eye are main ophthalmic features of ATL patients. The representative opportunistic infection in the eye is cytomegalovirus retinitis [27]. Cytomegalic cell infiltration and accompanied retinal necrosis can be seen. This ocular manifestation is similar to that of patients with AIDS, and is also associated with poor prognosis. Many case reports indicate that HTLV-1-infected leukocytes can infiltrate into the almost all tissue in the eye, which cause various ocular manifestations in such areas as orbita, cornea, iris, lens, vitreous, uvea, retina, sclera, optic nerve [28]. In addition to these regions, choroidal manifestation was newly reported and identified as a distinct ocular manifestation of ATL patient [28].

Investigation of the eye in HTLV-1-infected patients has progressed significantly in accordance with development of modern molecular biology technology such as microdissection, PCR, cytokine detection system and Flow cytometry. ATL cells are characterized by the expression of IL-2 receptor alpha (IL-2Rα) (CD25), which is not expressed in normal resting T cells. The recent technology showed that elevated levels of soluble IL-2Rα may suggest direct ocular infiltration of ATL cells, as ATL cells secrete soluble forms of IL-2Rα into the vitreous [28]. Detection of soluble IL-2Rα in vitreous may be a cue of ocular infiltration and prognosis [29].

KERATOCONJUNCTIVITIS SICCA

KCS is usually a part of Sjögren's syndrome. HTLV-1-associated tear film changes were first reported when investigating mice expressing the *Tax* gene and developing a Sjögren's syndrome -like clinical symptom [30]. The association between Sjögren's syndrome and HTL V-1 in humans was found in an endemic zone higher prevalence of this virus among the carriers of the syndrome than in the seronegative control group [31]. Development of clinical Sjögren's syndrome manifestations in HTL V-1 carriers have been explained by the activated autoreactive T cells, which break immunological tolerance and result in Sjögren's syndrome. However, KCS associated with HTLV-1 infection might differ from ocular manifestation in primary or secondary Sjögren's syndrome because it does not reveal immunological alteration related to a rheumatologic disease [19,32–34]. Therefore, the mechanism of HTLV-1-associated Sjögren's syndrome is still controversial. However, some patients have more than one of these HTLV-1-associated inflammatory conditions, that is overlap syndrome. HTLV-1 infection can change immunological status by T cell activation and various cytokines, which may contribute to the development of overlap syndrome. Infected activated T cells are thought to proliferate and infiltrate into not only the eye [26] but also other organs and secrete a variety of cytokines, including IL-1, IL-2, IL-3, IL-6, TNF-α and IFN-γ [35]. For example, a report presented that a HTLV-1 carrier had clinical and pathological features of overlap syndrome, which consisted of Sjögren's syndrome and dermatomyositis.

TREATMENT OF HUMAN T-CELL LYMPHOTROPIC VIRUS-1-RELATED OCULAR MANIFESTATION

HTLV-1 uveitis is considered to have caused by inflammatory cytokines produced by HTLV-1-infected $CD4^+$ T cells, which significantly accumulated in the eye of the patients, and, therefore, topical and/or oral corticosteroid treatment is effective to treat HTLV-1 uveitis patients by suppressing cytokine production of HTLV-1-infected $CD4^+$ T cells for their intraocular inflammation [4,5,13]. Clinical management should be performed according to their degree of ocular inflammation. HTLV-1

uveitis with mild degree of ocular inflammation can be managed by topical noncorticosteroid or corticosteroid drug. The sub-Tenon's injection of corticosteroids is used when the patients have moderate inflammatory activities in the vitreous cavity. If the vitreous inflammatory activity and the retinal vasculitis are severe, oral corticosteroids are given, but a long-term administration of systemic corticosteroid should be avoided [13]. Recent report indicates that tacrolimus [36] and 5-azacitidine [37] are useful for the resolution of autoimmune manifestations in HTLV-1-related overlap syndrome (deratomyositis/Sjögren's syndrome) and HTLV-1-related myelodysplastic syndrome, which might be potential alternative drugs to apply HTLV-1 uveitis complication in the future.

Despite advances of novel treatment agents, the prognosis for ATL remains poor. A variety of therapeutic approaches have been examined and effectiveness of them has been gradually improved. A combination of arsenic trioxide, zidovudine and IFN α achieved a significant remission rate with moderate toxicity [38]. Allogeneic hematopoietic stem cell transplantation (HSCT) is considered to be one of the best curative therapies [39–41], and a significant improvement of eye complications were observed in accordance with a decrease in the HTLV-1 proviral load after allogeneic HSCT. Recently, alternative treatments were reported, which showed that daclizumab (monoclonal antibody directed against the α chain of the IL-2R and denileukin diftitox (immunotoxin fusion protein that targets the IL-2R) improve ocular complications such as sclerotitis in patients with underlying ATL [42].

In cases of KCS, the first and most important mediation is lubricating for patients with mild and moderate severity. Lubricating drops are used to reduce tear film disorders and to prevent ocular complications such as superficial punctate keratitis and corneal ulcers [43].

RECENT BASIC RESEARCH

The recent basic researches have shown new insights of HTLV-1 infection and pathogenesis by pursuing molecular functions of HTLV-1 Tax HTLV-1 basic leucine zipper factor (HBZ) [44■]. Transgenic mice expressing HTLV-1 Tax develop an inflammatory arthropathy [45], and transgenic rats expressing HTLV-1 env-pX develop Sjögren's syndrome, arthropathy, vasculitis and polymyositis [46]. Regulatory T cells in HBZ transgenic mice were functionally impaired [47]. This implies that HTLV-1-induced regulatory T-cell dysfunction may be one of the mechanisms that induce immune activation by HTLV-1-infected T cells. Taken together, HTLV-1 Tax and HBZ are thought to be responsible for immune dysregulation and are potential target molecules for targeted therapies.

CONCLUSION

HTLV-1 infection causes sight-threatening problems not only in local endemic area but also metropolitan area. HTLV-1-related ocular manifestations are frequently associated with inflammation such as HTLV-1 uveitis. That is caused by the alternation of immune status the cytokine production from HTLV-1-infected nonmalignant cells. Therefore, corticosteroid is the first choice for treating these inflammatory diseases. Effectiveness of other drugs such as tacrolimus and 5-azacytidine on HTLV-1-related inflammatory disease has been reported, but careful attention should be paid.

ATL-related ocular manifestation is caused by HTLV-1-infected malignant cells, which infiltrate into ocular tissues and cause various ocular manifestations. The treatment is still difficult for these ATL patients. HSCT is considered to be the only curative therapy, but IL-2R treatment might be an alternative treatment in this ocular disorder.

Recent basic researches showed that HTLV-1 Tax and HBZ are responsible for immune disturbances so that they are one of the best candidates for future HTLV-1 therapy. Further endeavor is needed to investigate more detailed molecular mechanism to provide specific treatment of HTLV-1-related ocular diseases.

Acknowledgements

None

Conflicts of interest

There are no conflicts of interest.

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■■ of outstanding interest
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frontiers in
MICROBIOLOGY

MINI REVIEW ARTICLE
published: 24 July 2012
doi: 10.3389/fmicb.2012.00270

HTLV-1 uveitis

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Human T cell lymphotropic virus type 1 (HTLV-1) is the first retrovirus described as a causative agent of human disease. Following adult T cell leukemia/lymphoma and HLTV-1-associated myelopathy/tropical spastic paraparesis, HTLV-1 uveitis (HU) has been established as a distinct clinical entity caused by HTLV-1 based on seroepidemiological, clinical, and virological studies. HU is one of the most common causes of uveitis in endemic areas of Japan and can be a problematic clinical entity all over the world. HU occurs with a sudden onset of floaters and foggy vision, and is classified as an intermediate uveitis. Analysis of infiltrating cells in eyes with HU revealed that the majority of infiltrating cells were $CD3^{+}$ T cells, but not malignant cells or leukemic cells based on their T cell receptor usage. HTLV-1 proviral DNA, HTLV-1 protein, and viral particles were detected from infiltrating cells in eyes with HU. HTLV-1-infected $CD4^{+}$ T cell clones established from infiltrating cells in eyes with HU produced large amounts of various inflammatory cytokines, such as IL-1, IL-6, IL-8, TNF-α, and interferon-γ. Taken together, HU is considered to be caused by inflammatory cytokines produced by HTLV-1-infected $CD4^{+}$ T cells that significantly accumulate in eyes; therefore, topical and/or oral corticosteroid treatment is effective to treat intraocular inflammation in patients with HU. Further investigation is needed to establish a specific treatment for HU.

Keywords: HTLV-1, uveitis, ocular inflammation, $CD4^{+}$ T cell, T cell clone

INTRODUCTION

Retrovirus was first described in the 1970s (Temin and Baltimore, 1972), but its causal relationship with human diseases was not identified until the early 1980s when human T cell lymphotropic virus type 1 (HTLV-1) was identified as an etiologic agent of adult T cell leukemia/lymphoma (ATL; Poiesz et al., 1980; Hinuma et al., 1981; Yoshida et al., 1984). After the discovery of the link between HTLV-1 and ATL, HLTV-1 was also found to be a causal agent of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP; Gessain et al., 1985; Osame et al., 1986) and HTLV-1 uveitis (HU; Mochizuki et al., 1992a,b,c).

HTLV-1 uveitis, the third clinical entity of HTLV-1 infection, was established by a series of studies in the highly endemic area of southern Kyushu, Japan. Clinical case reports from this area suggested possible associations of HTLV-1 carriers with various ocular manifestations (Ohba et al., 1989). In the 1990s, the first set of evidence that indicated the causative implication of HTLV-1 in uveitis was reported by Mochizuki and colleagues. They showed clinical and laboratory data consisting of seroepidemiology, clinical features, detection of proviral DNA and mRNA of HTLV-1 from ocular tissues, and detection of viral particles from T cell clones (TCC) derived from the aqueous humor of the patient (Mochizuki et al., 1992a,b). Since then, it has been well established that uveitis is significantly related to HTLV-1. Here, we review historical findings that contributed to the establishment of the HU entity and recent advancements that deepen our understanding of HU.

SEROEPIDEMIOLOGY

HTLV-1 infection is known to have unique geographic distribution and is prevalent in Japan, Melanesia, the Caribbean Islands, Central America, South America, and Central Africa. It is estimated that 20 million people carry the virus worldwide (Watanabe, 2011). This virus is etiologically linked with HU, which is one of the most common causes of uveitis in the endemic area of Japan and can be a problematic clinical entity all over the world (Yoshimura et al., 1993; Takahashi et al., 2000; Merle et al., 2002; Pinheiro et al., 2006; Miyanaga et al., 2009). Uveitis is a sight-threatening inflammatory disorder affecting the intraocular tissues (Forrester, 1991) and is the third leading cause of blindness in developed countries. The etiology of uveitis is categorized as infectious or non-infectious and varies depending on the genetic background of the population and the prevalence of the pathogenic agent in the area. Clinically, the etiology of approximately 30% of cases could not be defined even when careful examinations were performed. A survey comparing the etiologies of uveitis in different areas of Japan demonstrated that the proportion of undefined etiologies was particularly high in southern Kyushu as compared to those in northern Kyushu and Tokyo. Seroepidemiological comparison studies (Mochizuki et al., 1992a,b; Shirao et al., 1993) in these highly endemic and non-endemic areas revealed that the HTLV-1 seroprevalence in patients with idiopathic uveitis was significantly higher than that in the following two control groups: patients with etiology-defined uveitis and patients with non-uveitic ocular diseases (**Figure 1**). This was the first clue suggesting that HTLV-1 infection is significantly related to uveitis. Uveitis is now recognized as a distinct clinical entity related to HTLV-1 and is designated as HU. The seroprevalence of HTLV-1 in the general Japanese population is known to have decreased after serological screening tests of HTLV-1 in blood donors started