ELSEVIER

CrossMark

The Spine Journal 14 (2014) 2847–2853

THE SPINE JOURNAL

Clinical Study

Voluntary driven exoskeleton as a new tool for rehabilitation in chronic spinal cord injury: a pilot study

Mirko Aach, MD[a,*], Oliver Cruciger, MD[a], Matthias Sczesny-Kaiser, MD[b], Oliver Höffken, MD[b], Renate Ch. Meindl, MD[a], Martin Tegenthoff, MD[b], Peter Schwenkreis, MD[b], Yoshiyuki Sankai, PhD[c], Thomas A. Schildhauer, MD[d]

[a]*Department of Spinal Cord Injuries, BG University Hospital Bergmannsheil, Bürkle-de-la-Camp-Platz 1, 44797, Bochum, Germany*
[b]*Department of Neurology, BG University Hospital Bergmannsheil, Bürkle-de-la-Camp-Platz 1, 44797, Bochum, Germany*
[c]*Faculty of Engineering, Information and Systems, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-0006, Japan*
[d]*Department of General and Trauma Surgery, BG University Hospital Bergmannsheil, Bürkle-de-la-Camp-Platz 1, 44797, Bochum, Germany*

Received 17 December 2013; revised 20 March 2014; accepted 28 March 2014

Abstract

BACKGROUND CONTEXT: Treadmill training after traumatic spinal cord injury (SCI) has become an established therapy to improve walking capabilities. The hybrid assistive limb (HAL) exoskeleton has been developed to support motor function and is tailored to the patients' voluntary drive.

PURPOSE: To determine whether locomotor training with the exoskeleton HAL is safe and can increase functional mobility in chronic paraplegic patients after SCI.

DESIGN: A single case experimental A-B (pre-post) design study by repeated assessments of the same patients. The subjects performed 90 days (five times per week) of HAL exoskeleton body weight supported treadmill training with variable gait speed and body weight support.

PATIENT SAMPLE: Eight patients with chronic SCI classified by the American Spinal Injury Association (ASIA) Impairment Scale (AIS) consisting of ASIA A (zones of partial preservation [ZPP] L3–S1), n=4; ASIA B (with motor ZPP L3–S1), n=1; and ASIA C/D, n=3, who received full rehabilitation in the acute and subacute phases of SCI.

OUTCOME MEASURES: Functional measures included treadmill-associated walking distance, speed, and time, with additional analysis of functional improvements using the 10-m walk test (10MWT), timed-up and go test (TUG test), 6-minute walk test (6MWT), and the walking index for SCI II (WISCI II) score. Secondary physiologic measures including the AIS with the lower extremity motor score (LEMS), the spinal spasticity (Ashworth scale), and the lower extremity circumferences.

FDA device/drug status: Not applicable.

Author disclosures: ***MA:*** Nothing to disclose. ***OC:*** Nothing to disclose. ***MS-K:*** Nothing to disclose. ***OH:*** Nothing to disclose. ***RCM:*** Nothing to disclose. ***MT:*** Nothing to disclose. ***PS:*** Nothing to disclose. ***YS:*** Royalties: University of Tsukuba (E); Stock Ownership (E, Paid directly to institution); Private Investments: (E, Paid directly to institution); Consulting: (E, Paid directly to institution); Speaking / Teaching Arrangements: (E, Paid directly to institution); Trips/Travel: (E, Paid directly to institution); Board of Directors: CYBERDYNE, Inc. (E); Grants: Cabinet Office (I). ***TAS:*** Nothing to disclose.

The disclosure key can be found on the Table of Contents and at www.TheSpineJournalOnline.com.

There has been no monetary study funding. The study was supported by a grant and has been supervised by the staff of BG University Hospital Bergmannsheil, Bochum.

Authors' contributions: MA and OC carried out the experiments and data analysis as well as drafting of the manuscript. RCM, PS and MT helped with the experimental set up. MS-K and OH contributed to the data analysis. TAS participated in study design and coordination of the study.

All authors read and approved the final manuscript.

YS is a founder, shareholder, and the CEO of Cyberdyne, Inc., which produces the HAL.

YS and Cyberdyne were neither involved in study funding, design, data collection, and analysis, nor in writing or submitting this article, therefore concluding in no specific influence on the trial. We certify that no party having a direct interest in the results of the research supporting this article has or will confer a benefit on us or on any organization with which we are associated.

YS and Cyberdyne as the manufacturer of the device provided exclusively technical and advisory support.

YS as the CEO of Cyberdyne has been involved exclusively in terms of an advisory capacity, regarding technical support and the limitations of the exoskeleton. Therefore, the inclusion and exclusion criteria have been modified (eg, body weight and contractures).

* Corresponding author. Department of Spinal Cord Injuries, BG University Hospital Bergmannsheil, Bürkle-de-la-Camp-Platz 1/44789, Bochum, Germany. Tel.: 81 29-853-2111.

E-mail address: bergmannsheil@bergmannsheil.de (M. Aach)

http://dx.doi.org/10.1016/j.spinee.2014.03.042

METHODS: Subjects performed standardized functional testing before and after the 90 days of intervention.
RESULTS: Highly significant improvements of HAL-associated walking time, distance, and speed were noticed. Furthermore, significant improvements have been especially shown in the functional abilities without the exoskeleton for over-ground walking obtained in the 6MWT, TUG test, and the 10MWT, including an increase in the WISCI II score of three patients. Muscle strength (LEMS) increased in all patients accompanied by a gain of the lower limb circumferences. A conversion in the AIS was ascertained in one patient (ASIA B to ASIA C). One patient reported a decrease of spinal spasticity.
CONCLUSIONS: Hybrid assistive limb exoskeleton training results in improved over-ground walking and leads to the assumption of a beneficial effect on ambulatory mobility. However, evaluation in larger clinical trials is required.

Keywords: Exoskeleton; Treadmill training; Rehabilitation; Paraplegia; Hybrid assistive limb; Spinal cord injury

Introduction

About 1,200 people suffer a traumatic spinal cord injury (SCI) each year in Germany. Recent statistics indicate that more than 50% of these injured patients have a motor incomplete lesion [1]. In patients with initial motor incomplete SCI, at least 75% regain some kind of ambulatory function. Better functional outcome is associated with age, level of lesion, and the classification in the American Spinal Injury Association (ASIA) Impairment Scale [2]. In the first 2 months after initial SCI, approximately half of the recovery occurs. Within the following 4 months, a decreasing rate of recovery has been observed. One year after injury, neurologic recovery is assumed to be nearly complete [3]. Although conventional rehabilitation programs enhance the performance of functional tasks, the loss of strength and coordination substantially limit one's capacity for over-ground ambulation training [4]. In the past two decades, body weight supported treadmill training (BWSTT) has been proposed as a useful adjunct to enhance locomotor function after motor incomplete SCI [5]. In patients with incomplete or complete SCI, a bilateral leg muscle activation combined with coordinated stepping movements can be induced in partially unloaded patients, standing on a moving treadmill. Body weight supported treadmill training enables early initiation of gait training and integration of weight-bearing activities, stepping and balance, by the use of a task-specific approach and a systematic gait pattern [6]. To facilitate the delivery of BWSTT in SCI patients, the locomotor training evolved over the last 12 years and a motorized robotic driven gait orthosis (DGO) has been developed [7]. The advantages over conventional BWSTT methods are considered to be less effort for attending physiotherapists [8], longer duration, more physiologic and reproducible gait patterns, and the possibility to measure a patients' performance. Several studies pointed out that DGO training improves over-ground walking [9–13]. However, there was no reported difference in the outcome of DGO training compared with conventional training. A significant switch in the ASIA classification has not been found [10,14].

Over the last 5 years, exoskeletal systems became available for SCI patients. These systems offer different possibilities. Three exoskeletons (Ekso [EksoBionics, Richmond, CA, USA], Rex [Rex Bionics, Aukland, New Zealand] and Re-Walk [ARGO Medical Technolgies, Israel]) allow SCI patients to stand up, walk with a defined pattern, and even climbing stairs mainly on a basis of passive range of motion (ROM). The exoskeleton hybrid assistive limb (HAL; Cyberdyne, Inc., Japan) offers the possibility of getting connected with the SCI patient through electromyography electrodes on the skin at the extensor/flexor muscle region of the lower extremities. This allows voluntary machine supported ROM of incomplete SCI patients by using minimal bioelectrical signals, recorded and amplified from hip and knee flexors and extensors [15–17]. More recently, these various exoskeletal systems allow the patients mobilization outside the treadmill. A former study by Kawamoto et al. [18] concerning locomotion improvement using HAL in chronic stroke patients, emphasized the feasibility for rehabilitation of these particular patients.

The aim of this pilot study was to evaluate the possibilities of exoskeletal locomotor training (HAL; Cyberdyne, Inc.) under voluntary control and identify beneficial effects on functional mobility of the patients. The hypothesis was that exoskeleton treadmill training is feasible and safe in application and capable of improving ambulatory mobility in chronic SCI patients.

Materials and methods

Patients

We enrolled eight patients (two women, six men). The mean±standard deviation age at the time of enrollment was 48±9.43 years. All patients were in the chronic stage of traumatic SCI according to the time since injury of 1 to 19 years (97.2±88.4 months). Inclusion criteria were traumatic SCI with chronic incomplete (ASIA B/C/D) or complete paraplegia (ASIA A) after lesions of the conus medullaris/cauda equine with zones of partial preservation. Independent of ASIA classification, the enrolled patients

must present motor functions of hip and knee extensor and flexor muscle groups to be able to trigger the exoskeleton. Exclusion criteria were as follows: nontraumatic SCI, pressure sores, severe limitation of ROM regarding hip and knee joints, cognitive impairment, body weight more than 100 Kg, nonconsolidated fractures, and mild or severe heart insufficiency. Two patients suffered from an incomplete thoracic SCI (ASIA C/D) from 3 to 13 years. Two patients suffered from an incomplete lumbar SCI (ASIA B/C) from 12 to 13 months and four patients had a complete SCI with zones of partial preservation in L3–S1 after lesions of the conus medullaris. The classification according to the ASIA was carried out before the treadmill training was initiated. The study was approved by Ethical Board Committee of Bergmannsheil Hospital and the University of Bochum and followed strictly the declaration of Helsinki.

All patients provided written informed consent. The study design was a single case experimental A-B (pre-post) design by repeated assessments of the same patients (Table 1).

Intervention

During this study, the patients underwent a BWSTT five times per week using the HAL exoskeleton (Cyberdyne, Inc., Japan). The study was performed between June 2013 and September 2013 in the BG University Hospital Bergmannsheil, Bochum.

Neither adverse nor severe adverse events occurred during the intervention.

The exoskeleton

The HAL robot suit (Cyberdyne, Inc., Japan) is an exoskeleton with a frame and robotic actuators that attach to the patients' legs. The joint movement is supported by electric motors. Voluntary initiated minimal bioelectrical signals recovered from extensor and flexor muscles of hip and knee are detected via electromyography electrodes (Fig. 1).

Through a cable connection between the exoskeleton and patient, this system allows voluntary robotic supported ROM (cybernic voluntary control mode). Also a passive, nonvoluntary ROM (cybernic autonomous control mode) is possible (Fig. 2).

The treadmill

The treadmill system (Woodway USA, Inc., Waukesha, WI, USA) includes a body weight support system with a harness. The speed can be adjusted from 0 Km/h to approximately 4.5 Km/h. During treatments, the velocity of the treadmill was set individually between comfortable and maximum speed tolerated by the patients. Approximately 50% of each patient's body weight needed to be supported by the harness system, individually reduced during the following sessions as tolerated without substantial knee buckling or toe drag.

EVIDENCE & METHODS

Context
The authors present a series of patients treated with an assistive exoskeleton developed to facilitate treadmill exercise in patients with spinal cord injury (SCI).

Contribution
In a series of eight patients with SCI graded ASIA A to C/D, improvements in walking time, distance and speed were noted after treatment with assistive exoskeleton.

Implications
This study is a case series of eight patients with heterogeneous clinical characteristics, including the severity of their spinal cord injury. The findings are limited to clinical contexts specific to these patients and clearly cannot be translated to the care of other individuals. This is simply a report that may show proof of concept. It should be noted that one of the authors reports a substantive conflict of interest (founder and shareholder of the company that produces the exoskeleton device).

—The Editors

The training

The patients underwent a 90-day period of HAL exoskeleton (Cyberdyne, Inc.) training (five per week), including a mean number of sessions of 51.75±5.6. The training was performed on a treadmill with individually adjustable body weight support and speed, recording walking speed, time, and distance. It included a 10-m walk test (10MWT) before and after each session and regular physiotherapy that lasted approximately 90 minutes. The training was supervised by a physiotherapist and a medical doctor.

Measurements

Walking capabilities and neurologic status

All patients were assessed on admission by medical doctors involved in this trial. The outcomes were assessed

Table 1
Subject demographics and clinical characteristics

Case	Sex	Age (y)	Time since trauma, y	Etiology	Level	ASIA/ZPP	WISCI II	Ashworth
1	M	40	13	# T7/T8	T8	C	13	4
2	M	63	1	# T12	L1	B/L3	6	0
3	M	36	1.16	# T11/T12	T12	A/L3	6	0
4	F	55	1.08	# L1	L1	C	13	0
5	M	42	16	# L1	L1	A/L3	9	0
6	M	52	10	# L3	L2	A/L3	6	0
7	F	40	19	# L1	T11	A/S1	9	0
8	M	53	3	# T12	T12	D	18	0

M, male; F, female; #, fracture; ASIA, American Spinal Injury Association; ZPP, zones of partial preservation; WISCI, walking index for spinal cord injury; T, thoracic; L, lumbal; S, sacral.

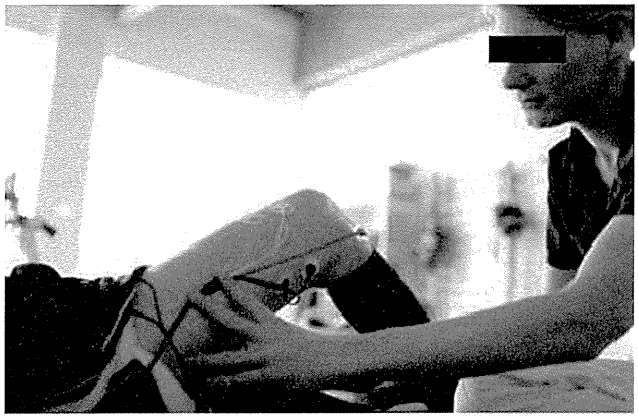

Fig. 1. Positioning of the electromyography electrodes on the knee extensor and flexor muscles.

by physiotherapists neither involved in the study design nor analysis after 45 days and on discharge from the training period. An assessment through the ASIA classification was already done on admission and on discharge from the SCI department, Bergmannsheil, Bochum, within the initial therapy after acute SCI. The 10MWT, done before and after each session, detected the needed time, the number of steps, and the required assistance to walk a 10 m distance [19,20]. The timed-up and go test (TUG test) describes the time and assistance required for standing up from the wheelchair, walk 3 m, turn around, walk back, and sit down. It was performed every 2 weeks. The 6-minute walk test (6MWT) was done at the beginning, at half time, and at the end if possible, depending on the patient. It evaluates the distance and assistance while walking for 6 minutes [21]. The main outcome was the functional motor assessment by the walking index for SCI II (WISCI II) [22,23]. The WISCI II score is a 20-item scale, measuring the walking capabilities of a patient based on the requirements of assistance because of walking aids, personal assistance, or braces. Grade 0 means that the patient has neither standing nor walking abilities. Grade 20 means that no assistance is needed to walk a distance of 10 m. The neurologic status was assessed using the ASIA Impairment Scale modified from the Frankel classification and classifies motor and sensory impairments that result from a SCI [3]. The lower extremity motor score (LEMS) acquired in this study was obtained by the addition of the impairment scores (0–5) of the lower extremity key muscles of both sides. Muscle volume was assessed by manual measurements, 20/10 cm above and 15 cm below inner knee gap.

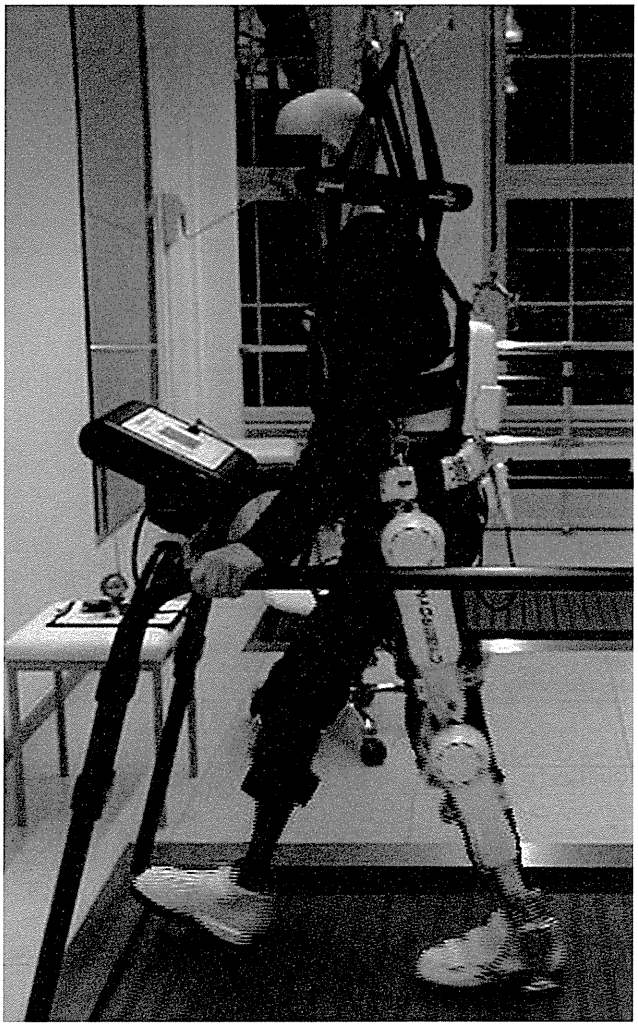

Fig. 2. Patient performing treadmill locomotion training with body weight support and hybrid assistive limb exoskeleton.

Statistical analysis

Descriptive analysis of the demographic and injury characteristics was done using frequency distribution for categorical data and mean for continuous variables. Differences between pre- and posttraining sessions were assessed by a paired t test (for continuous variables). Treatment effects on functional performance as the WISCI II are all ordinal scales. Medians were used as descriptive statistics for these outcomes, and nonparametric tests were used to assess the relative effect of the treatments.

Results were considered statistically significant when the p value was $\leq .05$.

Results

Treadmill associated results

All patients improved in treadmill training by using HAL (Cyberdyne, Inc., Japan). The mean walking speed increased from 0.91 ± 0.41 m/s (0.5–1.8 m/s) in the first session up to 1.59 ± 0.5 m/s (0.8–2.1 m/s) in the last session after 3 months. The progress in speed after 6 weeks of training was lower than in the first weeks. The range was located between 0 km/h and 0.8 km/h. The mean walking time at the beginning was 12.37 ± 4.55 minutes. The average walking time at the

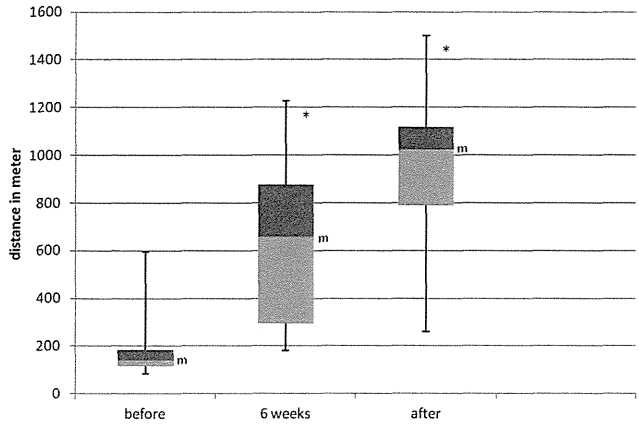

Fig. 3. Changes in treadmill-associated walking distance in pre-, mid-, and postevaluations. m, median. *pre-post difference, p<.05.

end was 31.97±9.45 minutes. The mean ambulated distance at the first session was 195.9±166.7 m and increased to 954.13±380.4 m on discharge (Fig. 3).

Functional outcome

Although the mean improvement concerning the WISCI II score was not statistically significant, three patients showed functional improvement in gait abilities. Two subjects needed braces, a walker, and support by a physiotherapist at the beginning and were able to walk after the training series only with a walker and braces (WISCI II score increased from 6 to 9). One patient increased from 9 to 12 and, therefore, was able to walk with two crutches and a brace compared with a walker and a brace before the training. At baseline, the mean WISCI II score was 10±4.3. At the end of the 90 days trial, the mean WISCI II was 11.13±6.68. Improvements in speed and endurance in over-ground gait assessments in all participants have been achieved. The 10MWT showed a significant increase in mean gait speed at the end of the training period compared with baseline (0.28±0.28 m/s vs. 0.50±0.34 m/s) (Fig. 4).

The improvement corresponded to a 44% faster walking than in initial evaluation. It also includes the reduction of

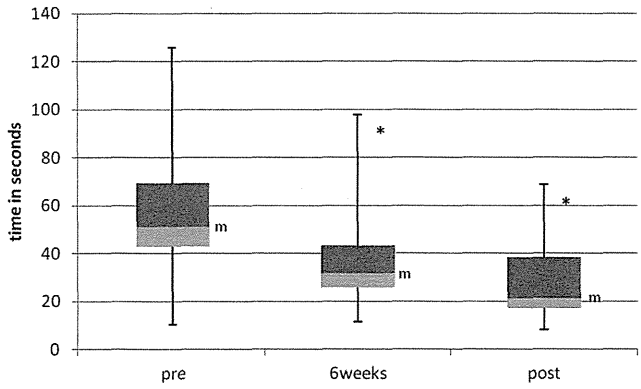

Fig. 4. Changes in 10-m walk test in pre-, mid-, and postintervention evaluations. m, median. *pre-post difference, p<.05.

Table 2
Comparison of pre- and postinterventions

Outcome measurements	Before training	After training	n
10MWT speed (m/s)	0.28±0.28	0.5±0.34*	8
Number of steps	29.88±7.85	19.38±3.16*	8
6MWT distance (m)	70.1±130	163.3±160.6*	8
TUG test (s)	55.34±32.20	38.18±25.98*	8
Distance (m)	195.88±166.71	954.13±380.35*	8
WISCI-II	10±4.34	11.12±3.68	8

10MWT, 10-m walk test; 6MWT, 6-minute walk test; TUG, timed-up and go; WISCI, walking index for spinal cord injury.
Note: Values are means±standard deviation.
* Pre-post difference, p<.05.

support needed detected by the WISCI II score. The mean number of steps decreased from 29.8±7.85 to 19.4±3.16. We observed significant increase in gait speed from pre- to midtraining and from mid- to posttraining assessments. Similar results were detected for the TUG test. The mean time needed for the TUG test decreased from 55.34±32.2 seconds to 38.18±25.98 seconds. The 6MWT was done with a constant walking time of 6 minutes without any break. Only three patients were able to perform the 6MWT before the training with a mean walking distance of 187±162.2 m. The subjects in this subgroup improved their performance and increased the walking distance to 287.3±229.4 m. After completing the training, all eight patients could be evaluated, therefore the overall mean distance increased from 70.1±130 m to 163.3±160.6 m (Table 2).

The LEMS increased in all patients. The mean LEMS before the training increased significantly from 21.75±8.3 to 24.38±7.6 after the intervention (Fig. 5).

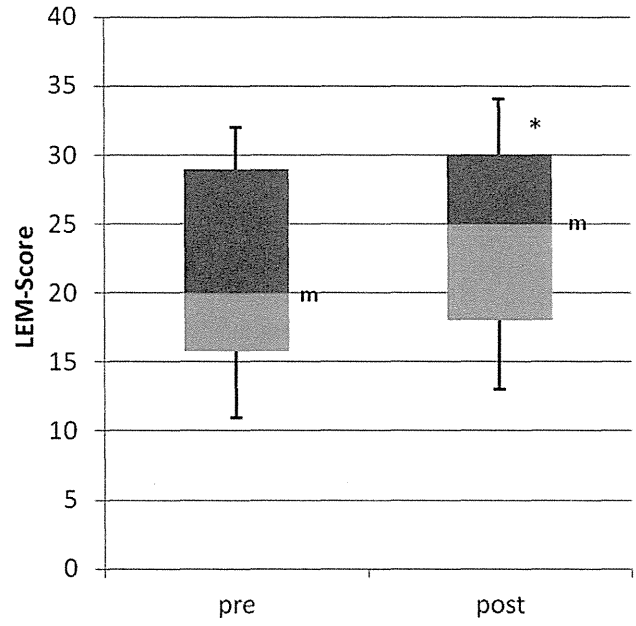

Fig. 5. LEMS in pre- and postevaluation. LEM, lower extremity motor score; m, median. *pre-post difference, p<.05.

One patient switched in the ASIA scale from ASIA B to C, he was at the beginning of the training 12 months posttrauma.

Others

To describe muscle volume, measurements of the circumferences 10/20 cm cranial of the inner knee joint gap and 15 cm distal of it have been done before and after the 90 days of training. Seven participants showed a gain of muscle circumference from 5 mm up to 50 mm. In one participant with edema in his lower legs, we observed a loss of circumference up to 25 mm. One patient suffering from a thoracic SCI presented a significant spinal spasticity. For spastic motor behaviors, we used the modified Ashworth scale to evaluate the involuntary resistance to passive stretch of the quadriceps muscle group. At pretraining evaluation, he showed an extensor spasm with high resistance to passive stretch according to Ashworth 4. After the training sessions, the resistance was reduced according to Ashworth 2. This level lasted for about 6 to 8 hours with a new maximum level at the next morning. All other patients showed no spastic motor behaviors.

Discussion

The objective of the study was to determine whether locomotor training with the exoskeleton HAL is feasible and safe in application, improves functional mobility, and increases motor functions in chronic paraplegic patients after SCI. The results obtained revealed a highly significant improvement for over-ground walking abilities evaluated by the 10MWT, the 6MWT, and the TUG test and the partial reduction of physical assistance and walking aids in the WISCI II score. Muscle strength, measured with the LEMS increased in all patients.

The results acquired in this clinical trial imply that HAL-supported locomotion training can improve walking abilities in terms of speed, gait, and distance. Furthermore, it improves motor functions.

Thus far there is insufficient evidence and only a few articles addressing the main hypothesis of this study that locomotor training improves walking function for patients with SCI [24].

The present study is according to the knowledge of the authors the first to investigate the impact of HAL-supported locomotor training in chronic SCI patients, where referring to the current state of knowledge no further functional improvements are to be expected.

In the subject population consisting of eight patients including patients suffering from SCI from 1 to 19 years (8.03±7.4 years), all patients improved significantly regarding treadmill-associated walking distance and speed and functional improvement was detected in the over-ground walking tests.

Although no significant influence was seen on the requirements of assistance in the 10MWT, three patients attained improvement in walking abilities according to the WISCI II, under condition of a comfortable and stable gait. A further reduction of assistance was not forced because of more pathologic gait or higher risk of falling [24].

Although the evidence is still insufficient, the effectiveness of automated locomotor training using the DGO in patients with chronic SCI is being investigated and considered promising in several systematic reviews including a Cochrane review [25,26]. The results mentioned previously add to the wealth of that data presuming that HAL-assisted locomotion training is useful in terms of functional mobility and a safe adjunct to the treatment of patient with chronic SCI.

Our study had several limitations: the relatively small number of patients (n=8) and the mixture of complete and incomplete SCIs.

However, all the patients were treated in the same facility by the same multidisciplinary team, according to a standardized protocol.

In summary, our study provides the first data demonstrating the clinical potential of HAL-locomotor training based on voluntary drive in patients suffering from chronic SCI.

It was proven to be a safe device for locomotion therapy as neither adverse nor severe adverse events occurred.

However, continued research in the form of large randomized trials to compare the efficacy of HAL-assisted training with well established, conventional therapies is necessary.

References

[1] Eng J, Teasell R, Miller W, et al. Spinal cord injury rehabilitation evidence: methods of the SCIRE systematic review. Top Spinal Cord Inj Rehabil 2007;13:1–10.

[2] Waters RL, Adkins R, Yakura J, Vigil D. Prediction of ambulatory performance based on motor scores derived from standards of the American Spinal Injury Association. Arch Phys Med Rehabil 1994;75:756–60.

[3] Piepmeier JM, Jenkins NR. Late neurological changes following traumatic spinal cord injury. J Neurosurg 1988;69:399–402.

[4] Gittler MS, McKinley WO, Stiens SA, et al. Spinal cord injury medicine. 3. Rehabilitation outcomes. Arch Phys Med Rehabil 2002;83:S65–71.

[5] Finch L, Barbeau H, Arsenault B. Influence of body weight support on normal human gait: development of a gait retraining strategy. Phys Ther 1991;71:842–55; discussion 855–6.

[6] Dietz V, Wirz M, Curt A, Colombo G. Locomotor pattern in paraplegic patients: training effects and recovery of spinal cord function. Spinal Cord 1998;36:380–90.

[7] Colombo G, Joerg M, Schreier R, Dietz V. Treadmill training of paraplegic patients with a robotic orthosis. J Rehabil Res Dev 2000;37:693–700.

[8] Winchester P, Querry R. Robotic orthosis for body weight-supported treadmill training. Phys Med Rehabil Clin N Am 2006;17:159–72.

[9] Colombo G, Wirz M, Dietz V. Driven gait orthosis for improvement of locomotor training in paraplegic patients. Spinal Cord 2001;39:252–5.

[10] Wirz M, Zemon DH, Rupp R, et al. Effectiveness of automated locomotor training in patients with chronic incomplete spinal cord

injury: a multicenter trial. Arch Phys Med Rehabil 2005;86: 672–80.
[11] Hornby TG, Zemon DH, Campbell D. Robotic assisted, body-weight-supported treadmill training in individuals following motor incomplete spinal cord injury. Phys Ther 2005;85:52–66.
[12] Hornby TG, Campbell DD, Zemon DH, Kahn JH. Clinical and quantitative evaluation of robotic-assisted treadmill walking to retrain ambulation following spinal cord injury. Top Spinal Cord Inj Rehabil 2005;11:1–17.
[13] Nooijen CF, Ter Hoeve N, Field-Fote EC. Gait quality is improved by locomotor training in individuals with SCI regardless of training approach. J Neuroeng Rehabil 2009;6:36–46.
[14] Schwartz I, Sajina A, Neeb M, et al. Locomotor training using a robotic device in patients with subacute spinal cord injury. Spinal Cord 2011;49:1062–7.
[15] Kawamoto H, Sankai Y. Power assist system HAL-3 for gait disorder person. Proceedings of ICCHP international conference on computers helping people with special needs. 2002; 196–203.
[16] Suzuki K, Mito G, Kawamoto H, et al. Intention-based walking support for paraplegia patients with robot suit hal. Advanced Robotics 2007;21:383–408.
[17] Yamawaki K, Kawamoto H, Eguchi K, et al. Gait training for a spinal canal stenosis patient using robot suit HAL: a case report-. Proceedings of the 6th World Congress of the International Society of Physical and Rehabilitation Medicine, 2011; 66–68.
[18] Kawamoto H, Kiyotaka K, Yoshio N, et al. Pilot study of locomotion improvement using hybrid assistive limb in chronic stroke patients. BMC Neurol 2013;13:141.
[19] Van Hedel HJ, Wirz M, Curt A. Improving walking assessment in subjects with an incomplete spinal cord injury: responsiveness. Spinal Cord 2006:44:352–6.
[20] Van Hedel HJ, Wirz M, Dietz V. Assessing walking ability in subjects with spinal cord injury: validity and reliability of 3 walking tests. Arch Phys Med Rehabil 2005:86:190–6.
[21] Harada ND, Chiu V, Stewart AL. Mobility-related function in older adults: assessment with a 6-minute walk test. Arch Phys Med Rehabil 1999:80:837–84.
[22] Ditunno JF, Ditunno PL. Walking index for spinal cord injury (WISCI II): scale revision. Spinal Cord 2001;39:654–6.
[23] Myeong OK, Burns AS, Ditunno JF, Marino RJ. The assessment of walking capacity using the walking index for spinal cord injury: self-selected versus maximal levels. Arch Phys Med Rehabil 2007;88:762–7.
[24] Nascimento LR, Caetan LC, Freitas DC, et al. Different instructions during the ten-meter walking test determined significant increases in maximum gait speed in individuals with chronic hemiparesis. Rev Bras Fisioter 2012;16:122–7.
[25] Mehrholz J, Kugler J, Pohl M. Locomotor training for walking after spinal cord injury. Cochrane Database Syst Rev 2012;11.
[26] Morawietz C, Moffat F. Effects of locomotor training after incomplete spinal cord injury: a systematic review. Arch Phys Med Rehabil 2013;94:2297–308.

J. Neurovirol.
DOI 10.1007/s13365-013-0192-8

REVIEW

Neuroimmunological aspects of human T cell leukemia virus type 1-associated myelopathy/tropical spastic paraparesis

Mineki Saito

Received: 19 April 2013 / Revised: 9 July 2013 / Accepted: 22 July 2013

Abstract Human T cell leukemia virus type 1 (HTLV-1) is a human retrovirus etiologically associated with adult T cell leukemia/lymphoma and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Only approximately 0.25–4 % of infected individuals develop HAM/TSP; the majority of infected individuals remain lifelong asymptomatic carriers. Recent data suggest that immunological aspects of host–virus interactions might play an important role in the development and pathogenesis of HAM/TSP. This review outlines and discusses the current understanding, ongoing developments, and future perspectives of HAM/TSP research.

Keywords HTLV-1 · HAM/TSP · Host immune response

Introduction

Human T cell leukemia virus type 1 (HTLV-1) is a replication-competent human retrovirus associated with two distinct types of disease: a malignancy of mature $CD4^+$ T cells called adult T cell leukemia/lymphoma (ATL) (Hinuma et al. 1981; Poiesz et al. 1980; Yoshida et al. 1984) and a chronic inflammatory central nervous system disease HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Gessain et al. 1985; Osame et al. 1986). Like human immunodeficiency virus (HIV), HTLV-1 is never eliminated from the host despite vigorous cellular and humoral immune responses. However, in contrast to HIV infection, few with HTLV-1 develop disease; only approximately 2–3 % of infected persons develop ATL (Tajima 1990), another 0.25–4 % develop HAM/TSP (Hisada et al. 2004; Kramer et al. 1995; Nakagawa et al. 1995; Osame et al. 1990), and the majority of infected individuals remain lifelong asymptomatic carriers (ACs). Therefore, evaluation of the individual risk of developing disease in ACs would certainly be of considerable importance, especially in HTLV-1 endemic areas.

The viral, host, and environmental risk factors as well as the host immune response against HTLV-1 infection appear to regulate the development of HTLV-1-associated diseases (Bangham and Osame 2005). In particular, a strong immune response, especially the cytotoxic T lymphocyte (CTL) response, to HTLV-1 is seen in patients with HAM/TSP and suggested to be strongly associated with the pathogenesis of HTLV-1-associated diseases (Matsuura et al. 2010; Saito et al. 2012). For more than two decades, the investigation of HTLV-1-mediated immunopathogenesis has focused on Tax, an HTLV-1-encoded viral oncoprotein, because Tax activates many cellular genes by binding to groups of transcription factors and co-activators and is necessary and sufficient for cellular transformation. However, recent reports have identified that another regulatory protein, HTLV-1 basic leucine zipper factor (HBZ), also has a critical role in the development of ATL and HAM/TSP (Matsuoka and Jeang 2011). This review summarizes past and recent studies of HAM/TSP, attempting to answer the following fundamental questions: Why do some HTLV-1-infected people develop disease whereas the vast majority remain healthy? How does HTLV-1 persist in the individual host despite a strong host immune response? How is the inflammatory lesion in HAM/TSP initiated and maintained?

History and epidemiology of HTLV-1

HTLV-1 belongs to the *Deltaretrovirus* genus of the *Orthoretrovirinae* subfamily and infects 10–20 million people worldwide (de The and Bomford 1993; Proietti et al. 2005; Uchiyama 1997). HTLV-1 can be transmitted through sexual contact (Roucoux et al. 2005), intravenous drug use (Proietti et al. 2005), and breastfeeding from mother to child (Hino

M. Saito (✉)
Department of Microbiology, Kawasaki Medical School,
577 Matsushima, Kurashiki 701-0192, Japan
e-mail: mineki@med.kawasaki-m.ac.jp

Published online: 14 August 2013

et al. 1985; Kinoshita et al. 1987). At present, the infection is endemic in southwest Japan, the Caribbean, Sub-Saharan Africa, and South America, with smaller foci in Southeast Asia, South Africa, and northeast Iran (Verdonck et al. 2007). 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 (Poiesz et al. 1980). It was the first retrovirus ever associated with cancer in a human. Three years before the isolation of HTLV-1, Takatsuki et al. reported ATL, a rare form of leukemia endemic to southwest Japan, as a distinct clinical entity (Uchiyama et al. 1977). In 1981, Hinuma et al. clearly demonstrated that ATL was caused by a new human retrovirus, originally termed ATLV (Hinuma et al. 1981; Miyoshi et al. 1981). Since then, 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 to a chronic progressive neurological disease, which was termed tropical spastic paraparesis in the Caribbean (Gessain et al. 1985) and HTLV-1-associated myelopathy in Japan (Osame et al. 1986). 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 (Hollsberg and Hafler 1993). To date, more than 3,000 cases of HAM/TSP have been reported in HTLV-1 endemic areas. Sporadic cases have also been described in non-endemic areas such as the USA and Europe, mainly in immigrants from an HTLV-1 endemic area (Araujo and Silva 2006). HTLV-1 can cause other chronic inflammatory diseases such as uveitis (Mochizuki et al. 1992), arthropathy (Nishioka et al. 1989), pulmonary lymphocytic alveolitis (Maruyama et al. 1988; Sugimoto et al. 1989; Sugimoto et al. 1987), polymyositis (Higuchi et al. 1992; Morgan et al. 1989), Sjögren syndrome (Terada et al. 1994), and infective dermatitis (LaGrenade et al. 1990), although there is no clear evidence for an etiological role of HTLV-1 in these diseases.

Clinical and pathological features of HAM/TSP

HAM/TSP is a chronic progressive myelopathy characterized by spastic paraparesis, sphincter dysfunction, and mild sensory disturbance in the lower extremities (Nakagawa et al. 1996). 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 that for the onset of ATL (Nakagawa et al. 1995; Olindo et al. 2006). HAM/TSP occurs both in vertically infected individuals and in those who become infected later in life, that is, 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 autoimmune diseases, the frequency of cases of HAM/TSP is greater in women than in men (the male-to-female ratio of occurrence is 1:2.3) (Nakagawa et al. 1995). In addition to HTLV-1 antibody (Ab) positivity both in serum and cerebrospinal fluid (CSF), the presence of atypical lymphocytes (the so-called "flower cells") in peripheral blood and CSF, a moderate pleocytosis, and raised protein content in CSF is observed in patients with HAM/TSP (Araujo and Silva 2006). Oligoclonal immunoglobulin bands in the CSF; raised concentrations of inflammatory markers such as neopterin, tumor necrosis factor (TNF)-α, interleukin (IL)-6, and interferon (IFN)-γ; and increased intrathecal Ab synthesis specific for HTLV-1 antigens have also been described (Jacobson 2002). Clinical progression of HAM/TSP is associated with an increase in the proviral load (PVL) in individual patients, and a high ratio of PVL in CSF cells/peripheral blood mononuclear cells (PBMCs) is also significantly associated with clinically progressive disease (Takenouchi et al. 2003). Thus, a pro-inflammatory environment associated with increased numbers of HTLV-1-infected cells is a characteristic immunological profile of HAM/TSP.

Pathological analysis of HAM/TSP autopsy materials showed the loss of myelin and axons in the lateral, anterior, and posterior columns of the spinal cord. These lesions are associated with perivascular and parenchymal lymphocytic infiltration with the presence of foamy macrophages, reactive astrocytosis, and fibrillary gliosis, predominantly at the thoracic level (Iwasaki 1990; Izumo et al. 2000; Yoshioka et al. 1993), suggesting that the immune response against HTLV-1 causes the inflammatory spinal cord damage seen in patients with HAM/TSP (Bangham 2000). In patients with active chronic lesions in the spinal cord, perivascular inflammatory infiltration with similar composition of cell subsets was also seen in the brain (Aye et al. 2000). The peripheral nerve pathology of patients with HAM/TSP with sensory disturbance showed varying degrees of demyelination, remyelination, axonal degeneration, regeneration, and perineural fibrosis (Bhigjee et al. 1993; Kiwaki et al. 2003).

Treatment of HAM/TSP

To date, no generally agreed standard treatment regimen has been established for HAM/TSP, and no treatment has proven to be consistently effective on a long-term basis. Therefore, clinical practice for treatment of patients with HAM/TSP is based on case series and open, nonrandomized, uncontrolled studies. Mild to moderate beneficial effects have been reported for a number of agents in open-label studies including corticosteroids (Nakagawa et al. 1996), danazol (Harrington et al. 1991), pentoxifylline (Shirabe et al. 1997), immunosuppressants such as ciclosporin A (Martin et al. 2012), high-dose intravenous gamma globulin (Kuroda et al. 1991), plasmapheresis (Matsuo et al. 1988), antibiotics (erythromycin and fosfomycin), and vitamin C (Nakagawa et al. 1996). It should be noteworthy that oral prednisolone was effective in 81.7 %

of 131 patients in a large-scale case series study (Nakagawa et al. 1996). However, the complications of corticosteroids limit their use, particularly in post-menopausal women, who are at higher risk for developing HAM/TSP. Multicenter double-blind randomized placebo-controlled trials for the IFN-α treatment indicate that IFN-α is an effective therapy with an acceptable side effects profile (Izumo et al. 1996), although the benefit of long-term IFN-α therapy has not been well studied. In regard to oral antiviral drugs zidovudine plus lamivudine, no evidence of significant benefit yet exists from randomized placebo-controlled trials (Taylor et al. 2006). Recently, oral administration of histone deacetylase inhibitor valproic acid (VPA) has been conducted as a single-center, open-label trial (Olindo et al. 2011). Although administration of VPA induced a transient increase of HTLV-1 expression to expose virus-positive cells to the host immune response, clinical measures and PVL were stable overall. It has also been reported that the antibiotic minocycline significantly inhibited spontaneous lymphocyte proliferation and degranulation/IFN-γ expression in $CD8^+$ T cells of patients with HAM/TSP, suggesting its potential for treatment (Enose-Akahata et al. 2012). Overall, more clinical trials with adequate power are needed in the future.

Risk factors for developing HAM/TSP

It is well-known that HAM/TSP occurs in only a minority of HTLV-1-infected individuals. A previous population association study in HTLV-1 endemic southwest Japan revealed that one of the major risk factors is the HTLV-1 PVL, because the PVL is significantly higher in patients with HAM/TSP than in ACs (Nagai et al. 1998). A higher PVL in patients with HAM/TSP than in ACs was also observed in other endemic areas such as the Caribbean (Manns et al. 1999), South America (Adaui et al. 2006), and the Middle East (Sabouri et al. 2005). In southwest Japan, it was suggested that genetic factors such as the human leukocyte antigen (HLA) genotype are related to the high PVL in patients with HAM/TSP and genetic relatives. Namely, possession of the HLA class I genes HLA-A*02 and Cw*08 was associated with a statistically significant reduction in both HTLV-1 PVL and the risk of HAM/TSP, whereas possession of HLA class I HLA-B*5401 and class II HLA-DRB1*0101 predispose to HAM/TSP in the same population (Jeffery et al. 2000; Jeffery et al. 1999). Because 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 PVL and the risk of HAM/TSP. In accordance with this observation, it has been reported that CTL spontaneously kill autologous HTLV-1-infected cells ex vivo (Hanon et al. 2000), granzymes and perforin are more highly expressed in individuals with a low PVL (Vine et al. 2004), and the lytic efficiency of the CD8+ T cell response (i.e., the fraction of autologous HTLV-1-expressing cells eliminated per CD8+ T cell per day) was inversely correlated with both PVL and the rate of spontaneous proviral expression (Kattan et al. 2009). Furthermore, the major histocompatibility complex (MHC) class I tetramer analysis of lymphocytes isolated from the CSF of patients with HAM/TSP showed even higher frequencies of HTLV-1 Tax11-19-specific, HLA-A*02-restricted CD8+ lymphocytes compared with PBMCs (Nagai et al. 2001b). These findings indicate that an increased proliferation or migration of HTLV-1-infected and/or HTLV-1-specific lymphocytes to the central nervous system might be closely associated with the pathogenesis of HAM/TSP (Hayashi et al. 2008a), and the CTLs against HTLV-1 reduce both PVL and the risk of HAM/TSP. Recently, using a combination of computational and experimental approaches, MacNamara et al. reported that a CTL response against HBZ restricted by protective HLA alleles such as HLA-A*02 or Cw*08, but not a response to the immunodominant protein Tax, also determines the outcome of HTLV-1 infection (Macnamara et al. 2010).

Meanwhile, analysis of non-HLA host genetic factors by candidate gene approaches revealed that non-HLA gene polymorphisms also affect the risk of developing HAM/TSP. Namely, the TNF-α promoter −863 A allele (Vine et al. 2002) and the longer CA repeat alleles of matrix metalloproteinase 9 promoter (Kodama et al. 2004) predisposed to HAM/TSP, whereas IL-10 −592 A (Sabouri et al. 2004), stromal-derived factor 1 +801A (Vine et al. 2002), and IL-15 +191 C alleles (Vine et al. 2002) conferred protection against HAM/TSP. The polymorphisms in the matrix metalloproteinase 9 and IL-10 promoters were each associated with differences in the HTLV-1 Tax-mediated transcriptional activity of the respective gene (Kodama et al. 2004; Sabouri et al. 2004). However, the contributions of these non-HLA genes to the pathogenesis of HAM/TSP are largely unknown and these data have not yet been reproduced in different populations. Further candidate gene studies together with genome-wide association studies in different ethnic populations in a larger sample size may provide evidence for the association of non-HLA genes with the pathogenesis of HAM/TSP.

It has been reported that the lifetime risk of developing HAM/TSP differs 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 versus 3 cases/100,000 population), with a two to three times greater risk for women in both populations (Hisada et al. 2004; Kramer et al. 1995; Nakagawa et al. 1995; Osame et al. 1990). Although most studies of HTLV-1 genotype have reported no association between variants of HTLV-1 and the risk of HAM/TSP, Furukawa et al. reported the association between HTLV-1 Tax gene variation and the risk of HAM/TSP (Furukawa et al. 2000). Tax subgroup A, which belongs to cosmopolitan

subtype A, was more frequently observed in patients with HAM/TSP, and this association was independent of the protective effect of HLA-A*02. Interestingly, HLA-A*02 appeared to give protection against only one of the two prevalent sequence variants of HTLV-1, Tax subgroup B, which belongs to cosmopolitan subtype B, but not against Tax subgroup A in the Japanese population (Furukawa et al. 2000). Jamaican subjects, who had a higher annual incidence of HAM/TSP, also have cosmopolitan subtype A, whereas approximately 80 % of Japanese subjects, who had a lower annual incidence of HAM/TSP, have cosmopolitan subtype B. Interestingly, HLA-A*02 did not appear to provide protection against HAM/TSP development with cosmopolitan subtype A in a population in Iran (Sabouri et al. 2005).

To test whether the genomic integration site determines the abundance and the pathogenic potential of an HTLV-1-positive T cell clone, Gillet et al. recently reported the results of high-throughput mapping and quantification of HTLV-1 proviral integration in the host genome (Gillet et al. 2011). They mapped >91,000 unique insertion sites (UISs) of the provirus in primary PBMCs from 61 HTLV-1-infected individuals and showed that a typical HTLV-1-infected host carries between 500 and 5,000 UISs in 10 μg of PBMC genomic DNA. They calculated an oligoclonality index to quantify the clonality of HTLV-1-infected cells in vivo and found that the oligoclonality index did not distinguish between ACs and patients with HAM/TSP and that there was no correlation between the oligoclonality index and HTLV-1 PVL in either ACs or patients with HAM/TSP. These results indicate that the higher PVL observed in patients with HAM/TSP was attributable to a larger number of UISs but not, as previously thought, to a difference in clonality. They also obtained evidence that the abundance of established HTLV-1 clones is determined by genomic features of the host DNA flanking the provirus. Namely, HTLV-1 clonal expansion in vivo is favored by a proviral integration site near a region of host chromatin undergoing active transcription or same-sense transcriptional orientation of the provirus. In contrast, negative selection of infected clones, probably by CTLs during chronic infection, favors establishment of proviruses integrated in transcriptionally silenced DNA, and this selection is more efficient in ACs than in HAM/TSP, indicating the selection of HTLV-1-infected T cell clones with low pathogenic potential. More recent reports indicate that circulating HTLV-1-positive cells each contain a single integrated proviral copy (Cook et al. 2012), and cells expressing HTLV-1 Tax protein (i.e., viral protein expression) were significantly more frequent in clones of low abundance in vivo, whereas certain transcription start sites immediately upstream of the viral integration site were associated with virus latency (i.e., no viral protein expression). In particular, Tax-expressing, more "pathogenic" clones were efficiently controlled by the immune response, especially CTLs, whereas non-Tax-expressing "invisible" infected clones were associated with mitotic clonal expansion in vivo (Melamed et al. 2013).

The innate immune response in HAM/TSP

Type I IFN is a key innate immune cytokine produced by cells in response to viral infection. The type I IFN response protects cells against invading viruses by inducing the expression of IFN-stimulated genes, which execute the antiviral effects of IFN (Samuel 2001). The IFN-stimulated genes then generate soluble factors including cytokines that activate adaptive immunity or directly inhibit the virus itself (Liu et al. 2011). In PBMCs of HTLV-1-infected individuals, the level of HTLV-1 messenger RNA is very low and viral protein is not detectable, but these molecules are rapidly expressed after a short time in culture in vitro (Hanon et al. 2000). However, the mechanisms of this phenomenon are largely unknown. Recently, it has been reported that HTLV-1 expression in HTLV-1-infected T cells is suppressed by stromal cells (i.e., epithelial cells and fibroblasts) in culture through type I IFNs (Kinpara et al. 2009). Namely, HTLV-1 Gag protein expression was suppressed when contacted with stromal cells and restored when separated from the stromal cells. Although neutralizing antibodies against human IFN-α/β receptor only partly abrogated this phenomenon, the results indicate that the innate immune system suppresses HTLV-1 expression in vitro and in vivo, at least through type I IFN. More recently, it has been reported that IFN-stimulated genes were overexpressed in circulating leukocytes and the expression correlated with the clinical severity of HAM/TSP (Tattermusch et al. 2012).

Previous reports indicated that patients with HAM/TSP had both a lower frequency and a lower activity of natural killer (NK) cells (especially the CD3+ CD16+ subset) than ACs, although the results were not normalized with respect to the PVL (Yu et al. 1991). Because an important mechanism of induction of NK cell-mediated killing is recognition by the NK cell of a complex of the non-polymorphic MHC molecule HLA-E bound to a peptide derived from the signal sequence of some other MHC class I molecules, the synthetic tetramers of HLA-E with the HLA-G signal sequence peptide were used to identify NK cells in patients with HAM/TSP (Saito et al. 2003). The results clearly showed a lower frequency of HLA-E tetramer-binding cells in patients with HAM/TSP than in ACs; as in the earlier studies (Yu et al. 1991), this reduction in frequency was particularly notable in the CD3+ cells, whereas there was no significant difference in the frequency of HLA-E tetramer-binding CD3− cells between patients with HAM/TSP and ACs (Saito et al. 2003). Recent reports also suggest that the frequency of invariant natural killer T (NKT) cells in the peripheral blood of patients with HAM/TSP is significantly decreased when compared with that in healthy subjects and/or ACs (Azakami et al. 2009; Ndhlovu et al. 2009). These findings indicated that the activity of the NK or NKT cell response was associated with the presence or absence of HAM/TSP. Interestingly, a previous uncontrolled preliminary trial of viable *Lactobacillus casei* strain Shirota-containing

fermented milk in patients with HAM/TSP resulted in a significant increase in NK cell activity with improvements in clinical symptoms (Matsuzaki et al. 2005). Thus, circulating NK and NKT cells might also play an important role in the disease progression and pathogenesis of HAM/TSP.

The acquired immune response in HAM/TSP

It has been reported that patients with HAM/TSP generally have higher anti-HTLV-1 Ab titers than ACs with a similar PVL (Ishihara et al. 1994; Kira et al. 1992; Nagasato et al. 1991), suggesting the existence of an augmented humoral immune response to HTLV-1. Interestingly, although Ab responses to the immunodominant epitopes of the HTLV-1 Envelope (Env) proteins were similar in all three clinical groups of HTLV-1 infection (HAM/TSP, ATL, and ACs), reactivity to four Tax immunodominant epitopes was higher in patients with HAM/TSP (71–93 %) than in patients with ATL (4–31 %) or ACs (27–37 %) (Lal et al. 1994). A recent report indicates that the Ab response against HBZ was associated with reduced CD4+ T cell activation in patients with HAM/TSP, and HBZ-specific Ab inhibited spontaneous in vitro lymphocyte proliferation in the PBMCs of patients with HAM/TSP (Enose-Akahata et al. 2013). Among these anti–HTLV-1 antibodies, anti-Env Ab is particularly important because some anti-Env Abs have neutralizing activity against HTLV-1. Antisera raised against recombinant HTLV-1 Env polypeptides (Kiyokawa et al. 1984; Nakamura et al. 1987), vaccinia virus containing the HTLV-1 env gene (Hakoda et al. 1995; Shida et al. 1987), immunization with neutralizing epitope peptides (Tanaka et al. 1994), and passive transfer of human immunoglobulin G that has neutralizing activity (Murata et al. 1996; Tanaka et al. 1993) were all shown to neutralize HTLV-1 infectivity. In HTLV-1 infection, the roles of HTLV-1 neutralizing Ab in vivo are still largely unknown. It will be interesting to examine whether HTLV-1 neutralizing Ab titers correlate with disease status and PVL in infected individuals. Because the mutation rate of HTLV-1 provirus is significantly lower than that of HIV-1, passive immunization with human monoclonal Ab may be a beneficial and effective method to prevent HTLV-1 infection.

Antiviral CD4+ T cell responses are of central importance in driving B cell and CD8+ T cell responses in vivo. The most common HTLV-1 antigen recognized by CD4+ T cells is the Env protein (Goon et al. 2004b; Kitze et al. 1998), in contrast to the immunodominance of Tax in the CD8+ T cell response (Goon et al. 2004a; Jacobson et al. 1990; Kannagi et al. 1991). At a similar PVL, patients with HAM/TSP had a significantly increased frequency of virus-specific CD4+ T cells compared with ACs (Goon et al. 2004b; Nose et al. 2007). The antiviral T-helper (Th) 1 phenotype is also dominant among HTLV-1-specific CD4+ T cells in both ACs and patients with HAM/TSP (Goon et al. 2002), and there is a higher frequency of IFN-γ, TNF-α, and IL-2 production by CD4+ T cells in patients with HAM/TSP compared with ACs of a similar PVL (Goon et al. 2002; Goon et al. 2003). A role for CD4+ T cells in initiating and causing HAM/TSP is also consistent with the immunogenetic observations that the possession of HLA-DRB1*0101, which restricts the immunodominant epitope of HTLV-1 Env gp21, was associated with susceptibility to HAM/TSP in independent HTLV-1-infected populations in southern Japan (Jeffery et al. 1999, 2000) and northeast Iran (Sabouri et al. 2005). Accordingly, a synthetic tetramer of DRB1*0101 and the immunodominant HTLV-1 Env380-394 peptide was used to analyze Env-specific CD4+ T cells directly ex vivo (Nose et al. 2007). The results showed that the frequency of tetramer+ CD4+ T cells was significantly higher in patients with HAM/TSP than in ACs with a similar PVL. Furthermore, direct ex vivo analysis of tetramer+ CD4+ T cells from two unrelated DRB1*0101-positive patients with HAM/TSP indicated that certain T cell receptor Vβs were utilized and antigen-specific amino acid motifs were identified in complementarity determining region 3 from both patients. These results suggest that the observed increase in virus-specific CD4+ T cells in patients with HAM/TSP, which may contribute to CD4+ T cell-mediated antiviral immune responses and to an increased risk of HAM/TSP, was not simply due to the rapidly growing HTLV-1-infected CD4+ T cells but was the result of in vivo selection by specific MHC–peptide complexes, as observed in freshly isolated HLA-A*0201/Tax11-19 tetramer+ CD8+ T cells (Saito et al. 2001) and muscle-infiltrating cells from patients with HAM/TSP and HTLV-1-infected patients with polymyositis (Saito et al. 2002).

Previous reports indicated that HTLV-1-specific CD8+ CTLs are typically abundant, chronically activated, and mainly targeted to the viral transactivator protein Tax (Bangham 2000). Further, as already mentioned, the median PVL in PBMCs of patients with HAM/TSP was more than 10 times higher than that in ACs, and a high PVL was also associated with an increased risk of progression to disease (Nagai et al. 1998). Furthermore, HLA-A*02 and HLA-Cw*08 genes were independently and significantly associated with a lower PVL and a lower risk of HAM/TSP (Jeffery et al. 2000; Jeffery et al. 1999), and CD8+ T cells efficiently kill autologous Tax-expressing lymphocytes in fresh PBMCs in HTLV-1-infected individuals (Hanon et al. 2000). These data have raised the hypothesis that the class I-restricted CD8+ CTL response plays a critical part in limiting HTLV-1 replication in vivo and that genetically determined differences in the efficiency of the CTL response to HTLV-1 account for the risk of developing HAM/TSP. The analysis of gene expression profiles using microarrays in circulating CD4+ and CD8+ lymphocytes indicated that granzymes and perforin are more highly expressed in individuals with a low PVL (Vine et al. 2004), suggesting that a strong CTL response is associated

with a low PVL and a low risk of HAM/TSP. In accordance with this observation, the lytic capacity of HTLV-1-specific CTLs in patients with HAM/TSP and ACs quantified by a CD107a mobilization assay showed significantly lower CD107a staining in HTLV-1-specific CTLs in patients with HAM/TSP than in ACs (Sabouri et al. 2008); this suggests that patients with HAM/TSP have a high frequency of HTLV-1-specific CD8+ T cells with poor lytic capacity, whereas ACs have a lower frequency of cells with high lytic capacity. Moreover, it has been reported that the high CTL avidity, which is closely associated with the lytic efficiency of CTLs, correlates with low PVL and proviral gene expression (Kattan et al. 2009), indicating that the efficient control of HTLV-1 in vivo depends on the quality of CTLs, which determines the position of virus–host equilibrium and also the outcome of persistent HTLV-1 infection. More recently, MacNamara et al. (Macnamara et al. 2010) showed that HLA class 1 alleles, which strongly bind oligopeptides from the HBZ protein, enable the host to have a more effective immune response against HTLV-1; therefore, such individuals have a lower PVL and are more likely to be asymptomatic. Another recent report showed the presence of HBZ-specific CD4+ and CD8+ cells in vivo in patients with HAM/TSP and in ACs and a significant association between the HBZ-specific CD8+ cell response and asymptomatic HTLV-1 infection (Hilburn et al. 2011). These findings provide strong evidence to support the hypothesis of the crucial role of CTLs and confirm the importance of HBZ for persistent infection. However, because the frequency of HTLV-1-specific CD8+ T cells was significantly elevated in patients with HAM/TSP compared with ACs (Greten et al. 1998; Nagai et al. 2001a), and these cells have the potential to produce proinflammatory cytokines (Kubota et al. 1998), there is debate on the role of HTLV-1-specific CD8+ T cells, namely, whether these cells contribute to the inflammatory and demyelinating processes of HAM/TSP or whether the dominant effect of such cells in vivo is protective against disease, although a protective role and a pathogenic role of CTLs are not mutually exclusive. Indeed, there are other examples of viral infections in which the virus-specific CTLs exert both beneficial (antiviral) and detrimental (inflammatory) effects, such as lymphocytic choriomeningitis virus infection in the mouse (Klenerman and Zinkernagel 1997). It is difficult to separate cause and effect in analyzing the association between T cell attributes and the efficiency of viral control in a persistent infection at equilibrium.

Regulatory T cells (Tregs) are important mediators of peripheral immune tolerance and play an important role in chronic viral infections. HTLV-1 preferentially and persistently infects CD4+ CD25+ lymphocytes in vivo (Yamano et al. 2005), which contain the majority of the Foxp3+ Tregs (Sakaguchi et al. 2006). In patients with HAM/TSP, the percentage of Foxp3+ Tregs in CD4+ CD25+ cells is lower than that in ACs and uninfected healthy controls (Oh et al. 2006; Yamano et al. 2005), whereas the percentage of Foxp3+ cells in the CD4+ population tends to be higher in patients with HAM/TSP than in ACs (Best et al. 2009; Hayashi et al. 2008b; Toulza et al. 2008). Because CD25 is induced by HTLV-1 Tax oncoprotein (Inoue et al. 1986), the proportion of Foxp3+ cells falls in the CD4+ CD25+ population, which contains both Tregs and activated non-Tregs, in HTLV-1-infected individuals, especially patients with HAM/TSP. Therefore, it is inappropriate to use CD25 as a marker of Tregs in HTLV-1 infection, and the best current working definition of Treg phenotype is CD4+ Foxp3+. The high frequency of CD4+ Foxp3+ T cells in HTLV-1-infected individuals is maintained by CCL22 produced by HTLV-1-infected PBMCs (Toulza et al. 2010). The frequency of HTLV-1-negative Foxp3+ CD4+ cells positively correlated with the HTLV-1 PVL (Hayashi et al. 2008a; Toulza et al. 2008), and the CTL activity negatively correlated with the frequency of HTLV-1-negative Foxp3+ CD4+ cells (Toulza et al. 2008). These results suggest that an increase in HTLV-1-negative FoxP3+ CD4+ Tregs is one of the chief determinants of the efficiency of T cell-mediated immune control of HTLV-1. If such Tregs reduce CTL activity, which in turn increases the HTLV-1 PVL, this activity increases the risk of developing HAM/TSP.

Dendritic cells and the other reservoirs of HTLV-1

Dendritic cells (DCs) are antigen-presenting cells that play a critical role in the regulation of the adaptive immune response. In HTLV-1 infection, it has been shown that the DCs from patients with HAM/TSP were infected with HTLV-1 (Macatonia et al. 1992), and the development of HAM/TSP is associated with rapid maturation of DCs (Ali et al. 1993). In vitro culture of lymphocytes from HTLV-1-infected individuals results in "spontaneous lymphocyte proliferation" (SLP), which is the in vitro proliferation of PBMCs without any exogenous stimuli such as antigen or mitogen. In patients with HAM/TSP, the levels of SLP reflect the severity of the disease (Ijichi et al. 1989; Itoyama et al. 1988). Interestingly, depletion of DCs from the PBMCs of patients with HAM/TSP abolished SLP, whereas supplementing DCs restores proliferation (Macatonia et al. 1992); supplementing B cells or macrophages had no effect. A DC-dependent mechanism of SLP was further supported by data showing that antibodies to MHC class II, CD86, and CD58 can block SLP (Makino et al. 1999). Recently, Jones et al. demonstrated that human-derived myeloid and plasmacytoid DCs are susceptible to infection with cell-free HTLV-1 and that HTLV-1-infected DCs can rapidly transfer virus to autologous primary CD4+ T cells (Jones et al. 2008). Furthermore, it was recently demonstrated that transmission of HTLV-1 from DCs to T cells was mediated primarily by DC-SIGN (Jain et al. 2009), and the DCs are the major cell type responsible for the generation and maintenance of Tax-specific CD8+ T cells both in vitro and in vivo (Manuel et al. 2009).

These findings suggest that the interaction of DCs with HTLV-1 is also crucial for the pathogenesis of HAM/TSP. Moreover, using transgenic mouse models that permit conditional transient depletion of CD11c+ DCs, and a chimeric HTLV-1 that carries the envelope gene from Moloney murine leukemia virus, Rahman et al. demonstrated the critical role of DCs in their ability to mount both innate and adaptive immune responses during early cell-free HTLV-1 infection (Rahman et al. 2011, 2010). Because HTLV-1 can impair the differentiation of monocytes into DCs (Nascimento et al. 2011), the interaction of DCs with HTLV-1 plays a central part in the persistence and pathogenesis of HTLV-1.

Conclusions

During the three decades since the discovery of HTLV-1, advances in research have successfully helped us to understand the clinical features of HTLV-1-associated diseases and the virological properties of HTLV-1, although the precise mechanism of disease pathophysiology is still incompletely understood and treatment is still unsatisfactory. Accumulating evidence suggests that the virus–host immunological interactions play a pivotal role in the pathogenesis of HAM/TSP. A genetically determined, less efficient CTL response against HTLV-1 may cause higher PVL and antigen expression in infected individuals, which lead to the activation and expansion of antigen-specific T cell responses, subsequent induction of large amounts of proinflammatory cytokines and chemokines, and progression of the development of HAM/TSP. Future studies should be conducted to identify the precise mechanism of disease development to allow effective treatment and prevention of disease. This will require the development of a humanized small animal model that could be exploited as a tool for screening and evaluation of HTLV-1-associated diseases.

Acknowledgment The author thanks the Ministry of Health, Labor and Welfare, Japan (Health Labour Sciences Research Grant on Intractable Disease, ref H22-013 and H23-126) and the Japan Society for the Promotion of Science (JSPS) (Grant-in-Aid for Scientific Research, ref 21590512 and 24590556), and the Novartis Foundation (Japan) for the Promotion of Science for financial supports.

References

Adaui V, Verdonck K, Best I, Gonzalez E, Tipismana M, Arevalo J, Vanham G, Campos M, Zimic M, Gotuzzo E (2006) SYBR Green-based quantitation of human T-lymphotropic virus type 1 proviral load in Peruvian patients with neurological disease and asymptomatic carriers: influence of clinical status, sex, and familial relatedness. J Neurovirol 12:456–465

Ali A, Patterson S, Cruickshank K, Rudge P, Dalgleish AG, Knight SC (1993) Dendritic cells infected in vitro with human T cell leukaemia/lymphoma virus type-1 (HTLV-1); enhanced lymphocytic proliferation and tropical spastic paraparesis. Clin Exp Immunol 94:32–37

Araujo AQ, Silva MT (2006) The HTLV-1 neurological complex. Lancet Neurol 5:1068–1076

Aye MM, Matsuoka E, Moritoyo T, Umehara F, Suehara M, Hokezu Y, Yamanaka H, Isashiki Y, Osame M, Izumo S (2000) Histopathological analysis of four autopsy cases of HTLV-I-associated myelopathy/tropical spastic paraparesis: inflammatory changes occur simultaneously in the entire central nervous system. Acta Neuropathol 100:245–252

Azakami K, Sato T, Araya N, Utsunomiya A, Kubota R, Suzuki K, Hasegawa D, Izumi T, Fujita H, Aratani S, Fujii R, Yagishita N, Kamijuku H, Kanekura T, Seino KI, Nishioka K, Nakajima T, Yamano Y (2009) Severe loss of invariant NKT cells exhibiting anti-HTLV-1 activity in patients with HTLV-1-associated disorders. Blood 114(15):3208–3215. doi:10.1182/blood-2009-02-203042

Bangham CR (2000) The immune response to HTLV-I. Curr Opin Immunol 12:397–402

Bangham CR, Osame M (2005) Cellular immune response to HTLV-1. Oncogene 24:6035–6046

Best I, Lopez G, Verdonck K, Gonzalez E, Tipismana M, Gotuzzo E, Vanham G, Clark D (2009) IFN-gamma production in response to Tax 161-233, and frequency of CD4+ Foxp3+ and Lin HLA-DRhigh CD123+ cells, discriminate HAM/TSP patients from asymptomatic HTLV-1-carriers in a Peruvian population. Immunology 128:e777–e786

Bhigjee AI, Bill PL, Wiley CA, Windsor IM, Matthias DA, Amenomori T, Wachsman W, Moorhouse D (1993) Peripheral nerve lesions in HTLV-I associated myelopathy (HAM/TSP). Muscle Nerve 16:21–26

Cook LB, Rowan AG, Melamed A, Taylor GP, Bangham CR (2012) HTLV-1-infected T cells contain a single integrated provirus in natural infection. Blood 120:3488–3490

de The G, Bomford R (1993) An HTLV-I vaccine: why, how, for whom? AIDS Res Hum Retrovir 9:381–386

Enose-Akahata Y, Matsuura E, Tanaka Y, Oh U, Jacobson S (2012) Minocycline modulates antigen-specific CTL activity through inactivation of mononuclear phagocytes in patients with HTLV-I associated neurologic disease. Retrovirology 9:16

Enose-Akahata Y, Abrams A, Massoud R, Bialuk I, Johnson KR, Green PL, Maloney EM, Jacobson S (2013) Humoral immune response to HTLV-1 basic leucine zipper factor (HBZ) in HTLV-1-infected individuals. Retrovirology 10:19

Furukawa Y, Yamashita M, Usuku K, Izumo S, Nakagawa M, Osame M (2000) Phylogenetic subgroups of human T cell lymphotropic virus (HTLV) type I in the tax gene and their association with different risks for HTLV-I-associated myelopathy/tropical spastic paraparesis. J Infect Dis 182:1343–1349

Gessain A, Barin F, Vernant JC, Gout O, Maurs L, Calender A, de The G (1985) Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 2:407–410

Gillet NA, Malani N, Melamed A, Gormley N, Carter R, Bentley D, Berry C, Bushman FD, Taylor GP, Bangham CR (2011) The host genomic environment of the provirus determines the abundance of HTLV-1-infected T-cell clones. Blood 117:3113–3122

Goon PK, Hanon E, Igakura T, Tanaka Y, Weber JN, Taylor GP, Bangham CR (2002) High frequencies of Th1-type CD4(+) T cells specific to HTLV-1 Env and Tax proteins in patients with HTLV-1-associated myelopathy/tropical spastic paraparesis. Blood 99:3335–3341

Goon PK, Igakura T, Hanon E, Mosley AJ, Asquith B, Gould KG, Taylor GP, Weber JN, Bangham CR (2003) High circulating frequencies of tumor necrosis factor alpha- and interleukin-2-secreting human T-lymphotropic virus type 1 (HTLV-1)-specific CD4+ T cells in patients with HTLV-1-associated neurological disease. J Virol 77:9716–9722

Goon PK, Biancardi A, Fast N, Igakura T, Hanon E, Mosley AJ, Asquith B, Gould KG, Marshall S, Taylor GP, Bangham CR (2004a) Human T cell lymphotropic virus (HTLV) type-1-specific CD8+ T cells:

frequency and immunodominance hierarchy. J Infect Dis 189:2294–2298

Goon PK, Igakura T, Hanon E, Mosley AJ, Barfield A, Barnard AL, Kaftantzi L, Tanaka Y, Taylor GP, Weber JN, Bangham CR (2004b) Human T cell lymphotropic virus type I (HTLV-I)-specific CD4+ T cells: immunodominance hierarchy and preferential infection with HTLV-I. J Immunol 172:1735–1743

Greten TF, Slansky JE, Kubota R, Soldan SS, Jaffee EM, Leist TP, Pardoll DM, Jacobson S, Schneck JP (1998) Direct visualization of antigen-specific T cells: HTLV-1 Tax11-19- specific CD8(+) T cells are activated in peripheral blood and accumulate in cerebrospinal fluid from HAM/TSP patients. Proc Natl Acad Sci U S A 95:7568–7573

Hakoda E, Machida H, Tanaka Y, Morishita N, Sawada T, Shida H, Hoshino H, Miyoshi I (1995) Vaccination of rabbits with recombinant vaccinia virus carrying the envelope gene of human T-cell lymphotropic virus type I. Int J Cancer 60:567–570

Hanon E, Hall S, Taylor GP, Saito M, Davis R, Tanaka Y, Usuku K, Osame M, Weber JN, Bangham CR (2000) Abundant tax protein expression in CD4+ T cells infected with human T-cell lymphotropic virus type I (HTLV-I) is prevented by cytotoxic T lymphocytes. Blood 95:1386–1392

Harrington WJ Jr, Sheremata WA, Snodgrass SR, Emerson S, Phillips S, Berger JR (1991) Tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM): treatment with an anabolic steroid danazol. AIDS Res Hum Retrovir 7:1031–1034

Hayashi D, Kubota R, Takenouchi N, Nakamura T, Umehara F, Arimura K, Izumo S, Osame M (2008a) Accumulation of human T-lymphotropic virus type I (HTLV-I)-infected cells in the cerebrospinal fluid during the exacerbation of HTLV-I-associated myelopathy. J Neurovirol 14(5):459–463. doi:10.1080/13550280802178538

Hayashi D, Kubota R, Takenouchi N, Tanaka Y, Hirano R, Takashima H, Osame M, Izumo S, Arimura K (2008b) Reduced Foxp3 expression with increased cytomegalovirus-specific CTL in HTLV-I-associated myelopathy. J Neuroimmunol 200:115–124

Higuchi I, Nerenberg M, Yoshimine K, Yoshida M, Fukunaga H, Tajima K, Osame M (1992) Failure to detect HTLV-I by in situ hybridization in the biopsied muscles of viral carriers with polymyositis. Muscle Nerve 15:43–47

Hilburn S, Rowan A, Demontis MA, MacNamara A, Asquith B, Bangham CR, Taylor GP (2011) In vivo expression of human T-lymphotropic virus type 1 basic leucine-zipper protein generates specific CD8+ and CD4+ T-lymphocyte responses that correlate with clinical outcome. J Infect Dis 203:529–536

Hino S, Yamaguchi K, Katamine S, Sugiyama H, Amagasaki T, Kinoshita K, Yoshida Y, Doi H, Tsuji Y, Miyamoto T (1985) Mother-to-child transmission of human T-cell leukemia virus type-I. Jpn J Cancer Res 76:474–480

Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto T, Kinoshita KI, Shirakawa S, Miyoshi I (1981) Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc Natl Acad Sci U S A 78:6476–6480

Hisada M, Stuver SO, Okayama A, Li HC, Sawada T, Hanchard B, Mueller NE (2004) Persistent paradox of natural history of human T lymphotropic virus type I: parallel analyses of Japanese and Jamaican carriers. J Infect Dis 190:1605–1609

Hollsberg P, Hafler DA (1993) Seminars in medicine of the Beth Israel Hospital, Boston. Pathogenesis of diseases induced by human lymphotropic virus type I infection. N Engl J Med 328:1173–1182

Ijichi S, Eiraku N, Osame M, Izumo S, Kubota R, Maruyama I, Matsumoto M, Sonoda S (1989) In vitro modulation of lymphocyte proliferation by prednisolone and interferon-alpha in patients with HTLV-I-associated myelopathy (HAM). J Neuroimmunol 23:175–178

Inoue J, Seiki M, Taniguchi T, Tsuru S, Yoshida M (1986) Induction of interleukin 2 receptor gene expression by p40x encoded by human T-cell leukemia virus type 1. EMBO J 5:2883–2888

Ishihara S, Okayama A, Stuver S, Horinouchi H, Shioiri S, Murai K, Kubota T, Yamashita R, Tachibana N, Tsubouchi H et al (1994) Association of HTLV-I antibody profile of asymptomatic carriers with proviral DNA levels of peripheral blood mononuclear cells. J Acquir Immune Defic Syndr 7:199–203

Itoyama Y, Minato S, Kira J, Goto I, Sato H, Okochi K, Yamamoto N (1988) Spontaneous proliferation of peripheral blood lymphocytes increased in patients with HTLV-I-associated myelopathy. Neurology 38:1302–1307

Iwasaki Y (1990) Pathology of chronic myelopathy associated with HTLV-I infection (HAM/TSP). J Neurol Sci 96:103–123

Izumo S, Goto I, Itoyama Y, Okajima T, Watanabe S, Kuroda Y, Araki S, Mori M, Nagataki S, Matsukura S, Akamine T, Nakagawa M, Yamamoto I, Osame M (1996) Interferon-alpha is effective in HTLV-I-associated myelopathy: a multicenter, randomized, double-blind, controlled trial. Neurology 46:1016–1021

Izumo S, Umehara F, Osame M (2000) HTLV-I-associated myelopathy. Neuropathology 20(Suppl):S65–S68

Jacobson S (2002) Immunopathogenesis of human T cell lymphotropic virus type I-associated neurologic disease. J Infect Dis 186(Suppl 2):S187–S192

Jacobson S, Shida H, McFarlin DE, Fauci AS, Koenig S (1990) Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. Nature 348:245–248

Jain P, Manuel SL, Khan ZK, Ahuja J, Quann K, Wigdahl B (2009) DC-SIGN mediates cell-free infection and transmission of human T-cell lymphotropic virus type 1 by dendritic cells. J Virol 83:10908–10921

Jeffery KJ, Usuku K, Hall SE, Matsumoto W, Taylor GP, Procter J, Bunce M, Ogg GS, Welsh KI, Weber JN, Lloyd AL, Nowak MA, Nagai M, Kodama D, Izumo S, Osame M, Bangham CR (1999) HLA alleles determine human T-lymphotropic virus-I (HTLV-I) proviral load and the risk of HTLV-I-associated myelopathy. Proc Natl Acad Sci U S A 96:3848–3853

Jeffery KJ, Siddiqui AA, Bunce M, Lloyd AL, Vine AM, Witkover AD, Izumo S, Usuku K, Welsh KI, Osame M, Bangham CR (2000) The influence of HLA class I alleles and heterozygosity on the outcome of human T cell lymphotropic virus type I infection. J Immunol 165:7278–7284

Jones KS, Petrow-Sadowski C, Huang YK, Bertolette DC, Ruscetti FW (2008) Cell-free HTLV-1 infects dendritic cells leading to transmission and transformation of CD4(+) T cells. Nat Med 14:429–436

Kannagi M, Harada S, Maruyama I, Inoko H, Igarashi H, Kuwashima G, Sato S, Morita M, Kidokoro M, Sugimoto M et al (1991) Predominant recognition of human T cell leukemia virus type I (HTLV-I) pX gene products by human CD8+ cytotoxic T cells directed against HTLV-I-infected cells. Int Immunol 3:761–767

Kattan T, MacNamara A, Rowan AG, Nose H, Mosley AJ, Tanaka Y, Taylor GP, Asquith B, Bangham CR (2009) The avidity and lytic efficiency of the CTL response to HTLV-1. J Immunol 182:5723–5729

Kinoshita K, Amagasaki T, Hino S, Doi H, Yamanouchi K, Ban N, Momita S, Ikeda S, Kamihira S, Ichimaru M et al (1987) Milk-borne transmission of HTLV-I from carrier mothers to their children. Jpn J Cancer Res 78:674–680

Kinpara S, Hasegawa A, Utsunomiya A, Nishitsuji H, Furukawa H, Masuda T, Kannagi M (2009) Stromal cell-mediated suppression of human T-cell leukemia virus type 1 expression in vitro and in vivo by type I interferon. J Virol 83:5101–5108

Kira J, Nakamura M, Sawada T, Koyanagi Y, Ohori N, Itoyama Y, Yamamoto N, Sakaki Y, Goto I (1992) Antibody titers to HTLV-I-p40tax protein and gag-env hybrid protein in HTLV-I-associated myelopathy/tropical spastic paraparesis: correlation with increased HTLV-I proviral DNA load. J Neurol Sci 107:98–104

Kitze B, Usuku K, Yamano Y, Yashiki S, Nakamura M, Fujiyoshi T, Izumo S, Osame M, Sonoda S (1998) Human CD4+ T lymphocytes recognize a highly conserved epitope of human T lymphotropic

virus type 1 (HTLV-1) env gp21 restricted by HLA DRB1*0101. Clin Exp Immunol 111:278–285

Kiwaki T, Umehara F, Arimura Y, Izumo S, Arimura K, Itoh K, Osame M (2003) The clinical and pathological features of peripheral neuropathy accompanied with HTLV-I associated myelopathy. J Neurol Sci 206:17–21

Kiyokawa T, Yoshikura H, Hattori S, Seiki M, Yoshida M (1984) Envelope proteins of human T-cell leukemia virus: expression in *Escherichia coli* and its application to studies of env gene functions. Proc Natl Acad Sci U S A 81:6202–6206

Klenerman P, Zinkernagel RM (1997) What can we learn about human immunodeficiency virus infection from a study of lymphocytic choriomeningitis virus? Immunol Rev 159:5–16

Kodama D, Saito M, Matsumoto W, Sabouri AH, Izumo S, Arimura K, Usuku K, Bangham CR, Osame M (2004) Longer dinucleotide repeat polymorphism in matrix metalloproteinase-9 (MMP-9) gene promoter which correlates with higher HTLV-I Tax mediated transcriptional activity influences the risk of HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP). J Neuroimmunol 156:188–194

Kramer A, Maloney EM, Morgan OS, Rodgers-Johnson P, Manns A, Murphy EL, Larsen S, Cranston B, Murphy J, Benichou J et al (1995) Risk factors and cofactors for human T-cell lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in Jamaica. Am J Epidemiol 142:1212–1220

Kubota R, Kawanishi T, Matsubara H, Manns A, Jacobson S (1998) Demonstration of human T lymphotropic virus type I (HTLV-I) tax-specific CD8+ lymphocytes directly in peripheral blood of HTLV-I-associated myelopathy/tropical spastic paraparesis patients by intracellular cytokine detection. J Immunol 161:482–488

Kuroda Y, Takashima H, Ikeda A, Endo C, Neshige R, Kakigi R, Shibasaki H (1991) Treatment of HTLV-I-associated myelopathy with high-dose intravenous gammaglobulin. J Neurol 238:309–314

LaGrenade L, Hanchard B, Fletcher V, Cranston B, Blattner W (1990) Infective dermatitis of Jamaican children: a marker for HTLV-I infection. Lancet 336:1345–1347

Lal RB, Giam CZ, Coligan JE, Rudolph DL (1994) Differential immune responsiveness to the immunodominant epitopes of regulatory proteins (tax and rex) in human T cell lymphotropic virus type I-associated myelopathy. J Infect Dis 169:496–503

Liu SY, Sanchez DJ, Cheng G (2011) New developments in the induction and antiviral effectors of type I interferon. Curr Opin Immunol 23:57–64

Macatonia SE, Cruickshank JK, Rudge P, Knight SC (1992) Dendritic cells from patients with tropical spastic paraparesis are infected with HTLV-1 and stimulate autologous lymphocyte proliferation. AIDS Res Hum Retrovir 8:1699–1706

Macnamara A, Rowan A, Hilburn S, Kadolsky U, Fujiwara H, Suemori K, Yasukawa M, Taylor G, Bangham CR, Asquith B (2010) HLA class I binding of HBZ determines outcome in HTLV-1 infection. PLoS Pathog 6

Makino M, Azuma M, Wakamatsu SI, Suruga Y, Izumo S, Yokoyama MM, Baba M (1999) Marked suppression of T cells by a benzothiophene derivative in patients with human T-lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis. Clin Diagn Lab Immunol 6:316–322

Manns A, Miley WJ, Wilks RJ, Morgan OS, Hanchard B, Wharfe G, Cranston B, Maloney E, Welles SL, Blattner WA, Waters D (1999) Quantitative proviral DNA and antibody levels in the natural history of HTLV-I infection. J Infect Dis 180:1487–1493

Manuel SL, Schell TD, Acheampong E, Rahman S, Khan ZK, Jain P (2009) Presentation of human T cell leukemia virus type 1 (HTLV-1) Tax protein by dendritic cells: the underlying mechanism of HTLV-1-associated neuroinflammatory disease. J Leukoc Biol 86:1205–1216

Martin F, Castro H, Gabriel C, Adonis A, Fedina A, Harrison L, Brodnicki L, Demontis MA, Babiker AG, Weber JN, Bangham CR, Taylor GP (2012) Ciclosporin A proof of concept study in patients with active, progressive HTLV-1 associated myelopathy/tropical spastic paraparesis. PLoS Negl Trop Dis 6:e1675

Maruyama I, Chihara J, Sakashita I, Mizoguchi R, Mori S, Usuku K, Jonosono M, Tara M, Matsumoto M, Niina S, Sonoda S, Yasaki S, Osame M (1988) HTLV-I associated bronchopneumonopathy—a new clinical entity? Am Rev Resp Dis 137:46

Matsuo H, Nakamura T, Tsujihata M, Kinoshita I, Satoh A, Tomita I, Shirabe S, Shibayama K, Nagataki S (1988) Plasmapheresis in treatment of human T-lymphotropic virus type-I associated myelopathy. Lancet 2:1109–1113

Matsuoka M, Jeang KT (2011) Human T-cell leukemia virus type 1 (HTLV-1) and leukemic transformation: viral infectivity, Tax, HBZ and therapy. Oncogene 30:1379–1389

Matsuura E, Yamano Y, Jacobson S (2010) Neuroimmunity of HTLV-I infection. J Neuroimmune Pharmacol 5:310–325

Matsuzaki T, Saito M, Usuku K, Nose H, Izumo S, Arimura K, Osame M (2005) A prospective uncontrolled trial of fermented milk drink containing viable *Lactobacillus casei* strain Shirota in the treatment of HTLV-1 associated myelopathy/tropical spastic paraparesis. J Neurol Sci 237:75–81

Melamed A, Laydon DJ, Gillet NA, Tanaka Y, Taylor GP, Bangham CR (2013) Genome-wide determinants of proviral targeting, clonal abundance and expression in natural HTLV-1 infection. PLoS Pathog 9:e1003271

Miyoshi I, Kubonishi I, Yoshimoto S, Akagi T, Ohtsuki Y, Shiraishi Y, Nagata K, Hinuma Y (1981) Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukaemic T cells. Nature 294:770–771

Mochizuki M, Yamaguchi K, Takatsuki K, Watanabe T, Mori S, Tajima K (1992) HTLV-I and uveitis. Lancet 339:1110

Morgan OS, Rodgers-Johnson P, Mora C, Char G (1989) HTLV-1 and polymyositis in Jamaica. Lancet 2:1184–1187

Murata N, Hakoda E, Machida H, Ikezoe T, Sawada T, Hoshino H, Miyoshi I (1996) Prevention of human T cell lymphotropic virus type I infection in Japanese macaques by passive immunization. Leukemia 10:1971–1974

Nagai M, Usuku K, Matsumoto W, Kodama D, Takenouchi N, Moritoyo T, Hashiguchi S, Ichinose M, Bangham CR, Izumo S, Osame M (1998) Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: high proviral load strongly predisposes to HAM/TSP. J Neurovirol 4:586–593

Nagai M, Kubota R, Greten TF, Schneck JP, Leist TP, Jacobson S (2001a) Increased activated human T cell lymphotropic virus type I (HTLV-I) Tax11-19-specific memory and effector CD8+ cells in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis: correlation with HTLV-I provirus load. J Infect Dis 183:197–205

Nagai M, Yamano Y, Brennan MB, Mora CA, Jacobson S (2001b) Increased HTLV-I proviral load and preferential expansion of HTLV-I Tax-specific CD8+ T cells in cerebrospinal fluid from patients with HAM/TSP. Ann Neurol 50:807–812

Nagasato K, Nakamura T, Shirabe S, Shibayama K, Ohishi K, Ichinose K, Tsujihata M, Nagataki S (1991) Presence of serum anti-human T-lymphotropic virus type I (HTLV-I) IgM antibodies means persistent active replication of HTLV-I in HTLV-I-associated myelopathy. J Neurol Sci 103:203–208

Nakagawa M, Izumo S, Ijichi S, Kubota H, Arimura K, Kawabata M, Osame M (1995) HTLV-I-associated myelopathy: analysis of 213 patients based on clinical features and laboratory findings. J Neurovirol 1:50–61

Nakagawa M, Nakahara K, Maruyama Y, Kawabata M, Higuchi I, Kubota H, Izumo S, Arimura K, Osame M (1996) Therapeutic trials in 200 patients with HTLV-I-associated myelopathy/ tropical spastic paraparesis. J Neurovirol 2:345–355

Nakamura H, Hayami M, Ohta Y, Ishikawa K, Tsujimoto H, Kiyokawa T, Yoshida M, Sasagawa A, Honjo S (1987) Protection of cynomolgus monkeys against infection by human T-cell leukemia virus type-I by

immunization with viral env gene products produced in *Escherichia coli*. Int J Cancer 40:403–407

Nascimento CR, Lima MA, de Andrada Serpa MJ, Espindola O, Leite AC, Echevarria-Lima J (2011) Monocytes from HTLV-1-infected patients are unable to fully mature into dendritic cells. Blood 117:489–499

Ndhlovu LC, Snyder-Cappione JE, Carvalho KI, Leal FE, Loo CP, Bruno FR, Jha AR, Devita D, Hasenkrug AM, Barbosa HM, Segurado AC, Nixon DF, Murphy EL, Kallas EG (2009) Lower numbers of circulating natural killer T (NK T) cells in individuals with human T lymphotropic virus type 1 (HTLV-1) associated neurological disease. Clin Exp Immunol 158(3):294–299. doi:10.1111/j.1365-2249.2009.04019.x

Nishioka K, Maruyama I, Sato K, Kitajima I, Nakajima Y, Osame M (1989) Chronic inflammatory arthropathy associated with HTLV-I. Lancet 1:441

Nose H, Kubota R, Seth NP, Goon PK, Tanaka Y, Izumo S, Usuku K, Ohara Y, Wucherpfennig KW, Bangham CR, Osame M, Saito M (2007) Ex vivo analysis of human T lymphotropic virus type 1-specific CD4+ cells by use of a major histocompatibility complex class II tetramer composed of a neurological disease-susceptibility allele and its immunodominant peptide. J Infect Dis 196:1761–1772

Oh U, Grant C, Griffith C, Fugo K, Takenouchi N, Jacobson S (2006) Reduced Foxp3 protein expression is associated with inflammatory disease during human t lymphotropic virus type 1 Infection. J Infect Dis 193:1557–1566

Olindo S, Cabre P, Lezin A, Merle H, Saint-Vil M, Signate A, Bonnan M, Chalon A, Magnani L, Cesaire R, Smadja D (2006) Natural history of human T-lymphotropic virus 1-associated myelopathy: a 14-year follow-up study. Arch Neurol 63:1560–1566

Olindo S, Belrose G, Gillet N, Rodriguez S, Boxus M, Verlaeten O, Asquith B, Bangham C, Signate A, Smadja D, Lezin A, Cesaire R, Willems L (2011) Safety of long-term treatment of HAM/TSP patients with valproic acid. Blood 118:6306–6309

Osame M, Usuku K, Izumo S, Ijichi N, Amitani H, Igata A, Matsumoto M, Tara M (1986) HTLV-I associated myelopathy, a new clinical entity. Lancet 1:1031–1032

Osame M, Janssen R, Kubota H, Nishitani H, Igata A, Nagataki S, Mori M, Goto I, Shimabukuro H, Khabbaz R et al (1990) Nationwide survey of HTLV-I-associated myelopathy in Japan: association with blood transfusion. Ann Neurol 28:50–56

Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC (1980) Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci U S A 77:7415–7419

Proietti FA, Carneiro-Proietti AB, Catalan-Soares BC, Murphy EL (2005) Global epidemiology of HTLV-I infection and associated diseases. Oncogene 24:6058–6068

Rahman S, Manuel SL, Khan ZK, Wigdahl B, Acheampong E, Tangy F, Jain P (2010) Depletion of dendritic cells enhances susceptibility to cell-free infection of human T cell leukemia virus type 1 in CD11c-diphtheria toxin receptor transgenic mice. J Immunol 184:5553–5561

Rahman S, Khan ZK, Wigdahl B, Jennings SR, Tangy F, Jain P (2011) Murine FLT3 ligand-derived dendritic cell-mediated early immune responses are critical to controlling cell-free human T cell leukemia virus type 1 infection. J Immunol 186:390–402

Roucoux DF, Wang B, Smith D, Nass CC, Smith J, Hutching ST, Newman B, Lee TH, Chafets DM, Murphy EL (2005) A prospective study of sexual transmission of human T lymphotropic virus (HTLV)-I and HTLV-II. J Infect Dis 191:1490–1497

Sabouri AH, Saito M, Lloyd AL, Vine AM, Witkover AW, Furukawa Y, Izumo S, Arimura K, Marshall SE, Usuku K, Bangham CR, Osame M (2004) Polymorphism in the interleukin-10 promoter affects both provirus load and the risk of human T lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis. J Infect Dis 190:1279–1285

Sabouri AH, Saito M, Usuku K, Bajestan SN, Mahmoudi M, Forughipour M, Sabouri Z, Abbaspour Z, Goharjoo ME, Khayami E, Hasani A, Izumo S, Arimura K, Farid R, Osame M (2005) Differences in viral and host genetic risk factors for development of human T-cell lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis between Iranian and Japanese HTLV-1-infected individuals. J Gen Virol 86:773–781

Sabouri AH, Usuku K, Hayashi D, Izumo S, Ohara Y, Osame M, Saito M (2008) Impaired function of human T-lymphotropic virus type 1 (HTLV-1)-specific CD8+ T cells in HTLV-1-associated neurologic disease. Blood 112:2411–2420

Saito M, Taylor GP, Saito A, Furukawa Y, Usuku K, Weber JN, Osame M, Bangham CR (2001) In vivo selection of T-cell receptor junctional region sequences by HLA-A2 human T-cell lymphotropic virus type 1 Tax11-19 peptide complexes. J Virol 75:1065–1071

Saito M, Higuchi I, Saito A, Izumo S, Usuku K, Bangham CR, Osame M (2002) Molecular analysis of T cell clonotypes in muscle-infiltrating lymphocytes from patients with human T lymphotropic virus type 1 polymyositis. J Infect Dis 186:1231–1241

Saito M, Braud VM, Goon P, Hanon E, Taylor GP, Saito A, Eiraku N, Tanaka Y, Usuku K, Weber JN, Osame M, Bangham CR (2003) Low frequency of CD94/NKG2A+ T lymphocytes in patients with HTLV-1-associated myelopathy/tropical spastic paraparesis, but not in asymptomatic carriers. Blood 102:577–584

Saito M, Jain P, Tsukasaki K, Bangham CR (2012) HTLV-1 infection and its associated diseases. Leuk Res Treat 2012:123637

Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, Shimizu J, Takahashi T, Nomura T (2006) Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 212:8–27

Samuel CE (2001) Antiviral actions of interferons. Clin Microbiol Rev 14:778–809, table of contents

Shida H, Tochikura T, Sato T, Konno T, Hirayoshi K, Seki M, Ito Y, Hatanaka M, Hinuma Y, Sugimoto M et al (1987) Effect of the recombinant vaccinia viruses that express HTLV-I envelope gene on HTLV-I infection. EMBO J 6:3379–3384

Shirabe S, Nakamura T, Tsujino A, Nishiura Y, Furuya T, Goto H, Suenaga A, Nakane S, Yoshimura T, Nagataki S (1997) Successful application of pentoxifylline in the treatment of HTLV-I associated myelopathy. J Neurol Sci 151:97–101

Sugimoto M, Nakashima H, Watanabe S, Uyama E, Tanaka F, Ando M, Araki S, Kawasaki S (1987) T-lymphocyte alveolitis in HTLV-I-associated myelopathy. Lancet 2:1220

Sugimoto M, Nakashima H, Matsumoto M, Uyama E, Ando M, Araki S (1989) Pulmonary involvement in patients with HTLV-I-associated myelopathy: increased soluble IL-2 receptors in bronchoalveolar lavage fluid. Am Rev Respir Dis 139:1329–1335

Tajima K (1990) The 4th nation-wide study of adult T-cell leukemia/lymphoma (ATL) in Japan: estimates of risk of ATL and its geographical and clinical features. The T- and B-cell Malignancy Study Group. Int J Cancer 45:237–243

Takenouchi N, Yamano Y, Usuku K, Osame M, Izumo S (2003) Usefulness of proviral load measurement for monitoring of disease activity in individual patients with human T-lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis. J Neurovirol 9:29–35

Tanaka Y, Ishii K, Sawada T, Ohtsuki Y, Hoshino H, Yanagihara R, Miyoshi I (1993) Prophylaxis against a Melanesian variant of human T-lymphotropic virus type I (HTLV-I) in rabbits using HTLV-I immune globulin from asymptomatically infected Japanese carriers. Blood 82:3664–3667

Tanaka Y, Tanaka R, Terada E, Koyanagi Y, Miyano-Kurosaki N, Yamamoto N, Baba E, Nakamura M, Shida H (1994) Induction of antibody responses that neutralize human T-cell leukemia virus type I infection in vitro and in vivo by peptide immunization. J Virol 68:6323–6331

Tattermusch S, Skinner JA, Chaussabel D, Banchereau J, Berry MP, McNab FW, O'Garra A, Taylor GP, Bangham CR (2012) Systems biology approaches reveal a specific interferon-inducible signature in HTLV-1 associated myelopathy. PLoS Pathog 8:e1002480

Taylor GP, Goon P, Furukawa Y, Green H, Barfield A, Mosley A, Nose H, Babiker A, Rudge P, Usuku K, Osame M, Bangham CR, Weber JN (2006) Zidovudine plus lamivudine in human T-lymphotropic virus type-I-associated myelopathy: a randomised trial. Retrovirology 3:63

Terada K, Katamine S, Eguchi K, Moriuchi R, Kita M, Shimada H, Yamashita I, Iwata K, Tsuji Y, Nagataki S et al (1994) Prevalence of serum and salivary antibodies to HTLV-1 in Sjogren's syndrome. Lancet 344:1116–1119

Toulza F, Heaps A, Tanaka Y, Taylor GP, Bangham CR (2008) High frequency of CD4+ FoxP3+ cells in HTLV-1 infection: inverse correlation with HTLV-1-specific CTL response. Blood 111:5047–5053

Toulza F, Nosaka K, Tanaka Y, Schioppa T, Balkwill F, Taylor GP, Bangham CR (2010) Human T-lymphotropic virus type 1-induced CC chemokine ligand 22 maintains a high frequency of functional FoxP3+ regulatory T cells. J Immunol 185:183–189

Uchiyama T (1997) Human T cell leukemia virus type I (HTLV-I) and human diseases. Annu Rev Immunol 15:15–37

Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H (1977) Adult T-cell leukemia: clinical and hematologic features of 16 cases. Blood 50:481–492

Verdonck K, Gonzalez E, Van Dooren S, Vandamme AM, Vanham G, Gotuzzo E (2007) Human T-lymphotropic virus 1: recent knowledge about an ancient infection. Lancet Infect Dis 7:266–281

Vine AM, Witkover AD, Lloyd AL, Jeffery KJ, Siddiqui A, Marshall SE, Bunce M, Eiraku N, Izumo S, Usuku K, Osame M, Bangham CR (2002) Polygenic control of human T lymphotropic virus type I (HTLV-I) provirus load and the risk of HTLV-I-associated myelopathy/tropical spastic paraparesis. J Infect Dis 186:932–939

Vine AM, Heaps AG, Kaftantzi L, Mosley A, Asquith B, Witkover A, Thompson G, Saito M, Goon PK, Carr L, Martinez-Murillo F, Taylor GP, Bangham CR (2004) The role of CTLs in persistent viral infection: cytolytic gene expression in CD8+ lymphocytes distinguishes between individuals with a high or low proviral load of human T cell lymphotropic virus type 1. J Immunol 173:5121–5129

Yamano Y, Takenouchi N, Li HC, Tomaru U, Yao K, Grant CW, Maric DA, Jacobson S (2005) Virus-induced dysfunction of CD4+ CD25+ T cells in patients with HTLV-I-associated neuroimmunological disease. J Clin Invest 115:1361–1368

Yoshida M, Seiki M, Yamaguchi K, Takatsuki K (1984) Monoclonal integration of human T-cell leukemia provirus in all primary tumors of adult T-cell leukemia suggests causative role of human T-cell leukemia virus in the disease. Proc Natl Acad Sci U S A 81:2534–2537

Yoshioka A, Hirose G, Ueda Y, Nishimura Y, Sakai K (1993) Neuropathological studies of the spinal cord in early stage HTLV-I-associated myelopathy (HAM). J Neurol Neurosurg Psychiatry 56:1004–1007

Yu F, Itoyama Y, Fujihara K, Goto I (1991) Natural killer (NK) cells in HTLV-I-associated myelopathy/tropical spastic paraparesis-decrease in NK cell subset populations and activity in HTLV-I seropositive individuals. J Neuroimmunol 33:121–128

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 30, Number 00, 2014

DOI: 10.1089/aid.2013.0214

Elimination of Human T Cell Leukemia Virus Type-1-Infected Cells by Neutralizing and Antibody-Dependent Cellular Cytotoxicity-Inducing Antibodies Against Human T Cell Leukemia Virus Type-1 Envelope gp46

Yuetsu Tanaka,[1] Yoshiaki Takahashi,[1] Reiko Tanaka,[1] Akira Kodama,[1] Hideki Fujii,[1] Atsuhiko Hasegawa,[2] Mari Kannagi,[2] Aftab A. Ansari,[3] and Mineki Saito[4]

Abstract

Human T cell leukemia virus type-1 (HTLV-1) is prevalent worldwide with foci of high prevalence. However, to date no effective vaccine or drug against HTLV-1 infection has been developed. In efforts to define the role of antibodies in the control of HTLV-1 infection, we capitalized on the use of our previously defined anti-gp46 neutralizing monoclonal antibody (mAb) (clone LAT-27) and high titers of human anti-HTLV-1 IgG purified from HAM/TSP patients (HAM-IgG). LAT-27 and HAM-IgG completely blocked syncytium formation and T cell immortalization mediated by HTLV-1 *in vitro*. The addition of these antibodies to cultures of $CD8^+$ T cell-depleted peripheral blood mononuclear cells (PBMCs) from HAM/TSP patients at the initiation of culture not only decreased the numbers of Tax-expressing cells and the production of HTLV-1 p24 but also inhibited the spontaneous immortalization of T cells. Coculture of *in vitro*-HTLV-1-immortalized T cell lines with autologous PBMCs in the presence of LAT-27 or HAM-IgG, but not an $F(ab')_2$ fragment of LAT-27 or non-neutralizing anti-gp46 mAbs, resulted in depletion of HTLV-1-infected cells. A 24-h ^{51}Cr release assay showed the presence of significant antibody-dependent cellular cytotoxicity (ADCC) activity in LAT-27 and HAM-IgG, but not $F(ab')_2$ of LAT-27, resulting in the depletion of HTLV-1-infected T cells by autologous PBMCs. The depletion of natural killer (NK) cells from the effector PBMCs reduced this ADCC activity. Altogether, the present data demonstrate that the neutralizing and ADCC-inducing activities of anti-HTLV-1 antibodies are capable of reducing infection and eliminating HTLV-1-infected cells in the presence of autologous PBMCs.

Introduction

HUMAN T CELL LEUKEMIA VIRUS type-1 (HTLV-1) is the first human retrovirus that was etiologically associated with adult T cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).[1–4] HTLV-1 is prevalent worldwide with foci of high prevalence in southwest Japan, the Caribbean islands, South America, and a part of Central Africa. The total number of HTLV-1 carriers is currently estimated to be 10–20 million.[5] The majority of HTLV-1 carriers remain asymptomatic throughout their lives, and approximately 5% of HTLV-1-infected individuals will develop either ATL or HAM/TSP after prolonged latency periods.

HTLV-1 is transmitted through contact with bodily fluids containing infected cells most often from mother to child through breast milk or via blood transfusion. It has been previously established that HTLV-1 efficiently spreads from cell to cell via the formation of virological synapses.[6] More recently, however, the formation of extracellular HTLV-1 viral particles similar to the formation of bacterial films has also been shown to be effective in viral transmission.[7] HTLV-1-antigen-expressing cells are difficult to detect at least in fresh peripheral blood mononuclear cells (PBMCs) from HTLV-1-infected individuals.[8] However, when these PBMCs are isolated from the blood and cultured *in vitro*, some T cells begin to produce HTLV-1 antigen[9,10] followed by spontaneous immortalization of the cells in media containing interleukin-2 (IL-2).[11]

[1]Department of Immunology, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan.
[2]Department of Immunotherapeutics, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.
[3]Department of Pathology, Emory University School of Medicine, Atlanta, Georgia.
[4]Department of Microbiology, Kawasaki Medical School, Okayama, Japan.

Although it has been suggested that HTLV-1 can stay dormant in infected cells and become resistant to immune effector mechanisms by ratcheting down its antigen production,[12] the continued presence of strong $CD8^+$ cytotoxic T lymphocyte (CTL) responses[13] and readily detectable levels of antibodies specific for HTLV-1 antigens in HTLV-1-infected people[14] indicates that persistent production of HTLV-1 must occur *in vivo* to maintain such effector mechanisms. Escape from immune effector mechanisms by spontaneous mutation of key residues is unlikely, due to the high degree of genomic stability that is characteristic of the HTLV-1 genome.[15] It has been suggested that HTLV-1-infected cells expressing HTLV-1 antigens occur at a low enough frequency that they are constantly being eliminated by HTLV-1-specific CTL *in vivo*[10] without leading to immune exhaustion. Besides CTL and virus neutralizing antibodies, there has been renewed interest in the potential role of antibody-dependent cellular cytotoxicity (ADCC) as an effector mechanism against a number of viral infections. This view has been highlighted by the recent demonstration of the potential role of ADCC in the only known partially successful human RV144 trial of a vaccine against human immunodeficiency virus type-1 (HIV-1).[16] The ADCC activity against HTLV-1 was first reported by Miyakoshi *et al.* in 1984[17] followed by a number of other reports.[18–21]

So far, several lines of evidence show that the HTLV-1 envelope gp46 antigen serves as a major target of ADCC.[22–24] Antibodies against gp46 antigen are commonly detected in the sera of HTLV-1-infected individuals.[25–27] However, the precise role of ADCC effector mechanism(s) in controlling HTLV-1 infection has been lacking. A possible involvement of anti-HTLV-1 antibodies in the suppression of spontaneous HTLV-1 antigen expression by HTLV-1-infected cells was first reported by Tochikura *et al.*[28] These investigators showed that serum IgG from HTLV-1-infected donors interfered with HTLV-1 antigen expression by *in vitro*-cultured PBMCs from both ATL patients and healthy HTLV-1 carriers. However, the precise mechanism by which this was mediated remained unclear.

In efforts to define the role of antibodies with neutralizing and ADCC-inducing activities in the control of HTLV-1 infection, we capitalized on the use of our previously defined rat anti-gp46 neutralizing monoclonal antibody (mAb) (LAT-27)[29] and pooled human anti-HTLV-1 IgG purified from HAM/TSP patients (HAM-IgG). Studies were conducted to evaluate the potential of these antibodies to block HTLV-1 infection and eliminate HTLV-1-infected cells from autologous T cell cultures that had previously been infected with HTLV-1 *in vivo* or *in vitro*. Results of these studies show that monoclonal LAT-27 and the polyclonal HAM-IgG are not only capable of mediating neutralization and ADCC, but are also highly effective in the elimination of HTLV-1-infected cells in the presence of fresh autologous PBMCs while preventing *de novo* infection with HTLV-1.

Materials and Methods

Reagents

The medium used throughout was RPMI 1640 medium (Sigma-Aldrich. Inc., St. Louis, MO) supplemented with 10% fetal calf serum (FCS), 100 U/ml of penicillin, and 100 μg/ml of streptomycin (hereinafter called RPMI medium). Anti-human CD3 (clone OKT-3) and anti-CD28 (clone 28.2) mAbs were purchased from the American Type Culture Collection (Rockville, MD) and Biolegend (San Diego, CA), respectively.

The rat and mouse mAbs utilized in the studies reported herein were produced and characterized by our laboratory previously.[29–34] These antibodies were rat IgG2b anti-gp46 (clones LAT-27 and LAT-25), rat IgG2a anti-gp46 (clone LAT-12), rat IgG2b anti-HCV (clone Mo-8), rat IgG2a anti HTLV-1 p24 (clone WAG-24), mouse IgG1 anti-HTLV-1 gp46 (clone MET-3), mouse IgG3 anti-HTLV-1 Tax (clone Lt-4), mouse IgG1 anti-p24 (clone NOR-1), and mouse IgG1 anti-HIV-1 p24 (clone 2C2). These in-house mAbs were purified from the ascites fluids of groups of CB.17-SCID mice carrying the appropriate hybridoma cell line. The ascites fluid was subjected to ammonium sulfate precipitation followed by gel filtration using Superdex G-200 (GE Healthcare, Tokyo, Japan). Aliquots of these mAbs were labeled with either fluorescein isothiocyanate (FITC), Alexafluor 488, Alexafluor 647, HRP (Dojindo, Kumamoto, Japan), or Cy-5 (GE Healthcare) according to the manufacturer's instructions. The FITC- or phycoerythrin (PE)-labeled mouse mAbs against human CD3, CD4, CD8, CD14, CD16, CD19, or CD56 and unlabeled mouse anti-CD16 and anti-CD32 mAbs were purchased from Abcam.

For cell depletion, magnetic beads labeled with anti-CD4, CD8, CD14, CD16, CD19, and antimouse IgG (Dynal) and those labeled with anti-CD56 mAb (LifeTec) were used according to the manufacturer's recommendations. Mitomycin-C (MMC) was commercially purchased from Kyowa Kirin (Tokyo, Japan) and used at 50 μg/ml in RPMI medium. A purified $F(ab')_2$ fragment of LAT-27 IgG generated by enzymatic digestion of LAT-27 IgG was purchased from IBL Inc. (Gunma, Japan). Human IgG was purified from pooled plasma from three normal donors (normal IgG) and three HAM patients (HAM-IgG) using protein-G affinity purification kits (GE Healthcare).

The protocols for the use of human PBMCs and animals were approved by the Human IRB and the Institutional Animal Care and Use Committee (IACUC) on clinical and animal research of the University of the Ryukyus prior to initiation of the present study.

Cell cultures

PBMCs were isolated from heparinized blood by standard density gradient centrifugation using Lympholyte (Cedarlane, Burlington, Canada). Some PBMCs were cryopreserved using a cell freezing media (Cell reservoir, Nakarai Tesque Inc., Kyoto, Japan). The method to activate PBMCs with anti-CD3 and CD28 mAbs has been described previously.[34] The HTLV-1-producing T cell lines utilized included MT-2, HUT102, IL-2-dependent $CD4^-CD8^+$ ILT-M1 cells derived from an HAM/TSP patient, $CD4^+CD8^-$ ILT-H2 cells, ATL-3 cells derived from ATL patients, and a number of other T cell lines derived from normal PBMCs following *in vitro* immortalization by cocultivation with MMC-treated ILT-M1 cells. These cell lines were maintained in culture using RPMI medium containing 20 U/ml IL-2.