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T cell vaccination in multiple sclerosis relapsing–remitting nonresponders patients

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Abstract

Myelin autoreactive T cells are involved in the pathogenesis of multiple sclerosis (MS) and lead to propagation of the disease. We evaluated the efficacy of T cell vaccination (TCV) therapy for patients with aggressive relapsing–remitting MS who failed to respond to immunomodulatory treatments. Twenty nonresponders relapsing–remitting MS patients were immunized with autologous attenuated T cell lines after activation with synthetic myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) encephalitogenic peptides. Each patient received three vaccinations in 6- to 8-week intervals. Annual relapse rate decreased from 2.6 to 1.1, $P = 0.026$. Neurological disability stabilized as compared with the 2- and 1-year pretreatment progression rates. Significant reduction in the number and volume of active lesions, as well as reduction in T2 lesion burden, was demonstrated by quantitative MRI analysis. No serious adverse events were observed. Our findings suggest that TCV has beneficial clinical effects in MS patients who, in spite of immunomodulatory treatments, continue to deteriorate. TCV could serve as a potential alternative therapy for this subgroup of nonresponders patients.

Keywords: T cell vaccination; Immunization; Multiple sclerosis; Immunomodulatory; Treatment; Autoreactive T cells

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) in which infiltrating mononuclear cells, predominantly T lymphocytes and macrophages, lead to damage of the myelin sheath [1,2]. The disease frequently affects young adults between 20 and 40 years and has a characteristic clinical course manifested by relapses and remissions in 85% of patients. The course of relapsing–remitting MS (RR–MS) is typified by attacks during which new neurologic symptoms and signs appear, or existing neurological symptoms and signs worsen. With each additional attack, the probability of complete clinical remission decreases, and neurological disability and handicap are more liable to develop [3,4]. Currently available immunomodulatory therapies for the treatment of RR–MS include three forms of recombinant interferon beta (two formulations of IFN beta-1a and one of IFN beta-1b), synthetic glatiramer acetate (GA) and intravenous immunoglobulins (IVIg). These treatments have been proved very effective in previously untreated patients with RR–MS and induce reduction in the frequency and severity of acute relapses and suppression of progression to disability [5–7]. However, there are patients that fail to respond to these treatments and continue to worsen over time, with the occurrence of additional relapses associated with neurological deterioration. These patients are defined as nonresponders and need new modes of therapeutic interventions to suppress the ongoing disease activity.

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The purpose of the present study was to evaluate T cell vaccination (TCV) therapy for nonresponders RR–MS patients. The rationale of TCV is based on the knowledge that, in MS, autoreactive T cells against myelin antigens like myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), or proteolipid protein (PLP) undergo activation and clonal expansion, infiltrate the blood–brain barrier, and induce local inflammation and demyelination [8,9].

During the process of TCV, autologous myelin reactive T cells are grown and inactivated in vitro to produce a vaccine that will induce regulatory immune response that is aimed to deplete autoreactive T cells and inhibit their activation. Previously, TCV was reported to prevent EAE [10,11] and to modulate disease activity in MS patients [12,13].

In the present study, we further evaluated the safety and efficacy of TCV in nonresponders RR–MS patients.

Methods

Patients selection

The study was performed at the MS Center, TCV Laboratory and Blood Bank, Sheba Medical Center, Tel-Hashomer, Israel, and was reviewed and approved by the Ethical Committee of the Israeli Ministry of Health. Informed consent was obtained from each patient before beginning the study. Inclusion criteria were definite MS according to Poser criteria [14]; relapsing–remitting disease course; age between 18–60 years; neurological disability assessed by the Expanded Disability Status Scale (EDSS) [15] ≤6, brain MRI compatible with MS; no effective treatment response to immunomodulatory treatments defined as increase in relapse rate and/or progression of at least 0.5 point of the EDSS score in the year before the study when the patient was under immunomodulatory treatment; not involved in other clinical trial; no other known systemic disease(s); negative pregnancy test; and written informed consent. Exclusion criteria for participation were steroid treatment within the last 3 months before the study; immunomodulatory treatment within the last 3 months before the study; known systemic disease; known allergic reaction to gadolinium (Gd) contrast material; and cognitive decline that interferes with patient's ability to understand the study.

Patient evaluations

All patients were given three subcutaneous injections with irradiated autologous MBP- and/or MOG-reactive T cells, within 6- to 8-week intervals. During the follow-up period of 1 year, patients were examined once every 3 months, and neurological disability was assessed by the EDSS. Clinical parameters related to relapse rate and progression to disability were compared with the patient's own pretreatment course. Time to progression was determined by an increase of at least 1.0 on the EDSS persisting for at least 3 months. Relapse was defined as the onset of new neurological symptoms or worsening of preexisting neurological symptoms lasting for at least 48 h, accompanied by objective change on neurological examination (worsening of at least 0.5 point on EDSS). Patients were instructed to report events between the scheduled regular visits and were examined by a neurologist if symptoms suggested a relapse. Safety assessments included vital signs, physical examination, and reports of adverse events, at the day of vaccination, 48 h, and 9 days thereafter, as well as at 3-month follow-up visits. Laboratory tests for blood count, liver function tests, electrolytes, kidney function tests, β-HCG, immunoelectrophoresis, and serology for hepatitis A, B, C, and HIV were performed at screening and upon study completion. In addition, blood count, liver function tests, electrolytes, kidney function tests, T cell subsets, and serum cytokine profile were performed once every 3 months along the study period.

Brain MRI procedure

Brain MRI was performed at baseline (within a week before the first vaccination) and upon study completion. Examinations were obtained using a 2.0 Tesla Imager (Elscint, Israel). For each MRI examination, the following data were acquired: (1) sagittal T1-weighted localizer images (500/12 TR/TE), 24-cm field of view, 256 × 180 matrix; (2) axial dual spin-echo (PD and T2) weighted sequences (5500/16–128 TR/TEs), 22-cm field of view, 256 × 256 matrix; (3) axial T1-weighted images (500/12 TR/TE), 24-cm field of view, 256 × 180 matrix before and after intravenous administration of 0.1 mmol/kg of gadolinium (Gd)-DTPA. All examinations covered the brain from the level of the foramen magnum to the higher convexity of the skull, using 3-mm slice thickness and no interslice gap, yielding 44 contiguous images. The number and volume of MS lesions were quantified by the MSAnalyse computerized software as previously described [16].

TCV preparation

Peripheral blood mononuclear cells were separated from 50 ml heparinized venous blood on ficoll hypaque, plated in round bottom 96-well microplates, 2×10^5 cells per well in the presence of 15 μg/ml synthetic peptides bearing encephalitogenic immunodominant epitope sequences of MBP (83–99, 87–110, 151–170) and MOG (6–26, 34–56), (Chiron, Mimmitopes, Australia). The cells were cultured in medium RPMI-1640 (Gibc) supplemented with 10% fetal bovine serum (HyClone), 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.1% glutamine in 37°C, 5% CO_2 incubator. Following cultivation for 7–14 days, the cultures were split and subcultures were prepared on 10^5 irradiated (400 Gy) autologous PBMC feeders and restimulated with the peptides, respectively. The index of cell stimulation (SI) in response to the MBP and MOG peptides was examined

Table 1
Demographic and clinical characteristics of the study patients

Age (years)	40.2 ± 11.3 (22.1–59.6)
Gender	M = 7 (35%), F = 13 (65%)
Age at MS onset (years)	28.9 ± 11.7
Age at MS diagnosis (years)	32.5 ± 11.7
Disease duration (years)	7.7 ± 5.7
Mean EDSS 2 years prior	2.8 ± 2.2
Mean EDSS 1 year prior	3.3 ± 2.1
Mean EDSS baseline (first vaccination)	4.0 ± 2.4
Mean EDSS 1 year after third vaccination	4.4 ± 2.4
Mean relapse rate 2 years prior	2.1 ± 2.4
Mean relapse rate 1 year prior	2.6 ± 1.6
Mean relapse rate 1 year after third vaccination	1.1 ± 1.6

after additional 72 h in culture using ^{3}H-thymidine incorporation assays (Amersham, Arlington Heights, IL).

Wells exhibiting a minimal SI > 3 (threefold increase in ^{3}H-thymidine incorporation relative to the average incorporation in reference control wells not stimulated with peptides) were selected for line propagation and expanded with IL-2 (50 IU/ml, Roche). The lines were examined for CD4/CD8 ratio, and only lines with a CD4 content > 60% were scaled up for vaccination, irradiated with 800 Gy, washed extensively, dispersed in saline, and used for immunization (up to 1.5×10^{7} cells per peptide with an overall cell count not exceeding 6×10^{7}). Patients were immunized only with T cell lines reactive to myelin epitopes as reflected by SI > 3 and a CD4 content of >60%.

HLA typing

Genomic DNA was extracted from PBMC using Qiagene spin columns (QIAGENE GmbH, Hilden, Germany), following the manufacturer's instructions. Low-resolution HLA class II typing was carried out by reverse SSOP (Amplicor, Roche, Branchburg, NJ).

Statistical methods

Paired *t* test and the nonparametric Wilcoxon signed rank test [17] were used to analyze the changes in relapse rate, neurological disability, and MRI lesion load. Univariate analyses for nonlinear variables were performed to analyze treatment effect on the change of the volume and number of MS lesions on brain MRI (calculated as percent of change from baseline values) and progression to neurological disability by the EDSS measurements. Linear regression analysis [18] was performed to calculate the predicted progression to disability over time in comparison with the observed data.

All tests were performed using the SAS Statistical Package SAS® Version 8.02 (SAS Institute, Cary, NC, USA), [19]. All reported *P* values are two-tailed; $P < 0.05$ was considered statistically significant.

Results

Pretreatment clinical parameters

Twenty MS patients (mean age 40.2 ± 11.3 years, 13 females) were included in the study. Mean disease duration was 7.7 ± 5.7 years. Mean relapse rate during the 2 years before study was 2.1 ± 2.4 and during the 1 year before study 2.6 ± 1.6. The mean EDSS 2 years before the study was 2.8 ± 2.2, and the mean EDSS 1 year before study was 3.3 ± 2.1 (Table 1). The increase in EDSS over the 2 years before the study and the corresponding high relapse rate are in agreement with the definition of these patients as non-responders in spite of treatment with immunomodulatory drugs. Previous immunomodulatory treatments during the year before the study included GA ($N = 5$), interferon beta-1b ($N = 7$), interferon beta-1a ($N = 6$), and IVIg ($N = 2$); all patients received more than one immunomodulatory treatment during their disease course.

Clinical outcome

Relapse rate decreased from a mean of 2.6 ± 1.6, 1 year before TCV, to 1.1 ± 1.6, 1 year after, yielding a decrease by 55% in the annual relapse rate ($P = 0.026$), while during the 2 years and 1 year before the study, the annual relapse rate increased by 27.5%. Neurological disability assessed by the EDSS score demonstrated that the study patients had progressed by 0.5 and 0.7 points in the EDSS during the 2 years and 1 year before TCV, respectively, in spite of immunomodulatory treatment, while 1 year after the last vaccination, the mean EDSS increased only by 0.4 (Table 1). Regression model analysis demonstrated that the expected EDSS calculated using the EDSS data of 2 years and 1 year before the study and at baseline was higher than the observed EDSS, suggesting modest effect of TCV therapy on neurological disability, although it did not reach statistical significance, $P = 0.118$ (Fig. 1). Mean change in number of relapses calculated per patient during 1 and 2 years before TCV and

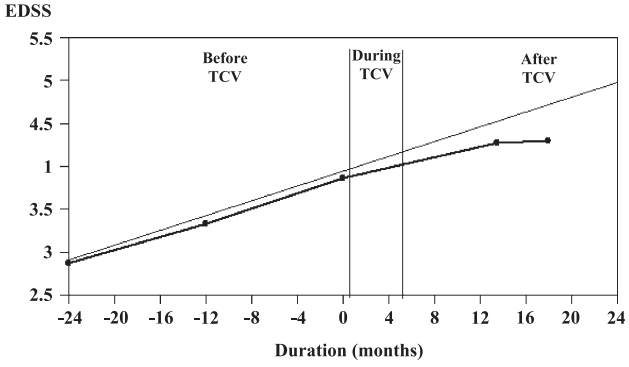

Fig. 1. Regression model analysis demonstrates TCV effect on neurological disability. The expected EDSS (dotted line) calculated using EDSS data 24 and 12 months before the study and at baseline differs from the observed EDSS (bold line), $P = 0.118$.

1 year after the third vaccination significantly decreased by 2.67, $P < 0.01$ (Fig. 2). Mean change in EDSS calculated per patient increased only by 0.03 1 year after the third vaccination, compared to 0.47 and 0.53, 2 years and 1 year before TCV, respectively, ($P = 0.02$), suggesting stabilization in neurological disability (Fig. 2).

DR and DQ HLA typing are presented in Table 2 along with the clinical outcome of patients (both relapse rate and disease progression). We could not find any correlations between the clinical response and the HLA typing. No significant correlations related to prior treatment with various immunomodulating drugs and responses to TCV were found.

MRI outcome

MRI data are presented in Table 3. There were fewer Gd-enhancing lesions on T1-weighted scans as well as fewer lesions on T2- and T1-weighted images. In accordance, the computerized analysis of the volume of lesions demonstrated decrease in lesion volume 12 months after the third vaccination compared to baseline, although not statistically significant. The median difference of changes in lesion volume corrected for the baseline volume decreased in T2- and Gd-weighted images, while it increased on T1-weighted images. These findings did not reach statistical significance but may suggest suppression of the inflammatory disease process.

Response to myelin encephalitogenic immunodominant epitope peptides

All patients responded to more than one immunodominant peptide; 45% responded to five peptides, 30% responded to four peptides, 20% responded to three peptides, and 5% responded to two peptides. The most frequent myelin peptides to which response was found in decreasing order were MBP 87–110 (90%), MOG 34–56 (90%), MOG 6–26 (85%), MBP 83–99 (75%), and MBP 151–170 (75%). The response patterns to the neuroantigens and the specifications of the T cell subsets used in the vaccine for each patient are presented in Table 4. No correlations were found between the clinical responses and any of the specific epitopes used to select the TCV.

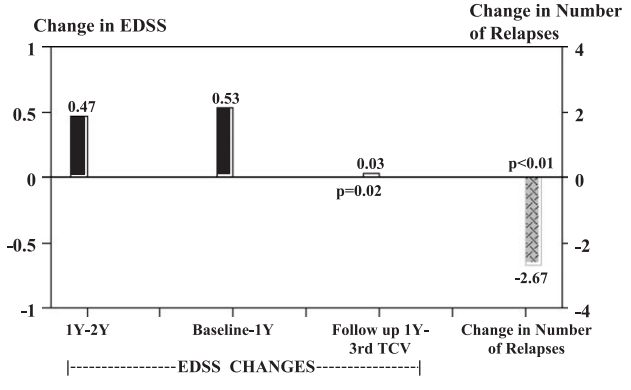

Fig. 2. Mean changes of EDSS and number of relapses before and after TCV.

Table 2
Clinical outcome and HLA typing of study patients

Patient (no.)	Response to immunodominant peptides (no.)	HLA typing		Effect of TCV on clinical outcome	
		DRB1	DQB1	Relapse rate	EDSS
1	3	13	03, 06	Decreased	Worsened
2	4	01, 15	05, 06	Decreased	Improved
3	3	11, 13	06, 03	Decreased	Stable
4	4	11, 13	02, 03	Decreased	Improved
5	3	11, 12	03	Decreased	Stable
6	5	01, 11	05, 03	Increased	Stable
7	5	11, 04	03	Decreased	Stable
8	3	07, 13	03	Decreased	Stable
9	5	11, 13	06, 03	Stable	Worsened
10	4	07,10	02, 05	Stable	Stable
11	5	04, 14	03, 05	Decreased	Stable
12	5	07, 13	02, 05	Decreased	Stable
13	4	03, 11	02, 03	Decreased	Stable
14	5	04, 11	03	Decreased	Stable
15	5	11, 14	03	Increased	Stable
16	5	04, 16	03, 05	Decreased	Stable
17	5	03, 11	02, 03	Decreased	Worsened
18	3	13, 15	05, 06	Decreased	Stable
19	4	11, 15	06, 02	Decreased	Stable
20	5	04, 13	03	Decreased	Stable
Total				Decreased = 16	Improved = 2
				Stable = 2	Stable = 15
				Increased = 2	Worsened = 3

Safety analysis

TCV procedure was safe and not associated with any laboratory significant abnormalities. No serious adverse events related to the treatment or clinically significant events constituting a significant hazard for patients occurred

Table 3
MRI findings

Variable	Baseline	12 Months after third vaccination
Median no. of brain lesions		
T2	60 (4–150)	51 (18–228)
T1	15 (3–33)	10 (5–45)
Gd-enhancing	9 (2–32)	4 (0–41)
Volume of brain lesions—mm^3		
Median (25th and 75th percentiles)		
T2	3429 (2524–4889)	2614 (1670–11,228)
T1	482 (281–1169)	356 (243–1878)
Gd-enhancing	153 (99–573)	82 (55–403)
[a]*Change in volume of lesions—mm^3*		*P*
Median (25th and 75th percentiles)		
T2	−34.5 (−117, 42)	0.264
T1	16 (−19, 79)	0.141
GD	−24 (−59, 24)	0.246

[a] Difference of changes by Wilcoxon signed rank test.

Table 4
Response patterns to immunodominant myelin antigens and the specifications of the T cells subsets used in the vaccine for each patient

Peptide No.	Vaccination profile									
	I		II		III		IV		V	
Patient	MBP 83–99	CD4/CD8, %	MBP 87–110	CD4/CD8, %	MBP 151–170	CD4/CD8, %	MOG 6–26	CD4/CD8, %	MOG 34–56	CD4/CD8, %
1	–		+	83/17	–		+	99/1	+	96/4
2	+	83/17	+	71/29	–		+	77/23	+	63/38
3	–		–		+	86/14	–		+	93/7
4	+	86/14	+	79/21	+	87/13	–		+	82/18
5	–		+	74/26	+	62/38	–		+	66/34
6	+	79/21	+	71/29	+	81/19	+	75/25	+	75/25
7	+	68/33	+	60/40	+	97/3	+	61/39	–	
8	–		+	96/4	+	87/13	+	98/2	–	
9	+	83/17	+	84/16	+	82/18	+	85/15	+	85/15
10	+	91/9	+	83/17	–		+	97/3	+	85/15
11	+	94/6	+	65/35	+	64/36	+	70/30	+	66/34
12	+	70/30	+	64/36	+	64/36	+	61/39	+	74/26
13	+	72/28	+	69/32	–		+	93/7	+	77/23
14	+	62/38	+	64/36	+	60/40	+	60/40	+	57/43
15	+	72/28	+	76/24	+	78/22	+	85/15	+	85/;15
16	+	73/27	+	80/20	+	76/24	+	64/36	+	70/30
17	+	81/19	+	66/34	+	88/12	+	90/10	+	86/24
18	–		–		+	60/40	+	67/23	+	72/28
19	+	72/28	+	81/19	–		+	82/16	+	77/23
20	+	61/39	+	69/31	+	93/7	+	85/15	+	71/29
Total no. of patients responsive	15		18		15		17		18	

during the study. The most frequently reported short-term adverse event was redness at the injection site in 55% of patients, mainly after the second or third vaccinations. This skin response was considered as a positive sign for acquired immunity to the vaccinated lines.

Discussion

In the present study, we assessed the effect of TCV in RR–MS patients with aggressive disease course and failure to respond to currently used immunomodulatory treatments. The pretreatment clinical parameters clearly demonstrate that these relatively young patients had deteriorating clinical course with frequent relapses and subsequent neurological disability within the first 5 years of their illness. TCV treatment induced favorable impact on both clinical variables, for example, annual relapse rate and progression to disability, as well as resulted in stabilization of MRI disease burden. These findings suggest a potential beneficial effect of TCV in nonresponders MS patients with aggressive relapsing–remitting course. Moreover, treatment was safe without associated adverse events, except for mild injection site reactions. Our results are in agreement with the findings reported in previous TCV studies in MS patients. Zhang et al. [20], in a pilot trial, vaccinated six MS patients (three with RR–MS, one with primary-progressive, and two patients with secondary-progressive disease course) with peripheral blood MBP-reactive T cell clones that were found to react against encephalitogenic MBP peptides and reported that vaccinations were not associated with adverse events and resulted in beneficial clinical effects. Medaer et al. [13] reported that after vaccination with irradiated T cells reactive to MBP, five out of eight MS patients had decrease in relapse rate and less increase in MRI lesion volume as compared with matched untreated MS patients (8% vs. 39.5% increase, respectively). Recently, Zhang et al. [12] reported the results of TCV in 54 patients with RR–MS (n = 28) or secondary-progressive MS (n = 26) that were immunized with irradiated autologous MBP-reactive T cells. Depletion of MBP-reactive T cells correlated with a reduction by 40% in the rate of relapses in RR–MS patients as compared with the pretreatment rate in the same cohort. However, the reduction in EDSS was minimal in RR–MS patients, while the EDSS slightly increased in secondary-progressive MS patients over a period of 2 years. Serial semiquantitative MRI examinations demonstrated stabilization in lesion activity as compared with baseline MRI. In our study, we found reduction by 55% in relapse rate as compared with the pretreatment year. This higher response maybe associated with the fact that, for the TCV procedure, we used not only MBP but also MOG encephalitogenic peptides and thus expanded the immune potential of the vaccine. Most of our relapsing–remitting RR–MS patients who were defined as nonresponders to the conventional immunomodulating treatments and had significant disease activity responded favorably to the TCV procedure; 75% (16 patients) had a decrease in their relapse rate, and 85% (17 patients) had either decrease or no change in their neurological disability.

Although the clinical trials on TCV in MS are still preliminary, as in the absence of placebo controls the clinical results were compared with the patient's own pretreatment status, they give important information related to the efficacy of TCV as a therapeutic procedure for a subgroup of MS with aggressive nonresponding disease and encourage further studies to evaluate the treatment efficacy of TCV in double-blind placebo-controlled clinical trials.

The mechanisms by which TCV can affect the disease are related to the autoimmune nature of MS where circulating autoreactive T cells react against myelin immunodominant epitopes and induce inflammation and myelin loss. Immunization with attenuated autoreactive T cell lines/clones which uses the patient's own activated T cells that are specific to the target antigens as effector cells, stimulate regulatory networks that induce direct depletion of the host pathogenic T cells by CD8 cytotoxic cells, as well as initiate CD4 Th2 antiinflammatory activity [20,21]. The generation of regulatory CD4 Th2 cells by TCV induces immunological shift from the proinflammatory Th1 to the antiinflammatory Th2 activity by production of antiinflammatory cytokines like IL10 and IL4. Other cell populations also expand upon stimulation with the vaccine including γδT cells and NK cells [22,23].

The duration of TCV effect is not clear yet, and the question whether additional vaccinations should be given is still unresolved. However, the findings of our study suggest that for patients with aggressive MS, in whom conventional therapy could not induce prolonged remission, vaccination with autologous autoreactive T cells should be considered.

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