ORIGINAL ARTICLE

Presence of glatiramer acetate-specific TH2 cells in the cerebrospinal fluid of patients with multiple sclerosis 12 months after the start of therapy with glatiramer acetate

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ABSTRACT

The proposed mechanism of action of glatiramer acetate (GA) from animal models is that GA-specific T cells enter the CNS to act there. To evaluate GA-specific T cells in patients with multiple sclerosis (MS), we tried to culture GA-specific T cell lines from the CSF of the identical patients with MS before and during GA therapy. Before treatment T cell lines could not be obtained, whereas after at least 1 year of GA treatment 12 GA-specific T cell lines secreting TH2 cytokines and BDNF could be generated from the CSF.

Key words: multiple sclerosis, glatiramer acetate, mechanism of action

INTRODUCTION

The current mechanism of action of glatiramer acetate (GA), approved for use in the immunomodulatory therapy of relapsing-remitting multiple sclerosis (MS), proposes that activated GA-specific TH2 cells penetrate the CNS and release anti-inflammatory cytokines and neurotrophic factors in situ.[1] Until now it is only known from animal experiments that activated GA-specific T cells are capable of entering the brain.[2] Studies involving areas beyond the blood-brain barrier are not available.

To demonstrate that GA-specific T cells are able to enter the human CNS compartment we cultured GA-specific T cells from the CSF of three identical patients with MS before treatment and at least 1 year after start of GA treatment, which were characterized in more detail.

METHODS

Three patients with relapsing-remitting MS underwent CSF puncture as part of their diagnostic procedure. All three received GA as their first immunomodulatory therapy. Twelve months after start of GA therapy, they received an additional CSF puncture with informed consent of the patients and approval of the ethical board. These patients on GA who enrolled in this longitudinal pilot study did not demonstrate any relapse or progression after starting their GA therapy.

After centrifugation of the CSF, 10^3 CSF cells were co-cultured with 2×10^5 irradiated autologous antigen presenting cells (APC) in the presence of GA (50 μg/mL) and IL-2 (2 U/mL) according to Neuhaus et al.[3] For our protocol, at least 100 wells were plated for T cell culture. T cells were cultured for 14 to 21 days until the next restimulation with GA (50 μg/mL) and 2×10^5 irradiated autologous APC. Until analysis, GA-specific T cell cultures underwent 5 to 7 rounds of restimulation.

For analysis, 10^5 washed GA-specific TCL cells were stimulated with 10^4 GA- or myelin-basic protein (MBP)-pulsed irradiated autologous PBLs. Supernatants were analyzed after 72 h for BDNF (Promega) and cytokine concentrations (interferon (IFN)-γ and interleukin (IL)-4) (Becton Dickinson) by ELISA. For proliferation assays, parallel cultures were labeled after 48 h with [methyl3H]thymidine (0.2 to 0.5 μCi per well; Amersham Buchler) and harvested 16 to 18 h later. [3H]Thymidine incorporation was measured with a direct β-counter. The intracellular flow cytometry analysis of BDNF production was performed 8 to 10 days after restimulation.[4]

RESULTS

We were not able to generate GA-specific T cell lines from the CSF of the three patients with MS before GA treatment. Although we cultured at least 100 wells for GA T cell culture of CSF cells, no GA-specific T cell lines could be obtained. Using the same protocol 12 months later, we were able to generate 12 GA-specific CSF T cell lines in the same patients. All 12 T cell lines were GA-specific as demonstrated by antigen specific proliferation. Eleven of 12 GA-specific cell lines demonstrated a TH2 or TH0 cytokine phenotype, and only one T cell line GA2-3

had a TH1 phenotype (Table 1). After stimulation with GA, all 12 GA-specific T cell lines secreted BDNF in various concentrations. For the T cell line GA2-4, we could demonstrate cross-reactivity with MBP at the level of cytokine and BDNF production, but not at the level of proliferation (Figure 1a).

To confirm that the CSF-derived GA-specific T cells are the source of the GA-induced BDNF release, we used intracellular staining technique suitable for flow cytometry and FACS analysis of intracellular BDNF production. This method allows the analysis of BDNF production by individual unstimulated und stimulated T cells. After stimulation with ionomycin and PMA, there was an increase of intracellular BDNF production. For all 12 GA-specific CSF-derived T cell lines, we were able to demonstrate intracellular BDNF production (given in Figure 1b for GA2-4). Our results here were in line with those obtained in studies on peripheral blood demonstrating a significant TH1-TH2-shift regarding GA-specific T cells (data not shown).

DISCUSSION

Results from animal and human in vivo studies indicate that GA induces regulatory T cells of the T-helper 2- (TH2)-type in the peripheral immune system outside the CNS.[1] When spleen cells from GA-treated mice were adoptively transferred into syngenic animals, these cells protected the animals from EAE induced by different CNS antigens.[5] Further support for the proposed protective role of GA-specific regulatory T cells comes from the demonstration that GA-specific TH2 cells are present in the CNS of GA-treated mice.[2] Some of these GA-specific TH2 cells are reactivated in the CNS because they cross react with locally presented myelin autoantigens.[3,5] The GA-specific T cells secrete anti-inflammatory cytokines such as IL-4, which, in turn, dampens the activity of any nearby autoaggressive T cells.[6] In addition to this anti-inflammatory bystander suppression, Aharoni et al.[1] demonstrated that GA treatment led to sustained augmentation in the expression of neurotrophic factors like BDNF in various brain regions as demonstrated by histological analysis of immunostained brain sections.

Until now, it was not known whether GA-specific T cells can migrate into the CNS in patients with MS who have been treated with this agent. Kim et al.[8] demonstrated that human TH1 and TH2 GA-reactive T cells could migrate across an artificial blood-brain barrier in vitro. In this pilot longitudinal study, we were able to demonstrate

Table 1. Proliferation (cpm), IFN-γ, IL-4, BDNF secretion (pg/mL) of the examined GA-specific T cell lines derived from CSF culture of three GA-treated patients with MS (GA1-3)

Patient	T cell line	Proliferation		IFN-γ		IL-4		TH profile	BDNF	
		No antigen	GA	No Antigen	GA	No Ag	GA		No antigen	GA
GA1	GA1-1	100 ± 12	3234 ± 678	10 ± 5	17 ± 4	5 ± 2	45 ± 5	TH2	521 ± 276	2365 ± 490
	GA1-2	155 ± 22	5223 ± 987	28 ± 7	34 ± 9	7 ± 2	65 ± 21	TH2	1345 ± 445	2145 ± 251
	GA1-3	300 ± 50	6213 ± 1245	8 ± 12	123 ± 34	11 ± 3	77 ± 11	TH0	834 ± 327	2542 ± 398
GA2	GA2-1	232 ± 27	3563 ± 543	35 ± 17	44 ± 12	15 ± 5	55 ± 21	TH2	1500 ± 211	2532 ± 654
	GA2-2	117 ± 23	2356 ± 765	23 ± 5	33 ± 5	8 ± 3	49 ± 7	TH2	467 ± 127	1589 ± 265
	GA2-3	99 ± 34	1200 ± 234	28 ± 12	33 ± 8	11 ± 4	77 ± 12	TH2	1278 ± 398	2000 ± 112
	GA2-4	115 ± 50	2377 ± 432	44 ± 3	55 ± 9	15 ± 6	55 ± 3	TH2	223 ± 98	812 ± 233
GA3	GA3-1	234 ± 37	4519 ± 399	8 ± 4	7 ± 5	9 ± 5	45 ± 2	TH2	499 ± 129	1736 ± 321
	GA3-2	334 ± 44	5200 ± 522	11 ± 9	234 ± 7	12 ± 3	15 ± 6	TH1	677 ± 381	2987 ± 455
	GA3-3	212 ± 12	3789 ± 687	33 ± 5	29 ± 6	19 ± 2	77 ± 5	TH2	324 ± 136	1566 ± 361
	GA3-4	120 ± 23	2245 ± 234	9 ± 4	15 ± 6	5 ± 3	57 ± 3	TH2	551 ± 269	2001 ± 471
	GA3-5	176 ± 10	5467 ± 469	13 ± 4	23 ± 4	7 ± 2	63 ± 4	TH2	431 ± 233	1991 ± 287

Values are mean of duplicates with standard deviation. The statistical significance between BDNF production without and with GA was $p < 0.0004$ (t-test).

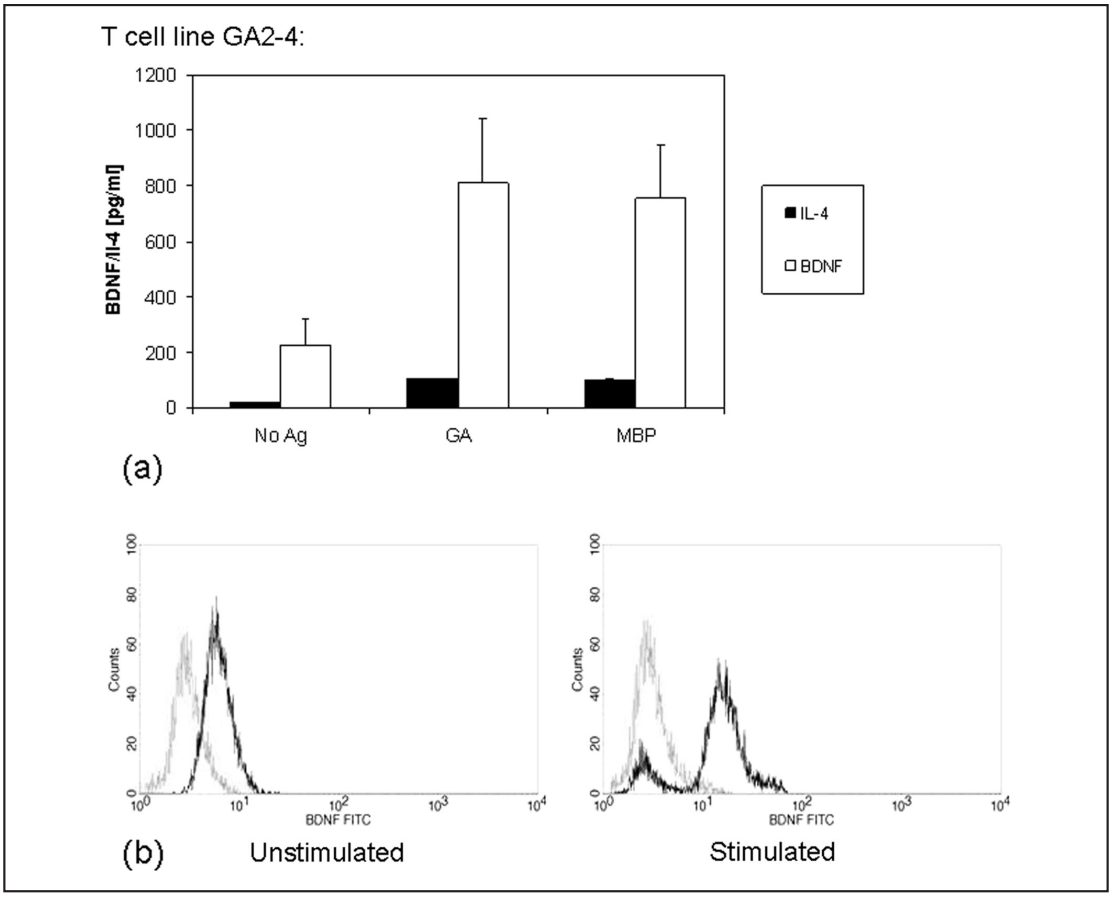

Figure 1. (a) IL-4 and BDNF production (pg/mL) by GA-specific T cell line GA2-4 measured by ELISA in supernatants of the same assay using no antigen, GA (50 µg/mL) and MBP (30 µg/mL). (b) Intracellular BDNF production of unstimulated (left panel) and PMA- and ionomycin-stimulated (right panels) GA-specific T cell line GA2-4. Fine lines represent isotype controls and bold lines represent the anti-BDNF-antibody.

for the first time that GA-specific T cells of TH2/TH0 phenotype are present in the CSF compartment of GA-treated patients after 12 months of GA treatment, whereas no GA-specific T cell lines could be obtained from the CSF compartment. Although GA is a polyclonal mitogen for T cell responses, no T cell responses could be demonstrated in the CSF compartment of untreated patients with MS.

This longitudinal study is only a pilot study with three enrolled patients. Beyond this limitation, these data are important and presented for the first time, which should be the basis for detailed longitudinal analysis of GA-specific T cell responses in the CSF. It has to be clarified whether peripheral immunological changes that are specific for GA like TH1-TH2-shift or GA-specific antibodies correlate with immunological changes in the CSF compartment, which is the only accessible compartment close to the CNS. The results obtained from the CSF should be representative of the CNS as we have already demonstrated that GA-specific T cells can enter the CNS and CSF in a comparable quantity using the murine model of experimental autoimmune encephalomyelitis.

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