Suppressor and cytolytic cell function in multiple sclerosis. Effects of cyclosporine A and interleukin 2.

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Research Article

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Suppressor and Cytolytic Cell Function in Multiple Sclerosis

Effects of Cyclosporine A and Interleukin 2

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Abstract

Patients with progressive multiple sclerosis (MS) demonstrated persistent reductions in levels of concanavalin A (Con A)–induced suppressor activity and heightened levels of in vitro pokeweed mitogen (PWM)–induced IgG secretion. The reduced Con A suppressor activity could not be reversed by addition of interleukin 2 (IL-2). Cyclosporine A (CsA) treatment did not alter the defect in Con A–induced suppressor activity, but did markedly inhibit $T8^+$ cell-mediated alloantigen directed cytolytic activity; this latter defect was reversible by in vitro addition of IL-2. CsA-treated patients did not differ from placebo-treated patients with regard to levels of PWM-induced IgG secretion or proliferative responses of their mononuclear cells to Con A. The results indicate that CsA treatment of MS patients reduces cytolytic function from baseline normal values, but does not alter aberrant suppressor cell function.

Introduction

It has been suggested that immune-mediated mechanisms contribute to the pathogenesis of lesion formation in multiple sclerosis (MS).[1] Studies of intrablood-brain barrier IgG synthesis (1) and of expression of activation antigens on T cells derived from the systemic circulation or cerebrospinal fluid (CSF) (2) indicate a state of persistent immune activation in MS with superimposed further activity at times of "active" clinical disease. Defects in suppressor cells have been demonstrated by several assay systems to be associated with periods of clinical disease activity and are postulated to underlie the aberrant immune reactivity. The consensus from studies enumerating T5/T8 phenotypic T suppressor subsets in patients with active disease, either acute flare-ups or progressive deterioration, suggests a modest decrease in the proportion of such cells in both the systemic circulation and the CSF (3–8). In our studies of in vitro pokeweed mitogen (PWM)–induced IgG secretion by peripheral blood mononuclear cells (PBMNCs) we found that reduced functional suppressor capability of the T8 cell subset rather than their proportion within the MNC population accounted for the high levels of in vitro IgG secretion found in the MS population (9, 10).

Defective suppressor function in active MS has also been demonstrated by us (11) and others (12–14) using concanavalin A (Con A) as a suppressor cell activator. Con A–induced suppression is mediated in normals by both T8 and T4 cell subsets as well as non–T cells (15–18). Whether a failure in a specific cell subset accounts for the defective suppressor cell function in the MS population remains unclear.

The purpose of the present study was to evaluate suppressor and cytolytic cell functions in a rigidly defined group of patients with progressive MS who were being treated with cyclosporine A (CsA), a drug that is reported to selectively inhibit induction of cytolytic T cells while permitting continued generation of suppressor cells (19–22). In our studies, suppressor function was assessed using both a Con A suppressor assay and a PWM-induced IgG secretion assay.

Methods

Patients

All patients had clinical evidence of progressive MS for at least 2 yr, with a Kurtzke disability rating between 3 and 7 (23). These patients were studied before and while participating in a double-blind placebo-controled clinical trial of CsA as a treatment for progressive MS. No clinical correlations were made as part of this study. Patients ranged in age from 27 to 52 yr. Control donors were healthy controls ranging in age from 28 to 54 yr. CsA-treated patients received 6–8 mg/kg of the drug daily. Placebo-treated patients received the olive oil base liquid required to dissolve the CsA.

Methods

All in vitro studies were conducted on PBMNCs isolated from fresh venous blood, using a Ficoll-Hypaque gradient (Pharmacia Fine Chemicals, Piscataway, NJ). All blood samples were coded at the time of study. Patients were studied in groups consisting of at least one placebo-treated patient, one CsA-treated patient, and a control.

CON A-INDUCED SUPPRESSOR ACTIVITY. This assay was performed as previously described (11, 18). Briefly, PBMNCs from the given donor were suspended at 10^6/ml in culture medium comprised of RPMI (Gibco, Grand Island, NY) with 10% fetal bovine serum plus gentamycin (0.1 mg/ml) and glutamine (4 mM). 5-ml aliquots of these cells were cultured with peak mitogenic dosages of Con A (usually 3 μg/ml) or without mitogen in 25-cm^2 flasks (Corning Glass Works, Corning, NY). After 48 h, 100-μl aliquots of stimulated and control cells were placed in microwells and pulsed for 5 h with [^{3}H]thymidine in order to assess the extent of Con A–induced proliferation. The remaining cells were treated with 50 μg/ml of mitomycin C (Sigma Chemical Co., St. Louis, MO), washed four times in Hanks' balanced salt solution (Gibco), and resuspended at 10^6/ml in fresh culture media. The Con A–activated mitomycin-treated cells are termed suppressor or S cells; the nonactivated mitomycin-treated cells are termed control or C cells. 100 μl of S or C cells were then cultured in microwells with 100 μl of 10^6/ml of PBMNCs, freshly isolated from a single allogeneic normal donor (responder or R cell), plus 3 μg/ml of Con A. After 72 h, cultures were pulsed for 5 h with [^{3}H]thymidine. Percent suppression was calculated as: 1 − cpm

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1. *Abbreviations used in this paper:* Con A, concanavalin A; CsA, cyclosporine A; CSF, cerebrospinal fluid; EBV, Epstein-Barr virus; E:T, effector:target ratio; IL-2, interleukin 2; MNC, mononuclear cell; MS, multiple sclerosis; NK, natural killer; PBMNC, peripheral blood MNC; PWM, pokeweed mitogen.

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(cultures of R cells + S cells + Con A)/(cultures of R cells + C cells + Con A) × 100%.

In some studies, 10 and 20% by volume of interleukin 2 (IL-2), derived as culture supernatant from the MLA 144 Gibbon monkey tumor cell line, were added to additional cultures in flasks on day 1 of the study. IL-2 activity of the supernatants was assayed using the IL-2–dependent CTLL cell line as described by Gillis et al. (24) and found to contain ~100 U of IL-2 per milliliter.

PWM-INDUCED IgG SECRETION. *(a) PWM-induced IgG secretion assay.* This assay was performed in microtiter trays as described previously (9). IgG levels were determined from supernatants of 7-d cultures of PWM-stimulated PBMNCs, using an enzyme-linked immunosorbent assay (ELISA).

(b) Alloantigen-specific cytotoxicity assays. Epstein-Barr virus (EBV)–transformed cell lines SB (HLA A12:B17, BW44:DRW2) and JY (HLA A2:B7:DR4,6) were obtained as a gift from Dr. Alan Krensky, Stanford University, Stanford, CA (25). These cell lines had been previously shown to be resistant to natural killer (NK) cell–mediated lysis. Cytotoxic cells from test donors were activated by co-culturing 7.5×10^5 donor PBMNCs with 3.75×10^5 irradiated (10^4 rad), transformed cells for 5 d in 24-well flat-bottom tissue culture plates (Costar, Cambridge, MA) at a final volume of 2 ml/well; medium used in these cultures consisted of RPMI 1640 supplemented with 5% human AB sera and 5×10^{-5} M 2-mercaptoethanol. In some studies, 10% IL-2 was added to the cultures on day 1. After 5 d, the cells from the culture were counted and resuspended in fresh medium at 1×10^6/ml. To perform the cytotoxicity assay, fresh EBV-transformed cells were incubated with ^{51}Chromium (^{51}Cr) (sodium chromate, Amersham Corp., Arlington Heights, IL) for one and one-half hours at 37°C, then washed twice and diluted to a final concentration of 1×10^5 cells/ml. 100-μl aliquots of these cells were placed in 96-well V-bottom microtiter plates (Diagnostic Concepts, Niles, IL). To these were added 100-μl aliquots of effector cells diluted to produce the required effector:target (E:T) ratios. All measurements were performed in triplicate. After a 5-h incubation at 37°C, plates were centrifuged, and the amount of ^{51}Cr released into 100-μl aliquots of cell-free supernatants was measured using a gamma counter. Percent specific lysis was determined as follows:

$$\frac{\text{cpm (target cells + effector cells)} - \text{cpm (spontaneous release by target cells)}}{\text{cpm (maximum lysis of target cells)} - \text{cpm (spontaneous release by target cells)}},$$

where spontaneous release is determined by the counts released by labeled target cells alone, and maximum release is determined by counts released by labeled targets treated with 5% Triton detergent.

Controls used in this assay included measurement of cytolytic activity of activated and nonactivated PBMNCs on NK cell–sensitive targets (K562 cells) and on an EBV-transformed cell line differing in HLA phenotype from the stimulator cells.

Data analysis

Data were analyzed both by comparing overall mean values between groups using a Student's *t* test and by a paired *t* test in which donors studied on the same day were compared or when baseline and follow-up data were compared. Mean values±SEM are given in the text.

Results

Con A–induced suppressor activity. As shown in Table I, baseline suppressor activity was significantly reduced in both the CsA-

Table I. Con A–activated Suppressor Cell Function in Progressive MS and Influence of CsA and IL-2

	Baseline study			Follow-up study						
				MS-placebo		MS-CsA		Control		
	MS-placebo	MS-CsA	Control		+IL-2		+IL-2		+IL-2	Duration of treatment
A	15%°	50%•	79%•[a]	11%	22	31%	13%	73%[a]	ND%	8 mo
	21°	51•	59°	23	23	21	18	73	ND	
B	−8°	−1°	47•	20	23	5	9			7 mo
	−8°	21°	37•	10	2	ND	ND			
			70°[b]					78[b]	57	
			58°							
C	12•	6•	50•	26	31	27	22			6 mo
	13°	ND	45•	30	16	24	9			
			38°[c]					77[c]	72	
			42°							
D	16•	20•	72•[d]	16	18	33	41	80[d]	82	4 mo
	24°	36°	78•	20	24	35	49			
			63°							
			65°							
E	ND	ND	ND	32	33	32	37	91	87	2 mo
	ND	ND	ND	21	29	22	52	87	86	
	15±2.0%*	25±7%	57±4%	21±2%	22±3%	26±3%	28±6%	80±2%	77±6%	

Data indicate percent suppression, calculated by the formula presented in Methods, for individual CsA-treated and placebo-treated MS patients and controls measured at baseline and at time duration (months) on treatment indicated. Patients are grouped (A–E) according to those begun on therapy at the same time. ° and • indicate the MS patients and controls studied at the same time to obtain baseline data. All donors within a group were studied within one week of each other. * Mean values±SEM. [a]-[d] indicate that these control donors were used in both baseline and follow-up studies. ND, not done.

treated and placebo groups compared with controls. For the entire MS groups, mean percent suppression was 20±4%, $n = 15$, compared with 57±4%, $n = 14$, for controls. MS and control donors did not differ with respect to the influence of their C cells on [^{3}H]thymidine uptake by R cell plus Con A cultures. In previous studies, we have found defective Con A–induced suppression in active MS patients whether freshly obtained autologous PBMNCs or normal donor PBMNCs were used as responder cells (26).

In serial studies conducted on the placebo-treated MS patients, a consistent suppressor deficit, relative to control values, was observed (Table II). When suppressor values of CsA-treated patients were compared with those of placebo-treated patients, no significant differences were observed. No differences were noted when baseline and on-treatment values were compared. The addition of exogenous IL-2, either 10% (Table I) or 20% (data not shown), did not enhance suppressor activity in either treated or untreated MS patients or controls.

Table II. Con A–induced Suppressor Cell Function in Progressive MS Patients

		Serial studies			
		Baseline	Follow-up studies		
		% Suppression	% Suppression at 1–3 mo	% Suppression at 4–6 mo	% Suppression at 7–9 mo
A	MS-placebo	15	19		37
	MS-placebo	21	ND		32
	MS-CsA	50	30		21
	MS-CsA	51	48		29
	Control	79	ND		73
	Control	69	76	ND	ND
B	MS-placebo	−8		−21	20
	MS-placebo	−8		−25	10
	Control	73		53	78
C	MS-placebo	16	20	16	
	MS-placebo	24	ND	20	
	MS-CsA	20	30	33	
	MS-CsA	36	20	35	
	Control	72	ND	82	
	Control	65	60	ND	
F	MS-placebo	12	24	26	
	MS-CsA	24	17	24	
	MS-CsA	6	29	27	
	Control	50	56	ND	
	Control	ND	ND	77	

Data indicate percent suppression for individual CsA-treated and placebo MS patients and control donors measured at baseline and at follow-up times indicated. Groups A–C correspond to those in Table I. Group F is not on Table I, as IL-2 studies were not performed on this group. There is no significant difference in the values of Con A–induced suppressor activity between baseline and follow-up results for the MS patients at any of the time periods studied, as determined by paired t test analysis. There is also no significant difference between suppressor values obtained at initial studies for control donors and values found at the longest follow-up time point (paired t test, $n = 6$). ND, not done.

Mean [^{3}H]thymidine uptake of the 48-h Con A–activated cells, which were then to be mitomycin treated, did not differ significantly between MS patients and controls (MS-CsA: 8,532±1,819 cpm, $n = 13$; MS-placebo: 6,451±1,720, $n = 11$; or control: 10,364±3,565, $n = 8$).

Alloantigen-directed cytolytic function. In the CsA-treated patient group, cytolytic activity (8±1%, $n = 8$) was markedly reduced compared with levels observed in placebo-treated patients (32±2%, $n = 6$, $P < 0.001$) and controls (31±4%, $n = 4$, $P < 0.001$). Cytolytic activity in the treated patient groups was also significantly reduced compared with their own baseline pretreatment values (7±1 vs. 25±3%, $n = 5$, $P < 0.001$). As shown in Table III, addition of 10% exogenous IL-2 restored the activity of the MS-CsA–treated patients. This effect of IL-2 on cytolytic function contrasts with the lack of augmentation of Con A suppressor activity.

PWM-induced IgG secretion (Table IV). Levels of IgG secretion did not differ between CsA- and placebo-treated MS patients; for both MS subgroups, IgG secretion levels were higher than those found for control donors. All MS patients studied were "high" responders (9, 10).

Discussion

The results of this study indicate a reproducible defect in Con A–induced suppressor activity in patients with progressive MS. Previous studies using this assay system have documented aberrant suppressor function in MS patients with both acute flare-ups and progressive disease (11–14). The data derived from the placebo-treated patients establishes the reproducibility and persistence of the finding. Previous data on patients in the recovery phase after acute flare-ups suggest that Con A–induced suppressor activity in MS can be increased at these times (11), and

Table III. Effects of CsA on Con A–induced Suppressor and Alloantigen-directed Cytolytic Activity in Patients with Progressive MS

			% Con A suppressor activity		% Cytolytic activity	
		Duration of treatment		+10% IL-2		+10% IL-2
A	MS-placebo		11	22	32	23
	MS-placebo		23	23	29	33
	MS-CsA	8 mo	31	13	2	29
	MS-CsA	8 mo	21	18	1	12
B	MS-placebo		20	23	40	41
	MS-placebo		10	2	38	38
	MS-CsA	7 mo	5	9	9	40
	MS-CsA	7 mo	ND	ND	10	33
	Control		78	57	39	39
C	MS-placebo		16	18	29	32
	MS-CsA	4 mo	33	41	9	29
	MS-CsA	4 mo	35	49	11	34

Data indicate the percent suppression and cytolytic activity at a 30:1 E:T ratio in the presence or absence of added exogenous IL-2. For groups A and B, suppressor and cytolytic assays were done within one month of each other; for group C, the assays were done at the same time.

Table IV. Effects of CsA on PWM-induced IgG Secretion by Mononuclear Cells from Patients with Progressive MS

	Baseline study			Follow-up study			
	MS-placebo	MS-CsA	Control	MS-placebo	MS-CsA	Control	Duration of treatment
A	4,000 ng/ml	5,100 ng/ml	2,000 ng/ml	4,750 ng/ml	4,900	2,200	5 mo
	4,400	5,600		ND	5,100		
B	6,000	6,700	600	ND	5,500	430	3 mo
	6,300	6,600	2,650	5,200	5,350	ND	
C	6,100	4,900	3,000	ND/6,800	4,850/7,000		2 mo/3 mo
	5,900	3,500	1,750	4,200/7,200	3,800/ND	2,800/2,100	

Data indicate levels of in vitro PWM-induced IgG secretion for individual CsA-treated and placebo-treated MS patients and controls. In groups A and B the same control donor was used in baseline and follow-up studies. For Group C, a different control donor was used in each of the serial studies. ND, not determined.

that the defective suppressor function is reversible. The lack of augmentation of Con A suppressor activity in the MS group by addition of exogenous IL-2 to the cultures suggests that the defect is not due to failure of IL-2 production. Conversely, the addition of IL-2 did not decrease suppressor activity in the controls, indicating that Con A suppressor activity is not a reflection of removal of IL-2 from the media as has been previously suggested (27, 28).

CsA has been reported by others to selectively spare or augment suppressor cell function while inhibiting cytolytic function (19–22). Treatment of MS patients with CsA does not restore Con A–induced suppressor activity to control values or reduce levels of PWM-induced IgG secretion, but does dramatically inhibit cytolytic cell function; this latter effect is reversed by addition of exogenous IL-2 in vitro to cultures. CsA has previously been demonstrated to inhibit IL-2 production by activated T cells. The observation that Con A–induced cell proliferation per se ([^{3}H]thymidine uptake by PBMNCs) is not inhibited by the CsA dosages used in this study implicates a specificity of CsA for cytolytic function. With regard to the PWM-induced IgG secretion assay results, our data do not permit us to state whether CsA further inhibits suppressor function, since levels of IgG secretion in the MS group are nearly equivalent to those seen when $T8^+$ cells are completely removed from the system, i.e. cultures of purified $T4^+$ plus $B(E^-)$ cells (9, 10).

Progressive MS patients provide a human disease model in which $T8^+$ cell-mediated cytolytic function is preserved, whereas suppressor function, as evaluated either by a PWM-induced IgG secretion assay or by a Con A–induced suppressor cell assay, is defective (29). In the PWM system, the $T8^+$ cell is established as the suppressor-mediating cell. $T8^+$ cells are also implicated as mediators of suppression in the Con A assay, although other cell subsets are also involved (17, 18). We have found that Con A–induced suppressor activity mediated by either E^+ cells isolated by rosetting techniques or by $T8^+$ cells isolated by a "panning" technique is defective in this group of MS patients. These studies were conducted in the presence of accessory cells (monocytes) derived from normal donors. Monocytes from the MS patients could support "normal" levels of suppression if co-cultured with normal donor E^+ or $T8^+$ cells (30). We do not, however, find a correlation between levels of Con A–induced suppression and levels of PWM-induced IgG secretion in controls. Whether the aberrant function in progressive MS reflects a single cellular mechanism remains to be defined.

The results of the present study indicate that the Con A suppressor defect is neither reversed nor augmented by CsA. Whether this combination of effects of CsA on immunologic functions will be associated with clinical efficacy of the drug awaits the results of clinical trials.

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