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ABSTRACT

Background: The modulation of costimulatory pathways represents an original therapeutic approach to regulate T cell–mediated autoimmune diseases by preventing or reducing autoantigen-driven T-cell activation in humans. Autoreactive $CD4^+$ T cells play a critical role in initiating the immune response leading to the chronic inflammation and demyelination characteristic of multiple sclerosis (MS).

Methods: We used IV infusions of CTLA4Ig to block the CD28/B7 T-cell costimulatory pathway in a phase 1 dose-escalation study in MS. Sixteen patients with relapsing-remitting MS received a single CTLA4Ig infusion and were monitored for up to 3 months after treatment. In an extension study, four additional subjects received four doses of CTLA4Ig.

Results: CTLA4Ig was well tolerated in patients with MS, and most adverse events were rated as mild. Immunologic assessment of the patients showed a reduction in myelin basic protein (MBP) proliferation within 2 months of infusion and decreased interferon-γ production by MBP-specific lines.

Conclusions: Inhibiting costimulatory molecule interactions by using CTLA4Ig seems safe in multiple sclerosis (MS), and the immunologic effects suggest that it may be a promising approach to regulate the inflammatory process associated with MS. *Neurology*® 2008;71:917–924

GLOSSARY

1M = 1 month after infusion; **2M** = 2 months after infusion; **3M** = 3 months after infusion; **8D** = 8 days after infusion; **AE** = adverse event; **AI** = Ambulation Index; **APC** = antigen-presenting cell; **BL** = baseline; **BL2** = second baseline; **BPF** = brain parenchymal fraction; **CTLA-4** = cytotoxic T lymphocyte–associated gene 4; **EDSS** = Expanded Disability Status Scale; **FDA** = Food and Drug Administration; **Gd+** = gadolinium-enhanced; **hMBP** = human myelin basic protein; **IDO** = indolamine 2,3-dioxygenase; **IFN** = interferon; **IL** = interleukin; **MBP** = myelin basic protein; **MS** = multiple sclerosis; **MSFC** = Multiple Sclerosis Functional Composite; **PBMC** = peripheral blood mononuclear cell; **RA** = rheumatoid arthritis; **TE** = echo time; **TR** = repetition time; **URI** = upper respiratory infection; **UTI** = urinary tract infection; **WBC** = white blood cell.

Multiple sclerosis (MS) is a chronic inflammatory disease characterized by the presence of perivascular infiltrates composed of mononuclear cells that lead to demyelination of the CNS white matter. T cells recognizing myelin antigens seem to be involved in the pathogenesis and perpetuation of the disease.[1,2]

Signaling through costimulatory molecules is essential in driving the activation of T cells. The binding of B7-1 (CD80) and B7-2 (CD86) expressed on the surface of antigen-presenting cells (APCs) to CD28 expressed on T cells augments the specific signal delivered through the T-cell receptor and promotes cell division and differentiation.[3,4] Alternatively, the engagement of B7 molecules with the receptor cytotoxic T lymphocyte–associated gene 4 (CTLA-4, CD152) that is up-regulated after T-cell activation conveys an inhibitory signal.[5,6]

CTLA4Ig is a chimeric molecule consisting of the extracellular domain of human CD152 and an IgG tail. CTLA4Ig binds to B7-1 and B7-2 molecules on APCs, thereby blocking the CD28-mediated costimulatory signal critical for T-cell activation. Blocking the

Supplemental data at www.neurology.org

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Table 1 MRI features

	Dose cohort				
	2 mg/kg	10 mg/kg	20 mg/kg	35 mg/kg	4 × 10 mg/kg
Total volume					
Screening	6.4 ± 3.4	4.6 ± 2.8	4.6 ± 4.4	2.1 ± 1.3	3.2 ± 2.7
Baseline	6.3 ± 2.9	4.5 ± 2.8	4.3 ± 4.5	2.2 ± 1.3	3.5 ± 2.7
Postinfusion day 7	6.3 ± 2.8	4.5 ± 2.5	4.8 ± 5.4	2.6 ± 1.5	3.4 ± 2.6
Postinfusion month 1	7.2 ± 3.3	4.5 ± 2.6	4.6 ± 4.4	2.4 ± 1.4	3.3 ± 2.8
Postinfusion month 3	6.2 ± 3.6	4.8 ± 2.6	4.7 ± 4.4	2.1 ± 1.4	3.4 ± 2.7
Total no. of Gd+ lesions					
Screening	0.3 ± 0.5	1.7 ± 2.4	0.0 ± 0.0	0.5 ± 0.6	0.5 ± 1.0
Baseline	1.5 ± 3.0	2.7 ± 2.8	0.2 ± 0.5	0.5 ± 0.6	2.0 ± 4.0
Postinfusion day 7	1.0 ± 2.0	3.2 ± 3.6	0.5 ± 0.6	0.5 ± 0.6	0.2 ± 0.5
Postinfusion month 1	3.0 ± 6.0	4.5 ± 5.4	0.2 ± 0.5	0.0 ± 0.0	0.2 ± 0.5
Postinfusion month 3	0.0 ± 0.0	3.0 ± 3.5	0.0 ± 0.0	1.0 ± 2.0	0.0 ± 0.0
BPF					
Screening	85.4 ± 7.3	87.7 ± 4.4	89.3 ± 4.9	85.5 ± 3.2	87.0 ± 2.7
Baseline	85.4 ± 6.0	87.8 ± 4.4	89.4 ± 4.7	85.3 ± 3.5	87.0 ± 2.6
Postinfusion day 7	85.8 ± 5.9	87.8 ± 4.5	89.1 ± 5.1	85.2 ± 3.5	87.0 ± 2.5
Postinfusion month 1	86.2 ± 5.9	87.7 ± 4.1	89.3 ± 4.6	85.1 ± 3.6	86.8 ± 2.5
Postinfusion month 3	83.0 ± 4.0	87.7 ± 4.7	89.8 ± 4.5	85.1 ± 3.3	87.1 ± 2.5

Gd+ = gadolinium-enhanced; BPF = brain parenchymal fraction.

CD28–B7 pathway is protective in experimental autoimmune encephalitis, a murine model of MS,[7-12] models of autoimmunity,[9,13,14] and transplantation.[15-20]

In human trials, CTLA4Ig was effective in improving the signs and symptoms of rheumatoid arthritis (RA)[21,22] and has been approved by the US Food and Drug Administration (FDA) for the treatment of RA. CTLA4Ig was also tested in patients with psoriasis, a T cell–mediated skin disorder, in a phase 1, open-label, dose-escalation trial[23] showing clinical improvement that correlated with decreased T-cell infiltrates and diminished epidermal proliferation.[24] A previous trial of CTLA4Ig (Abatacept) in MS was initiated by Bristol-Myers Squibb but was aborted because of treatment group imbalance that would have made interpretation of the study results difficult, but Abatacept at 10 mg/kg seemed safe and well tolerated.

We investigated the safety and tolerability of CTLA4Ig infusion in patients with relapsing–remitting MS and its effect on immune function of these patients.

METHODS

Study design. This study was approved by the institutional review board at the Brigham and Women's Hospital, Boston, Massachusetts. After signing informed consent and declining FDA-approved therapies, 20 treatment-naive patients with relapsing–remitting MS were enrolled in this phase 1 dose-escalation study. The patients' MRI characteristics are described in table 1. All the participants had relapsing–remitting MS with lesions mainly located in cerebrum and brainstem. Twelve participants had relapses within the previous 2 years. The median duration of disease was 43 months (range 3–223 months). Sixteen participants were assigned to one of four dose cohorts (2, 10.0, 20.0, or 35.0 mg/kg), each one consisting of 4 subjects. Patients were enrolled starting with the lowest dose (2.0 mg/kg) and proceeding to the next dose group after a 1-month safety evaluation was completed. The subjects received a single dose of CTLA4Ig, delivered as 1-hour infusion, and were followed up for 3 months. An extension study with 4 participants was performed at the end of the phase 1 study, including 4 participants who received four doses of 10 mg/kg CTLA4Ig (at days 0, 3, 14, and 28) and were followed up for 6 months. Safety of CTLA4Ig was evaluated by monitoring laboratory values, vital signs, neurologic and physical examinations, adverse events (AEs), and disease-specific events. AEs were recorded after physician examinations and subject reporting on day 1, day 2, day 8, month 1, and month 3 after infusion days (the schedule varied somewhat between single and multidose groups). The number of gadolinium-enhanced (Gd+) lesions and change from baseline T2 lesion volume on MRI were also examined at baseline, day 8, month 1, and month 3 after the infusion. Functional System Score, Expanded Disability Status Scale (EDSS), Ambulation Index (AI), and Multiple Sclerosis Functional Composite (MSFC) were measured at screening, at baseline, on day 8, and at months 1, 2, and 3. The

Table 2 Adverse events

	Dose cohort				
	2 mg/kg	10 mg/kg	20 mg/kg	35 mg/kg	4 × 10 mg/kg
Total events	9	2	13	17	21
Subjects with 1 or more AEs	3	2	3	4	4
Lymphadenopathy	1	1	4*	2	4
Palpitations	1*	0	0	0	1
Abdominal tenderness	0	0	1	0	1*
Nausea	1*	0	0	0	0
Weakness	0	0	0	2	0
UTI	1	0	2	1	0
URI	0	0	1	1	1
Temperature	1	0	0	0	0
Platelets increased	1	0	0	0	0
T cells increased	0	1	0	0	0
WBCs in urine	0	0	0	0	1
Back stiffness	0	0	0	1	0
Back pain	0	0	0	0	1
Muscle camps	0	0	0	0	1
Pain in limb	0	0	0	0	1
Heaviness	0	0	0	1	0
Hypesthesia	0	0	0	1	0
MS worse	1	0	0	1	0
Headache	0	0	0	1	0
Paraesthesia	0	0	1	0	0
Restless leg	0	0	0	1	0
Tremor	0	0	0	1	0
Visual field defect	1	0	0	0	0
Urinary hesitancy	0	0	0	1	0
Cough	0	0	1	0	0
Pulmonary congestion	0	0	0	0	1
Tonsillar hypertrophy	0	0	0	1	0
Night sweats	0	0	0	1	0
Increased sweating	1*	0	0	0	0

*Adverse events (AEs) that occurred within 24 hours of the infusion. Only one episode of lymphadenopathy occurred with 24 hours of infusion.
UTI = urinary tract infection; URI = upper respiratory infection; WBC = white blood cell; MS = multiple sclerosis.

patient's clinical scores are presented in table e-1 on the *Neurology*® Web site at www.neurology.org.

Disease-specific events. A relapse is defined as the occurrence of new neurologic symptoms lasting 24 hours, associated with an increase of at least 0.5 points on the EDSS or 1 point on the AI after a period of symptomatic stability of at least 29 days, in the absence of febrile illness or steroid withdrawal. Symptoms not associated with objective findings were classified as mild disease-specific events. A relapse associated with objective findings but not requiring treatment was classified as a moderate disease-specific event. A relapse requiring treatment with steroids or hospitalization was classified as a severe disease-specific event.

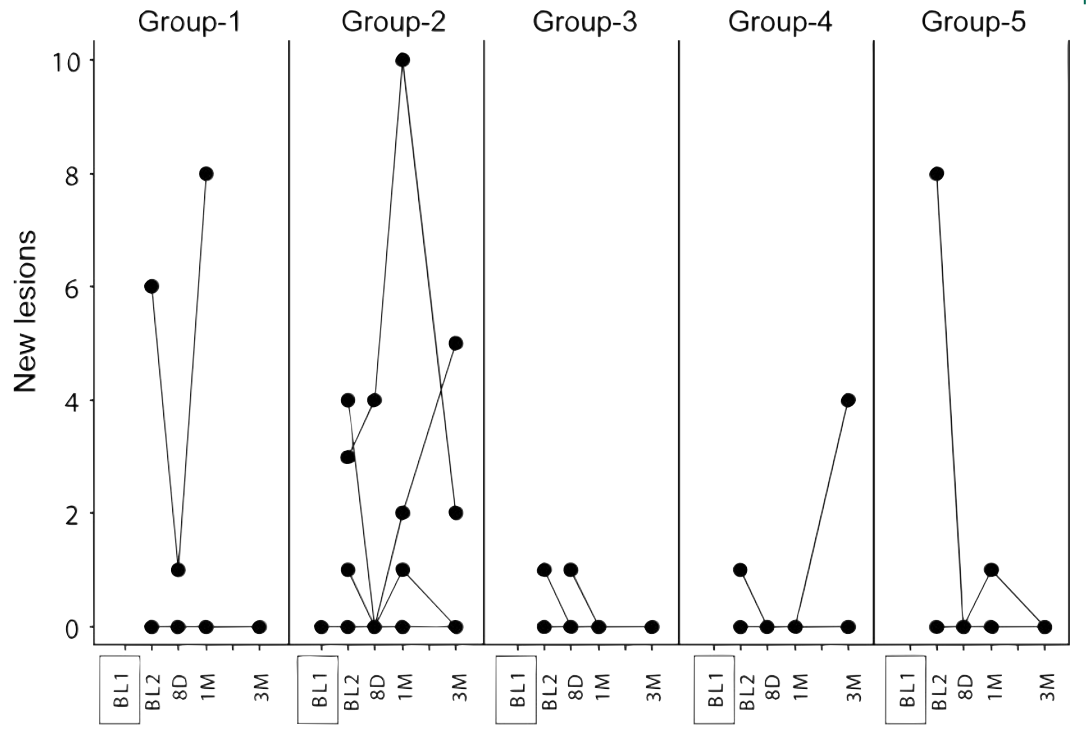
Study drug. CTLA4Ig was manufactured by Repligen as RG2077 as a recombinant CTLA4IgG4m fused to the heavy-chain constant region of the human IgG4 isotype. The gene sequence encoding the immunoglobulin portion has been altered to remove the functional properties of Fc receptor binding and complement fixation.

MRI. Head MRI was obtained using a 1.5-tesla scanner (GE Medical Systems, Milwaukee, WI). Slice thickness was 3 mm (no gap, interleaved acquisitions), and nominal in-plane image resolution was 0.9375 × 0.9375 mm. Dual-echo long repetition time (TR) conventional spin-echo images were acquired with TR = 3,000 msec, echo time (TE)1/TE2 = 30 msec/80 msec, and half-Fourier acquisitions, whereas precontrast ant postcontrast T1-weighted conventional spin-echo images were acquired with TR = 740 msec and TE = 19 msec. Postcontrast T1-weighted acquisitions were acquired 5 minutes after a bolus IV injection of 0.1 mmol/kg Gd-DTPA (Magnevist). The number of Gd+ lesions was assessed independently by two experienced readers, who subsequently reached consensus in a joint session. T2 lesion volumes were estimated using an automated template-driven image segmentation approach combined with a partial volume effect correction algorithm.[25] The occurrence of four or more new Gd+ lesions on the weekly or monthly MRI was documented as an MRI event.

Proliferation assay and measurement of cytokine. Peripheral blood mononuclear cells (PBMCs) were purified by Ficoll-Paque (Amersham Pharmacia Uppsala, Sweden) according to the manufacturer's protocol within 4 hours of blood drawing. PBMCs were cultured in 96-well microtiter plates at a concentration of 100,000 cells/well for 6 days in the presence of recombinant interleukin (IL)-7 (10 ng/mL) and human myelin basic protein (hMBP; 2 and 20 μg/mL) with 30 wells per experimental condition. We used Tetanus Toxoid (0.01, 0.1, and 1 μg/mL) as a control antigen. Supernatants (100 μL) were collected, and then the cells were pulsed with [^{3}H]-thymidine (1 μCi/well; NEN, Boston, MA) for an additional 18 hours to assess antigen response. Cell lines were considered positive when their cpm values were greater than the mean of control values (medium + IL-7 only) plus 3 SDs. Collected supernatants were diluted and analyzed to determine the cytokine profile by ELISA. Cell lines were considered positive when their values (pg/mL) were greater than the mean of control values (only medium, no antigen) plus 2 SDs. Lines producing interferon (IFN)-γ were called Th1, lines producing IL-13 were called Th2, and lines producing both cytokines were called Th0.

Statistical analysis. The repeated measurements on the subjects allowed investigation of treatment effects and trends over time. The MRI metrics and the percent positive lines for Th0, Th1, and Th2 and proliferation under the four conditions described above were compared using the Friedman test. Because this was an exploratory analysis, no corrections for multiple comparisons were completed for results from the mixed effects model and the Friedman test. Post hoc tests for the significant differences from the Friedman test were also completed to find specific pairwise differences using the Wilcoxon, Nemenyi, McDonald–Thompson test,[26] which includes a correction for multiple comparisons. Because the multiple-dose patients did not have a day 8 measurement, this time point was omitted from the extension analysis. Because of missing data on some patients, the 1-month time point was omitted from the Friedman test for some of the immunologic comparisons: the proliferation for each condition, Th0 for

Figure 1 Number of new Gd-enhanced lesions at various visits



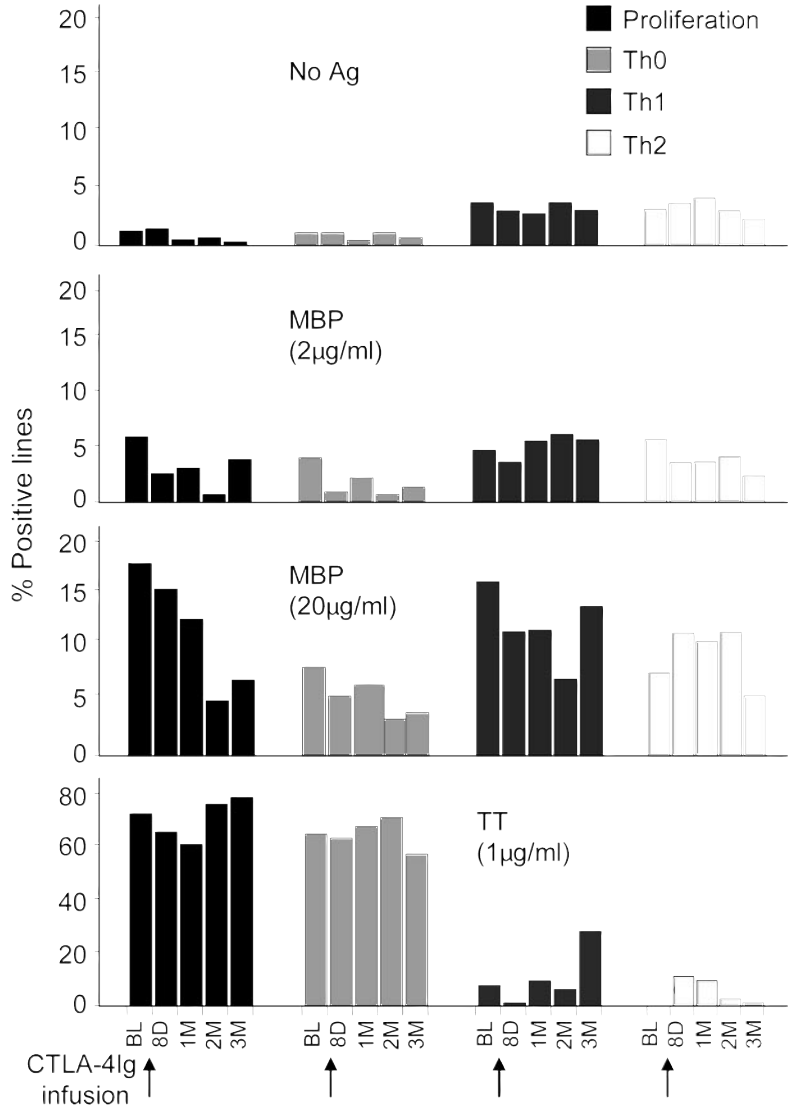
Visits included second baseline (BL2), 8 days after infusion (8D), 1 month after infusion (1M), and 3 months after infusion (3M). Each line represents 1 of the 20 patients in the five dose cohorts. A total of 9 participants had new gadolinium-enhanced (Gd+) lesions during the study, with 1 in the 2.0-mg/kg cohort, 4 in the 10.0-mg/kg cohort, 2 in the 20.0-mg/kg cohort, 1 in the 35.0-mg/kg cohort, and 1 in the 4 × 10.0-mg/kg cohort. Seven patients presented new Gd+ lesions at one of the baselines, and 6 of them showed improvement after CTLA4Ig infusion. Two patients who presented with new Gd+ lesions at the 1-month time point showed improvement by the end of the study.

hMBP2 and TT1, and Th1 and Th2 for TT1. All analyses were completed in the statistical package R.[27]

RESULTS **Safety evaluation.** All participants received one or multiple infusions of RG2077 and completed the study, and no participants discontinued early from the protocol. There was no apparent trend in the laboratory values, the means at each visit were within normal ranges, and no extreme values were observed. No serious AEs or death occurred during the study. A total of 16 participants (80%) experienced 63 AEs, including 59 mild and 4 moderate, and their relationship to the study drug was rated as 4 unrelated, 55 unlikely, and 4 possibly related. The commonly reported AEs (table 2) were lymphadenopathy (40%), urinary tract infection (15%), headache (15%), blurred vision (10%), upper respiratory tract infection (10%), worsening MS (10%), and weakness (10%). A total of 12 episodes of lymphadenopathy occurred in 8 participants during the study; all were transient in nature and were considered as mild and unrelated to dose, with 3 possibly and 9 unlikely related to the study drug. One episode occurred before infusion, 4 occurred 1 month after infusion, and 2 occurred 3 months after infusion. Four episodes occurred in the multidose group: 1 subject had lymphadenopathy 1 month after the last dose, and 1 subject had 3 episodes considered possibly related to drug 4 weeks after the third dose and 1 month and 3 months after the last dose. Three participants (15%) experienced a total of 4 moderate (grade 2) adverse events: 1 episode of back pain, 1 episode of anxiety, and 2 episodes of headache, and the 4 AEs were all unlikely related to the study drug and occurred in the 4 × 10.0-mg/kg group. Three participants reported uncomplicated genitourinary tract infection, with 1 each in 2.0-, 20.0-, and 35.0-mg/kg dose groups. Upper respiratory tract infection occurred in 2 participants.

A total of 10 participants (50.0%) reported 21 disease-specific events, with 3 occurring in the 2.0-mg/kg cohort, 4 in the 10.0-mg/kg cohort, 1 in the 20.0-mg/kg cohort, 8 in the 35.0-mg/kg cohort, and 5 in the 4 × 10.0-mg/kg cohort. Eleven of 21 disease-specific events were mild (symptoms not qualifying as relapses), 2 were moderate (1 of these met the criteria for a relapse and was associated with four new Gd+ lesions on MRI at month 3), and 8 were not classifiable (MRI events also listed in the MRI results section). One of the participants experienced 6 disease-specific events, including heaviness and weakness in the left leg, increased bladder symptoms–hesitancy, increased restless leg with poor sleep, numbness in the right lateral hand

Figure 2 Frequency of positive lines



Peripheral blood mononuclear cells derived from 20 patients were stimulated with different doses of myelin basic protein (MBP; 2 and 20 μg/mL) as well as the recall antigen TT (1 μg/mL). Percent of lines proliferating (black bars) and Th0 (producing both interferon [IFN]-γ and interleukin [IL]-13; light gray bars), Th1 (producing primarily IFN-γ; dark gray bars), and Th2 (producing primarily IL-13; white bars) lines are shown at each time point (BL = baseline; 8D = 8 days after infusion; 1M, 2M, and 3M: 1, 2, and 3 months after infusion). The frequency of positive lines decreased on average over the course of the study for both doses of human myelin basic protein (hMBP) but was significant for the higher concentration of hMBP ($p = 0.029$). A treatment effect on Th0 was observed for the lower concentration of hMBP ($p = 0.016$). *$p = 0.04$ for each. **$p = 0.0086$, #$p = 0.001$, ##$p = 0.025$.

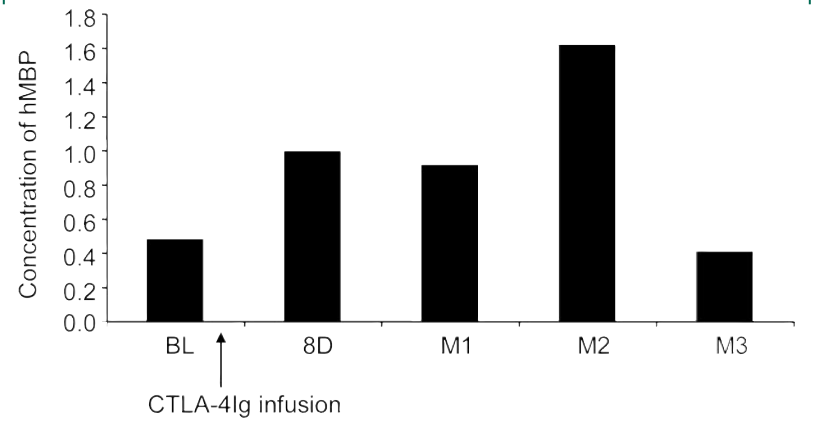
progressing to the right lateral thigh and left thorax, and stiffness in the sacral area of the spinal column.

MRI results. A total of 9 participants had new Gd+ lesions during the study, with 1 in the 2.0-mg/kg cohort, 4 in the 10.0-mg/kg cohort, 2 in the 20.0-mg/kg cohort, 1 in the 35.0-mg/kg cohort, and 1 in the 4 × 10.0-mg/kg cohort (figure 1). Seven patients presented new Gd+ lesions at one of the baselines, and 6 of them showed improvement after CTLA4Ig infusion. Two patients who presented with new Gd+ at the 1-month time point showed a decreased number of new lesions by the end of the study. No apparent correlations were found between new Gd+ lesions and EDSS, 25-Foot Walk Score, Paced Auditory Serial Addition Task 3 Score, Nine-Hole Peg Test Score, and MSFC score over time. No significant treatment effect was observed for T2 lesion volume or brain parenchymal fraction. The occurrence of four or more new Gd+ lesions on MRI was documented as an MRI event for the purpose of alerting the investigators and the Data Safety Monitoring Board. Eight MRI events were reported by 5 participants. Three events occurred at baseline, one event at day 8, one at month 1, and two at month 3. MRI events seemed to occur more frequently in the lower two dose cohorts.

Immunologic changes after CTLA4Ig treatment. The mean frequency of antigen-specific lines before CTLA4Ig infusion was 5.8% positive lines (ranging between 0 and 36%) when hMBP was used at 2 μg/mL. A higher mean frequency of 17.5% (ranging between 0 and 100%) was detected when the cultures were stimulated with 20 μg/mL hMBP (figure 2). The frequency of positive lines decreased on average over the course of the study for both doses of hMBP. The change over time was not significant for the lower concentration of hMBP and showed a trend for the higher concentration ($p = 0.088$), but the small sample size limited the ability to detect a significant difference. When the multiple-dose patients were included, the treatment effect showed a trend for a reduction in frequency of MBP-reactive lines at the lower antigen concentration ($p = 0.079$) and significant for the higher antigen concentration ($p = 0.029$).

Antigen-driven cytokine secretion was then examined. At baseline, the frequency of MBP-reactive lines (number of patients with any reactive lines) was 4% (9) Th0, 4.6% (6) Th1, and 5.6% (8) Th2 when stimulated with 2 μg/mL MBP, and 8% (10) Th0, 15.8% (12) Th1, and 7.5% (9) Th2 when stimulated with 20 μg/mL MBP. A treatment effect on Th0 was observed for the lower concentration of hMBP ($p = 0.016$). At any given post-treatment time point, 7 or 8 patients had a decrease in frequency of Th0 lines, 0 or 2 patients had an increase in frequency, and the remainder stayed the same. The comparisons of baseline with months 2 and 3 showed a significant decrease in the frequency of Th0 ($p = 0.04$ for each), and the comparison of baseline with day 8 showed a trend toward decrease ($p = 0.053$) (figure 2). The ratio Th2:Th1 for the high concentration was 0.4 at baseline and increased to 1.6 by month 2 after infusion (figure 3). As expected, most of the TT lines were Th0, secreting large amount of both IFN-γ and IL-13. No significant change in the Th0 response

Figure 3 Th2:Th1 ratio



An increase of Th2 lines occurred after CTLA4Ig treatment. The ratio Th2:Th1 for the high concentration of human myelin basic protein (hMBP) was 0.4 at baseline and increased to 1.6 by month 2 after infusion. BL = baseline; 8D = 8 days after infusion; M1 = 1 month after infusion; M2 = 2 months after infusion; M3 = 3 months after infusion.

was observed after CTLA4Ig infusion ($p = 0.364$). Interestingly, significant changes in the TT-positive lines were observed for both the Th1 phenotype ($p = 0.001$) and the Th2 phenotype ($p = 0.025$) (figure 2).

Induction of indoleamine 2,3-dioxygenase (IDO) by dendritic cells has been proposed as an additional mechanism of immune modulation by CTLA4Ig.[20] The ratio of the concentration of the product of IDO, kynurenine, vs the substrate tryptophan was calculated to estimate IDO activity. Thus, serum was obtained at several time points after infusion of CTLA4Ig. There were no significant changes in the serum levels of tryptophan or kynurenine after the CTLA4Ig infusion. Positivity for anti-CTLA4Ig antibodies was defined as a fourfold increase in titer compared with baseline. Only 2 subjects showed positivity at month 3, 1 subject in the 10-mg/kg group and 1 in the multidose group.

DISCUSSION Treatment with CTLA4Ig has been approved by the FDA for the treatment of RA and is under investigation for treatment of psoriasis and for organ transplantation. This phase 1 clinical trial is a safety and tolerability trial of CTLA4Ig infusion in patients with relapsing–remitting MS (a previous trial conducted by Bristol-Myers Squibb was terminated prematurely because of treatment group imbalance). Selective blockade of CD28–B7 costimulatory pathway through CTLA4Ig seems to be safe and well tolerated and may represent a valuable strategy to regulate T-cell activity and inflammation in MS. Continuous activation of T cells by the interaction between CD28 and the B7 molecules expressed on APCs and the subsequent secretion of cytokines seems crucial to the pathogenesis of MS and the perpetration of the inflammatory process. CTLA4Ig selectively blocks this interaction and may modulate the immune responses in MS without depleting T cells.

No significant changes in clinical parameters were observed during this study, although this is unremarkable given the short duration of observation. Similarly, no significant changes in total T2 lesion volume or brain parenchymal fraction were observed. During this study, 12 occurrences of asymptomatic lymphadenopathy were observed, some of which were apparent before the start of the treatment. The cause of this lymphadenopathy is unclear, and in only one subject was it thought to be possibly related to treatment.

The occurrence of infections represents one of the major risks in subjects undergoing immunosuppressive therapy or any treatment that is likely to interfere with the immune response to pathogens. The rate of infections observed in this study was not different from what is expected in the MS population, and no unusual infections were observed. One should be cautious in overinterpreting these data, however, given the small sample size and the single infusion received by most subjects.

Although this study was not powered to detect significant differences in immune responses among groups, some significant differences were found, and other trends were apparent. A decrease in the frequency of MBP-specific cell lines was detected at various time points after the CTLA4Ig infusion, and the frequency of IFN-γ–producing lines (Th0) decreased especially with the lower concentration of hMBP stimulation. An increased ratio of Th2:Th1 lines was observed after CTLA4Ig infusion. Because of limitation in the number of cells, only MBP reactivity was evaluated, but other myelin antigens would need to be evaluated in the future. Other studies have reported that stimulation of T cells in the presence of CTLA-4 blockade inhibits Th1 and increase Th2 cytokines.[28] They also found that strong T-cell receptor/costimulatory signals amplified by anti–CTLA-4 blockade results in preferential activation of a Th2 immune response and loss of a Th1 response. Although CD28–B7 signals are not absolutely required for Th1 and Th2 differentiation,[29] blockade of this pathway results in skewing of the immune response. This has been demonstrated in several animal models, with skewing toward Th2 responses in Th1-mediated diseases[9,30] although disease inhibition is not dependent on expression of Th2 cytokines.[31,32] Similarly, CTLA4Ig treatment skewed immune responses toward Th1 in Th2-mediated models.[33]

Another mechanism of CTLA4Ig action was reported to be by regulating tryptophan catabolism in B7-

expressing dendritic cells[20] through induction of IDO in the antigen-presenting cells, which would lead to inhibition of T-cell proliferation.[34] We did not detect any changes in IDO, tryptophan, or kynurenine in the serum after the infusion, but these changes likely occur in the local milieu between T cells and dendritic cells and may not be detectable in the serum.

Our findings demonstrate the safety and tolerability of CTLA4Ig infusions for the treatment of MS patients and contribute to the understanding of the mechanisms involved in the inflammatory process leading to MS.

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by B.H.

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