Reduction of CD8$^+$ T lymphocytes in multiple sclerosis patients treated with dimethyl fumarate

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ABSTRACT

Objective: To evaluate the influence of dimethyl fumarate (DMF, Tecfidera) treatment of multiple sclerosis (MS) on leukocyte and lymphocyte subsets.

Methods: Peripheral blood leukocyte and lymphocyte subsets, including CD3$^+$, CD4$^+$, and CD8$^+$ T cells; CD19$^+$ B cells; and CD56$^+$ natural killer (NK) cells, were obtained at baseline and monitored at 3 months, 6 months, and 12 months after initiation of DMF treatment.

Results: Total leukocyte and lymphocyte counts diminished after 6 months of DMF therapy. At 12 months, lymphocyte counts had decreased by 50.1% ($p < 0.0001$) and were below the lower limit of normal (LLN) in one-half of patients. CD3$^+$ T lymphocyte counts fell by 44.2% ($p < 0.0001$). Among subsets, CD8$^+$ T cell counts declined by 54.6% ($p < 0.0001$), whereas CD4$^+$ T cell counts decreased by 39.2% ($p = 0.0006$). This disproportionate reduction of CD8$^+$ T cells relative to CD4$^+$ T cells was significant ($p = 0.007$) and was reflected by a 35.5% increase in the CD4/CD8 ratio ($p = 0.007$). A majority of CD8$^+$ T cell counts, but not CD4$^+$ T cell counts, were below the LLN even when total lymphocyte counts were greater than 500 cells/μL. CD19$^+$ B cell counts were reduced by 37.5% ($p = 0.035$). Eosinophil levels decreased by 54.1% ($p = 0.006$), whereas levels of neutrophils, monocytes, basophils, and NK cells were not significantly altered.

Conclusion: Subsets of peripheral blood leukocytes and lymphocytes are differentially affected by DMF treatment of MS. Reduction of CD8$^+$ T cells is more pronounced than that of CD4$^+$ T cells. These findings may have implications for cell-mediated antiviral immunity during DMF treatment. *Neurol Neuroimmunol Neuroinflamm* 2015;2:e76; doi: 10.1212/NXI.0000000000000076

GLOSSARY

DMF = dimethyl fumarate; **FAE** = fumaric acid ester; **JC** = John Cunningham; **LLN** = lower limit of normal; **MS** = multiple sclerosis; **PML** = progressive multifocal leukoencephalopathy; **UCSF** = University of California, San Francisco.

Oral dimethyl fumarate (DMF, Tecfidera) was approved for treatment of relapsing forms of multiple sclerosis (MS) in 2013. Like Fumaderm, a fumaric acid ester (FAE) preparation of DMF and monoethyl fumarate used to treat psoriasis, DMF therapy in MS reduces peripheral blood lymphocyte counts.[1,2] Lymphopenia resulting from Fumaderm treatment has been associated with rare cases of progressive multifocal leukoencephalopathy (PML),[3–5] an opportunistic CNS infection caused by the John Cunningham (JC) virus. Recently, a fatal case of PML occurred in association with sustained lymphopenia that developed during DMF therapy for MS.[6] Both humoral and cellular immune responses are important in defense of viral infections. To our knowledge, the influence of DMF treatment of MS on lymphocyte subsets has not been reported. Due to the concern of potential immune suppression with DMF, we obtained lymphocyte subset counts before initiating DMF therapy in patients with MS and monitored them during treatment. We now report those results.
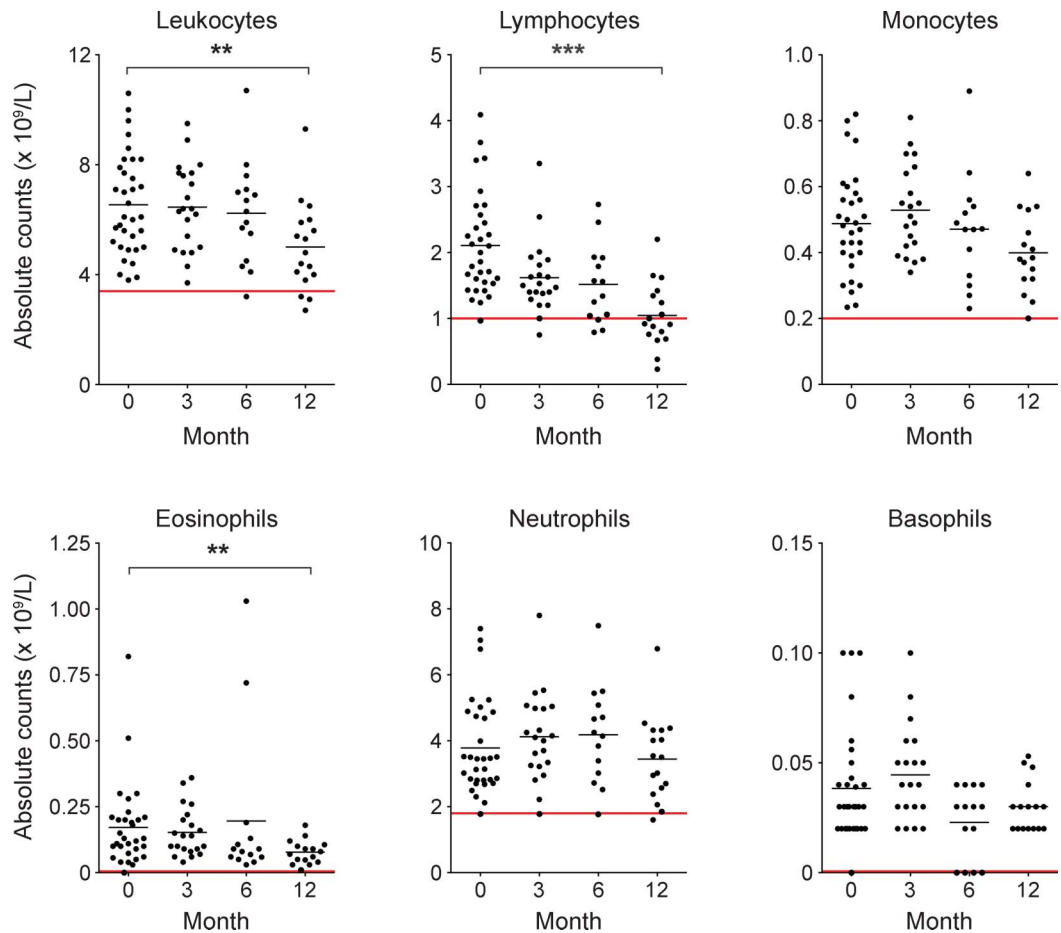
METHODS **Standard protocol approvals, registrations, and patient consents.** The University of California, San Francisco (UCSF) Institutional Review Board (UCSF Committee on Human Research) approved acquisition and reporting of data obtained in

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Figure 1 Peripheral blood leukocyte subset counts during dimethyl fumarate treatment

Complete blood cell counts were obtained at baseline (n = 34) and at month 3 (n = 21), month 6 (n = 15), and month 12 (n = 17) of treatment. Red lines represent lower limit of normal for each subset. Statistical significance was computed using Mann-Whitney U test. p Values less than 0.05 were considered statistically significant. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

this observational study. Thirty-five patients with relapsing forms of MS from the UCSF MS Center were included in this study between March 2013 and January 2015.

Complete blood counts were collected at baseline and 3 months, 6 months, and 12 months (±1.5 months) after initiating treatment with DMF. One of the investigators (S.S.Z.) obtained lymphocyte subsets on 25 of the patients before and during DMF treatment. At the time of analysis, 14 of those 25 patients had reached 12 months of treatment. Ages ranged from 21 to 67 years (mean age 46.1). A total of 71.4% of patients were women; 25.7% had been treated previously with glatiramer acetate, 28.6% with IM interferon β-1a, 22.9% with natalizumab, and 2.9% with fingolimod.

Complete blood cell counts and lymphocyte subsets were examined by cross-sectional analysis. For complete blood cell counts: baseline (n = 34), month 3 (n = 21), month 6 (n = 15), month 12 (n = 17); for lymphocyte subsets: baseline (n = 21), month 3 (n = 13), month 6 (n = 13), month 12 (n = 14). Paired longitudinal analyses were conducted for CD4 and CD8 counts at baseline and month 12 (n = 11). Absolute cell counts were used for subset analyses. Statistical analyses were conducted using Prism 6.0 (GraphPad Software, La Jolla, CA). Statistical significance for cross-sectional analyses was computed using Mann-Whitney U tests. Paired t tests were used for longitudinal analyses. p Values less than 0.05 were considered statistically significant.

RESULTS Total leukocyte counts diminished over time, and this reduction was statistically significant ($p = 0.004$) at month 12 of DMF therapy (figure 1, table). The absolute change in leukocytes primarily reflected a 50.1% decrease ($p < 0.0001$) in lymphocytes. At month 12, lymphocyte counts were below the lower limit of normal (LLN) in 50% of patients. While we observed a reduction in eosinophils ($p = 0.006$), a previous report indicated that DMF use may be associated with transient eosinophilia after the first 4 weeks of treatment.[7] Of interest, one patient who had a normal baseline eosinophil level exhibited eosinophilia at month 6 of DMF treatment, but this was not sustained. The level of neutrophils remained relatively stable, and although there were reductions in the numbers of monocytes and basophils, they were not significant.

Lymphocyte subsets also decreased at month 12 of DMF treatment (figure 2A). CD3^{+} T cell counts decreased by 44.2% ($p < 0.0001$). There was a 37.5% reduction of CD19^{+} B cells ($p = 0.035$). While CD4^{+} T cell counts diminished in a proportion (39.2%, $p = 0.0006$) similar to total CD3^{+}

Table Peripheral blood leukocyte and lymphocyte subset counts before and during dimethyl fumarate treatment

	Baseline Mean (SEM)	Month 3			Month 6			Month 12		
		Mean (SEM)	% Change[c]	p	Mean (SEM)	% Change[c]	p	Mean (SEM)	% Change[c]	p
Leukocytes[a]	6.544 (0.31)	6.46 (0.34)	−1.3	0.94	6.23 (0.48)	−4.8	0.6	5.01 (0.4)	−23.4	0.0043
Lymphocytes[a]	2.106 (0.14)	1.62 (0.12)	−23.1	0.008	1.52 (0.16)	−27.8	0.017	1.05 (0.12)	−50.1	<0.0001
CD3[b]	1,403 (97.5)	1,323 (114)	−5.7	0.63	1,081 (117.7)	−23.0	0.04	783.5 (89.4)	−44.2	<0.0001
CD4[b]	967.4 (71.2)	882.6 (67.2)	−8.8	0.49	763.1 (79.1)	−21.1	0.042	588.1 (66.5)	−39.2	0.0006
CD8[b]	431.7 (37.3)	434.6 (38.7)	+0.7	0.95	287.8 (40.7)	−33.3	0.013	196.1 (34)	−54.6	<0.0001
CD4/CD8 ratio	2.51 (0.21)	2.08 (0.11)	−17.1	0.26	2.95 (0.32)	+17.5	0.37	3.4 (0.26)	+35.5	0.007
CD19[b]	318.1 (33.6)	268 (40.9)	−15.7	0.27	215.6 (42.2)	−32.2	0.052	198.9 (35.1)	−37.5	0.035
Natural killer[b]	204.4 (23.5)	164.6 (25.7)	−19.5	0.22	165.5 (21.7)	−19.0	0.28	154.2 (31.4)	−24.6	0.12
Monocytes[a]	0.49 (0.028)	0.53 (0.03)	+8.2	0.42	0.47 (0.045)	−4.1	0.73	0.4 (0.03)	−18.4	0.058
Eosinophils[a]	0.17 (0.029)	0.15 (0.02)	−11.8	0.99	0.196 (0.079)	+15.3	0.19	0.078 (0.01)	−54.1	0.006
Basophils[a]	0.038 (0.005)	0.045 (0.005)	+18.4	0.15	0.023 (0.004)	−39.5	0.16	0.03 (0.003)	−21.1	0.41
Neutrophils[a]	3.78 (0.25)	4.12 (0.29)	+9.0	0.15	4.18 (0.395)	+10.6	0.33	3.4 (0.31)	−10.1	0.52

Statistical values were calculated using Mann-Whitney U test.
[a] Mean counts ($\times 10^9$/L).
[b] Mean counts ($\times 10^6$/L).
[c] Percentage change from baseline.

T cell counts, $CD8^+$ T cell counts decreased by 54.6% ($p < 0.0001$). This relative reduction in $CD8^+$ T cells was reflected by a 35.5% increase in the CD4/CD8 ratio ($p = 0.007$). A paired analysis of $CD4^+$ and $CD8^+$ T cells at baseline and month 12 demonstrated a disproportionate reduction of $CD8^+$ T cells relative to $CD4^+$ T cells ($p = 0.007$) (figure 2B). At month 12 of DMF therapy, $CD8^+$ T cell counts were below the LLN in a majority of patients. In contrast, $CD4^+$ T cell counts were above the LLN in most patients.

DISCUSSION DMF and other FAEs activate nuclear factor (erythroid-derived 2)–related factor 2 (Nrf2), a pathway that promotes expression of products that protect against oxidative damage and that can inhibit proliferation of lymphocytes and hematopoietic stem cells.[8–12] In the phase 3 MS clinical trials, DMF treatment was associated with a roughly 30% reduction in total lymphocytes.[1,2] In MS clinical practice, lymphocyte counts decreased below 500 cells/μL (grade 3 lymphopenia) in approximately 6% of patients.[13] T cells and B cells participate in cellular and humoral defense responses, respectively. $CD4^+$ T cells participate in cellular immune responses and direct B-cell differentiation of antibody-secreting plasma cells. $CD8^+$ T cells, which are dependent on interleukin-2 production from $CD4^+$ T cells, participate in antiviral immunity. Thus, it is important to evaluate how DMF influences each of these subsets. As part of our routine management for patients treated with DMF, we monitored lymphocyte subsets. Although we examined a relatively small number of patients, the reduction in $CD8^+$ T cells was prominent and more significant than the decrease in B cells or $CD4^+$ T cells. Of interest, in 1998 it was reported that FAE (combination of DMF and monoethyl fumarate) treatment of psoriasis vulgaris was associated with a larger reduction in peripheral $CD8^+$ T cells than $CD4^+$ T cells.[14] Our report is the second study showing that an FAE reduces $CD8^+$ T cells more prominently and the first study demonstrating that DMF monotherapy causes a more significant reduction in $CD8^+$ T cells than $CD4^+$ T cells. Therefore, it seems that this effect is a general property of FAEs and should not be attributed to differences in formulation, including changes in divalent cations used in FAE salt preparations.[5]

Immune suppression that occurs in HIV, treatment of malignancies, or transplant rejection increases the risk of PML.[15] The risk of PML with the use of FAEs, including DMF, may be quite low.[3,4] Currently, all PML cases associated with FAE treatment have been associated with sustained lymphopenia. Recently, one case of PML was reported in a patient with MS who was treated with DMF for 4.5 years and was

Figure 2   Peripheral blood lymphocyte subset counts during dimethyl fumarate treatment
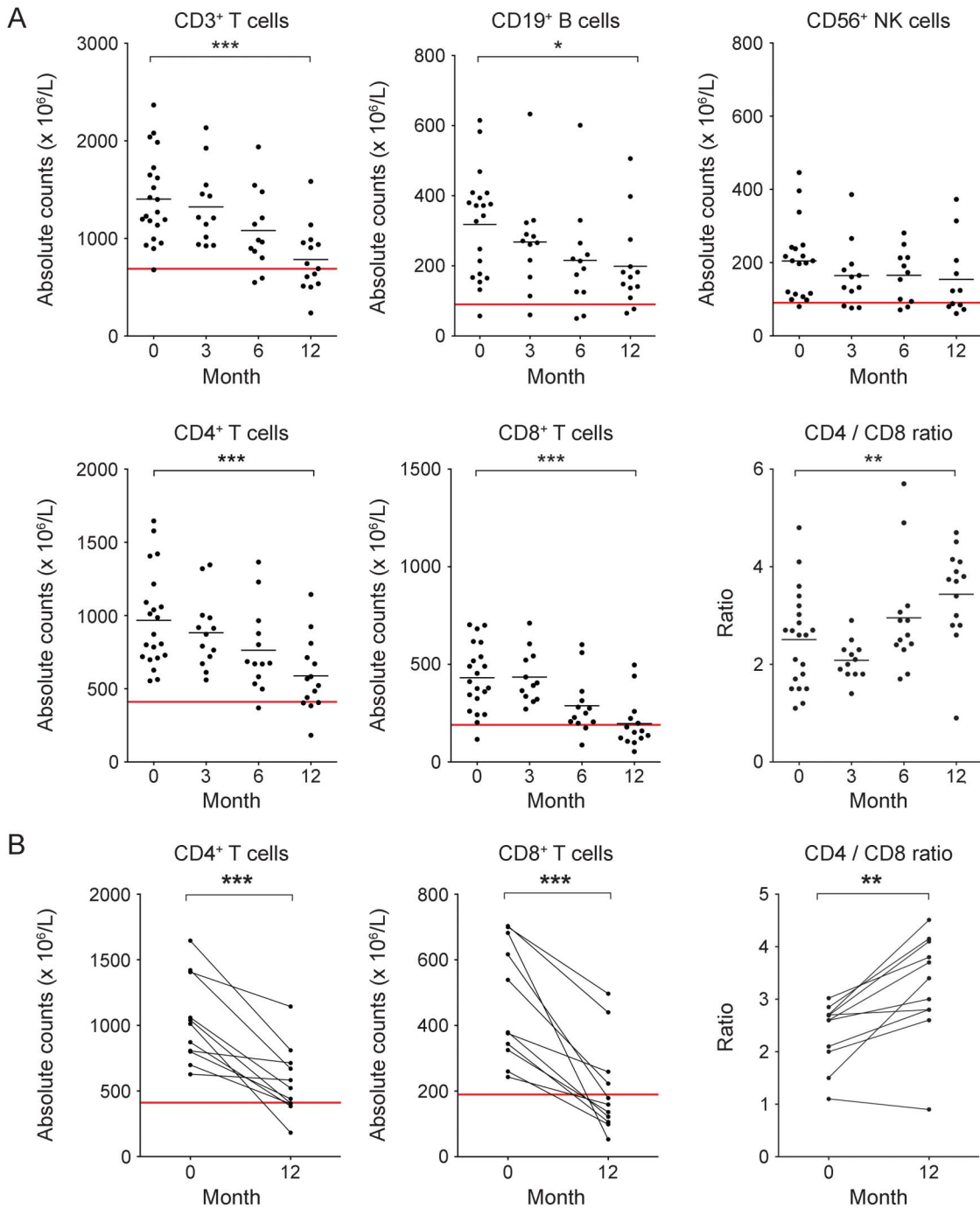
(A) Lymphocyte subsets were obtained at baseline (n = 21) and at month 3 (n = 13), month 6 (n = 13), and month 12 (n = 14) of treatment. Red lines represent lower limit of normal for each subset. Statistical significance was computed using Mann-Whitney U test. (B) Paired longitudinal analysis (n = 11) of CD4 counts, CD8 counts, and CD4/CD8 ratio between baseline and month 12. Percent reduction of CD4 and CD8 counts from baseline to month 12 was calculated for each individual. The mean reduction of CD8$^+$ T cells (55.5%) was significantly greater than the reduction in CD4$^+$ T cells (43.4%) (p = 0.007). The CD4/CD8 ratio increased by 38.3% at month 12. p Values less than 0.05 were considered statistically significant. *p < 0.05; **p < 0.01; ***p < 0.001.

lymphopenic for 3.5 years.[6] In this patient, absolute lymphocyte counts ranged from 290 to 580 cells/μL. Our results indicate that CD8$^+$ T lymphocytes, which are likely critical to defend against the JC virus,[16] are reduced to a greater extent than CD4$^+$ T lymphocytes during DMF treatment. While the risk of PML with DMF treatment of MS is exceptionally low, it is plausible that a sustained reduction in CD8$^+$ T cells could be one factor predisposing to JC virus activation.

There are limitations to our study. First, we examined a relatively small number of patients with MS. Second, we have not yet monitored sufficient numbers of patients beyond 12 months of DMF treatment. Third, our evaluation of peripheral blood

lymphocyte subsets in DMF treatment of MS may not reflect the influence of DMF on immune cells in other compartments, including the CNS. Nevertheless, our findings should provide incentive to conduct larger prospective investigations to examine lymphocyte subsets and immune function of CD8^{+} and CD4^{+} T cells in DMF treatment of MS.

Currently, the manufacturer recommends that lymphocyte counts be monitored every 6–12 months on DMF therapy and that interruption be considered when lymphocyte counts decrease below 500 cells/μL and remain below that level for more than 6 months.[17] We have observed that CD8^{+} T lymphocyte counts fell below the LLN in some patients at 12 months of DMF treatment, even though total lymphocyte counts were greater than 500 cells/μL. Monitoring T lymphocyte subsets during DMF treatment of MS may be beneficial.

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