Turkish Journal of Immunology - Open Access
www.turkishimmunology.org

Turk J Immunol 2019; 7(2):69–75

Comparison of Peripheral Blood Th17 Cells and Associated Cytokines in Fingolimod-Receiving and Untreated Multiple Sclerosis Patients

Fingolimod Alan ve Almayan Multiple Skleroz Hastalarının Perifierik Kanında Th17 Hücrelerinin Karşılaştırılması

Ahmet EKEN[1,2], Mehmet Fatih YETKİN[3], Fatma Zehra OKUS[1,2], Şerife ERDEM[1,2], Mustafa ÇAKIR[1,2], Yesim HALİLOĞLU[1,2], Zehra Busra AZİZOĞLU[1,2], Hamiyet DÖNMEZ ALTUNTAS[1,2], Meral MİRZA[3], Halit CANATAN[1,2]

Abstract

Introduction: Th17 cells are critical mediators of pathology in several autoimmune diseases including multiple sclerosis (MS). The aim of this study was to quantify Th17 cells and-associated cytokines in the peripheral blood of relapsing remitting multiple sclerosis patients (RRMS). We also aimed to compare those levels in fingolimod-treated, and untreated patients.

Material and Methods: Fifteen fingolimod administered RRMS, 9 untreated-RRMS patients and 6 healthy controls were evaluated. Their peripheral blood mononuclear cells (PBMCs) were isolated and sera separated. IL-17A$^+$, IL-22$^+$ and GM-CSF$^+$ T-cells were quantified via intracellular cytokine staining after stimulation using flouresecein activated cell sorter. Serum cytokine levels from all groups were measured via enzyme-linked immunosorbent assay (ELISA).

Results: Fingolimod-treated RRMS patients had reduced number of IL-17A$^+$ (p=0.02), IL-22$^+$ (p=0.05), and GM-CSF$^+$ (p=0.003) T cells in their peripheral blood compared to those of untreated RRMS patients. This is consistent with sequestration of lymphocytes in the secondary lymphoid organs after fingolimod use. However, the levels of same cytokines in the serum were statistically not different.

Conclusions: Fingolimod treatment reduced circulating IL-17A$^+$, IL-22$^+$ or GM-CSF$^+$ T cells in RRMS patients.

Keywords: Fingolimod, Multiple sclerosis, IL-17A, IL-22, GM-CSF

Öz

Giriş: Th17 hücreleri, multipl sklerozun da dahil olduğu birçok otoimmün hastalığın patogenezinde rol alan kritik bir hücre popülasyonudur. Bu çalışmada, fingolimod tedavisi alan ve almayan tekrarlayan-gerileyen multipl sklerozlu (TGMS) hastalarının kanındaki Th17 hücre sayısı ve ilişkili sitokinlerin miktarlarını irdelemeyi amaçladık.

Gereç ve Yöntemler: On beş fingolimod tedavisi alan TGMS hastası, ve tedavi almayan 9 TGMS hastası ile 6 sağlıklı kontrol çalışmaya dahil edildi. Kandan izole edilen periferik kan mononükleer hücreleri stimüle edilmiş, intraselüler hücre boyaması ve FACSAriaIII kullanılarak IL-17A, IL-22 ve GM-CSF üreten T hücreleri sayı ve yüzdeleri ölçüldü. Serum sitokin seviyeleri ELISA yöntemi ile saptandı.

Bulgular: Fingolimod tedavisi alan RRMS hastalarının kanındaki IL-17A$^+$ (p=0,02), IL-22$^+$ (p=0,05), GM-CSF$^+$ (p=0,003) T hücrelerinin mutlak sayılarında, fingolimod almayan TSMS hastalarına göre, önemli ölçüde azalma gözlendi. Bu sonuç fingolimod'un lenfositleri sekonder lenfoid organlarda tutma mekanizması ile uyumludur. ELISA ile serumdan ölçülen aynı sitokinlerin seviyerinde anlamlı azalma gözlenmedi.

Sonuç: Fingolimod dolaşımdaki IL-17A$^+$, IL-22$^+$ ve GM-CSF$^+$ üreten T hücre sayılarını azaltmaktadır.

Anahtar Kelimeler: Fingolimod, Multipl skleroz, IL-17A, IL-22, GM-CSF

[1]Erciyes University School of Medicine, Department of Medical Biology, 38030, Melikgazi, Kayseri, Turkey
[2]Betül-Ziya Eren Genome and Stem Cell Center (GENKOK), 38030, Melikgazi, Kayseri, Turkey
[3]Erciyes University School of Medicine, Department of Neurology, 38030, Melikgazi, Kayseri, Turkey

Correspondence:
Ahmet EKEN,
Erciyes University School of Medicine, Department of Medical Biology, 38030, Melikgazi, Kayseri,Turkey
E-mail: ahmet.eken@gmail.com
Phone: +90 545 377 3888

Received: Jan 01, 2019
Accepted: Jun 19, 2019

https://doi.org/10.25002/tji.2019.1007

©2019 Turkish Journal of Immunology. All rights reserved.

Introduction

Th17 cells are a lineage of CD4$^+$ helper T cells that differentiate from naïve CD4$^+$ T cells in the presence of proinflammatory cytokines IL-6, IL-1β, IL-23 along with TGF-β. [1,2] After their discovery, pathogenesis of many chronic inflammatory diseases including multiple sclerosis (MS), have been shown to associated with Th17 cells.[3–5] Th17 cell development is regulated by the master regulator transcription factor retinoic acid related

(RAR) orphan receptor gamma t (Rorγt), or Retinoic Acid Related Orphan Receptor C (RORC), in mice and humans, respectively.[1,3,6] Other transcription factors such as Stat3, Irf-4, Batf and AhR have also been shown to be critical for Th17 development in mice, however only STAT3 among those have been shown be required for human Th17 development or function.[2,3] Loss of function mutations in STAT3 is associated with immunodeficiency and reduced Th17 cell response and function.[7,8] In mice, deletion of Th17 cells by genetic means, or blockade of Th17 cells through biochemical means resulted in resistance to development of experimental autoimmune encephalomyelitis (EAE), a mouse model of MS.[9,10] In this regard, Rorγt deficient mice[9], Batf[11], Ahr[12], similarly, Il6-/-mice[13–15], Il23r-/-mice[16], Il23p19[17] or il23p40 deficient mice all have become resistant fully or partly to EAE development. Although a critical role for Th17 cells in EAE pathogenesis have been established by targeting genes required for Th17 cell development or function, the identities of Th17 cell-derived cytokines responsible for the pathogenesis remained somewhat controversial.[5] In fact, IL-17A-/-and IL-17F-/-mice still developed EAE. Similarly, neutralization of IL-17A with antibodies did not confer resistance to EAE. Likewise, IL-22-/-mice also develop EAE.[18] More recently, GM-CSF was reported to be essential for EAE pathogenesis[19–22], however, this was challenged by more recent studies suggesting a more supportive role for GM-CSF.[23] Nevertheless, Th17 cells and associated cytokines have been shown to be elevated in the peripheral blood of MS patients, and post-mortem analyzed CNS tissues.[5,24–27]

Fingolimod is the first orally administered agent approved for the treatment of MS.[28] Fingolimod is derived from myriocin, produced naturally by the fungus *Isaria sinclairii*. It is an analog of sphingosine 1 phosphate (S1P). S1P is endogenously produced by endothelial cells and is found at high concentrations in blood and lymph, and at low levels in the tissues.[29] Immune cells express five receptors for S1P, S1PR1–5.[29] Through S1PR1, T cells can migrate from secondary lymphoid and non-lymphoid tissues into lymph and blood.[29,30] S1P-S1PR1 binding triggers internalization of the receptor, thus cells are unable to sense the ligand when exposed to high levels of S1P. Similarly, fingolimod, after being phosphorylated *in vivo*, causes S1PR1 internalization, therefore when cells enter the lymph nodes they are trapped within and their egress is blocked as high fingolimod levels are maintained in the tissues through its intake by the patient.

In this study we aimed to quantify Th17 cell numbers and IL-17A, IL-22 and GM-CSF cytokine levels in the peripheral blood and serum of relapsing remitting MS (RRMS) patients treated in Neurology Clinic at Erciyes University School of Medicine, Kayseri, Turkey. Our results show that consistent with the previous reports in animal models and humans, fingolimod reduced IL-17A$^+$, IL-22$^+$ and GM-CSF$^+$ T cells in the RRMS patients' blood.

Methods

Patient information

After the informed consent was obtained from donors peripheral blood samples were drawn from patients at Gevher Nesibe Hospital, Erciyes University School of Medicine, Department of Neurology. The diagnosis of the patients was conducted based on the Revised McDonald Diagnostic Criteria for MS (Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria).[31] All subjects in the RRMS patient/no treatment group were recently diagnosed and off immunomodulatory or immunosuppressive drugs at the time of study for about 3 months before the blood was drawn. Fingolimod-receiving RRMS patients were under Fingolimod treatment for at least 3 months and were in remission not in relapse. Control subjects are free-of known autoimmune condition or a family history of autoimmune disease. The research protocols were approved by the Ethics Committee at Erciyes University (2015/346). All methods for human studies involving human samples were performed in accordance with the relevant guidelines and regulations.

Intracellular cytokine staining

Peripheral blood mononuclear cells (PBMCs) were isolated from 5 to 10 ml blood via Ficoll Paque (GE Healthcare) according to manufacturer's protocols. The cells were counted under hemocytometer using Trypan Blue exclusion before freezing and were stored in liquid nitrogen in 10% DMSO containing Fetal Bovine Serum. After thawing and counting PBMCs were resuspended in complete RPMI-1640 medium (containing 10% FBS and essential and non-essential amino acids, and Anti-Anti (Antibiotic-Antimycotic, Gibco)). PBMCs were plated at 10^6 cells/well density into 96-well round bottom plates and stimulated with Phorbol-Myristate-Acetate (PMA) / Ionomycin / Golgi Plug (50 ng/mL / 1 ug/mL / 1 ul/mL) for 4 hours at 37°C. The cells were surface stained

with anti-human FITC-TCRαβ (BioLegend) for 30 min following Fc block with Human TruStain FcX™. Cells were washed twice at 400 g, 5 min with PBS containing 2% FBS (Staining Buffer). The cells were fixed and permeabilized with BD Cytofix/Cytoperm™ Plus kit. Anti-human PE-GM-CSF, APC-IL-17A, PercpCy5.5-IL-22 (all from BioLegend) were added for 30 min. Cells were washed and run on a FACSAriaIII (BD Biosciences). Data analysis was performed using FlowJo and FACSDiva software (BD Biosciences).

ELISA

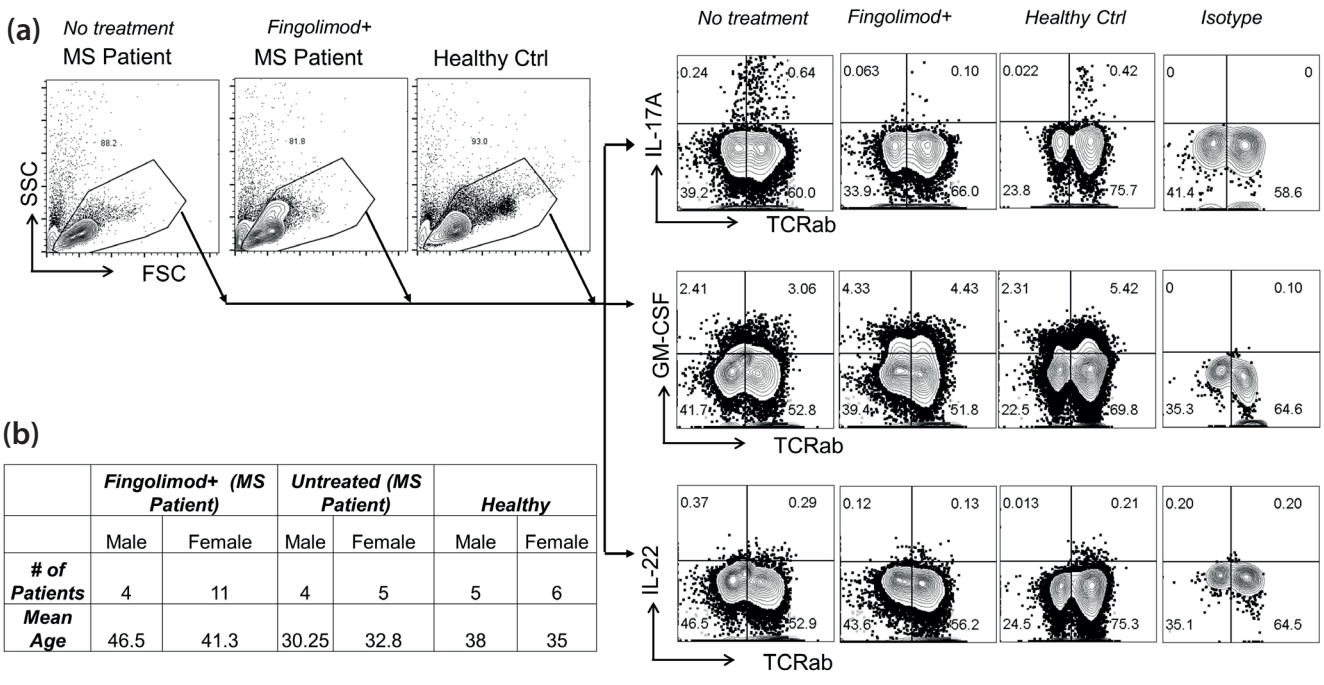
Biolegend Human IL-17A ELISA MAX, Human IL-22 ELISA MAX and Human GM-CSF ELISA MAX kits were used to run ELISA from sera collected from patient blood. Samples were diluted twice before use. The assays were performed based on the manufacturer's specified protocols.

Statistics

Th17-associated cytokines and cells in MS patient'peripheral blood, PBMCs were isolated and serum samples and the cells were frozen until sample collection was ended. The PBMCS were then thawed before use and stimulated with PMA/ Ionomycin/Golgi Plug for 4 hours. FITC TCRαβ, APCIL- 17A, PercpCy5.5-IL-22 and PE-GM-CSF antibodies were used to stain intracellular cytokines GraphPad Prism 6 program and ANOVA and Student's t test was used to calculate the statistics. The tests defined as statistically significant at p value <0.05.

Results

The gating strategy to define IL-17, IL-22 or GM-CSF producing T cells and a representative flow graph for each group is shown in Figure 1A. The patients' demographics (number of patients, age, gender, and mean age) were given in Figure 1B. To test whether fingolimod treatment reduced Th17-associated cytokines and cells in MS patient'peripheral blood. We detected significantly reduced absolute number of lymphocytes in the blood of RRMS patients treated with fingolimod compared to those not treated (Figure 2). Similarly, the absolute number of IL-17A$^+$TCRαβ+ as well as GM-CSF$^+$TCRαβ$^+$ cells were significantly reduced (Figure 2). IL-22$^+$TCRαβ$^+$ cell number was also reduced (p=0.05), however, possibly due to small sample size used for this study the significance was border line. Reduced percentage of IL-17A$^+$TCRαβ$^+$cells among lymphocytes was also noted in Fingolimod-receiving RRMS patients



(b)

	Fingolimod+ (MS Patient)		Untreated (MS Patient)		Healthy	
	Male	Female	Male	Female	Male	Female
# of Patients	4	11	4	5	5	6
Mean Age	46.5	41.3	30.25	32.8	38	35

Figure 1. Gating strategy and Patient Information. Representative flow plots are shown. Lymphocytes were gated and charted as cytokine versus TCRαβ$^+$ plots (a). Patient information summary table (b).

Turk J Immunol 2019; 7(2):69–75

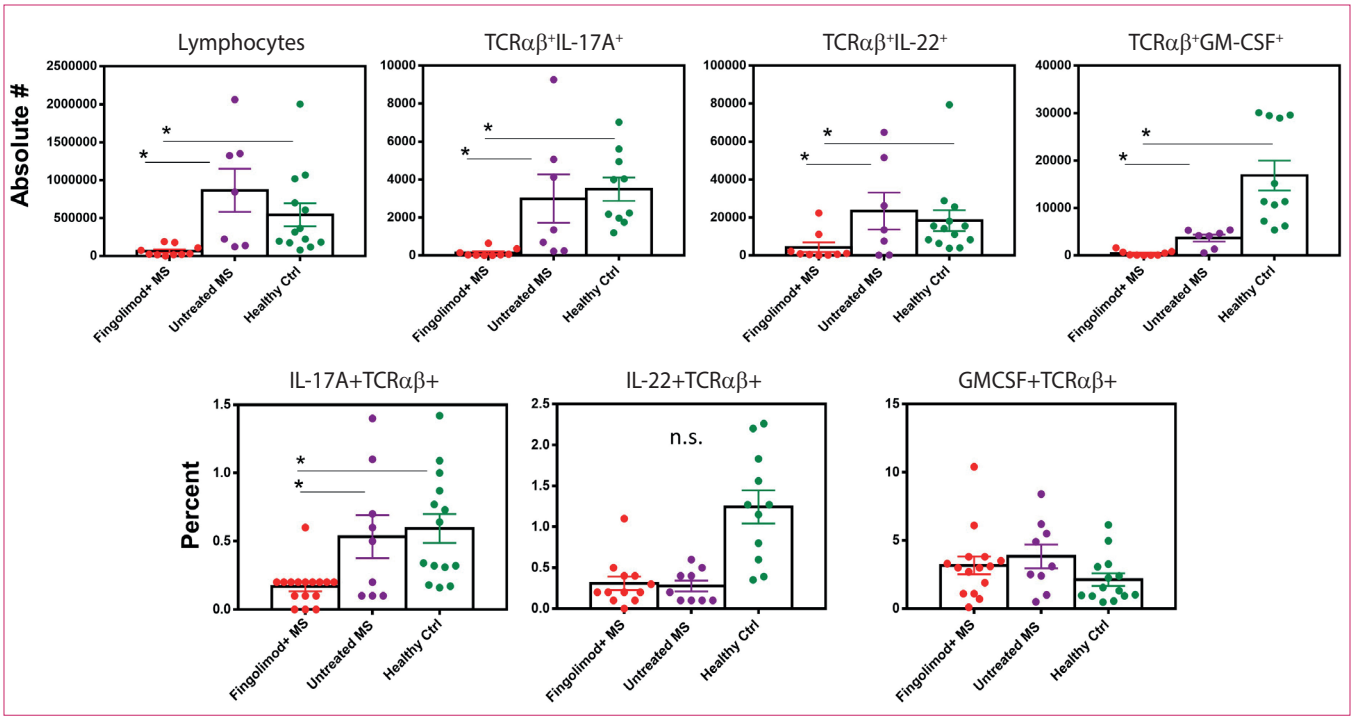


Figure 2. Absolute numbers of IL-17A+, IL-22+ and GM-CSF+T cells in the peripheral blood of fingolimod-receiving (n=9) and untreated RRMS patients (n=7) *(top)*. Percent IL-17A+, IL-22+ and GM-CSF+T cells in the peripheral blood of fingolimod-receiving (n=16) and untreated RRMS patients (n=9) *(bottom)*. PBMCSs were stimulated with PMA/Ionomycin/Golgi Plug for 4 h before staining (*, p<0.05; n.s, not significant).

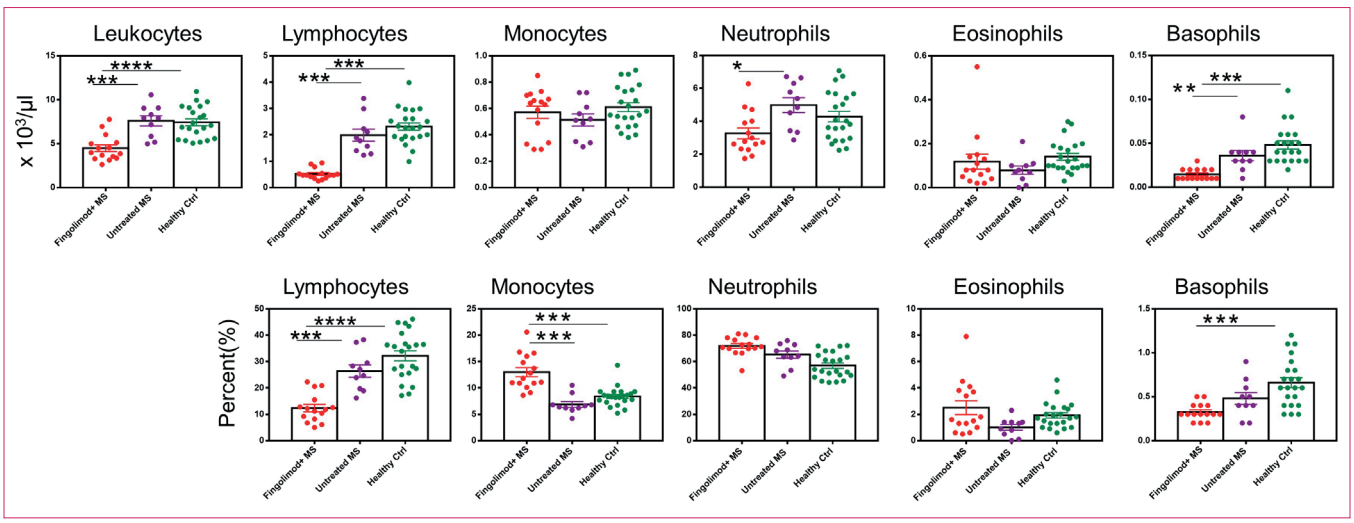


Figure 3. Leukocyte subset information (absolute and percent) from complete blood count reports of fingolimod-receiving (n=15), untreated RRMS patients (n=10) and healthy controls (n=21) (*, p<0.05**; **,** p<0.01; ***, p<0.001; ****, p<0.0001; n.s, not significant.

compared with those not treated (Figure 2). Percentages of IL-22+TCRαβ+ or GM-CSF+TCRαβ+ cells were comparable between Fingolimod-receiving and untreated RRMS groups (Figure 2). The reduction in absolute number of lymphocytes was also evident in complete blood count of RRMS patients treated with fingolimod compared to those untreated or healthy controls (Figure 3). Additionally, absolute numbers of neutrophils and basophils in the peripheral blood were significantly reduced after fingolimod use whereas monocytes and eosinophils remained unchanged (Figure 3). We also wanted to test serum cytokine levels. To this end we performed ELISA for IL-17A, IL-22, and GM-CSF using serum samples from healthy controls, Fingolimod-treated or untreated RRMS patients (Figure 4). Despite the reduction observed in the absolute number of Th17 cells with intracellular cytokine

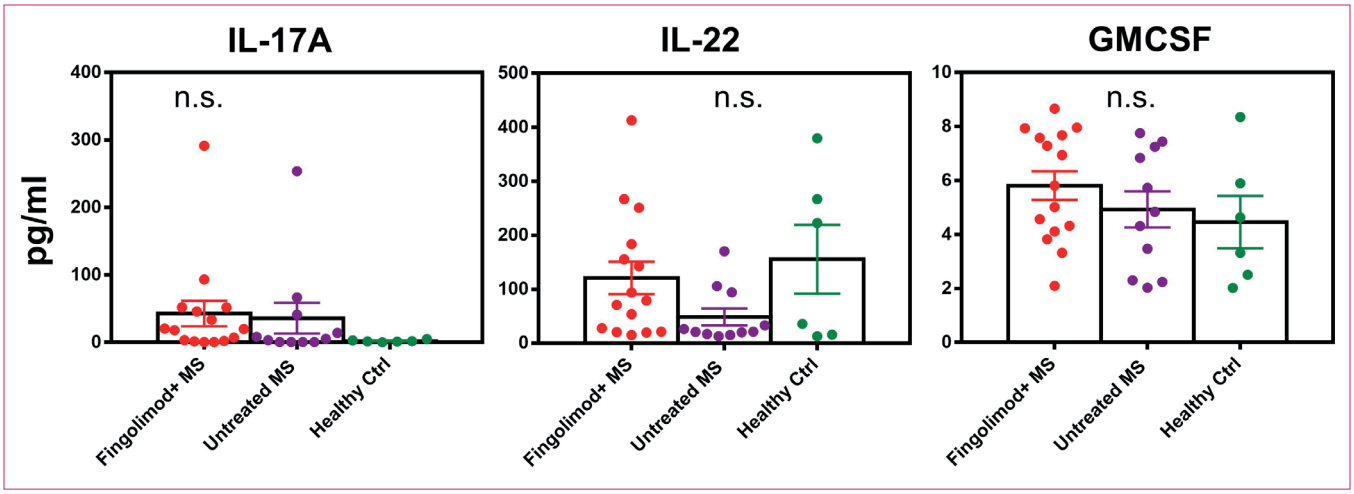


Figure 4. Serum IL-17A, IL-22 and GM-CSF levels in the peripheral blood of fingolimod-receiving (n=16) and untreated RRMS patients (n=9) (n.s, not significant).

staining, none of the cytokines were significantly different across the groups in the serum in our hands.

Discussion

MS is a neuroinflammatory and neurodegenerative disease. Immune-mediated pathology is partly mediated by Th17 cells. In humans, Th17 cells are increased in MS patients' blood as well as inflamed plaques in the CNS.[5,24,26,27,32] Fingolimod blocks T and B cells egress from secondary lymphoid organs and is used in MS treatment. Fingolimod also has immunomodulatory properties particularly on Th17 and Treg cells.[33–36]

In this study we compared Th17 cell number, and Th17-associated cytokines in the peripheral blood of untreated RRMS patients to those treated with fingolimod via flow cytometry-based assays. Previous studies have shown a reduction in IL-17 producing cells in the peripheral blood after Fingolimod treatment.[37,38] Our results from the current study are in line with those reports. Lymphocyte absolute numbers in our patients under Fingolimod treatment show reduction in the peripheral blood as evident in both CBC and flow cytometric assessment. Differences in CBC and flow cytometric counts could be attributed to loss of cells during freezing thawing for flow cytometric tests. Importantly CBC results also point to a significant reduction in neutrophils and basophil counts in patients treated with fingolimod. Eighty (80)% of the patients treated with fingolimod in this study were lymphopenic (lymphocyte counts were between 200 and 800/μl), however, none of the patients had less than 200/μL lymphocyte counts. These numbers are in line with the previously published values.[39–42] Up to 60% of patients have been reported to have lymphocyte counts below 600/μL.[40] Additionally, both absolute number and percentage of IL-17A$^+$ T cells were reduced in the peripheral blood after fingolimod treatment. Moreover, IL-22$^+$ as well as GM-CSF$^+$ T cell absolute numbers show a reduction in the peripheral blood. Percentages of these cells however did not show a significant difference (p=0.05). Although the absolute number of total lymphocytes, or IL-17A$^+$cells were assessed and explicitly reported in the previous literature, IL-22$^+$ T cells and GM-CSF+ T cell absolute numbers have not. Our study revealed that these cells also reduced in number. The reduction in the absolute number of IL-17$^+$ T cells in the peripheral blood might be due to sequestration of cells in the lymph nodes or due to immunomodulatory effects of fingolimod.[36,37,43,44] Reduction of central memory Th17 cells in the peripheral blood after fingolimod treatment have been reported previously although others have shown a subset of patients that had increased Th17 cells.[37,43] Fingolimod may also reduce polarization to Th17 by reducing Th17-polarizing cytokine production by dendritic cells[36] or suppressing IL-17 production by CD4$^+$ T cells.[44]

Lastly, we also compared the serum levels of IL-17A, IL-22, and GM-CSF between the fingolimod-receiving and untreated RRMS patient groups. Interestingly we did not observe a difference between healthy controls, fingolimod treated and untreated RRMS group. Previous studies showed an increase in MS patients' serum of cytokines IL-17A and IL-22.[24,26] Our sample size for each group

was modest, thus this is a limitation of our study. The lack of a significant difference in cytokine levels in the sera between RRMS patients and the healthy controls could be attributed to the small sample size.

In conclusion, our data show that fingolimod treatment decreases the number of circulating IL-17$^+$ T cells, IL-22$^+$ T cells and GM-CSF$^+$ T cells in RRMS patients' peripheral blood.

Ethics Committee Approval: The approval was obtained from Erciyes University's Clinical Research Ethics Committee (file no: 2015/346, date: 30.07.2015).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions: *Concept:* AE, MFY, HDA, MM, HC; *Design:* AE, MFY, HDA, MM, HC; *Data Collection or Processing:* FZO, SE, MC, ZBA, YH, MFY, AE; *Analysis or Interpretation:* FZO, MFY, AE; *Literature Search:* AE, MM, MFY, HC; *Writing:* AE; *Critical Review:* AE, FZO, SE, MC, ZBA, YH, MFY, MM, HAD, HC.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was supported by Erciyes University BAP grant TOA-2016-6130 to AE.

References

1. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol 2009;27:485–517. [CrossRef]
2. Zúñiga LA, Jain R, Haines C, Cua DJ. Th17 cell development: from the cradle to the grave. Immunol Rev 2013;252:78–88. [CrossRef]
3. Gaffen SL, Jain R, Garg AV, Cua DJ. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. Nat Rev Immunol 2014;14:585–600. [CrossRef]
4. Patel DD, Kuchroo VK. Th17 Cell Pathway in Human Immunity: Lessons from Genetics and Therapeutic Interventions. Immunity 2015;43:1040–51. [CrossRef]
5. Wagner CA, Goverman JM. Novel Insights and Therapeutics in Multiple Sclerosis. F1000Research 2015;4:517. [CrossRef]
6. Crome SQ, Wang AY, Levings MK. Translational mini-review series on Th17 cells: function and regulation of human T helper 17 cells in health and disease. Clin Exp Immunol 2010;159:109–19. [CrossRef]
7. Wilson RP, Ives ML, Rao G, Lau A, Payne K, Kobayashi M, et al. STAT3 is a critical cell-intrinsic regulator of human unconventional T cell numbers and function. J Exp Med 2015;212:855–64. [CrossRef]
8. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 Mutations in the Hyper-IgE Syndrome. N Engl J Med 2007;357:1608–19. [CrossRef]
9. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The Orphan Nuclear Receptor RORγt Directs the Differentiation Program of Proinflammatory IL-17+ T Helper Cells. Cell 2006;126:1121–33. [CrossRef]
10. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, et al. IL-6 programs T (H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol 2007;8:967–74. [CrossRef]
11. Schraml BU, Hildner K, Ise W, Lee WL, Smith WAE, Solomon B, et al. The AP-1 transcription factor Batf controls T (H)17 differentiation. Nature 2009;460:405–9. [CrossRef]
12. Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 2008;453:106–9. [CrossRef]
13. Samoilova EB, Horton JL, Hilliard B, Liu TS, Chen Y. IL-6-deficient mice are resistant to experimental autoimmune encephalomyelitis: roles of IL-6 in the activation and differentiation of autoreactive T cells. J Immunol 1998;161:6480–6.
14. Serada S, Fujimoto M, Mihara M, Koike N, Ohsugi Y, Nomura S, et al. IL-6 blockade inhibits the induction of myelin antigen-specific Th17 cells and Th1 cells in experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 2008;105:9041–6. [CrossRef]
15. Korn T, Mitsdoerffer M, Croxford AL, Awasthi A, Dardalhon VA, Galileos G, et al. IL-6 controls Th17 immunity in vivo by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells. Proc Natl Acad Sci 2008;105:18460–5. [CrossRef]
16. Awasthi A, Riol-Blanco L, Jäger A, Korn T, Pot C, Galileos G, et al. Cutting edge: IL-23 receptor GFP reporter mice reveal distinct populations of IL-17-producing cells. J Immunol 2009;182:5904–8. [CrossRef]
17. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 2003;421:744–8. [CrossRef]
18. Kreymborg K, Etzensperger R, Dumoutier L, Haak S, Rebollo A, Buch T, et al. IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. J Immunol 2007;179:8098–104. [CrossRef]
19. Haak S, Croxford AL, Kreymborg K, Heppner FL, Pouly S, Becher B, Waisman A. IL-17A and IL-17F do not contribute vitally to autoimmune neuro-inflammation in mice. J Clin Invest 2008;119:61–9. [CrossRef]
20. Codarri L, Gyülvészi G, Tosevski V, Hesske L, Fontana A, Magnenat L, et al. RORγt drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. Nat Immunol 2011;12:560–7. [CrossRef]
21. El-Behi M, Ciric B, Dai H, Yan Y, Cullimore M, Safavi F, et al. The encephalitogenicity of T (H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. Nat Immunol 2011;12:568–75. [CrossRef]
22. Ponomarev ED, Shriver LP, Maresz K, Pedras-Vasconcelos J, Verthelyi D, Dittel BN. GM-CSF production by autoreactive T cells is required for the activation of microglial cells and the onset of experimental autoimmune encephalomyelitis. J Immunol 2007;178:39–48. [CrossRef]
23. Pierson ER, Goverman JM. GM-CSF is not essential for experimental autoimmune encephalomyelitis but promotes brain-targeted disease. JCI Insight 2017;2:e92362. [CrossRef]
24. Bălaşa R, Bajko Z, Huţanu A. Serum levels of IL-17A in patients with relapsing-remitting multiple sclerosis treated with interferon-β. Mult Scler J 2013;19:885–90. [CrossRef]

25. Babaloo Z, Yeganeh RK, Farhoodi M, Baradaran B, Bonyadi M, Aghebati L. Increased IL-17A but Decreased IL-27 Serum Levels in Patients with Multiple Sclerosis. Iran J Immunol 2013;10:47–54. [CrossRef]
26. Perriard G, Mathias A, Enz L, Canales M, Schluep M, Gentner M, et al. Interleukin-22 is increased in multiple sclerosis patients and targets astrocytes. J Neuroinflammation 2015;12:119. [CrossRef]
27. Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, et al. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med 2002;8:500–8. [CrossRef]
28. Sharma S, Mathur AG, Pradhan S, Singh DB, Gupta S. Fingolimod (FTY720): First approved oral therapy for multiple sclerosis. J Pharmacol Pharmacother 2011;2:49–51. [CrossRef]
29. Cyster JG, Schwab SR. Sphingosine-1-Phosphate and Lymphocyte Egress from Lymphoid Organs. Annu Rev Immunol 2012;30:69–94. [CrossRef]
30. Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature 2004;427:355–60. [CrossRef]
31. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 2018;17:162–73. [CrossRef]
32. Garris CS, Wu L, Acharya S, Arac A, Blaho VA, Huang Y, et al. Defective sphingosine 1-phosphate receptor 1(S1P1) phosphorylation exacerbates TH17-mediated autoimmune neuroinflammation. Nat Immunol 2013;14:1166–72. [CrossRef]
33. Liu G, Yang K, Burns S, Shrestha S, Chi H. The S1P(1)-mTOR axis directs the reciprocal differentiation of T (H)1 and T (reg) cells. Nat Immunol 2010;11:1047–56. [CrossRef]
34. Eken A, Duhen R, Singh AK, Fry M, Buckner JH, Kita M, et al. S1P1 deletion differentially affects TH17 and Regulatory T cells. Sci Rep 2017;7:12905. [CrossRef]
35. Dominguez-Villar M, Raddassi K, Danielsen AC, Guarnaccia J, Hafler DA. Fingolimod modulates T cell phenotype and regulatory T cell plasticity in vivo. J Autoimmun 2019;96:40–9. [CrossRef]
36. Thomas K, Sehr T, Proschmann U, Rodriguez-Leal FA, Haase R, Ziemssen T. Fingolimod additionally acts as immunomodulator focused on the innate immune system beyond its prominent effects on lymphocyte recirculation. J Neuroinflammation 2017;14:41. [CrossRef]
37. Mehling M, Lindberg R, Raulf F, Kuhle J, Hess C, Kappos L, Brinkmann V. Th17 central memory T cells are reduced by FTY720 in patients with multiple sclerosis. Neurology 2010;75:403–10. [CrossRef]
38. Muls N, Dang HA, Sindic CJM, van Pesch V. Fingolimod increases CD39-expressing regulatory T cells in multiple sclerosis patients. PLoS One 2014;9:e113025. [CrossRef]
39. Paolicelli D, Manni A, D'Onghia M, Direnzo V, Iaffaldano P, Zoccolella S, et al. Lymphocyte subsets as biomarkers of therapeutic response in Fingolimod treated Relapsing Multiple Sclerosis patients. J Neuroimmunol 2017;303:75–80. [CrossRef]
40. Rojas JI, Patrucco L, Miguez J, Cristiano E. Real-World Safety and Patient Profile of Fingolimod in Relapsing-Remitting Multiple Sclerosis. Clin Neuropharmacol 2017;40:251–4. [CrossRef]
41. Francis G, Kappos L, O'Connor P, Collins W, Tang D, Mercier F, Cohen JA. Temporal profile of lymphocyte counts and relationship with infections with fingolimod therapy. Mult Scler J 2014;20:471–80. [CrossRef]
42. Johnson TA, Shames I, Keezer M, Lapierre Y, Haegert DG, Bar-Or A, Antel J. Reconstitution of circulating lymphocyte counts in FTY720-treated MS patients. Clin Immunol 2010;137:15–20. [CrossRef]
43. Sato DK, Nakashima I, Bar-Or A, Misu T, Suzuki C, Nishiyama S, et al. Changes in Th17 and regulatory T cells after fingolimod initiation to treat multiple sclerosis. J Neuroimmunol 2014;268:95–8. [CrossRef]
44. Dominguez-Villar M, Raddassi K, Danielsen AC, Guarnaccia J, Hafler DA. Fingolimod modulates T cell phenotype and regulatory T cell plasticity in vivo. J Autoimmun 2019;96:40–9. [CrossRef]