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

Turk J Immunol 2020; 8(2):50–56

Influence of İnterferon α-2a Treatment on Monocyte Subsets in Patients with Uveitis

Üveit Hastalarında Interferon α-2a Tedavisinin Monosit Alt Grupları Üzerine Etkisi

Fehim ESEN[1,2], Özlem TÜRKYILMAZ[2], Veysel AYKUT[2], Haner DİRESKENELİ[3], Günnur DENİZ[1], Halit OĞUZ[2], Esin ÇETİN AKTAŞ[1]

Abstract

Introduction: Interferon α is a type-I interferon molecule with antiviral and antineoplastic properties. Interferon α treatment is also used in the management of uveitis and believed to have immunomodulatory properties demonstrated by increased number of regulatory T cells. The aim of this study was to define the influence of interferon α-2a treatment on monocyte subsets in patients with uveitis.

Materials and Methods: Six patients with uveitis and 7 healthy control subjects were included in this study. Blood samples were obtained from the uveitis patients before treatment with interferon α-2a and at the 1st month of treatment (or at clinical remission). Peripheral blood mononuclear cells were stained with anti-CD16, anti-CD14, anti-PD-1, anti-CTLA-4, anti-LAG-3, anti-TIM-3 and anti-TIGIT antibodies and analyzed with flow cytometry. Data were analyzed with FlowJo software and statistical analysis was performed with SPSS 21.0 software.

Results: Six patients with uveitis (5 Behçet's, 1 Eales disease, mean age: 29.0±3.8 years, 4 male, 2 female) and 7 healthy control subjects (mean age: 28.4±4.9 years, 3 male, 4 female) were included. The number of $CD14^+CD16^-$ classical monocytes were increased and $CD16^+$ non-classical monocytes were decreased in patients with active uveitis compared to controls (p=0.037 and p=0.045) and this difference disappeared after treatment with interferon α-2a. There was no difference in immune checkpoint receptor expressions between groups at baseline. PD-1 expression increased significantly after interferon α-2a treatment (p=0.01).

Conclusion: Interferon α-2a treatment increases the ratio of non-classical monocytes and their expression of PD-1. These changes may be associated with the previously demonstrated immunomodulatory effects of interferon α-2a in patients with uveitis.

Keywords: Monocyte, Behçet's disease, uveitis, interferon α, PD-1

Öz

Giriş: İnterferon α antiviral ve antineoplastik etkileri olan bir tip 1 interferon molekülüdür. İnterferon α ayrıca üveit tedavisinde de kullanılmakta olup, bu tedavinin immünomodulatuvar etkisi olduğu düşünülmektedir. Interferon α-2a tedavisinin bu hastalarda regülatör T hücrelerini arttırdığı bildirilmiştir. Bu çalışmanın amacı interferon α-2a tedavisinin üveit hastalarında monosit alt grupları üzerindeki etkisini tanımlamaktır.

Gereç ve Yöntemler: Çalışmaya üveiti olan 6 hasta ile 7 sağlıklı gönüllü dahil edildi. Üveit hastalarından kan örnekleri interferon α-2a tedavisine başlanılmadan önce ve tedavinin 1. ayında (veya klinik remisyon sonrasında) alındı. Periferik kan mononükleer hücreleri anti-CD16, anti-CD14, anti-PD-1, anti-CTLA-4, anti-LAG-3, anti-TIM-3 ve anti-TIGIT antikorları ile boyandı ve akan hücre ölçer ile analiz edildi. Veriler FlowJo yazılımı ile değerlendirildi ve istatistiksel analiz için SPSS 21.0 yazılımı kullanıldı.

Bulgular: Üveiti olan 6 hasta (5 Behçet, 1 Eales hastalığı, yaş ortalaması: 29.0 ±3.8 yıl, 4 erkek, 2 kadın) ile 7 sağlıklı kontrol (ortalama yaş: 28.4 ±4.9 yıl, 3 erkek, 4 kadın) çalışmaya katıldı. Aktif üveiti olan hastalarda sağlıklı bireylere kıyasla $CD14^+CD16^-$ klasik monosit alt grubu artmış ve $CD16^+$ klasik olmayan monosit alt grubu ise azalmıştı (p=0.037 ve p=0.045), ancak bu fark interferon α-2a tedavisi ardından kayboldu. Başlangıçta gruplar arasında immün kontrol noktası reseptörü ekspresyonu açısından anlamlı fark yoktu. İnterferon α-2a tedavisi ardından PD-1 ekspresyonu anlamlı olarak artış gösterdi (p=0.01).

Sonuç: İnterferon α-2a tedavisi klasik olmayan monosit alt grubunu ve monositlerin yüzeyinde PD-1 ifadesini artırmıştır. Monositlerde tespit edilen bu değişimler interferon alfa-2a tedavisinin üveit hastalarındaki immünomodulatuvar etkisi ile ilişkili olabilir.

Anahtar Sözcükler: Monosit, Behçet hastalığı, üveit, interferon alfa, PD-1

[1]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkey,
[2]Istanbul Medeniyet University Medical Faculty, Department of Ophthalmology, Istanbul, Turkey
[3]Marmara University, School of Medicine, Department of Internal Medicine, Division of Rheumatology, Istanbul, Turkey

Correspondence:
Esin ÇETİN AKTAŞ
Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Vakif Gureba Caddesi, Sehremini 34393, Istanbul, Turkey
E-mail: esinaktas@yahoo.com

Received: May 21, 2020
Accepted: Jul 28, 2020

https://doi.org/10.25002/tji.2020.1261

Available online at
http://www.turkishimmunology.org

Introduction

Uveitis is a sight threatening intraocular inflammation, which is responsible for up to 10–15% of blindness in developed world.[1] Biologic agents such as interferon α and tumor necrosis factor-alpha (TNF-α) inhibitors are successfully used for the management of sight threatening uveitis refractory to conventional immunosuppressive treatments.[1–3]

Interferon-α is an immune-regulating signaling protein with antiviral and antineoplastic properties.[4] Interestingly, it is also successfully used for the management of sight threatening, non-infectious uveitis and believed to have immunomodulatory properties.[5] A recent study demonstrated that interferon α-2a treatment increased the number of regulatory T cells and reduced Th17 cells in patients with Behçet's uveitis.[6] Interferon α-2a treatment also reduced TLR expression in $CD4^+$ T cells and monocytes in patients with Behçet's uveitis.[6]

Two distinct population of monocytes were identified according to their distinct surface markers and functional properties; the classical monocytes ($CD14^+CD16^-$) and nonclassical monocytes ($CD14^{dim}CD16^+$).[7] Classical monocytes have a more proinflammatory phenotype, secrete proinflammatory cytokines and can differentiate to monocyte derived dendritic cells.[8] Therefore, classical (proinflammatory) monocytes have also the ability to influence adaptive immune responses by their ability of antigen presentation and have an important role in shaping the inflammation and its resolution in tissues. On the other hand, non-classical (anti-inflammatory, pro-repair) monocytes are involved in complement and FcR-mediated phagocytosis, transendothelial migration, antiviral immune responses and wound healing.[9,10] Investigations on monocyte developmental pathways demonstrated classical monocytes were produced from the bone marrow first, whereas non-classical monocytes are derived from the pool of classical monocytes.[11]

Immune checkpoint receptors are initially defined as regulators of T cells responses and later their presence have been described in other lymphocyte groups such as B cells and natural killer (NK) cells.[12,13] PD-1 expression in monocytes is associated with IL-10 secretion and known to contribute to HIV-specific T cell exhaustion.[14] Similarly, expression of TIM-3 in monocytes is also associated with production of IL-10 and suppression of interferon-gamma secreting T cells.[15]

With this background, we had the hypothesis that some of the immunomodulatory effects of interferon α-2a treatment leading to increases in regulatory T cell differentiation, may be associated with the changes in antigen presenting cells (APC). Monocytes represent the major group of APCs in peripheral blood and can potentially interact with T cells to influence their immune responses. The aim of this study was to understand whether interferon α treatment would induce a change in the distribution of monocyte subsets and their expression of immune checkpoint receptors.

Materials and Methods

Study Population

We included six patients with posterior or panuveitis diagnosed at Istanbul Medeniyet University Goztepe Education and Research Hospital, Department of Ophthalmology, that were refractory to corticosteroids and/or conventional immunosuppressive treatments and switched to interferon α-2a (Roferon-A, Roche Pharmaceuticals, Nutley, NJ, USA), and 7 healthy, age-matched blood donors as healthy control subjects. None of the patients had any systemic disease other than the one causing uveitis or did not have any other comorbidity such as malignancy, diabetes mellitus, coexistent autoimmune disease and allergies. The study protocol was approved by the institutional ethics review board. The study was conducted according to the tenets of declaration of Helsinki and all patients gave a written informed consent.

All patients had a detailed ophthalmological examination including determination of best corrected visual acuity, slit lamp microscopy, indirect ophthalmoscopy and optical coherence tomography at all visits. Fundus fluorescein angiography was performed when needed. Patients with an acute uveitis attack under conventional immunosuppressive medications were switched to interferon α-2a treatment. Patients were treated with the previously described low-dose and dose-escalating regimen.[2] During the acute attack all patients received a subcutaneous injection of interferon α-2a 3 MIU (million international units) per day and the dose was reduced to 3 MIU every other day after the uveitis attack was controlled (usually the second week of treatment). Other immunosuppressive medications were discontinued after the initiation of interferon α-2a treatment. Clinical remission was defined as absence of macular edema, less than 1 (+) vitreous haze, absence of inflammatory cells in the anterior chamber and posterior segment findings of active uveitis.[16]

Collection of Blood Samples

Peripheral venous blood samples (10 ml) were collected in heparin coated blood collection tubes (BD Bioscience, San Jose, CA, USA) immediately before starting the interferon

α-2a treatment and at the 1st month of treatment if clinical remission was obtained or after clinical remission was observed. Only a single blood sample was collected from the healthy control subjects.

Flow Cytometric Characterization of Monocytes

Peripheral blood (10 ml) was collected in heparinized tubes. Cell surface staining was processed following whole blood lysis. Freshly drawn peripheral whole blood samples of 100 µL were stained with fluorochrome labeled monoclonal antibodies: anti-human CD223 (LAG-3)-FITC, anti-human CD366 (TIM-3)-PerCP/Cy5.5, anti-human CD279 (PD-1)-APC/Cy7, anti-human CD16-Alexa Flour700, anti-human TIGIT-PE, anti-human CD3-Pacific blue, anti-human CD152 (CTLA-4)-APC, anti-human CD14-PE/Dazzle (All from Biolegend, San Diego, CA) and incubated for 30 minutes at room temperature in dark. Autofluorescent tubes were used as isotypic control for the analysis. Following staining, erythrocytes were lysed with FACS lysing solution (BD Biosciences, San Jose, CA). Cells were span with PBS solution at 2000 rpm for 5 minutes. Cells were re-suspended in 500 µL of PBS with 1% paraformaldehyde and analyzed on FACSAriaII (BD Biosciences, San Jose, CA) running FACSDiva software. Data analysis was performed with FlowJoTM10.2 software (Tree Star Inc., USA).

Statistical Analysis

Statistical comparisons were performed with SPSS 21.0 software (IBM, Chicago, IL, USA). Intergroup comparisons were performed with Mann-Whitney test and repeated samples were compared with Wilcoxon test. GraphPad Prism 7.0 software (GraphPad Software Inc., La Jolla, USA) was used for the figures.

Results

Demographic and Clinical Characteristics of the Patients

Six patients with posterior or panuveitis and 7 age-matched healthy control subjects were included in the study. Five of the patients had Behçet's disease and one had Eales disease. All patients responded well to interferon α-2a treatment and clinical remission was achieved before taking the second blood sample. The baseline clinical characteristics of the study participants were summarized in Table 1.

Table 1. Clinical characteristics of the patients and controls

	Patients (n=6)	Healthy controls (n=7)
Gender (M:F)	4:2	3:4
Age, years (Mean ± SD)	29.0 ± 3.8	28.4 ± 4.9
Diagnosis		
- Behçet's disease, n(%)	5 (83.3)	0 (0)
- Eales disease, n(%)	1 (16.7)	0 (0)
Medications used at study recruitment		
- Azathioprine, n(%)	5 (83.3)	0 (0)
- Cyclosporine-A, n(%)	5 (83.3)	0 (0)
- Low dose steroids*, n(%)	6 (100)	0 (0)

M, male; F, female; SD, standard deviation.
*equivalent to 10 mg prednisolone or less.

Distribution of Monocyte Subsets

Phenotypic analysis of the monocytes was performed for the characterization of $CD14^{+}CD16^{-}$ classical and $CD16^{+}$ non-classical monocytes. Non-classical monocytes were diminished in the monocyte pool during the acute attack of the uveitis and were significantly less compared to healthy control subjects (6.8±7.7 vs. 19.8±5.2, p=0.045) (Figure 1 d and 1f). The number of the non-classical monocytes increased and the difference between the healthy subjects disappeared after interferon α-2a treatment (16.5±5.3 vs. 19.8±5.2, p=0.63) (Figure 1d and 1f). Consistent with these findings, the ratio of the classical monocytes were increased in the monocyte pool compared to healthy controls (93.0±7.8 vs. 76.6±6.0, p=0.037) (Figure 1c and 1e) and this difference also disappeared after interferon α-2a treatment (83.3±5.3 vs. 76.6±6.0, p=0.42) (Figure 1c and 1e). These changes in the monocyte subset were given in detail at Figure 1.

Immune Checkpoint Receptor Expressions in Monocytes

Immune checkpoint receptor status was analyzed with flow cytometry in the monocyte population. There was no statistically significant difference between the uveitis patients and healthy control subjects before interferon α-2a treatment (Figure 2a–2e). However, PD-1 expression significantly increased after interferon α-2a treatment in uveitis patients, both compared to healthy controls (21.2±8.5 vs. 0.52±0.18, p=0.010) and the levels during the uveitis attack (21.2±8.5 vs. 7.4±6.7, p=0.028) (Figure 2a). There was also an insignificant trend towards an increase in CTLA-4 and LAG-3 expressions (Figure 2c

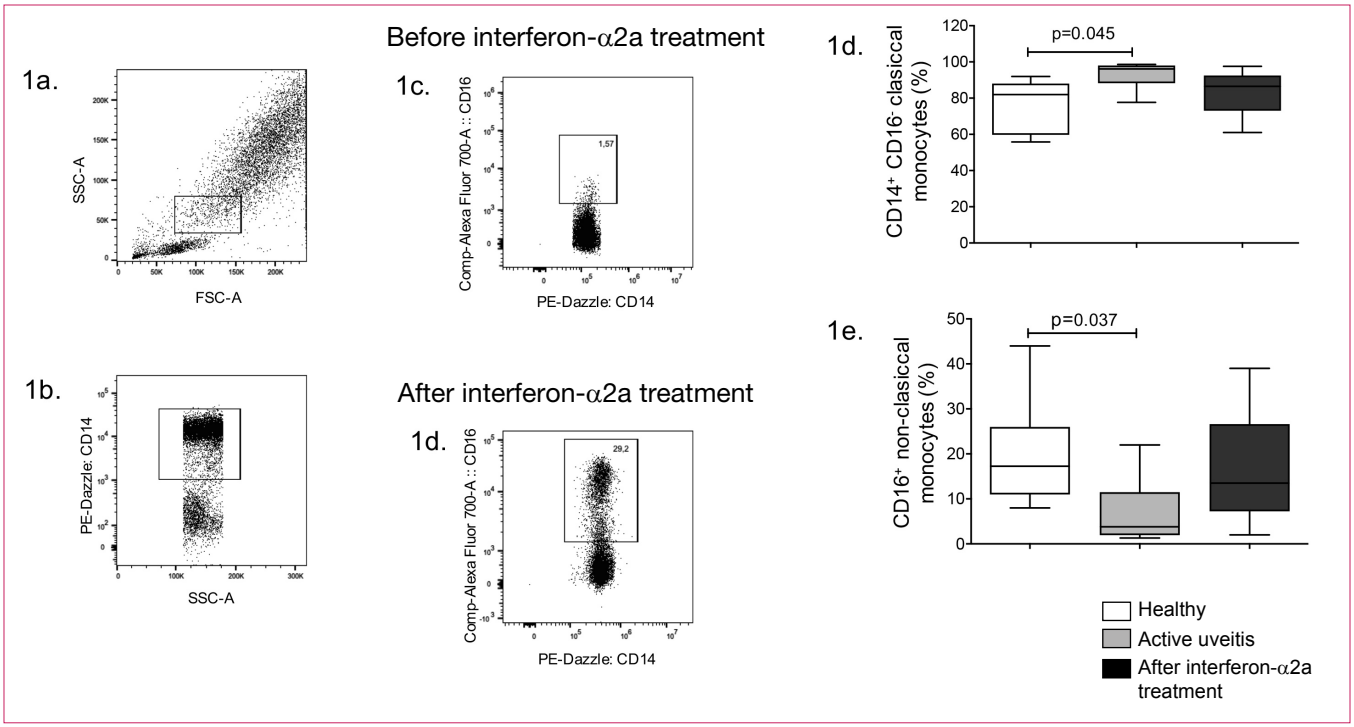

Figure 1 a–f. The distribution of monocyte subsets in patients and controls subjects. Whole blood samples were stained with anti human – CD14-PE-Dazzle and anti-human-Alexa Flour 700 mAbs and analyzed using flow cytometry. Gating of monocytes was based on the side scatter **(a)** and CD14 dot plots **(b)**. Representative dot-plot analyses were given for the characterization of $CD14^{+}CD16^{-}$ classical and $CD14^{+}CD16^{+}$ non-classical monocytes in a patient with uveitis before **(c)** and after interferon α-2a treatment **(d)**. The numbers indicate the proportion of CD16 expressing monocytes. Bar graphs represent the median (and interquartile range) proportions of classical **(e)** and non-classical monocytes in the groups **(f)**.

and 2e). The details of the immune checkpoint receptor expression in the monocyte population were given in Figure 2.

Immune Checkpoint Receptor Expressions in Monocyte Subsets

Immune checkpoint receptor distribution was analyzed in the monocyte subsets to better understand changes in these

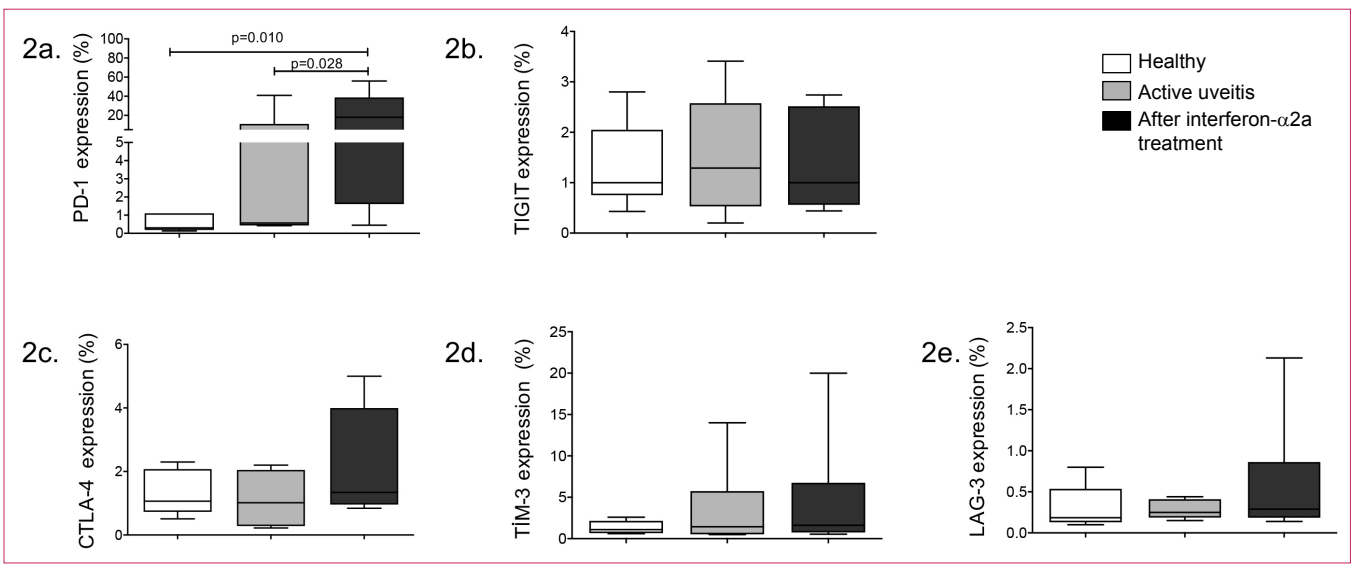

Figure 2. a–e. Immune checkpoint receptor expressions in the monocytes of the patients and control subjects. Immune checkpoint receptor expressions of CD14 expressing monocytes were shown in healthy controls, patients with active uveitis and patients treated with interferon α-2a **(a–e)**. Bar graphs represent median and interquartile range.

Turk J Immunol 2020; 8(2):50–56

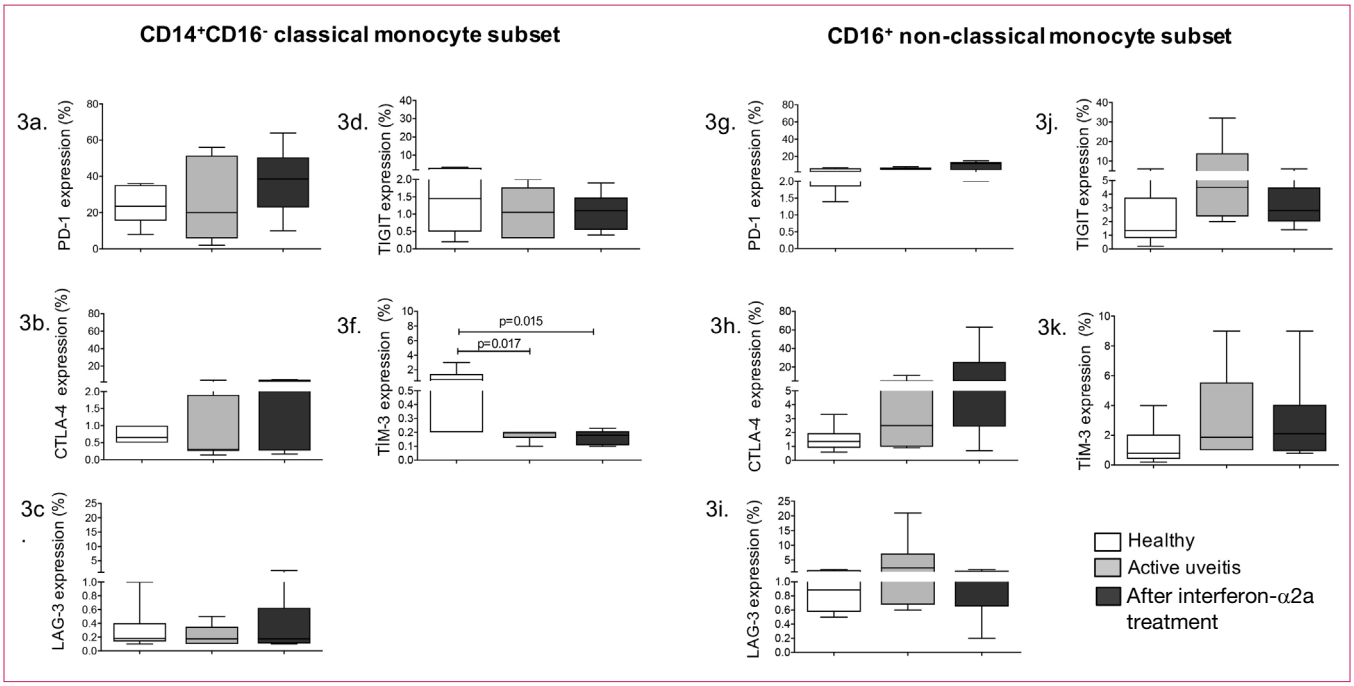

Figure 3. a–k. PD-1, TIM-3, CTLA-4, TIGIT and LAG-3 expressions in classical and non-classical monocyte subsets. Immune checkpoint receptor expressions on $CD14^+CD16^-$ classical **(a–e)** and $CD14^+CD16^+$ non-classical **(f–k)** monocyte subsets were given. Bar graphs represent median and interquartile range.

individual groups. In the non-classical monocytes, there was an insignificant trend towards an increased expression of PD-1 and CTLA-4 (Figure 3g and 3h), while there was a trend towards decline in LAG-3 and TIGIT expressions after interferon α-2a treatment (Figure 3i and 3j). In the classical monocytes, TIM-3 expression was significantly reduced in patients with active uveitis (p=0.026) and this difference remained statistically significant after treatment (p=0.015) (Figure 3f). There was also an insignificant trend towards an increase in CTLA-4 and LAG-3 expression in the classical monocyte subset after interferon α-2a treatment (Figure 3b and 3 c). The details of the changes in the immune checkpoint receptors in monocyte subsets were given in Figure 3.

Discussion

Many of the T cell responses are shaped by antigen presentation and costimulation from innate cells as well as cytokines coming from these cells. The changes in costimulatory roles of the antigen presenting cells can either induce effector T cells responses or skew T cells to a regulatory phenotype.[17] T cells are known as the master regulators of the immune system and the role of the APCs are rather neglected in many inflammatory diseases.

Behçet's disease is the most common cause of uveitis in Turkey and accounts around 32% of the uveitis cases in tertiary care.[18] Behçet's disease is a systemic vasculitis with autoimmune and autoinflammatory characteristics.[19] Monocytes from Behçet's uveitis patients have increased secretion of proinflammatory cytokine IL-1β in response to LPS stimulation.[20] Epigenome-wide study of patients with Behçet's disease also revealed changes in the cytoskeletal remodeling genes in monocytes.[21] Interferon regulatory factor 8 is associated with the regulatory function of the dendritic cells and suppression of Th1/Th17 responses. Aberrant methylation of interferon regulatory factor 8 (IRF8) was demonstrated in monocyte derived dendritic cells of uveitis patients with Behçet's disease and Vogt-Koyanagi-Harada disease.[22,23] Monocytes from patients with Behçet's disease also have an increased activation of JAK/STAT pathway, indicating a proinflammatory genetic signature.[24] A recent GWAS demonstrated that Behçet's disease associated SNPs in CCR1 and IL-10 loci contributed to a M1 macrophage-predominant inflammation in Behçet's disease.[25] Another recent study in patients with Behçet's uveitis also demonstrated that interferon α-2a treatment reduced the TLR expression in T cells and monocytes, and increased the number of regulatory T cells.[6]

The non-classical monocyte subset, a monocyte group that is known by their anti-inflammatory and tissue repair inducing properties, was reduced in number among active uveitis patients compared to healthy control subjects. Interferon α-2a treatment was able to restore the population of this anti-inflammatory monocytes and reduce the proportion of the proinflammatory classical macrophages in the peripheral blood of uveitis patients. Classical monocytes secrete high amounts of TNF-α, a proinflammatory cytokine and blockade of TNF-α is also proven to be an effective treatment for non-infectious uveitis.[26]

Immune checkpoint receptors have important roles in the regulation of many cells of the immune system including $CD4^+$ and $CD8^+$ T cells, B cells, NK cell and macrophages. Expression of PD-1 and TIM-3 are associated with the secretion of IL-10 in macrophages, a cytokine which is also associated with the effective functions of the regulatory T cells.[14,15] These PD-1 and TIM-3 expressing macrophages have an immunoregulatory phenotype and inhibit Th1 mediated inflammation.[15] In this study, interferon α-2a treatment induced an increase in PD-1 expression among patients with uveitis, which may potentially skew the monocyte population of these patients to a regulatory phenotype. There was also an insignificant trend towards increased expression of CTLA-4 in monocytes after interferon α-2a treatment, which might affect T cell co-stimulation and contribute to the previously-demonstrated increase in regulatory T cell population after this treatment.

This study has some shortcomings despite demonstrating significant changes in macrophage phenotype and immune checkpoint receptor expressions. The main limitations of this study include the relatively small sample size and lack of functional tests to demonstrate changes in macrophage functions. We were also unable to perform gene expression studies due to some technical problems during sample collection. We believe that future studies on the interaction of monocyte/macrophages and T cells would contribute to the better understanding of the immunomodulatory effects of interferon α-2a.

In conclusion, this study demonstrated for the first time that interferon α-2a treatment expanded the non-classical monocytes in the peripheral blood of patients with uveitis and also increased the expression of PD-1 in monocytes of these patients. We believe that these changes in the monocyte populations are associated with the immunomodulatory effects of interferon α-2a treatment.

Ethics Committee Approval: Istanbul Medical Faculty Ethics Board on Clinical Research (Date: 09.12.2019, Document No: 2019/1459)

Conflict of interest: The authors declared that there were no conflicts of interest.

Contribution of authors: Concept: FE, OT, VA, GD, HD, HO, EAC; Design: FE, VA, GD, HD, HO, EAC; Data Collection or Processing: FE, OT, EAC; Analysis or Interpretation: FE, VA, EAC; Literature Search: FE, VA, EAC; Writing: FE, OT, VA; Critical Review: EAC, HO, GD, HD.

Acknowledgements: This work was supported by the Research Fund of Istanbul Medeniyet University Project Number: 2019-1466.

References

1. Jaffe GJ, Dick AD, Brézin AP, Nguyen QD, Thorne JE, Kestelyn P, et al. Adalimumab in patients with active noninfectious uveitis. N Engl J Med 2016;375:932–43. [Crossref]
2. Onal S, Kazokoglu H, Koç A, Akman M, Bavbek T, Direskeneli H, Yavuz S. Long-term efficacy and safety of low-dose and dose-escalating interferon alfa-2a therapy in refractory Behcet uveitis. Arch Ophthalmol 2011;129:288–94. [Crossref]
3. Oray M, Onal S, Uludag G, Akbay AK, Tugal-Tutkun I. Interferon Alpha for the Treatment of Cystoid Macular Edema Associated with Presumed Ocular Tuberculosis. J Ocul Pharmacol Ther 2017;33:304–12. [Crossref]
4. Vidal P. Interferon alpha in cancer immunoediting: From elimination to escape. Scand J Immunol 2020;91:e12863. [Crossref]
5. Deuter C, Stübiger N, Zierhut M. Interferon-alpha therapy in noninfectious uveitis. Dev Ophthalmol 2012;51:90–7. [Crossref]
6. Albayrak O, Oray M, Can F, Kirimli GU, Gul A, Tugal-Tutkun I, Onal S. Effect of Interferon alfa-2a Treatment on Adaptive and Innate Immune Systems in Patients with Behcet Disease Uveitis. Invest Ophthalmol Vis Sci 2019;60:52–63. [Crossref]
7. Kapellos TS, Bonaguro L, Gemünd I, Reusch N, Saglam A, Hinkley ER, Schultze JL. Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. Front Immunol 2019;10:2035. [Crossref]
8. Menezes S, Melandri D, Anselmi G, Perchet T, Loschko J, Dubrot J, et al. The Heterogeneity of Ly6C (hi) Monocytes Controls Their Differentiation into iNOS(+) Macrophages or Monocyte-Derived Dendritic Cells. Immunity 2016;45:1205–18. [Crossref]
9. Gren ST, Rasmussen TB, Janciauskiene S, Hakansson K, Gerwien JG, Grip O. A Single-Cell Gene-Expression Profile Reveals Inter-Cellular Heterogeneity within Human Monocyte Subsets. PLoS One 2015;10:e0144351. [Crossref]
10. Schmidl C, Renner K, Peter K, Eder R, Lassmann T, Balwierz PJ, et al. Transcription and enhancer profiling in human monocyte subsets. Blood 2014;123:e90–9. [Crossref]
11. Patel AA, Zhang Y, Fullerton JN, Boelen L, Rongvaux A, Maini AA, et al. The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. J Exp Med 2017;214:1913–23. [Crossref]
12. Khan M, Arooj S, Wang H. NK Cell-Based Immune Checkpoint Inhibition. Front Immunol 2020;11:167. [Crossref]
13. Riva A, Mehta G. Regulation of Monocyte-Macrophage Responses in Cirrhosis-Role of Innate Immune Programming and Checkpoint Receptors. Front Immunol 2019;10:167. [Crossref]

14. Said EA, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M, et al. Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. Nat Med 2010;16:452–9. [Crossref]
15. Li X, Chen Y, Liu X, Zhang J, He X, Teng G, Yu D. Tim3/Gal9 interactions between T cells and monocytes result in an immunosuppressive feedback loop that inhibits Th1 responses in osteosarcoma patients. Int Immunopharmacol 2017;44:153–9. [Crossref]
16. Jabs DA, Nussenblatt RB, Rosenbaum JT; Standardization of Uveitis Nomenclature (SUN) Working Group. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. Am J Ophthalmol 2005;140:509–16. [Crossref]
17. Domogalla MP, Rostan PV, Raker VK, Steinbrink K. Tolerance through Education: How Tolerogenic Dendritic Cells Shape Immunity. Front Immunol 2017;8:1764. [Crossref]
18. Kazokoglu H, Onal S, Tugal-Tutkun I, Mirza E, Akova Y, Özyazgan Y, et al. Demographic and clinical features of uveitis in tertiary centers in Turkey. Ophthalmic Epidemiol 2008;15:285–93. [Crossref]
19. Yılmaz F, Ergun T, Bıçakçıgil M, Şahin, Direskeneli H, Direskeneli G. NALP3 Q705K Gene Polymorphism Frequency in Behçet's Disease Patients in Turkey. Turk J Immunol 2016;4:25–9. [Crossref]
20. Liu X, Wang C, Ye Z, Kijlstra A, Yang P. Higher expression of Toll-like receptors 2, 3, 4, and 8 in ocular Behcet's disease. Invest Ophthalmol Vis Sci 2013;54:6012–7. [Crossref]
21. Hughes T, Ture-Ozdemir F, Alibaz-Oner F, Coit P, Direskeneli H, Sawalha AH. Epigenome-wide scan identifies a treatment-responsive pattern of altered DNA methylation among cytoskeletal remodeling genes in monocytes and CD4+ T cells from patients with Behcet's disease. Arthritis Rheumatol 2014;66:1648–58. [Crossref]
22. Qiu Y, Zhu Y, Yu H, Yi S, Su W, Cao Q, et al. Ocular Behcet's disease is associated with aberrant methylation of interferon regulatory factor 8(IRF8) in monocyte-derived dendritic cells. Oncotarget 2017;8:51277–87. [Crossref]
23. Qiu Y, Yu H, Zhu Y, Ye Z, Deng J, Su W, et al. Hypermethylation of Interferon Regulatory Factor 8(IRF8) Confers Risk to Vogt-Koyanagi-Harada Disease. Sci Rep 2017;7:1007. [Crossref]
24. Tulunay A, Dozmorov MG, Ture-Ozdemir F, Yilmaz V, Eksioglu-Demiralp E, Alibaz-Oner F, et al. Activation of the JAK/STAT pathway in Behcet's disease. Genes Immun 2015;16:170–5. [Crossref]
25. Nakano H, Kirino Y, Takeno M, Higashitani K, Nagai H, Yoshimi R, et al. GWAS-identified CCR1 and IL10 loci contribute to M1 macrophage-predominant inflammation in Behcet's disease. Arthritis Res Ther 2018;20:124. [Crossref]
26. Suhler EB, Adan A, Brezin AP, Fortin E, Goto H, Jaffe GJ, et al. Safety and Efficacy of Adalimumab in Patients with Noninfectious Uveitis in an Ongoing Open-Label Study: VISUAL III. Ophthalmology 2018;125:1075–87. [Crossref]