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Original Investigation

Immune and inflammatory gene expressions are different in Behçet's disease compared to those in Familial Mediterranean Fever

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Abstract

Objective: The immune classification of Behçet's disease (BD) is still controversial. In this study, we aimed to compare the immune/inflammatory gene expressions in BD with those in familial Mediterranean fever (FMF), an autoinflammatory disorder with innate immune activation.

Material and Methods: CD4+ T cells and CD14+ monocytes were isolated from the peripheral blood mononuclear cells of Behçet's disease patients (n=10), FMF (n=6) patients, and healthy controls (n=4) with microbeads, and then, the mRNA was isolated. The expressions of 440 genes associated with immune and inflammatory responses were studied with a focused DNA microarray using a chemiluminescent tagging system. Changes above 1.5-fold and below 0.8-fold were accepted to be significant.

Results: In BD patients, in the CD4+ T-lymphocyte subset, interleukin 18 receptor accessory protein (1.7-fold), IL-7 receptor (1.9-fold), and prokineticin 2 (2.5-fold) were all increased compared to those in FMF patients, whereas chemokine (C-X3-C motif) receptor-1 (CX3CR1) (0.7-fold) and endothelial cell growth factor-1 (0.6-fold) were decreased. In the CD14+ monocyte population, the V-fos FBJ murine osteosarcoma viral oncogene homolog (1.5-fold), Interleukin-8 (IL-8) (2.1-fold), and Tumor Necrosis Factor alpha (TNF-α) (1.8-fold) were all increased, whereas the chemokine (C-C motif) ligand 5 (CCL5) (0.6-fold), C-C chemokine receptor type 7 (0.6-fold), and CX3CR1 (0.7-fold) were decreased, again when compared to those in FMF. Compared to healthy controls in the CD4+ T-lymphocyte population, in both BD and FMF patients, pro-platelet basic protein and CD27 had elevated expression. In BD and FMF patients, 24 and 19 genes, respectively, were downregulated, with 15 overlapping genes between both disorders. In the CD14+ monocytes population, chemokine (C-C motif) receptor-1 (CCR1) was upregulated both in BD and FMF patients compared to that in the controls, whereas CCL5 was downregulated.

Conclusion: Immune and inflammatory gene expressions seem to be variable in both the innate (CD14+) and adaptive (CD4+) immune responses in BD and FMF patients compared to those in controls, suggesting differences in immune regulation between the two disorders.

Keywords: Behçet's disease, familial Mediterranean fever, gene expression

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Introduction

Behçet's Disease (BD) is an inflammatory disease with recurrent oral aphthous and genital ulcers with skin lesions. Repeated attacks of uveitis and involvement of the gastrointestinal tract, central nervous system, and large vessels are also observed in BD. In the pathogenesis of BD, there is a mixed genetic background leading to proinflammatory attacks with an onset of innate immune system activation and later adaptive immune responses with autoantigens and environmental factors (1, 2).

Microarray techniques make the evaluation of a large number of gene transcriptions (mRNA) on a single array possible, thus allowing the assessment of immune system changes comprehensively (3). Various immune disorders, such as psoriasis, systemic lupus erythematosus (SLE), and renal vasculitides, have been studied with this method, generating informative data about their pathogenesis (4-6). Recently, BD was proposed as an autoinflammatory disease (7). "Autoinflammation" is a concept defined for diseases such as familial Mediterranean fever (FMF) that are limited to innate immune system activation without an autoimmune T- or B-cell response (8). A relationship seems to exist between BD and FMF with some common clinical manifestations; however, BD involves longer attacks and different manifestations, such as vascular or central nervous system involvement (9).

Table 1. Immune/Inflammatory gene profile

Cytokines
Chemokines: CCL1, CCL11, CCL13, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL3L1, CCL4, CCL4L1, CCL5, CCL7, CCL8, CKLF, CX3CL1, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL2, CXCL3, CXCL5, CXCL6, CXCL9, CYP26B1, IL13, IL8, PF4V1, PPBP, PXMP2, XCL1.
Other Cytokines: AREG, BMP1, BMP2, BMP3, BMP7, CAST, CD40LG, CER1, CKLFSF1, CKLFSF2, CLC, CSF1, CSF2, CSF3, CTF1, CXCL16, EBI3, ECGF1, EDA, EPO, ERBB2, ERBB2IP, FAM3B, FASLG, FGF10, FGF12, FIGF, FLT3LG, GDF2, GDF3, GDF5, GDF6, GDF8, GDF9, GLMN, GPI, GREM1, GREM2, GRN, IFNA1, IFNA14, IFNA2, IFNA4, IFNA8, IFNB1, IFNE1, IFNG, IFNK, IFNW1, IFNWP2, IK, IL10, IL11, IL12A, IL12B, IL15, IL16, IL17, IL17B, IL17C, IL17D, IL17E, IL17F, IL18, IL19, IL1A, IL1B, IL1F10, IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, IL1RN, IL2, IL20, IL21, IL22, IL23A, IL24, IL26, IL27, IL28B, IL29, IL3, IL32, IL4, IL5, IL6, IL7, IL9, INHA, INHBA, INHBB, KITLG, LASS1, LEFTY1, LEFTY2, LIF, LTA, LTB, MDK, MIF, MUC4, NODAL, OSM, PBEF1, PDGFA, PDGFB, PRL, PTN, SCGB1A1, SCGB3A1, SCYE1, SDCBP, SECTM1, SIVA, SLCO1A2, SLURP1, SOCS2, SPP1, SPRED1, SRGAP1, THPO, TNF, TNFRSF11B, TNFSF10, TNFSF11, TNFSF13, TNFSF13B, TNFSF14, TNFSF15, TNFSF18, TNFSF4, TNFSF7, TNFSF8, TNFSF9, TRAP1, VEGF, VEGFB, YARS.
Cytokine Receptors: CNTFR, CSF2RA, CSF2RB, CSF3R, EBI3, EPOR, F3, GFRA1, GFRA2, GHR, IFNAR1, IFNAR2, IFNGR1, IFNGR2, IL10RA, IL10RB, IL11RA, IL12B, IL12RB1, IL12RB2, IL13RA1, IL13RA2, IL15RA, IL17R, IL17RB, IL18R1, IL1R1, IL1R2, IL1RAP, IL1RAPL2, IL1RL1, IL1RL2, IL20RA, IL21R, IL22RA1, IL22RA2, IL28RA, IL2RA, IL2RB, IL2RG, IL31RA, IL3RA, IL4R, IL5RA, IL6R, IL6ST, IL7R, IL8RA, IL8RB, IL9R, LEPR, LIFR, MPL, OSMR, PRLR, TTN.
Chemokine Receptors: BLR1, CCL13, CCR1, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCRL1, CCRL2, CX3CR1, CXCR3, CXCR4, CXCR6, IL8RA, IL8RB, XCR1.
Cytokine Metabolism: APOA2, ASB1, AZU1, B7H3, CD28, CD4, CD80, CD86, EBI3, GLMN, IL10, IL12B, IL17F, IL18, IL21, IL27, IL4, INHA, INHBA, INHBB, IRF4, NALP12, PRG3, S100B, SFTPD, SIGIRR, SPN, TLR1, TLR3, TLR4, TLR6, TNFRSF7, TNFSF15.
Cytokine Production: APOA2, ASB1, AZU1, B7H3, CD28, CD4, CD80, CD86, EBI3, GLMN, IL10, IL12B, IL17F, IL18, IL21, IL27, IL4, INHA, INHBA, INHBB, INS, IRF4, NALP12, NFAM1, NOX5, PRG3, S100B, SAA2, SFTPD, SIGIRR, SPN, TLR1, TLR3, TLR4, TLR6, TNFRSF7.
Other Genes involved in Cytokine–Cytokine Receptor Interaction: ACVR1, ACVR1B, ACVR2, ACVR2B, AMH, AMHR2, BMPR1A, BMPR1B, BMPR2, CCR1, CD40, CRLF2, CSF1R, CXCR3, IL18RAP, IL23R, LEP, TGFB1, TGFB2, TGFB3, TGFBR1, TGFBR2, TNFRSF1A, TNFRSF1B, TNFRSF21, TNFRSF8, TNFRSF9, XCR1.
Acute-Phase Response: AHSG, APCS, APOL2, CEBPB, CRP, F2, F8, FN1, IL22, IL6, INS, ITIH4, LBP, PAP, REG-III, SAA2, SAA3P, SAA4, SERPINA1, SERPINA3, SERPINF2, SIGIRR, STAT3.
Inflammatory Response: ADORA1, AHSG, AIF1, ALOX5, ANXA1, APOA2, APOL3, ATRN, AZU1, BCL6, BDKRB1, BLNK, C3, C3AR1, C4A, CCL1, CCL11, CCL13, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL3, CCL3L1, CCL4, CCL4L1, CCL5, CCL7, CCL8, CCR1, CCR2, CCR3, CCR4, CCR7, CD14, CD40, CD40LG, CD74, CD97, CEBPB, CHST1, CIAS1, CKLF, CRP, CX3CL1, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL16, CXCL2, CXCL3, CXCL5, CXCL6, CXCL9, CYBB, DOCK2, EPHX2, F11R, FOS, FPR1, GPR68, HDAC4, HDAC5, HDAC7A, HDAC9, HRH1, ICEBERG, IFNA2, IL10, IL10RB, IL13, IL17, IL17B, IL17C, IL17D, IL17E, IL17F, IL18RAP, IL1A, IL1B, IL1F10, IL1F5, IL1F6, IL1R1, IL1RAP, IL1RN, IL20, IL22, IL31RA, IL5, IL8, IL8RA, IL8RB, IL9, IRAK2, IRF7, ITCH, ITGAL, ITGB2, KNG1, LTA4H, LTB4R, LY64, LY75, LY86, LY96, MEFV, MGLL, MIF, MMP25, MYD88, NALP12, NCR3, NFAM1, NFATC3, NFATC4, NFE2L1, NFKB1, NFRKB, NFX1, NMI, NOS2A, NR3C1, OLR1, PAP, PARP4, PLA2G2D, PLA2G7, PRDX5, PREX1, PRG2, PRG3, PROCR, PROK2, PTAFR, PTGS2, PTPRA, PTX3, REG-III, RIPK2, S100A12, S100A8, SAA2, SCUBE1, SCYE1, SELE, SERPINA3, SFTPD, SN, SPACA3, SPP1, STAB1, SYK, TACR1, TIRAP, TLR1, TLR10, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TNF, TNFAIP6, TOLLIP, TPST1, VPS45A, XCR1.
Humoral Immune Response: BATF, BCL2, BF, BLNK, C1R, C2, C3, C4A, CCL16, CCL18, CCL2, CCL20, CCL22, CCL3, CCL7, CCR2, CCR6, CCR7, CCRL2, CCRL2, CD1B, CD1C, CD22, CD28, CD40, CD53, CD58, CD74, CD86, CLC, CR1, CRLF1, CSF1R, CSF2RB, CXCR3, CYBB, EBI3, FADD, GPI, IL10, IL12A, IL12B, IL12RB1, IL13, IL18, IL1B, IL2, IL26, IL4, IL6, IL7, IL7R, IRF4, ITGB2, LTF, LY86, LY9, LY96, MAPK11, MAPK14, MCP, NFKB1, NR4A2, PAX5, POU2AF1, POU2F2, PTAFR, RFXANK, S100B, SERPING1, SFTPD, SLA2, TNFRSF7, XCL1, XCR1, YY1.

In our study, we compared the immune/inflammatory gene expressions of two main cell types, namely CD4+ T lymphocytes and CD14+ monocytes, in peripheral blood mononuclear cell populations in BD and FMF patients. In this way, we attempted to evaluate two characteristic cell types of the innate and adaptive immune system that are known to be activated in BD patients at the same time using microarray technology.

Materials and Methods

Patients and controls

Ten BD patients (F/M: 6/4 (F: Female, M: Male), mean age: 36.7 years, active/inactive: 5/5) who fulfilled the 1990 International Study Group classification criteria for Behçet's disease; six FMF patients (F/M: 3/3, mean age: 29.2 years, active/inactive: 3/3) who were followed in Marmara University School of Medicine, Rheumatology Clinics; and four healthy controls (HC) (F/M: 2/2, mean age: 32.4 years) were enrolled in the study (10). All patients and controls provided their informed consent before participation and the study was approved by the Ethics Committee of Marmara University School of Medicine.

Cells and RNA extractions

Peripheral blood mononuclear cells (PBMC) were separated from blood samples obtained from the normal healthy controls and patients using density gradient centrifugation (Amersham Biosciences; Uppsala, Sweden). Then, the CD4+ T lymphocytes and CD14+ monocytes were isolated with microbeads (MicroBeads human, Company MiltenyiBiotec; Bergisch Gladbach, Germany) according to the kit protocol and separated by autoMACS separator. A purity of >90% was shown with flow cytometry.

RNA was also isolated with the RNeasy kit (Qiagen; Valencia, CA, USA) and was measured for concentration and purity at 260 and 280 nm absorbance (>50 ng/uL). The samples were stored at -80°C until use in the microarray study.

Microarray

Human Inflammatory Response and Autoimmunity Microarray (OligoGEArray, SABiosciences; OHS-803, USA) was used in our study. The microarray was composed of a set of distinct, gene-specific, nucleic acid probes immobilized on a solid support. During a microarray experiment, RNA is enzymatically converted to labeled cDNA (complementary

DNA) or cRNA, and then hybridized to the immobilized nucleic acid probe. The labeled target bound at each gene-specific spot is typically detected using chemiluminescent, fluorescent, or radioactive methods. The signal produced at each spot is representative of the amount of message in the original RNA sample. The genes (n=440) involved in this array represent the expression of inflammatory cytokines, chemokines, and their receptors and comprise genes related to cytokine metabolism, the production of cytokines, and cytokine–cytokine receptor interactions. Thoroughly researched panels of genes involved in the acute-phase response, inflammatory response, and humoral immune responses are represented as well. The transcripts are shown in Table 1. cDNA synthesis from the RNA samples and further processes were performed in SAB-Biosciences Lab, France.

Analysis

The Interquaritle Normalization option normalizes each spot brightness to the mean intensity value of all the spots in the middle half (50%) of the range that remains after ignoring the most and least intense quarters (25%) of the entire data set. The measurements of patients that were read in the immune/inflammatory array were compared with the healthy controls. Upregulated (>1.5) and downregulated (<0.8) gene expressions were accepted as significant.

Results

The results were analyzed separately for the CD4+ T lymphocytes and CD14+ monocytes.

CD4+ T-lymphocyte population

When the CD4+ T lymphocytes were analyzed in patients compared to controls (Table 2, 3), among eight increased transcripts in BD patients, the Burkitt lymphoma receptor 1 (BLR1) (p=0.03) and toll-interleukin 1 receptor (TIR) domain containing adaptor protein (TIRAP) (p<0.001) were significantly increased. There were also six increased transcripts in FMF patients; however, only the Activin A receptor type I (ACVR1) reached statistical significance (p=0.05). Pro-Platelet basic protein (PPBP) and CD27 were both increased in the two groups compared to the control group but did not reach significance. Twenty four genes in BD and 19 genes in FMF were decreased compared to the controls. Fifteen genes were common in both disease groups (Table 4, 5).

When T-lymphocytes responses were compared between BD and FMF (Table 6, 7), IL-7 receptor (IL-7R) was observed to be significantly different between the groups (p=0.05).

Table 2. Increased gene expressions of CD4+ T lymphocytes in Behçet's Disease compared to those in controls

	Fold	p
Burkitt lymphoma receptor 1 (BLR1)	2.07	0.03
Interleukin 7 receptor (IL7R)	1.91	0.09
Lymphotoxin beta (LTB)	1.59	0.11
Nuclear factor erythroid 2-related factor 1 (NFE2L1)	3.47	0.56
Pro-platelet basic protein (PPBP)	1.98	0.29
Prokineticin 2 (PROK2)	1.75	0.58
Toll-interleukin 1 receptor (TIR) domain containing adaptor protein (TIRAP)	1.60	0.00
CD27	1.81	0.14

Table 3. Increased gene expressions of CD4+ T lymphocytes in FMF compared to those in controls

	Fold	p
Activin A receptor, type I (ACVR1)	1.56	0.05
C-C chemokine receptor type 7 (CCR7)	2.15	0.21
CD40 ligand (CD40LG)	1.52	0.15
Fms-related tyrosine kinase 3 ligand (FLT3LG)	1.62	0.33
Pro-Platelet basic protein (PPBP)	1.93	0.33
CD27	1.88	0.16

Table 4. Decreased gene expressions of CD4+ T lymphocytes in Behçet's Disease compared to those in controls

	Fold	p
Allograft inflammatory factor 1 (AIF1)	0.43	0.00
Arachidonate 5-Lipoxygenase (ALOX5)	0.47	0.03
B-cell lymphoma 6 (BCL6)	0.49	0.09
Chemokine (C-C motif) ligand (CCL3)	0.34	0.01
Chemokine (C-C motif) ligand 3-like 1 (CCL3L1)	0.41	0.11
CD14	0.50	0.09
CD74	0.55	0.00
CD86	0.46	0.02
CCAAT/enhancer binding protein (C/EBP), beta (CEBPB)	0.47	0.03
NACHT, LRR and PYD domains-containing protein 3 (NLRP3)	0.65	0.11
Colony-stimulating factor 1 receptor (CSF1R)	0.47	0.03
Colony-stimulating factor 3 receptor (CSF3R)	0.48	0.24
Cytochrome b-245, beta polypeptide (CYBB)	0.49	0.02
Endothelial Cell Growth Factor 1 (ECGF1)	0.42	0.00
V-fosFBJ murine osteosarcoma viral oncogene homolog (FOS)	0.23	0.01
Formyl peptide receptor 1 (FPR1)	0.50	0.21
Interleukin 13 receptor, alpha 1 (IL13RA1)	0.63	0.06
Interleukin 8 (IL8)	0.64	0.57
Lactotransferrin (LTF)	0.44	0.39
S100 calcium binding protein A12 (S100A12)	0.48	0.03
S100 calcium binding protein A8 (S100A8)	0.53	0.09
Spleen tyrosine kinase (SYK)	0.64	0.03
Tumor necrosis factor receptor superfamily, member 1A (TNFRSF1A)	0.66	0.00
Tumor necrosis factor (ligand) superfamily, member 13b (TNFSF13B)	0.58	0.05

Table 5. Decreased gene expressions of CD4+ T lymphocytes in FMF compared to those in controls

	Fold	p
Allograft inflammatory factor 1 (AIF1)	0.56	0.10
Arachidonate 5-lipoxygenase (ALOX5)	0.62	0.24
B-cell CLL/lymphoma 6 (BCL6)	0.51	0.12
Chemokine (C-C motif) ligand 3 (CCL3)	0.40	0.03
Chemokine (C-C motif) ligand 3-like 1 (CCL3L1)	0.25	0.07
Chemokine (C-C motif) ligand 4-like 1 (CCL4L1)	0.61	0.04
CD14	0.51	0.04
CD86	0.53	0.13
CCAAT/enhancer binding protein (C/EBP), beta (CEBPB)	0.48	0.02
Colony-stimulating factor 1 receptor (CSF1R)	0.59	0.21
Colony-stimulating factor 3 receptor (CSF3R)	0.35	0.12
Chemokine (C-X-C motif) receptor 4 (CXCR4)	0.62	0.21
FBJ murine osteosarcoma viral oncogene homolog (FOS)	0.19	0.01
Formyl peptide receptor 1 (FPR1)	0.37	0.02
Interleukin 13 receptor, alpha 1 (IL13RA1)	0.61	0.05
Interleukin 1 receptor antagonist (IL1RN)	0.62	0.11
Interleukin 8 (IL8)	0.17	0.01
Oncostatin M (OSM)	0.52	0.12
Tumor necrosis factor (ligand) superfamily, member 13b (TNFSF13B)	0.60	0.07

FMF: Familial Mediterranean fever

Table 6. Increased gene expressions of CD4+ T lymphocytes in BD compared to those in FMF

	Fold	p
Interleukin 18 Receptor Accessory Protein (IL18RAP)	1.69	0.26
Interleukin 7 Receptor (IL7R)	1.86	0.05
Prokineticin 2 (PROK2)	2.52	0.37

BD: Behçet's disease; FMF: Familial Mediterranean fever

Table 7. Decreased gene expressions of CD4+ T lymphocytes in BD compared to those in FMF

	Fold	p
Chemokine (C-X3-C Motif) Receptor 1 (CX3CR1)	0.66	0.27
Endothelial cell growth factor (ECGF1)	0.60	0.15

BD: Behçet's disease; FMF: Familial Mediterranean fever

Table 8. Increased gene expressions of CD14+ monocytes in BD compared to those in controls

	Fold	p
C-C Chemokine Receptor 1 (CCR1)	2.14	0.01
V-fosFBJ murine osteosarcoma viral oncogene homolog (FOS)	1.88	0.04
Tumor Necrosis Factor Alpha (TNF-α)	1.85	0.15

BD: Behçet's disease

Table 9. Increased gene expressions of CD14+ monocytes in FMF compared to those in controls

	Fold	p
C-C Chemokine Receptor 1 (CCR1)	1.51	0.15
Prokineticin 2 (PROK2)	1.76	0.06

FMF: Familial Mediterranean fever

Table 10. Decreased gene expressions of CD14+ monocytes in BD compared to those in controls

	Fold	p
Chemokine (C-C motif) ligand 5 (CCL5)	0.39	0.11
C-C Chemokine Receptor 7 (CCR7)	0.59	0.38
Interleukin 2 receptor, gamma (IL-2RG)	0.63	0.20
Lymphotoxin beta (TNF superfamily, member 3)(LTB)	0.59	0.34
Vascular endothelial growth factor B(VEGFB)	0.60	0.21

BD: Behçet's disease

Table 11. Decreased gene expressions of CD4+ T lymphocytes in FMF compared to those in controls

	Fold	p
Chemokine (C-C motif) ligand 5 (CCL5)	0.62	0.48

FMF: Familial Mediterranean fever

Table 12. Increased gene expressions of CD14+ monocytes in BD compared to those in FMF

	Fold	p
V-fosFBJ murine osteosarcoma viral oncogene homolog (FOS)	1.54	0.06
Interleukin 8 (IL8)	2.06	0.21
Tumor necrosis factor alpha (TNFa)	175	0.17

BD: Behçet's disease; FMF: Familial Mediterranean fever

Table 13. Decreased gene expressions of CD14+ monocytes in BD compared to FMF

	Fold	p
Chemokine (C-C motif) ligand 5 (CCL5)	0.63	0.37
Chemokine (C-C motif) receptor 7 (CCR7)	0.58	0.43
Chemokine (C-X3-C motif) receptor 1 (CX3CR1)	0.66	0.01

BD: Behçet's disease; FMF: Familial Mediterranean fever

Prokineticin 2 (PROK2) had an increased expression in BD CD4+ T lymphocytes compared to both the FMF and control groups, but did not reaching significance.

CD14+ monocyte population Chemokine (C-C motif) receptor-1 (CCR1) was increased in both BD and FMF, compared to HC, but was only statistically significant in BD. The V-fosFBJ murine osteosarcoma viral oncogene homolog (FOS) was also significantly increased in BD (Table 8, 9). Five genes in BD and only the chemokine (C-C motif) ligand 5

Table 14. Decreased gene expressions of CD4+ T lymphocytes in FMF compared to those in controls

	Fold	p
Behçet vs control group	NFE2L1 (3,47), IL-7R (1,91), LTB (1,59), BLR1 (2,07), PPBP (1,98), PROK2 (1,75), TIRAP (1,60), CD27 (1,81)	AIF1 (0,56), ALOX5 (0,62), BCL6 (0,51), CCL3 (0,40), CCL3L1 (0,25), CCL4L1 (0,61), CD86 (0,53), CEBPB (0,48), CSF1R (0,59), CSF3R (0,35), CXCR4 (0,62), FOS (0,19), FPR1 (0,37), IL13RA1 (0,61), IL1RN (0,62), IL8 (0,17), OSM (0,52), TNFSF13B (0,60)
FMF vs control group	ACVR1 (1,56), CCR7 (2,15) CD40LG (1,52), FLT3LG (1,62), PPBP (1,93) CD27 (1,88)	AIF1 (0,43), ALOX5 (0,47), BCL6 (0,49), CCL3 (0,34), CCL3L1 (0,41), CD14 (0,50), CD74 (0,55), CD86 (0,46), CEBPB (0,47), NLRP3 (0,65), CSF1R (0,47), CSF3R (0,48), CYBB (0,49), ECGF1 (0,42), FOS (0,23), FPR1 (0,50), IL13RA1 (0,63), IL8 (0,64), LTF (0,44), S100A12 (0,48), S100A8 (0,53), SYK (0,64), TNFRSF1A (0,66), TNFSF13B (0,58)
Behçet vs FMF	PROK2 (2,52), IL-7R (1,86), IL-18RAP (1,69)	CX3CR1 (0,66), ECGF1 (0,60)

FMF: Familial Mediterranean fever

Table 15. Genes over- and underexpressed in CD14+ monocytes

	Fold	p
Behçet vs control group	CCR1 (2,14), FOS (1,88), TNF (1,85)	CCL5 (0,39), CCR7 (0,59), IL-2RG (0,63), LTB (0,59), VEGFB (0,60)
FMF vs control group	CCR1 (1,51), PROK2 (1,76)	CCL5 (0,62)
Behçet vs FMF	FOS (1,54), IL-8 (2,06), TNF (1,75)	CCL5 (0,63), CCR7 (0,58), CX3CR1 (0,66)

FMF: Familial Mediterranean fever

(CCL5) in FMF were decreased compared to HC, but without reaching significance. CCL5 was decreased in both diseases compared to HC (Table 10, 11).

When we compared BD with FMF, similar to HC, while the FOS and Tumor Necrosis Factor (TNF) expressions were increased, CCL5 expression was decreased (Table 12, 13).

The gene over- and underexpressions for CD14+ monocytes and CD4+ T lymphocytes are given in Table 14, 15. The gene expressions of Interleukin 18 Receptor Accessory Protein (IL-18RAP), IL-7R, and PROK2 were higher in BD patients compared to in the FMF group in the CD4+ T population. In the CD14+ monocytes, FOS, Interleukin-8 (IL-8), and TNF-α were upregulated in BD compared to in FMF, whereas chemokine (C-X3-C motif) receptor-1 (CX3CR1), CCL5, and C-C chemokine receptor type 7 (CCR7) were observed to be downregulated. When compared with the controls, BLR1, Interleukin 7 Receptor (IL7R), Lymphotoxin Beta (LTB), Nuclear Factor, Erythroid 2-Like 1 (NFE2L1), PPBP, PROK2, TIRAP, and CD27 expression in BD and the ACVR1, CCR7, CD40 Ligand (CD40LG), Fms-Related Tyrosine Kinase 3 Ligand (FLT3LG), PPBP, and CD27 expressions in FMF group were higher in the CD4+ T-lymphocyte population. On the other hand, the CCL5, CCR7, Interleukin 2 Receptor, Gamma (IL-2RG), LTB, and vascular endothelial growth factor B (VEGFB) levels of the BD group and the CCL5 levels of the FMF group were lower in the CD14+ monocyte population when compared to the controls.

Discussion

Inflammation in immune disorders progresses with the activation of many inflammatory molecules and common signaling pathways. RNA gene expression analyses with microarray techniques have become widespread methods to estimate these complex systems, as they enable one to analyze many genes at the same time. In our study, we used a "superarray" microarray that contained 440 gene products. In recent years, this methodology allowed researchers to determine candidate molecules of various diseases like rheumatoid arthritis and juvenile idiopathic arthritis (11, 12). It was previously shown that cytokines, such as Interleukin 1 (IL-1), Interleukin 6 (IL-6), Interleukin 12 (IL-12), Interleukin 18 (IL-18), Tumor Necrosis Factor alpha (TNF-α), and Interferon gamma (IFN-γ); chemokines, such as IL-8; cytokine receptors, such as soluble Interleukin-2 receptor (sIL2R); and chemokine antagonist and receptors were increased in peripheral blood and tissue samples of BD patients (13-15). However, even the largest comprehensive study could only investigate 17 inflammatory molecules at the same time with a microbead system in BD (16). Our study is therefore the most comprehensive study in this category.

PROK2 and IL-7R were found to be increased in the BD group compared to in both FMF and HC groups in a CD4+ T-lymphocyte population in our study. PROK2 is a chemokine with five disulfide bonds and was shown to be expressed in inflamed tissues (17). It was observed that the secretion of PROK2 from lymphocytes stimulate IL-10 and this process causes an increase in angiogenesis (18). An increase in PROK2 may play a role in BD neoangiogenesis with a vascular pattern. Interleukin-7 (IL-7) is a cytokine that has pleotropic roles. While IL-7 plays a role in T cells evolution in the thymus, it also increase naive and memory T cells survivals by releasing anti-apoptotic Bcl-2 in the periphery (19). It was shown that an increase of IL-7R expression in synovial has an association with resistant treatment by biologic agents (20). It was also shown that IL-7R expression in peripheral blood is associated with a bad prognosis in Anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitides (21).

Another salient finding in the CD4+ T-lymphocyte population is the decreased expression in 15 common transcripts of both BD (n=24) and FMF (n=19) compared to HC. This finding shows that some common T-lymphocyte genes become inactivated in inflammation of both diseases and also shows that they use common adaptive pathways.

In our study, IL-8 and TNF-α genes expression were increased in BD compared to FMF in a CD14+ monocyte population. IL-8 has been shown in many studies to be an important kemokine in BD and to be associated with neutrophil migration to the tissues (22, 23). IL-8 secretion is also increased in CD4+ T lymphocytes of BD (24). The increase of TNF-α in BD has also been shown in many studies and the inhibition of TNF-a with anti-TNFa agents is currently one of the major progresses in BD treatment (25).

The expression of FOS, which is an oncogene protein, increased in BD patients compared to that in both FMF patients and HC in our experiment. It is known that the c-fos oncogene group interacts with many stimulants and plays a role in cell growth and development (26). Generally, increased expressions of FOS in malignant tumors were also observed in fibroblasts when they were in stress. This interesting finding suggests that FOS also plays a role in BD oral ulcer pathogenesis (27). Expression of the c-fos proto-oncogene in the bone, cartilage, and tooth-forming tissues during mouse development may also show the association of FOS with BD (28).

The main limitation of our study is the low patient and control numbers. The heterogeneity of BD patients for different clinical manifestations also limits the applicability of our results.

As a conclusion, we showed, by a comprehensive immune/inflammatory microarray analysis, that CD4+ T lymphocytes and CD14+ monocytes in BD patients have different gene expression profiles compared to those in FMF patients and HC. However, increased and decreased transcripts in this study need to be confirmed by other studies. These days, high-dose immunosuppressives, such as corticosteroids, azathioprine, and cyclosporine A, are used for organ damage, such as damage of the eyes, vessels, and central nervous system, in BD patients (1). New therapies are also needed for resistant patients, intolerance to drugs, and side effects of drugs. This detailed analysis of an immune/inflammatory gene profile might be helpful for new therapeutic targets in BD patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Marmara University School of Medicine.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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