

Basic Science

P1.

INCREASED SERUM ANTIBODY TITER AGAINST HPV-16 ANTIGEN IN PATIENTS WITH BEHÇET'S DISEASE

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Objectives. It was reported that quadrivalent Human Papillomavirus (HPV) vaccine was significantly associated with Behçet's disease (BD). There was no report that HPV infection can be an one of the possible causes to develop BD. The objective of this study to evaluate whether the anti-HPV antibody titer in BD would increase.

Methods. Sera from 93 Korean BD patients, who fulfilled the diagnostic criteria of the International Study Group for BD were used for ELISA. The clinical activity of BD was evaluated at the time of blood sampling. HPV 16 L1 VLP antigen was used in this study for ELISA.

Results. Patients with BD had significantly higher antibody titer against HPV 16 (OD:0.210-3.675; mean 0.992) than that of healthy controls (OD:0.248-0.762; mean 0.517) ($p < 0.001$). Using ROC analysis from analysis, the cut-off value for anti-HPV antibody titer of 0.578 OD was determined in order to differentiate BD patients from healthy controls. When we compared the clinical features of BD between the two groups, articular involvement of BD could be more likely in patients with anti-HPV 16 antibody titer < 0.578 OD ($p = 0.035$). In addition, patients with anti-HPV 16 antibody titer < 0.578 were significantly younger than patients with anti-HPV 16 antibody titer ≥ 0.578 OD.

Conclusion. There might be a possibility that HPV can be an one of the extrinsic triggering possible infectious agent for the development of BD.

P2.

IL-27 GENE POLYMORPHISMS IN IRANIAN PATIENTS WITH BEHÇET'S DISEASE

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Background. Behçet's Disease (BD) is a chronic systemic inflammatory disease of unknown etiology, principally characterized by relapsing periods of a broad range of clinical symptoms. Cytokines play fundamental roles in the pathogenesis of BD. Polymorphisms within cytokine genes have been found to play a pathogenic role in the development of autoimmune/inflammatory disorders. Interleukin 27 (IL-27), a new pro/anti-inflammatory cytokine, is a great candidate for chronic inflammatory disease studies. The purpose of this study was to investigate a possible association between polymorphisms in the IL-27 gene and susceptibility to BD.

Methods. Fifty Iranian patients with BD and one hundred healthy individuals were examined for rs153109A/G and rs181206T/C IL-27 gene single nucleotide polymorphisms using RFLP-PCR and ARMS-PCR, respectively. Allele and genotype distributions were compared between groups using chi-square or Fisher's exact test.

Results. Frequencies of the rs153109AA genotype and rs153109A allele were statistically higher in BD patients comparing with the control group ($p = 0.034$ and $p = 0.011$, respectively). The genotype and allele frequencies of rs181206 T/C polymorphism in BD patients were not significantly different from those of healthy controls.

Conclusions. Present findings demonstrate for the first time that IL-27 gene rs153109 A/G SNP may be involved in susceptibility to BD in the Iranian population.

P3.

FUNCTIONAL ANALYSIS OF M1 AND M2 MACROPHAGE IN BEHÇET'S DISEASE

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Introduction. The recent GWAS have identified susceptible loci encompassing chemokine CCR1 and anti-inflammatory cytokine IL10, genes highly expressed in macrophages, suggesting its pathological roles in Behçet's disease (BD). Interestingly, reduced expression of CCR1 is associated with risk for BD. Recently, inflammatory macrophage M1 and anti-inflammatory M2 polarization has gained attention in the immunology field.

Objectives. To compare features of in vitro differentiated M1 and M2 macrophages from peripheral blood between BD and healthy controls (HC).

Methods. Differentiation into M1 or M2 macrophages (M ϕ) was induced in vitro from peripheral monocytes in the presence of GM-CSF or M-CSF, respectively. Expressions of CD68, CD163, and CCR1 were determined by real-time PCR and flow cytometric analyses. For the M ϕ that were treated with LPS for 24 hours, supernatants were analyzed for cytokine profiles using beads assay. GWAS identified IL10 SNP rs1518111 was genotyped.

Results. As previously shown, differentiated M2 expressed conventional M2 marker CD163 protein and mRNA, but not M1, confirming validity of our assay to differentiate M2. M1 M ϕ produced higher amounts of IL-6, whereas only M2 secreted IL-10 cytokine, although we could not find significant difference of cytokine production between HC and BD in our assay. Both mRNA and protein analysis of CCR1 revealed higher expression in M2 compared to M1 M ϕ . In comparison between HC, CCR1 protein in M1 was higher in BD. Finally, we found significant association between IL-10 mRNA expression and rs15181111 SNP genotypes in M2M ϕ from HC.

Conclusion. We found that CCR1 and IL-10 are highly expressed in M2M ϕ . GWAS-identified SNP genotypes could affect on expression of CCR1 and IL-10 in M2M ϕ , resulting in lower migration of anti-inflammatory cells to site of active inflammation.

P4.

SEQUENCING OF 16S RRNA REVEALS A DISTINCT SALIVARY MICROBIOME SIGNATURE IN BEHÇET'S DISEASE

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Objective. Behçet's disease (BD) is a multisystem inflammatory disorder characterized by recurrent oro-genital ulcers, mucocutaneous lesions and serious organ involvement. This study was undertaken to investigate the structure of the salivary microbiome in BD patients.

Methods. Stimulated saliva samples were collected from 31 BD patients and 15 healthy controls, and detailed oral health indices were recorded. In 9 BD patients a second oral health evaluation and saliva collection was performed following dental and periodontal treatment. High-throughput sequencing of the 16S rRNA V4 region in saliva samples was performed. Sequences were rigorously filtered and grouped into phylogenetically-related operational taxonomic units (OTUs), used to measure bacterial community diversity and richness. OTUs were classified using a 16S rRNA reference database at the species-level. AMOVA and LEfSe analyses were used to measure differences between patients and controls at the community- and species-level, respectively.

Results. Sequence analysis identified a total of 908 OTUs present across all samples. Patients had a microbial community structure that is significantly different and less diverse compared to healthy controls. The most overabundant species in BD patients compared to controls was Haemophilus parainfluenzae, while the most depleted included Alloprevotella rava and species in the genus Leptotrichia. Patients receiving periodontal treatment showed improvements in oral health indices, but no short-term differences in bacterial community structure. Neither the BD-associated genetic risk locus within the HLA-B/MICA region nor being on immunosuppressive medications explained the differences between patients and controls.

Conclusion. This is the first high-throughput sequencing-based evaluation of the salivary microbiome in BD. These findings demonstrate that the salivary microbiome of BD patients has a specific signature characterized by changes at the community and species level.

P5.

INCREASED EXPRESSION OF ARYL HYDROCARBON RECEPTOR IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF PATIENTS WITH ACTIVE BEHÇET'S DISEASE

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Behçet's disease (BD) is characterized by multi-systemic vasculitis. Although the pathogenesis of BD remains elusive, low frequency of regulatory T cells (Treg) and high frequency of T helper 17 cells (Th17) have been suggested to contribute to BD pathogenesis. Given that aryl hydrocarbon receptor (Ahr) is involved in the induction of Treg and Th17 cells, we analyzed the expression of Ahr and indoleamine-2,3-dioxygenase 1 (IDO1) producing endogenous Ahr ligand in the peripheral blood mononuclear cells (PBMCs) of patients with BD. Both protein

and mRNA expression of Ahr was elevated in the ex-vivo PBMCs of patients with active BD compared to healthy controls, but protein levels of IDO1 were not. Notably, IDO1 expression was significantly increased in patients with recurrent aphthous ulcer compared to healthy controls. Stimulation of PBMCs with lipopolysaccharides (LPS) and/or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) did not alter the expression level of Ahr and IDO1 in patients with BD. Further study is warranted to evaluate the role of Ahr in the pathophysiology of BD and as a biomarker for monitoring BD patients.

P6.

TRIM21 IN MONOCYTE ENHANCES TH1/TH17 INFLAMMATION IN BEHÇET'S DISEASE

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Background. Behçet's disease (BD) is a chronic, multisystem vasculitis and autoinflammatory disorder, characterized by oral ulcers, genital ulcers, various inflammatory skin lesions, and uveitis. The exact cause of BD is still not known, many study suggest that this disease triggered by environmental factor including infectious agents such as herpes simplex virus (HSV) and streptococcus sanguinis. Tripartite motif containing 21 (TRIM21), an E3 ligase protein, regulates the production of cytokine by ubiquitination of transcriptional factors such as interferon regulatory factor (IRF) family and NF-κB. Moreover TRIM21 can present antiviral properties, mediating antiviral defense mechanisms to DNA/RNA viruses.

Objective. The purpose of this study was to elucidate TRIM21 proteins expression in monocyte of BD patient, and to identify the role of TRIM21 on immune dysregulation in BD.

Methods. Monocytes and T cells were isolated from peripheral blood using Ficoll-Hypaque density-gradient centrifugation and relevant immunomagnetic negative selection method. Protein levels were measured using western blot, ELISA, and flow cytometry. Co-cultures between monocyte and responder T cells (1:1 to 1:2 ratio) were maintained in media for 7~14 days. Knock-down of TRIM21 was performed using siRNA technique on THP-1 or freshly isolated monocytes.

Results. TRIM21 expression was increased in peripheral blood monocytes from BD patients compared to healthy control. The expression of IRF8, a representative ubiquitination target of TRIM21, was also decreased in BD monocyte. BD monocyte promoted secretion of pro-inflammatory cytokines including Th17 promoting cytokine such as IL-6, IL-1β and IL-23 following NF-κB activation. BD monocytes promoted Th17 polarization after co-culture with responder T cells from healthy donors, and IL-17A production from these co-cultured T cells was also increased. Known-down of TRIM21 in monocyte using siRNA prevents NF-κB activation and decreased Th1 and Th17 polarization of responder T cell in co-culture system.

Conclusion. From our results suggest that BD monocytes facilitate Th1/Th17 differentiation of naive T cells and TRIM21 may regulate the secretion of pro-inflammatory cytokine from monocytes.

P7.

THE ROLE OF CD206 IN PATIENTS AND MOUSE MODEL OF BEHÇET'S DISEASE

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Although the exact etiology of Behçet's disease (BD) remains unclear, a complex interaction of T cells and antigen presenting cells is known to be important in the immunopathogenesis of BD. This study was aimed to identify the role of CD206, one of pattern recognition receptors, in BD. CD206 positive cell frequencies were analyzed by flow cytometry in patients and mouse model, and several serum cytokine levels were detected by ELISA in mice. The ligand of CD206 treatment down-regulated the frequencies of CD206 in vitro culture of PBMC and in vivo treatment of normal mice. The treatment with a ligand of CD206 also decreased disease severity score and induced improvement of symptoms accompanied with regulation of cytokines in mice. CD206 positive cell frequencies were different between ligand responsive BD mice and non-responsive BD mice. All these findings showed that expression of CD206 was correlated to the BD symptoms.

P8.

SERUM LIPOPOLYSACCHARIDE LEVELS ARE ASSOCIATED WITH DISEASE ACTIVITY OF THE ORAL MUCOUS MEMBRANE IN BEHÇET'S DISEASE.

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Purpose. Gut microbial dysbiosis is capable of inducing systemic, extra-intestinal and ocular inflammation in murine models. We hypothesise that the translocation of gut microbial endotoxins through the dysfunctional mucous membrane drives disease. We investigated the link between serum lipopolysaccharide (LPS) levels, as a surrogate marker of increased gut permeability, and disease activity in Behçet's Disease (BD).

Methods. BD patients attending the multidisciplinary Behçet's clinic at the Birmingham and Midland Eye Centre, UK, underwent complete clinical assessment for evidence of disease activity using the validated BD Current Activity Form. Active ocular inflammation was defined as at least a 2+ increase in intraocular cells between clinic visits or the presence of a hypopyon as assessed by slit lamp, whilst active oral lesions were defined as the appearance of new ulceration of the oral mucous membrane. Serum LPS levels were quantified by ELISA with Limulus Amebocyte Lysate chromogenic endpoint assay. Serum LPS levels in patients with BD (n=23) were compared with serum samples from healthy volunteers (n=10) and disease controls (ocular mucous membrane pemphigoid (OcMMP; n=15)). We obtained longitudinal serum samples from BD patients (n=7) to monitor changes in serum LPS with disease phenotype and progression. Non-parametric statistical analyses were analysed by Mann-Whitney U test and Kruskal-Wallis test.

Results. There was no statistically significant difference in the LPS levels between patients with BD [median: 0.244 EU/ml; interquartile range (IQR): 0.108-0.778], OcMMP [0.175; 0.140-0.202], and healthy controls [0.200; 0.164-0.251] (p=0.504). BD patients with inactive oral lesions had significantly higher levels of LPS (0.462; 0.168-0.856) compared to those with active oral lesions (0.119; 0.057-0.148) and healthy controls (0.200; 0.164-0.251) (p=0.012). There was no difference in the LPS levels of BD patients with active vs. inactive ocular inflammation (p=0.142).

Conclusions. Serum LPS levels are associated with oral mucous membrane disease activity and indicates a potential role for microbial translocation in the inflammatory pathophysiology of BD.

P9.

CXCL1, CXCR1 AND IL-17A

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Purpose. Behçet's disease (BD) is a chronic inflammatory disease that is characterized by the recurrent involvement of oral and genital ulceration, cutaneous, ocular, and vascular lesions. The pathogenesis of BD is unknown, however, genetic and environmental factors participate in the inflammatory processes. IL-8, IL-12 and IL-17 participates in the pathogenesis of BD. Dysregulation of cytokines such as TNF-IL-8/ CXCL1 is a leukocyte chemoattractant factor and accumulates neutrophils into the lesional skin. CXCL1 is produced by macrophage, endothelial cells, neutrophils and keratinocytes. Susceptibility of single nucleotide polymorphism of CXCL1 in BD has been mentioned. IL-17 protein secretion by peripheral blood mononuclear cells was influenced by different allele of the IL-17A gene. However, little is known about the association of IL-17A with each of the clinical phenotypes of BD.

Materials and methods. Genomic DNA was analyzed by PCR with specific primers. The PCR products were sequenced using an ABI Big Dye cycle sequencing termination kit (Applied Biosystems, Foster City, CA). Fisher's test was performed to examine the statistical correlations. P-values of <0.05 were considered to be statistically significant.

Results. Genotype frequency of CXCL1 (-353T/A) SNP in BD and healthy controls was TT:62.0%, TA:32.2%, AA:5.7% in healthy donor (n=87), and TT:50%, TA:44.3%, AA:4% in BD patients (n=70). The frequency of TA genotype of BD patients did not accept significant difference with healthy donor. There were no significant difference of allele frequencies of CXCR-1 (+2607G/C), CXCR2 (+785C/T, +1208T/C) SNP in BD patients and healthy controls.

There were no significant difference in the genotype and allele frequency of IL-17A gene SNP between BD patients and controls. No significant differences in the genotype frequency of IL-17 gene SNP were identified between populations with or without clinical signs, such as skin involvement, ocular involvement, vascular involvement, arthropathy, epididymitis and central nervous involvement. However there was a higher tendency of IL-17 A genotype A frequency in BD group concerning interstitial involvement. Thus, these results suggest the possibility that there may be some association between IL-17A gene mutation and gastrointestinal tract formation in BD. The biological function of IL-17 towards ulcer formation in the gastrointestinal tract will be required in the future experiments.

Conclusion. Our result suggests the possibility that there may be an association between the IL-17A gene polymorphism and gastrointestinal symptoms in patients with BD, although there was not a significant difference.

P10.

SERUM AMYLOID A STIMULATED PRODUCTION OF PRO-INFLAMMATORY CYTOKINES BY PERIPHERAL BLOOD MONOCYTES IN PATIENTS WITH BEHÇET'S DISEASE

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Background. Behçet's disease (BD) is a systemic inflammatory disorder characterised by an abnormal innate and adaptive immune response with consequent hyper-activation of pro-inflammatory mediators. The main clinical features of BD are represented by recurrent oral aphthosis, genital ulcers and chronic relapsing bilateral uveitis configuring the wide known "triple symptom complex". Serum amyloid-A (SAA) is an inflammatory biomarker recently associated to BD, whose production is influenced by mediators of inflammation such as IL-6, IL-1 and TNF- α . Moreover, it has been shown that several innate immune cells including neutrophils produce pro-inflammatory cytokines through inflammatory activation after SAA stimulation.

Objectives. The aim of this study was to assess the putative role of SAA in the pathogenesis of Behçet's disease.

Methods. Monocytes obtained from heparinised venous blood of Behçet's disease patients (BD, n=14) and healthy controls (HC, n=7) have been stimulated or not with SAA, and serum cytokine levels of IL-1 β , IL-18, IL-6 and TNF- α have been consequently assessed using a multiplex bead analysis. Statistical approaches including two-tailed Mann-Whitney test (for two non-parametric groups) and Student's t-test (for two parametric groups) have been used for statistical comparisons between groups. Correlations have been demonstrated using Spearman's correlation (two-tailed p-value) analysis.

Results. We noticed an increased production of IL-1 β ($p=0.0017$), TNF- α ($p=0.0003$) and IL-6 ($p=0.0003$) in BD monocytes after SAA stimulation. The amount of pro-inflammatory cytokines production did not differ between HC group and BD group. We also found that IL-1 β levels were positively correlated with IL-6 ($r=0.842$, $p<0.001$), and TNF- α ($r=0.889$, $p<0.001$), whilst a positive correlation between TNF- α levels and IL-6 levels ($r=0.894$, $p<0.001$) was shown. Also IL-18 showed a positive trend with no significant differences between the two groups.

Conclusions. SAA might trigger peripheral blood monocytes of BD patients to overproduce pro-inflammatory mediators, thus contributing to the inflammatory manifestations typically observed in this disorder.

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P11.

INCREASED SENESCENT CD8+ T CELLS IN THE PERIPHERAL BLOOD MONONUCLEAR CELLS OF BEHÇET'S DISEASE PATIENT

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Background. Behçet's disease (BD) is a chronic inflammatory disease characterized by recurrent mucocutaneous ulceration and complications such as blindness and large vessel inflammation. Immunosenescence, aging of immune system, is related to increased susceptibility to infectious diseases, vaccine failure, and chronic low grade systemic inflammation. The role of immunosenescence in BD is not well understood.

Objective. We investigated the differences in the frequencies of immunosenescent cells in peripheral blood mononuclear cells in BD patients and controls.

Methods: Peripheral blood mononuclear cells were extracted from age-matched active BD patients (n=19), inactive BD patients (n=20), disease controls (n=15) and healthy controls (n=15). Using flow cytometry, the frequencies of senescent CD4+ T cells (CD3+ CD4+ CD27- CD28- cells), CD8+ T cells (CD3+ CD8+ CD27- CD28- cells), and B cells (CD19+ CD27- IgD- cells) were analyzed. The differences among the groups, the correlation with age in normal controls, and whether the steroid treatment or specific organ involvement affected the frequencies of senescent immune cells were investigated.

In addition, senescent-associated β galactosidase (SA- β -Gal) activity was investigated in CD8+ T cells, using flow cytometry with 5-Dodecanoylamino fluorescein Di- β -D-Galactopyranoside (C12FDG).

Results. In active BD patients, the frequency of CD3+ CD8+ CD27- CD28- cells was significantly higher than in disease controls and in healthy controls, respectively. Also, the frequency of CD3+ CD8+ CD27- CD28- cells increased significantly with the age in normal controls, in accordance with the previous literature. Other senescent immune cells did not show significant differences. Neither the steroid treatment nor specific organ involvement had significant influence on frequencies of senescent immune cells. Frequencies of SA- β -Gal+ cells among CD8+ T cells were significantly higher in active BD and in inactive BD compared to those in disease controls and healthy controls HC, respectively. **Conclusion.** CD8+CD28- T cells, or senescent CD8+ T cells, are increased in peripheral blood mononuclear cells of patients with BD.

P12.

IL-17 EXPRESSION BY LYMPHOCYTES IS HIGHER IN BEHÇET'S DISEASE COMPARED TO TAKAYASU'S ARTERITIS

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Objectives. Interleukin-17 (IL-17) has been associated with the pathogenesis of various autoimmune/inflammatory diseases. The aim of this study was to investigate the expression of Th17-related immunity in two phenotypically different disorders: Behçet's disease (BD) with innate and Takayasu's arteritis (TAK) with adaptive immune responses.

Methods. Peripheral blood mononuclear cells (PBMC) from 37 patients (age: 38.5 \pm 9.8 years) with BD, 25 patients (age: 42.7 \pm 15.5 years) with TAK and 25 HC (age: 39.1 \pm 9.3 years) were cultured in Th17 inducing conditions (IL-6, PHA, IL-1 β and IL-23) for 6 days. Cultured cells were stained with CD4, CD8, CD3, TCR γ /delta, CD19, IFN- γ and IL-17 antibodies to determine the intracellular cytokine secretion by flow cytometry.

Results. In BD patients, IL-17 expression by CD4+ T cells was observed to be higher than TAK patients ($p=0.02$). IL-17 expression by CD8+ and $\gamma\delta$ + T cells was also higher in BD compared to HC ($p=0.004$, $p=0.003$ respectively). No differences were observed between the groups in the IL-17 production by B cells. Under Th17 inducing conditions, production of IFN- γ by CD4+, CD8+, and $\gamma\delta$ + T cells were also higher in BD compared to TAK patients and HC ($p<0.05$ in all). **Conclusion.** Our results suggest that under Th17 stimulating conditions, T cells express higher IL-17 levels in BD. More prominent IL-17 and IFN- γ production by all lymphocyte subsets in BD might be associated with the increased innate responses and early tissue neutrophil infiltrations in BD, which is not observed in TAK.

Key words. Behçet's disease, Takayasu's arteritis, interleukin-17, Th17 cells

P13.

A SERUM METABOLOMIC ANALYSIS IN BEHÇET'S DISEASE: A PRELIMINARY STUDY

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Background. Behçet's disease (BD) is a chronic inflammatory disorder of unknown etiology. The diagnosis of BD is mainly based on clinical manifestations and remains a challenge in clinical practice, due to the fact that there are no diagnostic biomarkers available currently. Recently metabolomics has been applied in discovering and validating biomarkers of inflammatory diseases. This study aims to identify serum metabolites associated with BD diseases and to search for the metabolites responsive to treatment using metabolomics approach.

Methods. Medical records and serum samples of 24 pre-treated BD patients and 12 post-treated patients at Peking Union Medical College Hospital were collected. 25 gender and age matched healthy volunteers serums were also collected. Metabolomics and lipidomics profiling were carried out by using UPLC-QTOFMS and UPLC-QTOFMS respectively. Raw mass spectrometric data were processed using Progenesis Q1 software. Statistical analysis and putative ion identification on the post-processed data were conducted utilizing MetaboLyzer.

Results. 24 BD patients (15 men and 9 women) were all Han Chinese population. Their mean age was 35.83 \pm 11.96 years old. Their disease duration of BD was 120 months (range 13–379). Unsupervised principal component analysis (PCA) plots of the lipidomics and metabolomics data showed separation of profiles

from BD patients and healthy controls. Statistical analysis of the data revealed statistically differential metabolites between BD patients and healthy controls. Identification of selected metabolites was confirmed by comparing MS/MS fragmentation pattern with authentic standards. It is of interest to note that treatment recovered some but not all of these differential metabolites.

Conclusions. Our study suggests that the altered levels of the metabolomics profile may be indicative in the diagnosis of BD. Some of the metabolites may provide insights for therapeutic effects.

P14.

BEHÇET'S DISEASE UNDER MICROBIOTIC SURVEILLANCE?

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Background. Behçet's disease (BD) is an idiopathic systemic disease driven by excessive T-cell response, possibly triggered by infectious antigens in genetically susceptible hosts. Crohn's Disease (CD) resembles BD in symptoms and intestinal inflammation, and recent studies demonstrated intestinal dysbiosis in CD patients. We, therefore, hypothesized that intestinal dysbiosis affects the immune system and BD.

Methods. Fecal and oral samples of 22 patients and 24 age, sex and ethnicity matched controls were collected and analyzed using 16S RNA sequencing

Results. Principle covariant analysis did not reveal distinction of different clusters of fecal samples and oral swabs from patients and healthy controls. We identified enrichment of fecal samples with Ruminococcus and of oral samples with Porphyromonas gingivalis in patients compared to controls. From subgroup analysis of patient samples we identified enrichment of fecal samples with Rikenellaceae and loss of Prevotella copri associated with uveitis. In addition, Enrichment of oral samples with Streptococcus sp and Neisseriaceae was associated with uveitis.

Conclusion. We show distinct deviations in both oral and fecal microbiota of patients with BD compared to healthy controls, and between BD patients with and without uveitis. Although these results suggest a possible role for dysbiosis in the pathogenesis of BD and could implicit new treatment strategies, these results should be confirmed in a separate BD cohort, and validation of causal relations of microbial shifts and BD need subsequent investigation in for instance eye models.

P15.

CD16+Vδ2 T CELLS AND THEIR FUNCTIONAL POTENTIAL IN BEHÇET'S DISEASE

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Background. Behçet's disease (BD) is a multisystem inflammatory disorder characterized by oral and genital ulcerations, ocular, joint and skin lesions with episodes of exacerbation and remissions. The pathogenesis remains inconclusive but immuno-dysregulation involving $\gamma\delta$ T cells (V δ 2 subsets) have been reported. V δ 2 T cells are the major subset of $\gamma\delta$ T cells which is a prototype of atypical T cells responsible for bridging the innate and adaptive immunity. CD16 (Fc γ RIII) differentiates these V δ 2 T cells into distinct functional subsets which are less studied in BD. This study investigated the proportion of CD16+V δ 2 T cells in BD and their potential roles on the induction and/or maintenance of pro-inflammatory characteristics of the disease.

Methods. PBMCs from BD patients and healthy controls (HC) were analysed by flow cytometry for the expression of V δ 2, CD16 and CD56. Intracellular IFN γ , IL17, Perforin and TNF α expression and the effect of phosphoantigen (HMB-PP) stimulation on CD16+V δ 2 T cells was also investigated.

Results. CD16+V δ 2 T cells were significantly increased in BD compared to HC. CD16 expression was higher in CD56+ rather than CD56-V δ 2 T cells and CD56+CD16+V δ 2 T cells were significantly increased in BD. CD16+V δ 2 T cells showed greater potential to express TNF- α and IL17 whereas CD16-V δ 2 T cells expressed greater IFN γ . When compared with HC, CD16+V δ 2 T cells showed significantly increased IL17 whilst IFN γ expression was significantly decreased in BD with no significant differences in Perforin and TNF- α expression. Finally, CD16+V δ 2 T cells were up-regulated following phosphoantigen stimulation.

Conclusion. Increased CD16+V δ 2 subset found in BD in association with altered cytokine expression underpins a potential role of these cells in the disease pathogenesis which might be responsible for inducing and/or maintaining the pro-inflammatory characteristic of BD.

P16.

DIFFERENTIAL DIAGNOSTIC PROBLEMS IN PATIENTS WITH ADAMANTIADIS-BEHÇET'S DISEASE

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The 48 year-old female patient presented in our emergency department with aching mucosal erosions in the mouth and lip area, itchy lesions on hands and knees and in a bad general condition. In addition, she reported a recurrent herpes labialis (approximately every 2 months) over several years. The patient suffers from Adamantiadis-Behçet's disease with oculo/mucocutaneous manifestations: bilateral recurrent panuveitis, macular oedema, oral aphthous ulcers, recurrent genital ulcers, and arthralgia. The oral mucosa of the patient was covered with ulcers and small blisters on an erythematous base. The right lower lip area was covered by honey-yellow crusts. On the left forearm and elbow, both femur and tibia, gluteal and genital area multiple small vesicles on erythematous base or erythematous papules and plaques were detectable. The patient has acutely being treated with oral prednisolone because of the history of recurrent uveitis and the suspicion of a disease recurrence. Because of the untypical clinical picture of the ulcers biopsies were performed. The histology of the papules on the left elbow and femur detected an erythema exsudativum multiforme. The laboratory examination showed an active Cocksackie infection. This case is a good example of how misleading the history of a patient may be and the importance to know and recognise the special features of Adamantiadis-Behçet's disease

P17.

OSTEOPONTIN LEVELS AND OTHER IN OCULAR BEHÇET'S DISEASE: A CONTROLLED STUDY

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Aim. Osteopontin (OPN) is a cytokine involved in inflammatory and autoimmune mechanisms. In this study, we aimed to investigate OPN in active and inactive Behçet's uveitis

Patients and methods. Twenty-two patients with Behçet Disease (BD) were assessed by the same ophthalmologist for eye involvement. Blood samples from each patients were taken twice: first during an eye attack and after about three months, after the attack subsided. OPN, Interleukin 12 (IL-12) and interleukin 10 (IL-10) levels were measured. Eighteen patients with active systemic lupus erythematosus (SLE) along with 18 age and sex-matched healthy subjects were also studied.

Results. OPN levels in patients with active Behçet's uveitis (96.79 ± 34.09 ng/mL) and SLE group (119.88 ± 66.55 ng/mL) were higher than in healthy controls (67.29 ± 24.29 ng/mL) ($p < 0.05$, $p < 0.01$, respectively) while there was no significant differences in OPN levels between patients with active Behçet's uveitis and SLE. No significant difference in the levels of OPN was found between active and inactive periods of patients with Behçet's uveitis. OPN showed positive correlation with C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels in SLE patients ($p < 0.05$, $p < 0.05$, respectively). No significant differences were found in the levels of IL-10 and IL-12 between patients with active Behçet's uveitis and healthy controls. In SLE patients, IL-10 and IL-12 levels were significantly higher compared with healthy controls ($p < 0.001$, $p < 0.001$, respectively), and active uveitis group ($p < 0.01$, $p < 0.001$, respectively). There were no significant differences in IL-10 and IL-12 levels between patients with active Behçet's uveitis and those with inactive Behçet's uveitis.

Conclusions. No differences in OPN levels were observed not only between active and inactive Behçet's uveitis, but also between active Behçet's uveitis and SLE. These results suggest that OPN might not be an important cytokine at least in eye disease in BD.

P124.

IMMUNOLOGICAL PROFILING OF AQUEOUS HUMOR IN BEHÇET'S DISEASE PATIENTS WITH ACTIVE OCULAR INVOLVEMENT

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Background. Behçet's disease (BD) is a systemic inflammatory disorder whose clinical hallmark are recurrent oral and genital ulcers, variably associated with various organ involvement. Uveitis and retinal vasculitis are among the most common manifestations, occurring in 60-80% of patients during the disease course. The pathogenesis of BD is still unclear. Some HLA-I residues have recently been shown to influence antigen binding and regulate the activation of both Natural Killer (NK) cells and CD8+ cytotoxic T lymphocytes. Higher levels of Natural Killer T (NKT) cells (CD3+ CD56+) have previously been found in the aqueous humor (AH) of patients with BD-related uveitis as compared to other types of uveitis. The aim of our study was to perform an immunological profiling of AH in BD patients with active uveitis, comparing it to that of AH from patients with active Vogt-Koyanagi-Harada (VKH) disease and subjects with cataract undergoing surgery.

Patients and Methods. AH of 8 adult patients with BD (according to 1990 ISGB criteria) and active uveitis, and of 8 patients with active VKH were analyzed. Patients were defined as having active uveitis when ≥ 2 cells in the anterior chamber (Hogan scale, 1950), and/or 2+ vitritis (Nussenblatt scale, 1990), papillitis, macular edema supported by optical coherence tomography and retinal vasculitis with active 'photo fundus', were found. AH from 5 subjects undergoing cataract surgery were included as controls. Cytokines' concentrations were determined with the Bio-Plex Pro Human cytokine 27-plex assay (Bio-Rad®). Frequency of NK and NKT cells was determined by flow cytometry using anti-CD3, -CD56, -CD16 antibodies.

Preliminary Results. Levels of IL-1 β , IL-1RA, IL-5, IL-7, IL-6, G-CSF, IFN- γ , IP-10, TNF- α were higher in AH from patients with BD and VKH compared to controls. In particular, we found a 3000-fold increase in IL-6 levels; G-CSF and IFN- γ were detected in AH from BD and VKH patients, but not in the control group. Lower levels of GM-CSF were found in BD and VKH patients as compared to controls. No differences were detected between BD and VKH patients regarding cytokine levels. However, HA from 4 BD patients showed a peculiar distinct pattern in terms of cytokine levels, when analyzed by unsupervised cluster analysis. The frequency of NKT (CD3+ CD56+) cells was higher in BD patients as compared to VKH, while that of NK (CD56+ CD3neg) and T cells (CD56neg CD3+) was similar. Finally, no difference was found between NKT and NK subsets in terms of proportion of CD16+ cells in both BD and VKH groups.

Discussion. Our preliminary results confirm the previous observation of increased NKT cells levels in BD uveitis as compared to VKH. In addition, AH of both BD and VKH groups showed increased levels of IL-6, G-CSF and IFN- γ , which might suggest their potential role in the immune-pathogenesis of those types of uveitis. A distinct cytokine profile able to distinguish the two conditions remains to be identified.

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P18.

HLA REVISITED IN EGYPTIAN PATIENTS WITH BEHÇET'S SYNDROME: NEW ASSOCIATIONS OF HLA ALLELES WITH SUSCEPTIBILITY, PROTECTION, PRESENTATION AND SEVERITY OF THE DISEASE

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Background. Behçet's syndrome is a multisystem autoimmune syndrome. Its manifestations usually start in the young adulthood affecting mainly the skin, eyes, Brain and blood vessels (1)

Objectives. The aim of the study was to perform HLA class I genotyping in a cohort of Egyptian patients with Behçet's syndrome and comparing them with HLA genotyping in healthy population (control group) to estimate the syndrome susceptibility and possible association between HLA and syndrome presentations.

Methods. Fifty-seven Egyptian patients with Behçet's syndrome fulfilling the International study group criteria for Behçet's syndrome (2) were recruited from the Rheumatology department, Cairo University teaching hospitals. HLA class I genotyping was done for all patients via sequence specific oligonucleotides probes at the National Cancer Institute. HLA class I genotyping data of normal control group was obtained from case control studies done on Egyptian population with a total of 221 individual (3-4).

Results. The studied 57 patients were divided into 50 males and 7 females. The mean age of patients was 35.28 ± 9.73 years with mean disease duration of 9.26 ± 7.36 years. The main clinical features were oral ulcers (100%), genital ulcers (100%), eye involvement (54%) neurological involvement (29%) and vascular involvement (36%) furthermore (33%) had bilateral visual acuity $\leq 6/60$ fulfilling the diagnosis of legal blindness. Certain HLA genotypes were significantly associated with susceptibility for Behçet's syndrome, the odds ratio (OR) for HLA-A68 was 8.4 (CI=2.9-25.9), HLA-B15 was 6.7 (CI=2.9-15.6), HLA-B51 was 6.6 (CI=3.4-13.0), HLA-A24 was 4.0 (CI=1.5-10.2) and HLA-A2 was 2.1 (CI=1.1-3.8). On the other hand HLA A3 genotype was found to be significantly protective with odds ratio of 0.003 (CI=0.01-0.6). HLA B51 was significantly associated with ocular disease with odds ratio of 3.47. Furthermore HLA B51 was associated with legal blindness with a significant odds ratio of 5.21.

Conclusions. HLA A68, B15, B51, A24 and A2 are associated with Behçet's syndrome susceptibility in the present cohort of Egyptian patients and A3 was found to be protective. HLA B51 is associated with ocular involvement and more important the blinding eye disease and can be considered as a poor prognostic marker for ocular disease.

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P20.

HLA-B5/51 GENOTYPE: AN ASSOCIATION WITH THE CLINICAL MANIFESTATIONS OF BEHÇET'S DISEASE

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Objective. To estimate the contribution of HLA-B5/51 genotype to the clinical manifestations and risk of Behçet's disease (BD) in two ethnic groups.

Subjects and methods. 146 BD patients fulfilling the International Criteria for BD (ICBD) were divided into two ethnic groups: 1) 86 patients from Dagestan (representatives of 8 ethnic nationalities in this region) with mean age 30.7 ± 9.6 years; disease duration – 8.8 ± 10.1 years; 2) 60 ethnic Russian patients, non-residents of Dagestan with mean age 32.9 ± 11.1 years; disease duration – 11.2 ± 10.1 years. All patients were examined at the V.A. Nasonova Research Institute of