Characteristics of T Cell Receptor Beta Repertoires Associated With Behçet's Syndrome Patients With Major Organ Involvements

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Abstract

Background

T lymphocytes are one of the major components of the adaptive immunity in Behçet's syndrome (BS) pathology. To further understand of the role of T cells in Behçet's syndrome (BS), we explore disease related T cell receptor (TCR) repertoires.

Methods

We performed a bulk sequencing of the TCR beta chain (TRB) of peripheral T-cells collected from 45 BS patients with panuveitis, intestinal involvement, and cardiovascular lesions and 10 cases of healthy controls (HC). Data analysis included peptide sequences, diversity analysis, variable (V)-joining (J) gene usage and K-nearest neighbor algorithm.

Results

We found significant differences in V, J and V-J combinations between the BS patients and HC. The decrease in TCR clone diversity indicates clonal expansion in BS. Although no significant difference in TCR clone diversity was observed among patient subgroups, the patients with panuveitis displayed the highest heterogeneous TCR distribution. In addition, a set of V-J genes could effectively discriminate between active and inactive BS patients with an area under the receiver operating characteristics (ROC) curve of 0.88 (95% CI: 0.77-1.00).

Conclusions

Clonal T cell expansion has been observed in BS patients. TCR profiles could help in the discrimination between active and inactive BS.

Background

Behçet's syndrome (BS) is a complex, heterogeneous inflammatory disorder, whose manifestations include recurrent oral aphthae, genital ulcers, skin lesions and ocular involvement [1]. In addition, patients with BS can present with a variety of major organ involvements, which represent the heterogeneous characteristics of the disorder [2]. Previously, by hierarchical clustering analysis using clinical variables, we identified five phenotype subgroups [3]. The subgroups of uveitis, gastrointestinal involvement, and cardiovascular involvement represent the most common patterns of major organ involvement.

Although the pathogenic mechanisms of BS remain obscure, host genetic background, a strong association with human leukocyte antigen (HLA)-B51, infectious agents and loss of immune homeostasis are regarded as potential risk factors for BS [4]. Adaptive T cells, innate T cells, myeloid cells, and their predominant cytokines are the key regulators of immune responses in the development of BS [5]. Substantial evidence has demonstrated that T cells play an essential role in immune dysfunction in BS pathogenesis and progression. T helper (Th) 1 and Th17 cells and their correspondent cytokines are associated with the activity of BS [4, 6, 7]. GWAS studies showed the variants in the IL23R-IL12RB2 region were related to BS susceptibility [8, 9]. Predominantly expressed on T cells [10], IL-23R is required for the terminal differentiation of IL-17-producing T-helper cells [11]. Genetic and molecular dysregulation of the IL-23/IL-17 signaling pathway plays an essential role in the inflammatory

responses in BS [12, 13]. Moreover, the aberrance of regulatory T (Treg) cells had an impact on the biological outcome of the immune response and could lead to an impaired inflammatory status in BS patients [14].

T cell receptor (TCR) is a heterodimer of membrane proteins that participate in the activation of T-cells in response to an antigen [15]. Most T cells in the blood express $\alpha\beta$ TCR chains [16], and each chain has a variable region with three hypervariable complementarity-determining regions (CDRs) - the exact region directly contacting with antigen peptide/MHC complex [17]. Each unique CDR3 sequence is a proxy for T cell clonotype and determines TCR diversity [18]. Deep profiling of T-cell repertoires could help us to better understand dynamic adaptive immune changes during the onset and progression of diseases [19].

Since no specific laboratory test is available for BS, the diagnosis is based primarily on clinical manifestations [20]. Exploring the TCR repertoire in immune-mediated diseases without a certain antigen could give us clues to trace the disease-specific antigen and provide biomarkers for early detection, diagnosis, and monitoring of diseases.

Previous studies examined the features of TCR in BS by flow cytometry with monoclonal antibodies for a TCR gene segment, as well as a reverse transcriptase polymerase chain reaction (RT-PCR) with specific primers followed by Southern blotting and hybridization with radiolabeled probes [21, 22]. Several preliminary observations have been made: a strict bias usage of Jbeta gene indicating T cell clonality expansions and its correlation with disease activity [21], and oligoclonal TCR Vbeta expressed in most BS [22]. Due to the immense diversity of TCR, the aforementioned techniques were limited by insufficient depth of coverage, providing a low-resolution overview of TCR profile and a lack of sequence information [23, 24]. Moreover, both studies were restricted by the small number of BS patients and the lack of information on organ involvement. Accordingly, a comprehensive diversity analysis could barely be conducted. The diversification of TCR profiles in BS remains unclear. Here, we explore and characterize disease-specific TCR repertoire signatures, by high-throughput TCR beta chain (TRB) sequencing of peripheral blood samples from BS patients with major organ involvement.

Materials And Methods

Patients and sample collection

The study was approved by the local Ethical Committee. A written informed consent for the collection of peripheral blood was signed by each of the participants. BS patients were enrolled from the division of Rheumatology and Immunology in Huadong Hospital. The International Criteria for BS (ICBD) [25] were selected for inclusion. The TCR repertoire data of healthy controls (HC) were derived from individuals with no history of infectious diseases, autoimmune diseases or cancer. The disease activity was assessed by Behçet's Disease Current Activity Form (BDCAF) [26].

We enrolled 45 BS patients with major organ involvement (uveitis, intestinal ulceration and cardiovascular involvement). As shown in Table 1, the median age of patients was 33 years. The sex ratio was M:F = 1.5:1. Uveitis and intestinal ulcers were the most common major organ involvements, followed by cardiac and vascular lesion, and central nervous system involvement as well. On the other hand, we collected blood samples from 10 sex- and age-matched HC subjects.

Table 1
Clinical characteristics of patients with Behçet's syndrome

	BS patients	HC
No. of patients	45	10
Sex (M/F)	27/18	6/4
Age (IQR), years	33 (29-45.5)	33 (28-42)
Disease duration (IQR), years	7 (5-11.5)	-
Clinical manifestation		-
Oral ulceration (%)	97.8	-
Genital ulceration (%)	64.4	-
Erythema nodosum (%)	44.4	-
Folliculitis (%)	22.2	-
Arthritis/Arthralgia (%)	8.9	-
Major organ involvement		
Uveitis (%)	35.6	-
Intestinal involvement (%)	35.6	-
Vascular involvement (%)	20	-
Cardiac lesion (%)	22.2	-
Central nervous system involvement (%)	8.9	-
BS: Behçet's syndrome; HC: healthy control; IQR: interquartile range;		

Definition of major organ involvement

Behçet's uveitis (BU) was defined as posterior, or panuveitis [27]. Diagnosis of intestinal BS (IBS) was confirmed by the characteristic endoscopic and radiological features, which helped to distinguish intestinal BD from other diseases [28]. Vascular involvement was defined as deep venous thrombosis, major vein (vena cava, hepatic) thrombosis and arterial thrombosis or aneurysms, detected by doppler ultrasonography and (or) magnetic resonance imaging (MRI) and (or) computerized tomography (CT) [29]. Cardiac lesions were documented as valvular regurgitation, intracardiac thrombi [30] and coronary artery disease [31], detected by echocardiography or coronary angiography and (or) CT. Cardiovascular Behçet's syndrome (CVB) refers to patients with cardiac lesions or vascular involvement or both.

TCR repertoire sequencing

DNA samples were analyzed by high-throughput sequencing of TRB using the ImmuHub TCR profiling system at a deep level (ImmuQuad Biotech, Hangzhou, China). Briefly, a multiplex PCR amplification protocol was used.

Thirty cycles of first round PCR (PCR-1) amplification were carried out by using of 1 µg DNA with 31 TRBV and 12 TRBJ primers with the Multiplex PCR Kit (QIAGEN, Germany) to amplify the third complementarity-determining region (CDR3) of TRB. The PCR was performed under the conditions of 30 s at 94°C, 30 s at 68°C, and 60 s at 72°C, plus a final extension for 10 min at 72°C. The amplified TRB products were purified with the Agenecourt AMPure XP beads (A63882, Beckman Coulter, Inc.). To prepare final libraries compatible with the Illumina sequencing platform, a twenty-cycle 2nd round PCR (PCR-2) was applied and the Illumina sequencing indices were added. Final PCR products were purified by the Agenecourt AMPure XP beads (A63882, Beckman Coulter, Inc.) again. Purified final PCR products were then analyzed by Agilent 2100 Bioanalyzer System (Agilent) to determine the molar concentration for dilution required for sequencing sample pool preparation. Sequencing was performed on an Illumina NovaSeq system with PE150 mode (Illumina).

Raw Data processing

The raw sequencing data were then aligned with IMGT VDJ database with IgBLAST (NCBI) and PCR amplification and sequencing error correction based on clone frequency. The resulting nucleotide and AA sequences of TRB CDR3 were determined and those with out-of-frame and stop codon sequences were removed from the identified TRB repertoire. We further defined amounts of each TRB clonotype by adding numbers of TRB clones sharing the same nucleotide sequence of CDR3.

Statistical analysis

All statistical analysis was performed using R software (version 3.6.1). Indexes of normal distribution were expressed by mean $\pm$ standard deviation. A T-test for independent samples was performed on comparison between groups. Indexes of non-normal distribution were expressed by median and interquartile range (IQR; 25th-75th percentile). Principal component analysis (PCA) was used for clustering analysis. The diversity of the TCR repertoire was calculated based on Shannon's Entropy index. To identify the signature clonotypes, K-nearest neighbor analysis (by using the "kkn" package in R software) together with leave-one-out cross-validation was performed to estimate the area under the receiver operating characteristics (ROC) curve and the importance of individual variables.

Results

Repertoire Diversity in BS patients

First, we analyzed the abundance and diversity of TCR clonotypes between groups. On the basis of their frequency in the samples, the CDR3 sequence frequency was divided into four groups (<0.001, 0.001-0.005, 0.005-top 11, and top 10, respectively). Our results demonstrated that top 10 clones were more abundant in BS patients, while the distributions of low abundance clones (frequency <0.1 and 0.1-0.5%) were sparser (Figure 1A), which suggested putatively decreased TCR diversity. By calculating Shannon's entropy index, we found that the TRB diversity of BS patients was significantly lower than that of control subjects (Figure 1B). By applying principal component analysis (PCA) with the V and J gene segments of all samples, BS patients were clearly separated from the HC (Figure 1C). The above results suggested the TCR repertoire characterized the immune profiles of BS status, which could efficiently discriminate patients from healthy subjects.

TCR diversity among subgroups

We compared the characteristics and TCR diversity among the three BS subgroups (Table 2). No significant difference was found in the demographic variables as well as in the number and proportion of T, B, and Nature Killer cells between subgroups. As shown in Figure 2, although there was no substantial difference in TCR diversity among subgroups, the inter-group similarity of the V-J pairing was significantly lower in the BU subgroup (Figure 1C), indicating the relatively higher heterogeneity of TCR gene usage in the BU subgroup.

Table 2
Comparison of clinical features and flow cytometry among Behcet's syndrome subgroups

	BU	IBS	CVB	P
Sex (M/F)	8/7	9/6	10/5	NS
Age (IQR), years	30 (26, 41)	33 (26, 46)	44 (32, 49)	NS
Duration (IQR), years	8 (5, 17)	6 (2, 10)	6 (5, 11)	NS
Clinical manifestation				
Oral ulceration (%)	93.3	100	100	NS
Genital ulceration (%)	60	60	73.3	NS
Erythema nodosum (%)	53.3	20	60	NS
Folliculitis (%)	20	26.7	20	NS
Arthritis/Arthralgia (%)	6.7	13.3	6.7	NS
Major organ involvement				
Uveitis (%)	100	6.7	0	NS
Intestinal involvement (%)	6.7	100	0	< 0.001
Vascular involvement (%)	0	0	60	< 0.001
Cardiac lesion (%)	0	6.7	60	< 0.001
Central nervous system involvement (%)	6.7	0	20	NS
CD3 + T cells	1127.7 ± 688.5	1428.5 ± 545.1	1436.8 ± 739.8	NS
CD3 + T cells %	74.2 ± 10.5	76.9 ± 8.7	76.3 ± 8.9	NS
CD4 + T cells	524.1 ± 340.6	710.0 ± 289.8	791.0 ± 513.5	NS
CD4 + T cells %	34.2 ± 7.8	37.9 ± 7.3	41.0 ± 9.3	NS
CD8 + T cells	427.5 (267.5, 651.0)	534 (465, 924)	545 (342, 723)	NS
CD8 + T cells %	34.1 ± 7.8	34.8 ± 9.1	33.9 ± 9.7	NS
NK cells	196.5 ± 152.5	210.6 ± 138.2	149.4 ± 60.5	NS
NK cells%	13.0 (6.5, 16.0)	11.0 (7.0, 13.0)	15.0 (9.0, 17.4)	NS
Lymphocyte count	1545 (761, 1921)	1810 (1150, 2946)	1529 (1210, 1980)	NS

BU: Behçet's uveitis; IBS: intestinal Behçet's syndrome; CVB: Cardiovascular Behçet's syndrome; IQR: interquartile range; NK: nature killer; NS: Not significant

	BU	IBS	CVB	P
B cells	139.5 (74.3, 212.5)	172.0 (94.0, 297.0)	176.0 (156.0, 250.0)	NS
B cells%	12.5 (8.0, 19.0)	10.0 (7.0, 15.0)	12.0 (9.0, 13.0)	NS
BU: Behçet's uveitis; IBS: intestinal Behçet's syndrome; CVB: Cardiovascular Behçet's syndrome; IQR: interquartile range; NK: nature killer; NS: Not significant				

Association between TCR repertoire and disease activity

Finally, we compared the TCR repertoire between patients with active and inactive disease. A series of clonotypes were identified for differential abundance in the two groups (Table 3). We also developed a K-nearest neighbor model (see Materials and Methods) to evaluate whether the TRB profile could help differentiate active from inactive BS. The leave-one-out cross-validation yielded an area under the receiver operating characteristics (ROC) curve (AUC) of 0.88 (95% CI: 0.77-1.00; Figure 3).

Table 3
Differential clonotypes identified between active and inactive Behçet's syndrome

	No. of positive samples in active group (20)	No. of positive samples in inactive group (23)	<i>P</i>
TGTGCCAGCAGTTTATTTTTATCTTCACCCCTCCACTTT	0/20	8/15	0.00425
TGTGCCACCAGCGGAATAGCGGGAGAGGCGCAGTATTTT	6/14	0/23	0.00636
TGTGCCAGCAGTTACTTGGCTAGCGGGCCCAATGAGCAGTTCTTC	6/14	0/23	0.00636
TGTGCCAGTAAGACTAGCCCCAACACCGGGGAGCTGTTTTTT	6/14	0/23	0.00636
TGTGCCAGCAGTTACCAGGGGAGTCAGCCCCAGCATTTT	0/20	7/16	0.01001
TGTGCCAGCAGTTACTCGACGCCGCTCATGGAGCAGTTCTTC	0/20	7/16	0.01001
TGTGCCAGTAGTGTCCTACTAGCGGGAGTGCTTAATGAGCAGTTCTTC	0/20	7/16	0.01001
TGTGCCAGCAAACCTCCGGGACAGAACACCGGGGAGCTGTTTTTT	0/20	7/16	0.01001
TGTGCCAGCAGTTCCTACAATGAGCAGTTCTTC	5/15	0/23	0.01611
TGTGCCAGCAGTGAAGCCGCATCTGGGGCCAACGTCCTGACTTTC	5/15	0/23	0.01611
TGTGCCACCAGTGATTTGCAGTTGGACACCGGGGAGCTGTTTTTT	5/15	0/23	0.01611
TGTGCCAGCAGTGAAGGGGGCTCTGGGGCCAACGTCCTGACTTTC	5/15	0/23	0.01611
TGTGCCAGCAAGAAAGCGCCGAACACCGGGGAGCTGTTTTTT	5/15	0/23	0.01611
TGTGCCAGCAGCTTAGCAGCGGGATTGCAGTTCTTC	7/13	1/22	0.01655
TGTGCCACCAGTGATTTCTGGGGACAGAGGCACAGATACGCAGTATTTT	7/13	1/22	0.01655
TGTGCCAGCAGTGAAGGGACTAGCGGGAGCAATGAGCAGTTCTTC	7/13	1/22	0.01655
TGTGCCAGCAGCAGGCGGGAGAGGAGGGAGCTGTTTTTT	0/20	6/17	0.02292
TGTGCCAGCAGTTACGGGGGAAAAGGGAACATTTCAGTACTTC	0/20	6/17	0.02292
TGTGCCAGCAGCAGGGGACAGGGGCAACTATGGCTACACCTTC	0/20	6/17	0.02292
TGTGCCAGCAGTTACTCCCAGGACAGGGGGCGAACTATGGCTACACCTTC	0/20	6/17	0.02292
TGTGCCAGTAGTATAACCGGGCCACTGACCGGGGAGCTGTTTTTT	0/20	6/17	0.02292
TGTGCCAGCTCACCACCAGGCGGATCAGATACGCAGTATTTT	0/20	6/17	0.02292
TGTGCCAGCAGCTGGGACCCAGAGCAGGTCACCGGGGAGCTGTTTTTT	0/20	6/17	0.02292
TGTGCCAGCAGCTCCCCCGGGAGAACTACAATGAGCAGTTCTTC	0/20	6/17	0.02292
TGTGCCAGTAGTATGTCAGCGAACTATGGCTACACCTTC	6/14	1/22	0.03768
TGCAGCGCCCGACCGGGGGGAAATGAGCAGTTCTTC	6/14	1/22	0.03768

	No. of positive samples in active group (20)	No. of positive samples in inactive group (23)	<i>P</i>
TGTGCCAGCTCAGGACAGGACACTAGCACAGATACGCAGTATTTT	6/14	1/22	0.03768
TGTGCCAGCAGTTACTCGACAGGGGGCTATGGCTACACCTTC	4/16	0/23	0.03926
TGTGCCACTACTATGGACAGGGATGGGGCTGGGGCCAACGTCCTGACTTTC	4/16	0/23	0.03926
TGTGCCAGCAGCCCACCAGGTACCTCTGGGGCCAACGTCCTGACTTTC	4/16	0/23	0.03926
TGTGCCAGCAGCTTGGGTCAGGGGGAAGTAGGCTACACCTTC	4/16	0/23	0.03926
TGTGCCAGCAGTTACTCCGGCAGTGCCCAAGAGACCCAGTACTTC	4/16	0/23	0.03926
TGTGCCTGGAGTGTACAAAGCGGGAGTACTGAGCAGTTCTTC	4/16	0/23	0.03926
TGTGCCAGCAGTAGCGGCGTCCGGGAGCTGTTTTTT	4/16	0/23	0.03926
TGTGCCAGCAGCTTGGGGGGAGGGAATCAGCCCCAGCATTTT	4/16	0/23	0.03926
TGTGCCATAAAACCGGGACAGACCACAGATACGCAGTATTTT	4/16	0/23	0.03926
TGCAGCGTTGAAGTTACCAGGGATTACCCCCTCCACTTT	4/16	0/23	0.03926
TGCAGCGTTGATTTGCAATACAATGAGCAGTTCTTC	4/16	0/23	0.03926
TGCAGCGCAGGGGCTTACAATGAGCAGTTCTTC	4/16	0/23	0.03926
TGTGCCAGTAGCATTATCGGCAATGAGCAGTTCTTC	4/16	0/23	0.03926
TGTGCCAGCAGCTTGACAGGGACCCTCTATGGCTACACCTTC	4/16	0/23	0.03926
TGTGCCAGCAGTGACTCAGCGGGAGGGCCAGATACGCAGTATTTT	4/16	0/23	0.03926
TGTGCCAGCAGCGAGATGGAACATCAGCCCCAGCATTTT	4/16	0/23	0.03926
TGTGCCAGCAAAGTAGGACAGGGCGATGAGCAGTTCTTC	4/16	0/23	0.03926
TGCAGCGTCCCCTCCGGACAGGGGGCAAGGGGCTACACCTTC	4/16	0/23	0.03926
TGTGCCAGCAGTTTGGTAGAATACAATGAGCAGTTCTTC	4/16	0/23	0.03926
TGTGCCACCAGCAGGACCTATGGGGGGGATCAGCCCCAGCATTTT	4/16	0/23	0.03926
TGTGCCAGCAGCTTAGCGGGGGTCTAACCAGGGGAGCTGTTTTTT	4/16	0/23	0.03926
TGTGCCAGCAGCTTGGCCGGGACAAACACCGGGGAGCTGTTTTTT	4/16	0/23	0.03926
TGTGCCAGCAGTTTTTTCGGGACAGCCTTATGGCTACACCTTC	4/16	0/23	0.03926
TGTGCCAGCAGTGAATTATGGGCGGGGGGAAAGATACGCAGTATTTT	4/16	0/23	0.03926
TGCAGCGTTGAAGATCAGGATTCGTAAGTACGATACGCAGTATTTT	4/16	0/23	0.03926

Discussion

BS is a complex disorder with aberrant T-cell activation in the initiation and perpetuation of the disease [4]. The lack of a specific diagnostic test impedes the early detection of BS. TCR, a distinct immune biomarker of T cells, has been applied to better understand and track the potential disease driven peptide [32]. Since TCR repertoire can change greatly with the onset and progression of diseases, depicting TCR profile could help to illustrate the diverse immune status [18], and predict the outcome of disease [23].

For the first time, we employed high-through sequencing to uncover the TRB repertoire in BS with certain major organ involvements - namely panuveitis, intestinal and cardiovascular involvement [33]. As with other autoimmune diseases [19, 34-36], a diminished diversity and increased clonality of TCR repertoire were demonstrated in our cohort, which might indicate an ongoing autoreactive clonotype expansion in BS. By using V-J pairing index, we identified a diagnostic tool to discriminate BS from HC. To note, the difference of V-J pairing and the inter-group similarity among subgroups of BS reveal distinct T cell signatures among BS patients with varied organ involvements, which partially explains the immune pathogenesis of the clinical heterogeneity of BS [3]. Last but not least, we identified the preference of clones and V-J combinations between active and inactive disease, which could help developing TCR biomarkers for detecting active BS.

Our study suggested that the differences in V-J pairing and specific clones between BS patients and HC could represent the disease-associated T cells' responses. TCR repertoire is shaped by biases during V(D)J recombination and by the subsequent expansion and deletion of certain clones upon antigen recognition during T cells' development in the thymus and later in the periphery [37, 38]. The V(D)J recombination mechanism generates many more antigen receptor sequences than others [38]. Before further interpretation, we should better consider the context. First, TCR clonal sharing is determined by the same epitope, which means the antigen is presented by the same or similar MHC-alleles [23]. Although the exact mechanism by which TCR repertoire interacts with peptide/MHC has remained controversial [39], MHC Class II loci play a crucial role in determining thymic selection of the V-gene usage in TCR repertoire [40, 41]. However, due to the constraint of sample size, we could not apply expression quantitative trait locus (eQTL) mapping to test for associations between genetic variation and TCR V-gene usage in the current study. Moreover, the immense level of receptor degeneracy [42] and cross-reactivity of TCRs could underestimate the presence of TCR responses to common autoantigens between patients [43]. Notably, TRBJ2-7 gene usage was more enriched in patients with intestinal involvement and cardiovascular lesions than in patients with uveitis. Previous studies have proved the TRBJ2-7 segment was abnormally expressed in patients with systemic lupus erythematosus [36, 44], and chronic hepatitis B [45]. Collectively, public TCR clones existing in BS subgroups could suggest that distinct antigen-specific T cell cloning and expansion is associated with different phenotypes.

Additionally, our study demonstrated the diverse V-J combinations and distinct clone usage between different disease activity groups. However, the association of distinct TCR and disease activity is not conclusive in other connective tissue diseases. Lu et al.[46] found the Gini index of the V-J gene combinations and SLE-associated clones was correlated with the disease activity, while a longitudinal study of TCR clonotypes revealed that the repertoire diversity remained unchanged in 9/11 patients with either active or quiescent SLE [19]. In RA, TCR diversity and the abundance of differentiated Th17 T cells were significantly correlated with disease activity [35]. Analyses of collagen-stimulated blood cells from early HLA-DR4 RA patients showed that TRBV25-bearing T cells were more frequently expanded in those with moderate to high disease activity [47].

Our study has several limitations. First, due to the rarity of patients with major organ involvement, the sample size was relatively small, which could impair the statistical power. Considering potential factors that may confound the TCR characteristics, future studies of larger cohorts and long-term outcome measurements in BS patients and matched controls are required to better understand the immunological significance of TCR changes [48, 49]. Second, the current study did not include patients with only skin and (or) articular lesions, who make up the majority of BS. An essential biomarker might predict which patients with only skin and (or) articular manifestations are prone to develop major organ involvement. Thus, subsequent research should find the specific TCR profiles of BS patients with major organ involvement. Currently we investigated TCR profiles at the pan-T-cell level, which could mix the distinct features of TRB from different cell subsets.

In summary, our study highlights the essential roles and dynamic complexity of the T cell immune responses in BS pathophysiology. BS patients exhibit a skewed TRB repertoire. The V-J combination, along with unique clones, could distinguish active from inactive BS, which suggests clonal expansions during disease activation. TCR repertoires could provide novel quantitative parameters for disease monitoring in BS.

Abbreviations

AUC area under the receiver operating characteristics (ROC) curve

BDCAF Behçet's Disease Current Activity Form

BS Behçet's syndrome

BU Behçet's uveitis

CVB Cardiovascular Behçet's syndrome

CT computerized tomography

CDRs complementarity-determining regions

eQTL expression quantitative trait locus

HC healthy controls

HLA human leukocyte antigen

IBS intestinal BS

ICBD The International Criteria for BS

MRI magnetic resonance imaging

PCA Principal component analysis

ROC receiver operating characteristics

RT-PCR reverse transcriptase polymerase chain reaction

TCR T cell receptor

Th T helper

TRB TCR beta chain

Treg regulatory T

Declarations

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Conflicts of Interest:

The authors declare no conflict of interest.

Author Contributions:

JZ contributed to the conception and design. JY, HB, CH, LJ, DL, and HM acquired the samples. JC and YS performed experiments. JG gave critical guidance during the project. JZ performed the analysis of the data and drafted the manuscript. All authors approved the final version of the manuscript.

Data Availability Statement:

The datasets generated for this study are available on request to the corresponding author.

Institutional Review Board Statement:

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Huadong Hospital, Fudan University.

Informed Consent Statement:

Informed consent was obtained from all subjects involved in the study.

References

1. Yazici H, Seyahi E, Hatemi G, Yazici Y: **Behçet syndrome: a contemporary view.** *Nature reviews Rheumatology* 2018, **14**(2):107-119.
2. Bettiol A, Hatemi G, Vannozzi L, Barilaro A, Prisco D, Emmi G: **Treating the Different Phenotypes of Behçet's Syndrome.** *Frontiers in immunology* 2019, **10**:2830.
3. Zou J, Luo JF, Shen Y, Cai JF, Guan JL: **Cluster analysis of phenotypes of patients with Behçet's syndrome: a large cohort study from a referral center in China.** *Arthritis research & therapy* 2021, **23**(1):45.
4. Leccese P, Alpsoy E: **Behçet's Disease: An Overview of Etiopathogenesis.** *Frontiers in immunology* 2019, **10**:1067.

5. Tong B, Liu X, Xiao J, Su G: **Immunopathogenesis of Behcet's Disease.** *Frontiers in immunology* 2019, **10**:665.
6. Aktas Cetin E, Cosan F, Cefle A, Deniz G: **IL-22-secreting Th22 and IFN- γ -secreting Th17 cells in Behçet's disease.** *Modern rheumatology* 2014, **24**(5):802-807.
7. Sonmez C, Yucel AA, Yesil TH, Kucuk H, Sezgin B, Mercan R, Yucel AE, Demirel GY: **Correlation between IL-17A/F, IL-23, IL-35 and IL-12/-23 (p40) levels in peripheral blood lymphocyte cultures and disease activity in Behcet's patients.** *Clinical rheumatology* 2018, **37**(10):2797-2804.
8. Remmers EF, Cosan F, Kirino Y, Ombrello MJ, Abaci N, Satorius C, Le JM, Yang B, Korman BD, Cakiris A *et al*: **Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behçet's disease.** *Nature genetics* 2010, **42**(8):698-702.
9. Mizuki N, Meguro A, Ota M, Ohno S, Shiota T, Kawagoe T, Ito N, Kera J, Okada E, Yatsu K *et al*: **Genome-wide association studies identify IL23R-IL12RB2 and IL10 as Behçet's disease susceptibility loci.** *Nature genetics* 2010, **42**(8):703-706.
10. Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, Pflanz S, Zhang R, Singh KP, Vega F *et al*: **A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R.** *Journal of immunology* 2002, **168**(11):5699-5708.
11. McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, Blumenschein WM, McClanahan TK, O'Shea JJ, Cua DJ: **The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo.** *Nature immunology* 2009, **10**(3):314-324.
12. Chi W, Zhu X, Yang P, Liu X, Lin X, Zhou H, Huang X, Kijlstra A: **Upregulated IL-23 and IL-17 in Behçet patients with active uveitis.** *Investigative ophthalmology & visual science* 2008, **49**(7):3058-3064.
13. Hamzaoui K, Bouali E, Ghorbel I, Khanfir M, Houman H, Hamzaoui A: **Expression of Th-17 and RORgammat mRNA in Behcet's Disease.** *Medical science monitor : international medical journal of experimental and clinical research* 2011, **17**(4):Cr227-234.
14. Ahmadi M, Yousefi M, Abbaspour-Aghdam S, Dolati S, Aghebati-Maleki L, Eghbal-Fard S, Khabbazi A, Rostamzadeh D, Alipour S, Shabani M *et al*: **Disturbed Th17/Treg balance, cytokines, and miRNAs in peripheral blood of patients with Behcet's disease.** *Journal of cellular physiology* 2019, **234**(4):3985-3994.
15. **Chapter 8 - The T Cell Receptor: Proteins and Genes.** In: *Primer to the Immune Response (Second Edition)*. Edited by Mak TW, Saunders ME, Jett BD. Boston: Academic Cell; 2014: 181-196.
16. Chiffelle J, Genolet R, Perez MA, Coukos G, Zoete V, Harari A: **T-cell repertoire analysis and metrics of diversity and clonality.** *Current opinion in biotechnology* 2020, **65**:284-295.
17. Davis MM, Bjorkman PJ: **T-cell antigen receptor genes and T-cell recognition.** *Nature* 1988, **334**(6181):395-402.
18. Rosati E, Dowds CM, Liaskou E, Henriksen EKK, Karlsen TH, Franke A: **Overview of methodologies for T-cell receptor repertoire analysis.** *BMC biotechnology* 2017, **17**(1):61.
19. Thapa DR, Tonikian R, Sun C, Liu M, Dearth A, Petri M, Pepin F, Emerson RO, Ranger A: **Longitudinal analysis of peripheral blood T cell receptor diversity in patients with systemic lupus erythematosus by next-generation sequencing.** *Arthritis research & therapy* 2015, **17**(1):132.
20. Davatchi F, Chams-Davatchi C, Shams H, Shahram F, Nadji A, Akhlaghi M, Faezi T, Ghodsi Z, Sadeghi Abdollahi B, Ashofteh F *et al*: **Behcet's disease: epidemiology, clinical manifestations, and diagnosis.** *Expert review of clinical immunology* 2017, **13**(1):57-65.

21. Esin S, Gül A, Hodara V, Jeddi-Tehrani M, Dilsen N, Koniçe M, Andersson R, Wigzell H: **Peripheral blood T cell expansions in patients with Behcet's disease.** *Clinical and experimental immunology* 1997, **107**(3):520-527.
22. Direskeneli H, Eksioğlu-Demiralp E, Kibaroglu A, Yavuz S, Ergun T, Akoglu T: **Oligoclonal T cell expansions in patients with Behçet's disease.** *Clinical and experimental immunology* 1999, **117**(1):166-170.
23. Minervina A, Pogorelyy M, Mamedov I: **T-cell receptor and B-cell receptor repertoire profiling in adaptive immunity.** *Transplant international : official journal of the European Society for Organ Transplantation* 2019, **32**(11):1111-1123.
24. Liu X, Wu J: **History, applications, and challenges of immune repertoire research.** *Cell biology and toxicology* 2018, **34**(6):441-457.
25. **The International Criteria for Behcet's Disease (ICBD): a collaborative study of 27 countries on the sensitivity and specificity of the new criteria.** *Journal of the European Academy of Dermatology and Venereology : JEADV* 2014, **28**(3):338-347.
26. Bhakta BB, Brennan P, James TE, Chamberlain MA, Noble BA, Silman AJ: **Behcet's disease: evaluation of a new instrument to measure clinical activity.** *Rheumatology* 1999, **38**(8):728-733.
27. Tugal-Tutkun I, Onal S, Altan-Yaycioglu R, Huseyin Altunbas H, Urgancioglu M: **Uveitis in Behcet disease: an analysis of 880 patients.** *Am J Ophthalmol* 2004, **138**(3):373-380.
28. Cheon JH, Kim WH: **An update on the diagnosis, treatment, and prognosis of intestinal Behcet's disease.** *Current opinion in rheumatology* 2015, **27**(1):24-31.
29. Seyahi E: **Behçet's disease: How to diagnose and treat vascular involvement.** *Best practice & research Clinical rheumatology* 2016, **30**(2):279-295.
30. Lee I, Park S, Hwang I, Kim M-J, Nah S-S, Yoo B, Song J-K: **Cardiac Behçet disease presenting as aortic valvulitis/aortitis or right heart inflammatory mass: a clinicopathologic study of 12 cases.** *The American journal of surgical pathology* 2008, **32**(3):390-398.
31. Chen H, Zhang Y, Li C, Wu W, Liu J, Zhang F, Zheng W: **Coronary involvement in patients with Behçet's disease.** *Clinical rheumatology* 2019, **38**(10):2835-2841.
32. Pacholczyk R, Ignatowicz H, Kraj P, Ignatowicz L: **Origin and T cell receptor diversity of Foxp3+CD4+CD25+ T cells.** *Immunity* 2006, **25**(2):249-259.
33. Hatemi G, Christensen R, Bang D, Bodaghi B, Celik AF, Fortune F, Gaudric J, Gul A, Kötter I, Leccese P *et al*: **2018 update of the EULAR recommendations for the management of Behçet's syndrome.** *Annals of the rheumatic diseases* 2018, **77**(6):808-818.
34. Hanson AL, Nel HJ, Bradbury L, Phipps J, Thomas R, KA LC, Kenna TJ, Brown MA: **Altered Repertoire Diversity and Disease-Associated Clonal Expansions Revealed by T Cell Receptor Immunosequencing in Ankylosing Spondylitis Patients.** *Arthritis & rheumatology* 2020, **72**(8):1289-1302.
35. Jiang X, Wang S, Zhou C, Wu J, Jiao Y, Lin L, Lu X, Yang B, Zhang W, Xiao X *et al*: **Comprehensive TCR repertoire analysis of CD4(+) T-cell subsets in rheumatoid arthritis.** *Journal of autoimmunity* 2020, **109**:102432.
36. Ye X, Wang Z, Ye Q, Zhang J, Huang P, Song J, Li Y, Zhang H, Song F, Xuan Z *et al*: **High-Throughput Sequencing-Based Analysis of T Cell Repertoire in Lupus Nephritis.** *Frontiers in immunology* 2020, **11**:1618.
37. Jung D, Alt FW: **Unraveling V(D)J recombination; insights into gene regulation.** *Cell* 2004, **116**(2):299-311.

38. Schatz DG, Ji Y: **Recombination centres and the orchestration of V(D)J recombination.** *Nature reviews Immunology* 2011, **11**(4):251-263.
39. Baker BM, Evavold BD: **MHC Bias by T Cell Receptors: Genetic Evidence for MHC and TCR Coevolution.** *Trends in immunology* 2017, **38**(1):2-4.
40. Sharon E, Sibener LV, Battle A, Fraser HB, Garcia KC, Pritchard JK: **Genetic variation in MHC proteins is associated with T cell receptor expression biases.** *Nature genetics* 2016, **48**(9):995-1002.
41. Garcia KC, Adams JJ, Feng D, Ely LK: **The molecular basis of TCR germline bias for MHC is surprisingly simple.** *Nature immunology* 2009, **10**(2):143-147.
42. Wooldridge L, Ekeruche-Makinde J, van den Berg HA, Skowera A, Miles JJ, Tan MP, Dolton G, Clement M, Llewellyn-Lacey S, Price DA *et al*: **A single autoimmune T cell receptor recognizes more than a million different peptides.** *The Journal of biological chemistry* 2012, **287**(2):1168-1177.
43. Frankild S, de Boer RJ, Lund O, Nielsen M, Kesmir C: **Amino acid similarity accounts for T cell cross-reactivity and for "holes" in the T cell repertoire.** *PloS one* 2008, **3**(3):e1831.
44. Sui W, Hou X, Zou G, Che W, Yang M, Zheng C, Liu F, Chen P, Wei X, Lai L *et al*: **Composition and variation analysis of the TCR β -chain CDR3 repertoire in systemic lupus erythematosus using high-throughput sequencing.** *Molecular immunology* 2015, **67**(2 Pt B):455-464.
45. Qu Y, Huang Y, Liu D, Huang Y, Zhang Z, Mi Z, An X, Tong Y, Lu J: **High-Throughput Analysis of the T Cell Receptor Beta Chain Repertoire in PBMCs from Chronic Hepatitis B Patients with HBeAg Seroconversion.** *The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale* 2016, **2016**:8594107.
46. Liu X, Zhang W, Zhao M, Fu L, Liu L, Wu J, Luo S, Wang L, Wang Z, Lin L *et al*: **T cell receptor β repertoires as novel diagnostic markers for systemic lupus erythematosus and rheumatoid arthritis.** *Annals of the rheumatic diseases* 2019, **78**(8):1070-1078.
47. Di Sante G, Tolusso B, Fedele AL, Gremese E, Alivernini S, Nicolò C, Ria F, Ferraccioli G: **Collagen Specific T-Cell Repertoire and HLA-DR Alleles: Biomarkers of Active Refractory Rheumatoid Arthritis.** *EBioMedicine* 2015, **2**(12):2037-2045.
48. Liu YY, Yang QF, Yang JS, Cao RB, Liang JY, Liu YT, Zeng YL, Chen S, Xia XF, Zhang K *et al*: **Characteristics and prognostic significance of profiling the peripheral blood T-cell receptor repertoire in patients with advanced lung cancer.** *International journal of cancer* 2019, **145**(5):1423-1431.
49. Spassova I, Ugurel S, Terheyden P, Sucker A, Hassel JC, Ritter C, Kubat L, Habermann D, Farahpour F, Saeedghalati M *et al*: **Predominance of Central Memory T Cells with High T-Cell Receptor Repertoire Diversity is Associated with Response to PD-1/PD-L1 Inhibition in Merkel Cell Carcinoma.** *Clinical cancer research : an official journal of the American Association for Cancer Research* 2020, **26**(9):2257-2267.

Figures

Fig 1A

Fig 1B

Fig 1C

Figure 1

The abundance and diversity of T cell receptor (TCR) clonotype. (A) The frequency distribution of different clonotypes. (B) The D50 index at the level of the V-J pairing indicated the TCR diversity of each group. (C) Principal component analysis (PCA) based on the abundance of T cell receptor (TCR) clones. BU: Behçet's uveitis; IBS: intestinal Behçet's disease; CVB: cardiovascular Behçet's disease; HC: healthy control.

Figure 2

Heat maps of average TRBV gene expression levels in BS and control groups. BU: Behçet’s uveitis; IBS: intestinal Behçet’s syndrome; CVB: cardiovascular Behçet’s syndrome; HC: healthy control.

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Figure 3

Receiver operating characteristic (ROC) curves showing the performance of differentiation active and inactive groups using the V gene, J gene and V-J combinations.