Molecular Immunology 116 (2019) 167–173

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

No difference in TCRβ repertoire of CD4+ naive T cell between patients with primary biliary cholangitis and healthy control subjects

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ARTICLE INFO

Keywords:
Primary biliary cholangitis
High-throughput sequencing
T cell receptor

ABSTRACT

Primary biliary cholangitis (PBC) is considered as a model of organ-specific autoimmune disease based on the serological findings of anti-mitochondrial antibodies (AMA), infiltrates of T cells, and selective destruction of epithelial cells in the liver. T-cell-mediated autoimmune mechanisms are considered to be involved in the pathogenesis of primary biliary cholangitis (PBC). In this context, we used a combination of multiplex-PCR, Illumina sequencing and IMGT/HighV-QUEST for a standardized analysis of the T cell receptor β-chain (TCRβ) repertoire of $CD4^+$ naive T cells in PBC patients compared with healthy volunteers. Nonfunctional TCRs were used to study the pre-selection TCR repertoire, as they are not subject to functional selection (positive and negative selection). Functional TCRs were used to study the post-selection TCR repertoire. The results showed that there was not significant difference between PBC patients and healthy volunteers in TCRβ diversity, CDR3 length distributions, degree of sequence sharing, and usage frequency of TRBV and TRBJ segments, no matter in Pre-selection or Post-selection repertoires. In conclusion, early events in thymic T cell development and repertoire generation are not abnormality in PBC patients. The breakdown of self-tolerance to autoantigen may be derived from other immunological dysregulation or environmental agents.

1. Introduction

Primary biliary cholangitis (PBC) is an autoimmune chronic cholestatic liver disease characterized by the presence of progressive inflammation, antimitochondrial antibodies (AMA), and destruction of interlobular bile ducts in the liver (Tanaka et al., 2019). The major antigens recognized by AMA have been identified as the E2 component of the pyruvate dehydrogenase complex (PDC-E2) (Younossi et al., 2018). Although the causative factors of PBC are still unknown, susceptibility genes and environmental risk factors such as infections (i.e. *Escherichia coli*) have been reported as important for the development of the disease (Koutsoumpas et al., 2014; Im et al., 2018). The mechanisms involved in the breakdown of self-tolerance is one of the most important issues in defining the basis of PBC. T lymphocytes play a pivotal role in this process (Jiang et al., 2018). The ability of the human T cell repertoire to recognize vast array of pathogens and initiate specific adaptive immune responses depends on the versatility of the T cell receptor (TCR), which consists of two disulphide- linked α and β chains both containing a variable (V), joining (J) and constant region together with a diversity (D) region in the β-chain (Hou et al., 2016a, b, c). Three hypervariable complementary determining regions (CDRs) (CDR1, CDR2, CDR3) have been found in the variable regions of β-chain. The CDR3 loops, which are somatically hypervariable, are generated by recombination of the germline V, D, and J gene segments and the deletion and insertion of nucleotides at the V(D)J junctions (Hou et al., 2016a, b, c). As CDR3 interacts most closely with the antigenic peptide, the diversity of CDR3 sequences provide a measure of T cell diversity. It is worth noticing that TCR locus recombination events can produce nonfunctional (out-of-frame) TCRs with frameshifts or stop codons (Zvyagin et al., 2014; Larimore et al., 2012). In this case, the T cell tries to arrange the second allele, and if the successful (in-frame) TCR formation occurs, the T cell carries both functional and nonfunctional TCR genes (Zvyagin et al., 2014). Although the nonfunctional TCR genes do not translate into functional TCRβ chains, the corresponding RNA is still present in the T cell at some concentration. Nonfunctional TCRs can be representative of the pre-selection TCR repertoire, as they are not

Abbreviations: PBC, primary biliary cholangitis; CDR3, complementarity-determining region 3; TCRβ, T cell receptor β-chains; AMA, anti-mitochondrial antibody; TRBV, TCR beta chain variable gene; TRBJ, TCR beta chain joining gene; HTS, high-throughput sequencing

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https://doi.org/10.1016/j.molimm.2019.09.019
Received 13 October 2018; Received in revised form 14 August 2019; Accepted 24 September 2019
Available online 04 November 2019

Table 1
Clinical data of patients with PBC.

Case	Age	Sex	ALT (U/L)	AST (U/L)	GGT (U/L)	ALP (U/L)	AMA (RU/ml)
1	64	F	32	41	82	131	1079
2	48	F	15	18	78	70	266
3	55	F	15	23	96	107	765
4	65	F	46	58	652	274	537
5	44	F	16	18	162	64	125
6	60	F	26	27	174	102	542
7	41	F	7	17	146	52	916
8	51	F	41	33	184	169	576

ALT, alanine transaminase, normal: 5 ~ 35U/L; AST, aspartate transaminase, normal: 8 ~ 40U/L; GGT, Gamma-glutamyl transpeptidase, normal: 7 ~ 32U/L; ALP, alkaline phosphatase, normal: 40 ~ 150U/L; AMA, antimitochondrial antibody, normal < 20RU/ml.

subject to functional selection (positive and negative selection) (Murugan et al., 2012; Robins et al., 2010, 2009). In addtion, functional TCRs can be used to study the post-selection TCR repertoire.

A recent study shown that type 1 diabetes (T1D) patients present alterations in the pre-selection TCRβ repertoire, indicating that early events in thymic T cell development and repertoire generation are abnormal in T1D (Gomez-Tourino et al., 2017). As similar to T1D, PBC is a chronic inflammatory autoimmune disease. In this study, we used deep sequencing technologies to study the TCRβ CDR3 repertoire of peripheral CD4 + naive T cells in PBC patients compared with healthy volunteers, and focused on both functional and nonfunctional TCRβ sequences, to clarify whether the early differentiation and development of T cell is abnormal in PBC patients.

2. Material and methods

2.1. Study group

Eight female patients with PBC of early histological stages (stage I-II) were studied. All the patients were diagnosed according to established criteria (Carey et al., 2015), and did not have other serious diseases or health problems, such as tumors, hepatitis infection, obesity, diabetes, circulatory system diseases, cardiorenal dysfunction, or nervous system diseases. All of the PBC patients were AMA-positive (mean age 54 years, range 41–65). Patient characteristics at the time of study were shown in Table 1. In addition, some patients underwent ultrasonically guided liver biopsy, which also showed cholestatic features

Table 2
TCRβ CDR3 sequence statistics.

Sample name	Total reads (pair)	Filter rate (%)	All reads number	Total input sequences	Total good sequences	Unique CDR3 nucleotide sequences	Out of frame Clones (%)
PBC-1	6095193	0.13%	6087072	5999209	3163341	218575	4.58
PBC-2	8099529	0.14%	8088305	7962843	4385175	563370	3.64
PBC-3	6855542	0.11%	6847993	6767743	3961804	299799	6.11
PBC-4	6438729	0.17%	6427496	6328257	6209473	579667	5.82
PBC-5	7232730	0.15%	7221705	7109229	4414385	195672	4.72
PBC-6	7629463	0.19%	7615296	7492513	7349347	652858	4.71
PBC-7	8093578	0.14%	8082523	7955482	3404486	354860	4.66
PBC-8	7889480	0.20%	7873842	7713814	7571239	466052	7.23
HC-1	8781091	0.14%	8768909	8632742	4487429	347615	7.31
HC-2	9385995	0.12%	9374287	9231362	5234419	307521	5.37
HC-3	8297001	0.16%	8283897	8116398	3876389	311737	5.47
HC-4	5741439	0.19%	5730349	5668463	5569711	453239	5.55
HC-5	3602206	0.11%	3598274	3551444	3412236	540826	5.68
HC-6	6893352	0.18%	6880663	6783193	6014041	523693	4.81
HC-7	8339048	0.16%	8325850	8213666	4897343	218476	7.30

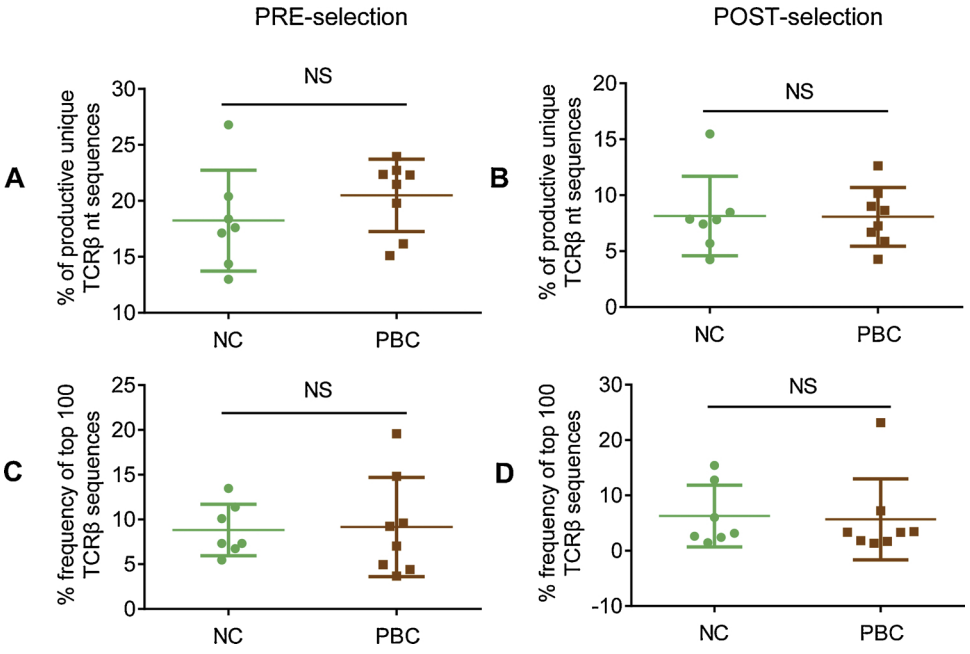

Fig. 1. Diversity indices of TCRβ repertoire of CD4 + naive T cells in PBC and NC group. **A, B** Frequency of unique TCRβ sequences identified in each group. Data points represent the percentage of unique sequences in the total Pre-selection (**A**) or Post-selection (**B**) TCR repertoires of each individual, and bars depict the mean (± SEM) of the groups. Data was normally distributed, and differences between groups were compared using student's *t*-test **C, D** Percentage frequency of top 100 TCRβ nucleotide sequences in each group. Data points represented the percentage of the top 100 TCRβ sequences in the total Pre-selection (**C**) or Post-selection (**D**) TCR repertoire of each individual.

(Fig. S1). Seven healthy, volunteer members of staff (mean age 55 years, range 43–63, five female), all AMA negative, were tested as normal controls.

This study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University, China (Ref No 2015-313). All the participating individuals provided their written informed consent.

2.2. T cell isolation and RNA extraction

Peripheral blood mononuclear cells (PBMCs) were isolated from 20 mL of whole blood from healthy volunteers and PBC patients using Ficoll gradients (Hou et al., 2017). $CD4^+CD45RA^+$ T cells were isolated using the BD FACSAria cell-sorting system (BD Biosciences) with the following antibodies: anti-CD4 PerCP-Cy5.5 (OKT4) and anti-CD45RA APC (HI100) (Fig. S2). All sorted populations were over 1.5 million cells and > 95% pure as confirmed by FACS-analysis. RNA was immediately extracted from sorted cells using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. The final product was air dried and dissolved in diethypyrocarbonate-treated water for construction of the library and high-throughput sequencing (HTS).

2.3. Sequencing of TCRβ repertoires and bioinformatic analyses

We purified RNA (RNEasy, QIAGEN), generated a cDNA template (Superscript III, Invitrogen). To generate the template library for the Illumina HiSeq machine (Illumina, San Diego, Calif), a multiplex PCR system was designed to amplify all possible rearranged TCRβ CDR3 regions from the whole cDNA sample. The method used 32 forward primers specific to all TCR Vβ gene segments and 13 reverse primers specific to all TCR Jβ gene segments. The details of the method of library construction and high-throughput sequencing were derived from earlier published work (Hou et al., 2016a, b, c). MiTCR software was used to correct the sequencing errors and PCR amplification bias (Bolotin et al., 2013). In addition, algorithms to eliminate PCR and sequencing errors for the Illumina platform was executed according to the previous description (Hou et al., 2016a, b, c; Zhang et al., 2015; Bolotin et al., 2012). Both the TCRβ CDR3 region was delineated according to the definition established by the International ImMunoGeneTics collaboration (Lefranc, 2003). Sequences that did not match CDR3 sequences were excluded from the analysis. A standard

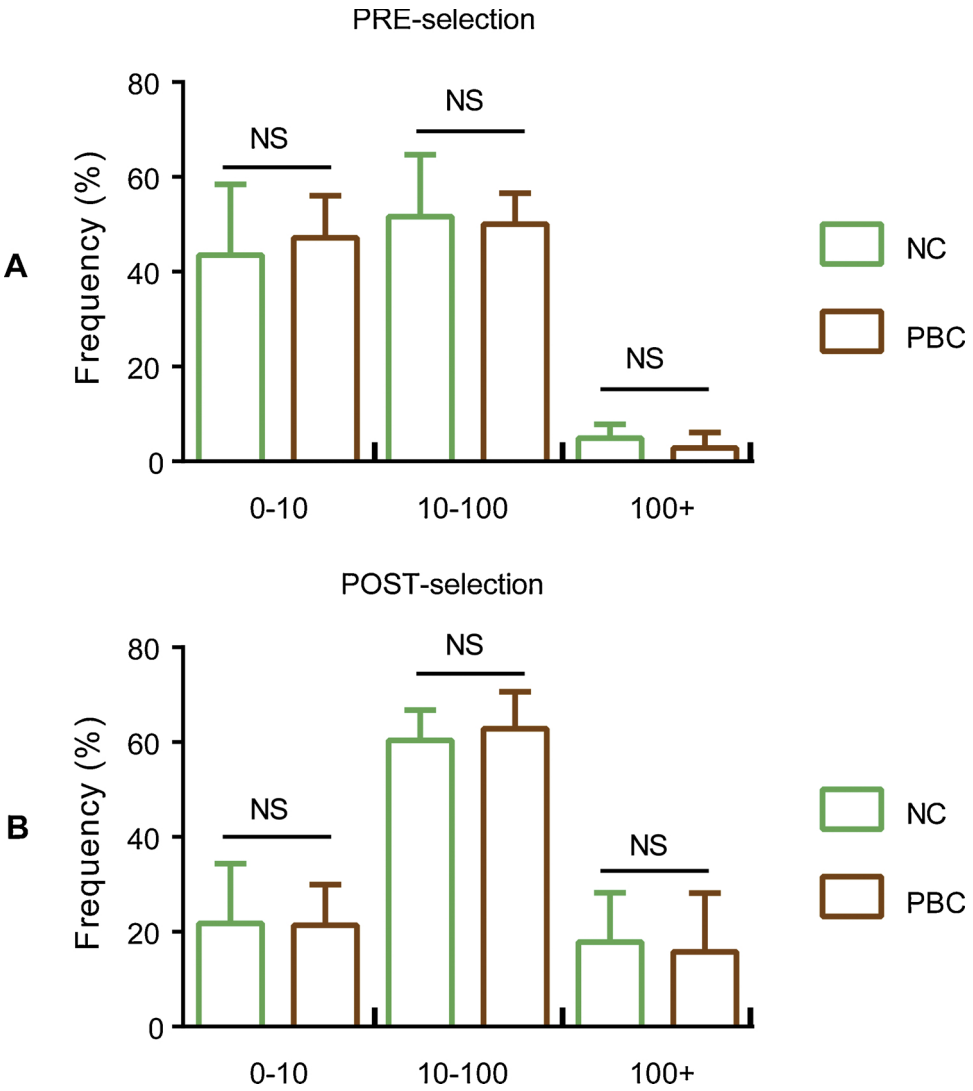

Fig. 2. Clonal abundance distribution of CD4+ naive T cells in patients with PBC and healthy individuals. The abundance of these TCRβs was characterized further in 3 groups based on the reads detected by sequencing per distinct TCRβ: low (1–10 reads), medium (11–100 reads), and high (> 100 reads), as the percentages of total distinct TCRβ sequences in the Pre-selection (**A**) and Post-selection repertoires (**B**). Data were presented as the mean ± SEM values for each group, and compared using the *t*-test.

algorithm was used to identify which V, D, and J segments contributed to each TCRβ CDR3 sequence (Yousfi et al., 2004). In addition, the frequency of expression of each distinct DNA sequence, amino acid sequence, and V-J combination was determined. The diversity of the TCR repertoire was assessed based on earlier published work (Liaskou et al., 2016).

2.4. Statistical analysis

If not otherwise stated, data were presented as the mean ± SD values or as percentages (%). Statistical significance was calculated using the unpaired *t*-test or two-way ANOVA test by SPSS20. P values < 0.05 were considered significant.

3. Results

Using HTS, we sequenced the TCRβ CDR3 repertoires from $CD4^+CD45RA^+$ T cells collected from the peripheral blood of PBC patients (PBC group) and healthy individuals (NC group). We collected an average of 7.29 million reads, which met our quality requirements. Low-quality reads were filtered for quality using previously described criteria. On average, 0.15% (range, 0.11–0.20%) of reads were filtered out, yielding an average of 6.527 (range, 3.132 ~ 9.549) million reads per sample. The identified number of clonotypes was different for each individual, varying from ~190,000 to nearly 650,000. A portion of each library was comprised by the out-of-frame clonotypes representing the nonfunctional TCR sequences formed during the recombination step. The percentage of such sequences was different for each sample, varying in most cases from 3.64% to 7.31% (mean value, 5.53%). A detailed description of the reads and clones distribution was displayed in Table 2.

3.1. Similar diversity of T-cell repertoire

To quantify TCR diversity in each group, several evaluation methods were implemented. The percentage of unique clonotypes in the total TCRβ repertoire was calculated in each of the samples. This percentage was 20.49 ± 3.23% and 8.07 ± 2.62% in the Pre-selection and Post-selection repertoires of PBC patients, respectively, and it was similar for healthy individuals, with 18.24 ± 4.51% and 8.14 ± 3.55% in the Pre-selection (Fig. 1A) and the Post-selection repertoires (Fig. 1B), respectively. Moreover, clonal expansion was

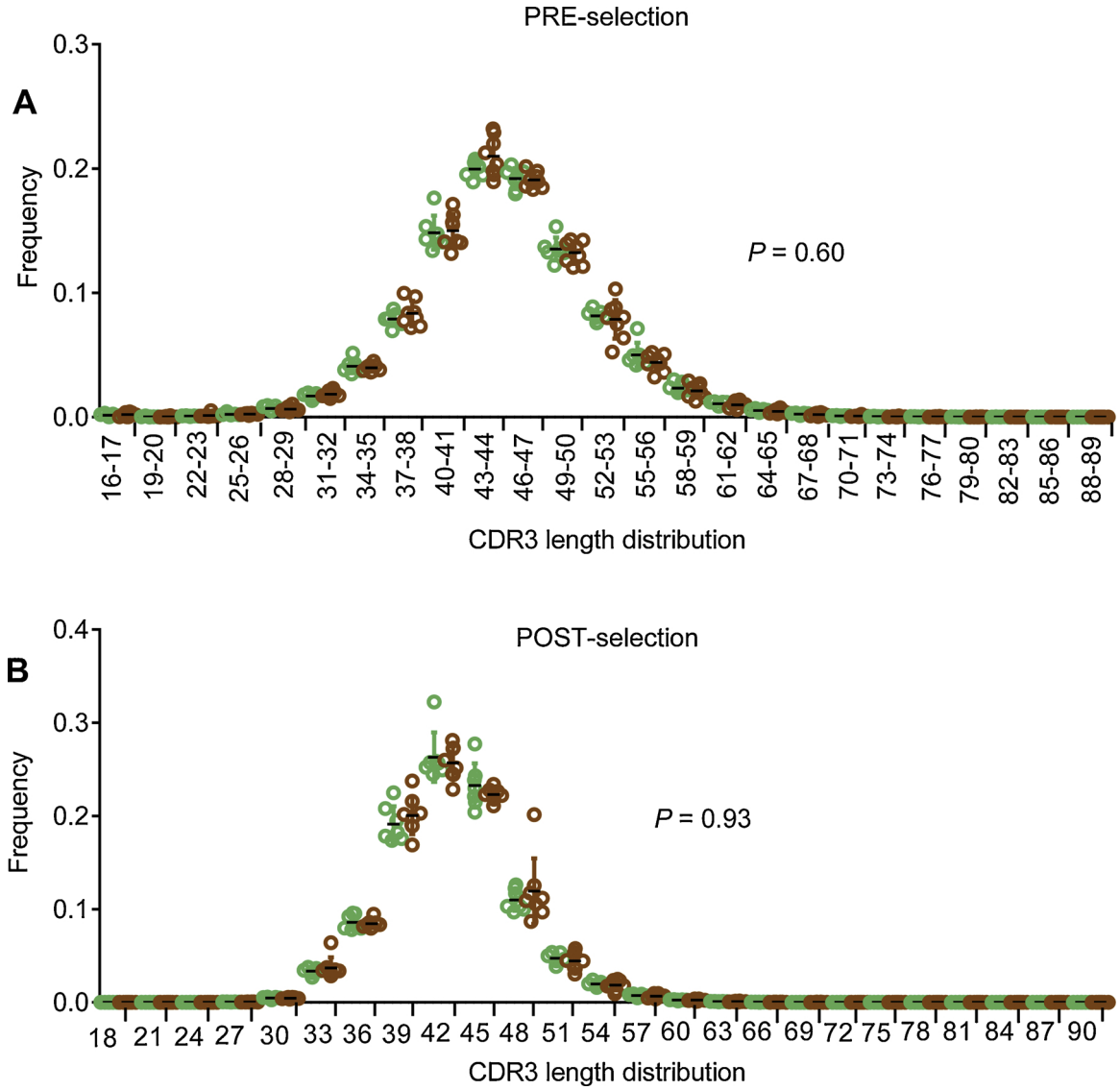

Fig. 3. The distribution of CDR3 nucleotide lengths was similar between PBC and NC group. Comparison of TCRβ CDR3 nucleotide length between the PBC patients and healthy individuals in the Pre-selection (**A**) and Post-selection repertoires (B). The mixed effects two-way ANOVA calculated *P* values for overall differences between PBC and NC group.

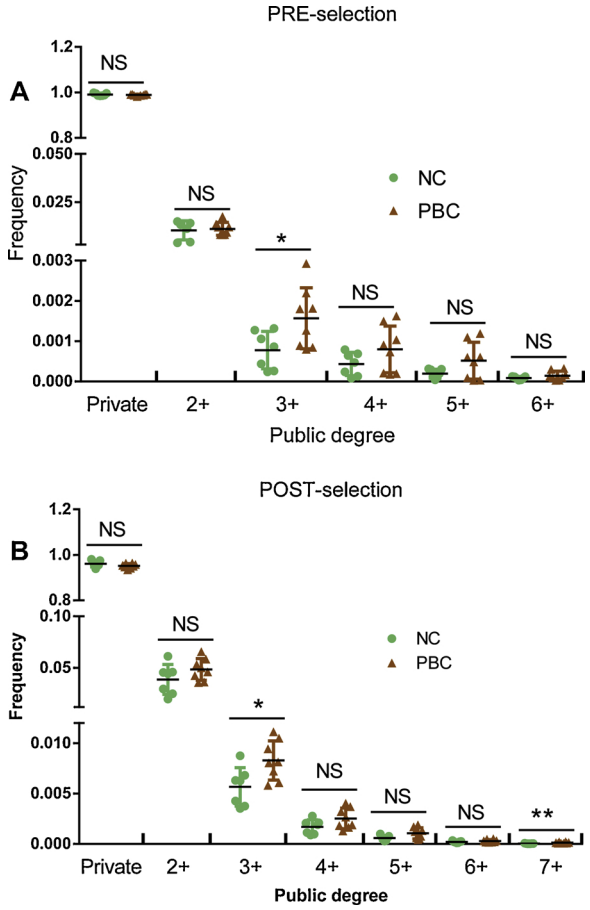

Fig. 4. Overlap indices were compared between PBC and NC group. Overlap indices were calculated for the Pre-selection (A) and Post-selection (B) TCRβ repertoires from 7 healthy donors and 8 PBC patients. The TCRβ overlap was calculated based on the number of common nucleotide sequences in each sharing category (summed across all subjects in which this sequence was found)—(Percentage(n)= number of common nucleotide sequences appeared in ≥ n subjects / total number of nucleotide sequences in sample). Data were presented as the mean ± SD values, and compared using the *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$.

assessed by calculating the cumulative percentage of the repertoire that was constituted by the top 100 TCRβ nucleotide sequences. We found that the average fraction of the top 100 TCRβ sequences was also similar for PBC group (9.16% and 5.67%) and NC group (8.83% and 6.27%) in the Pre-selection (Fig. 1C) and Post-selection repertoires (Fig. 1D), respectively. The abundance of TCRβs was further characterized in 3 groups based on the reads detected by sequencing per distinct TCRβ: low (1–10 reads), medium (11–100 reads), and high (> 100 reads), as the percentages of total distinct TCRβ sequences. We found that there was no difference in each group, no matter in Pre-selection (Fig. 2A) or Post-selection repertoires (Fig. 2B). Therefore, the diversity of T-cell repertoire did not differ significantly between the PBC and NC groups.

3.2. CDR3 length distributions and overlap degree was similar

TCRβ CDR3 loops can vary in both length and sequence, allowing for the ability to recognize diverse antigens. The distribution of CDR3 sequence lengths is another feature that provides an overall view of repertoire composition. However, in this study, we found that the distributions of CDR3 lengths were similar between the PBC group and NC groups, no matter in Pre-selection (Fig. 3A) or Post-selection repertoires (Fig. 3B). Previous studies have shown that TCRβ CDR3 length was associated with the degree of sequence sharing (Gomez-Tourino et al., 2017). To examine this we calculated the overlap indices of TCRβ nucleotide sequences in each group. As shown in Fig. 4, the overlap degree in PBC group did not differ significantly from that of controls, no matter in Pre-selection (Fig. 4A) or Post-selection repertoires (Fig. 4B), except one or two subclass were different.

3.3. Similar usage frequency of TRBV and TRBJ segments

Then, to identify whether there was a skewed and restricted VDJ segment usage in PBC patient, we compared the TRβV (Fig. 5) and TRβJ repertoires (Fig. 6) between PBC group and NC group. First, to avoid the distortion by dominant clones (high frequency clones), we carried out on unique nucleotide sequences (irrespective of each clonotype frequency). We found that the expression patterns of TRβV and TRβJ repertoires did not differ significantly between PBC group and NC groups, no matter in Pre-selection or Post-selection repertoires, and *P* values were close to 1. In addition, we assessed the expression patterns of TRβV and TRβJ repertoires across the overall TCR-β repertoires

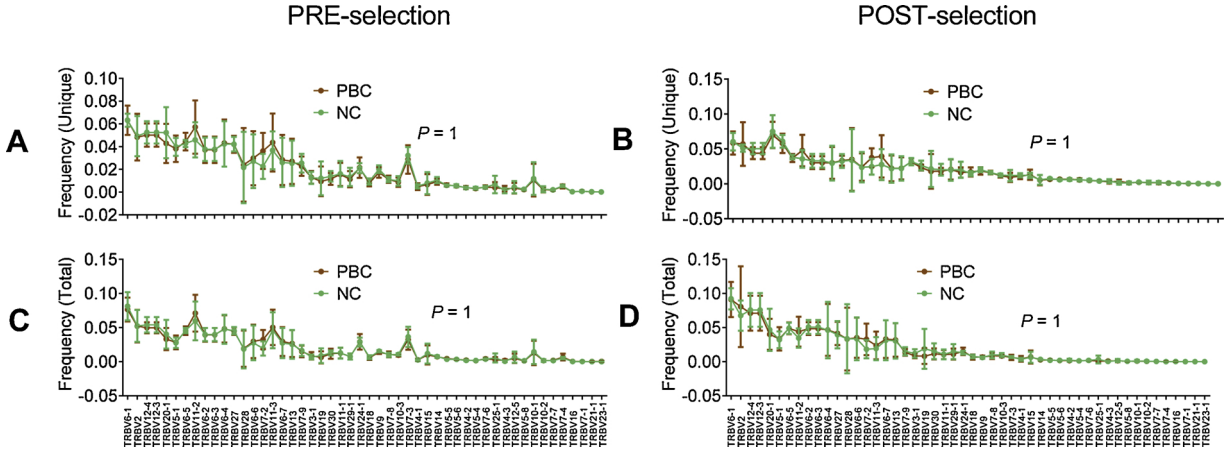

Fig. 5. Usage frequency of TRBV segments was similar between PBC and NC group. Comparison of the usage frequency of TRBV segments in PBC patients and healthy individuals in the Pre-selection (A) and Post-selection repertoires (B). The mixed effects two-way ANOVA calculated *P* values for overall differences between PBC and NC group. Significant differences of TRBV segment usage between PBC and NC group were also indicated (unpaired *t*-test, mean ± SD), After Bonferroni correction, none of TRBV segments was found to be different.

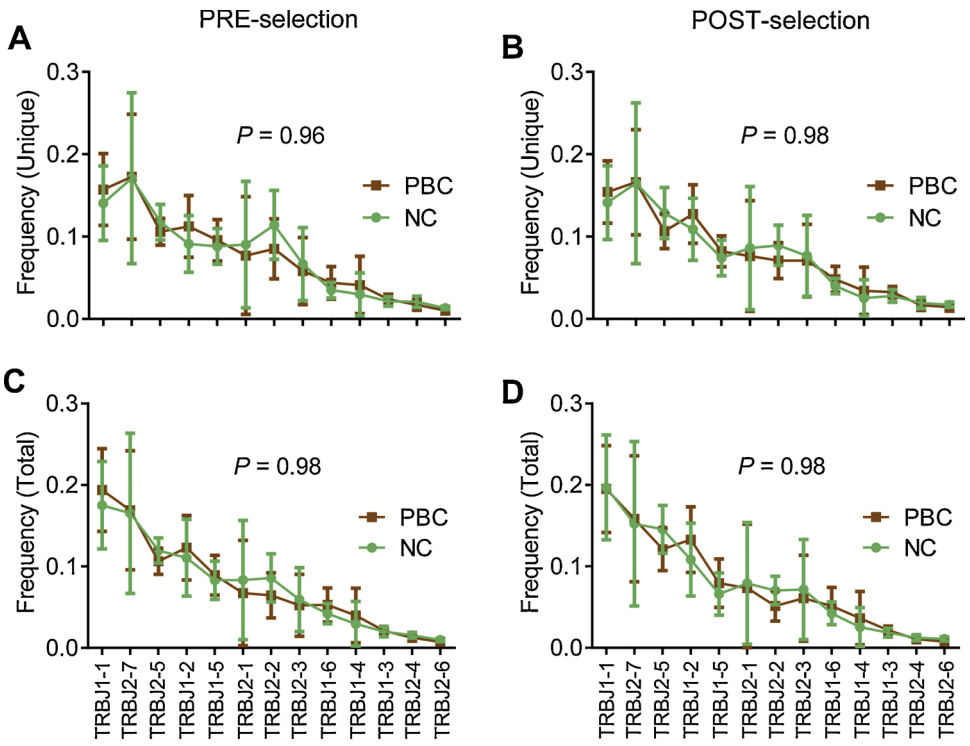

Fig. 6. Usage frequency of TRBJ segments was similar between PBC and NC group. Comparison of the usage frequency of TRBJ segments in PBC patients and healthy individuals in the Pre-selection (A) and Post-selection repertoires (B). The mixed effects two-way ANOVA calculated *P* values for overall differences between PBC and NC group. Significant differences of TRBJ segment usage between PBC and NC group were also indicated (unpaired *t*-test, mean ± SD), After Bonferroni correction, none of TRBJ segments was found to be different.

(including the abundance of each clonotype). There was still no significant difference between the two.

4. Discussion

An essential characteristic of T lymphocytes is their ability, as a population, to recognize an enormous number of peptide antigens. This capability is essential to the function of the adaptive immune system and is attributable to the diversity of the TCR they express (Hou et al., 2016a, b, c). A recent study shown that early events in thymic T cell development and repertoire generation are abnormal in type 1 diabetes. Primary biliary cholangitis (PBC) is also a chronic inflammatory autoimmune disease (Gomez-Tourino et al., 2017). In the present study, we used next-generation sequencing to comprehensively characterize the TCRβ CDR3 repertoire of peripheral CD4 + naive T cells in PBC patients and healthy volunteers. We found that there was not significant difference between PBC patients and healthy volunteers in TCRβ diversity, CDR3 length distributions, degree of sequence sharing, and usage frequency of TRBV and TRBJ segments, no matter in Pre-selection or Post-selection repertoires. Therefore, the TCRβ repertoire of CD4 + naive T cells was no abnormality in PBC patients, which might indicate that the early differentiation and development of T cells, including somatic V(D)J recombination and selection process (positive and negative selection), was not abnormality in PBC patients.

To our knowledge, the data presented here are the first global TCRβ CDR3 repertoire analysis of PBC patients at the CD4 + naive T cells level. Relevant to this investigation, Bao and co-workers reported that the PBC patients showed a lower level of diversity among the peripheral $CD4^+$ TCR Vβ CDR3 than the healthy volunteers, and patients with higher level progression of the disease showed a greater lack of diversity (Bao et al., 2015). In addition, several reports have been available concerning the TCR analysis of T cells infiltrating in the liver. Tsai et al. (1996) found the preferential Vβ gene usage and conserved residues at the CDR3 regions of the TCR β-chain in the liver infiltrating T cells of two patients with PBC. Here are several possible explanations for this discordance. Work carried out by Bao et al. (2015). (2015) studied the TCRβ CDR3 repertoire at $CD4^+$ T cells level, which including naive T cells, effector T cells, and memory T cells. Following exposure to antigen, naive cells proliferate rapidly and differentiate into effector cells, and a small number of them enter the memory pool. Exogenous antigen (or autoantigen) challenge cause the excessive abnormal cloning of one or a few TRBV segments to produce a clone of daughter cells all encoding the same TCR. Our previous experiments demonstrated that TCRβ repertoire of CD4+/CD8+ memory T cells was abnormal in PBC patients, and provided evidence of *Escherichia coli* as triggering events in the breakdown of self-tolerance (Hou et al., 2019). The dominant form of the cloned T cell may suppress the cloning of other T cells, which may result in a skewed repertoire and decreased immune function. With regard to the repertoire of T cells infiltrating in the liver, the autoimmune process in PBC is main restricted to the liver, and TCRβ CDR3 repertoire is further shaped by the disease-causing antigen. However, glycine (G) were common in the CDR3 nDn regions whether in peripheral blood lymphocyte (PBL) or liver tissue of PBC patients. In addition, in a study by Okamoto et al., they compared the TCRβ repertoire between the liver and PBL, and demonstrated that both CD4 and CD8 T cell clones were expanded.

In conclusion, we used high-throughput sequencing to study the TCRβ repertoires of $CD4^+$ naive T cells from PBC patients, and demonstrated that early events in thymic T cell development and repertoire generation was not abnormality in PBC patients. Other susceptible genes and environmental factors, lead to breakdown of tolerance to PDC-E2 molecule at the T-cell level. Based on our research, TCR-based immunotherapy of PBC may be more effective than that of type 1 diabetes because the origin of TCRβ repertoire is normal.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

This work was supported by funds received from the National Natural Science Foundation of China (No. 81571953), the major national S&T projects for infectious diseases (2018ZX10301401-005).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.molimm.2019.09.019.

References

Bao, J., Xu, Q., Zou, Y., Gao, F., Li, F., Li, Y., Gao, K., Peng, X., Pang, S., Chen, Y., et al., 2015. Deep sequencing of the T cell receptor Vb CDR3 repertoire of peripheral CD4+T cells in primary biliary cirrhosis. Zhonghua Gan Zang Bing Za Zhi 23, 580–585.

Bolotin, D.A., Mamedov, I.Z., Britanova, O.V., Zvyagin, I.V., Shagin, D., Ustyugova, S.V., Turchaninova, M.A., Lukyanov, S., Lebedev, Y.B., Chudakov, D.M., 2012. Next generation sequencing for TCR repertoire profiling: platform-specific features and correction algorithms. Eur. J. Immunol. 42, 3073–3083.

Bolotin, D.A., Shugay, M., Mamedov, I.Z., Putintseva, E.V., Turchaninova, M.A., Zvyagin, I.V., Britanova, O.V., Chudakov, D.M., 2013. MiTCR: software for T-cell receptor sequencing data analysis. Nat. Methods 10, 813–814.

Carey, E.J., Ali, A.H., Lindor, K.D., 2015. Primary biliary cirrhosis. Lancet 386, 1565–1575.

Gomez-Tourino, I., Kamra, Y., Baptista, R., Lorenc, A., Peakman, M., 2017. T cell receptor beta-chains display abnormal shortening and repertoire sharing in type 1 diabetes. Nat. Commun. 8, 1792.

Hou, X.L., Wang, L., Ding, Y.L., Xie, Q., Diao, H.Y., 2016a. Current status and recent advances of next generation sequencing techniques in immunological repertoire. Genes Immun. 17, 153–164.

Hou, X., Liang, Y., Chen, J., Wei, Y., Zeng, P., Wang, L., Lu, C., Diao, H., 2017. Expression profiling of cellular MicroRNA in asymptomatic HBsAg carriers and chronic hepatitis B patients. Biomed Res. Int. 2017, 6484835.

Hou, X., Lu, C., Chen, S., Xie, Q., Cui, G., Chen, J., Chen, Z., Wu, Z., Ding, Y., Ye, P., et al., 2016b. High throughput sequencing of t cell antigen receptors reveals a conserved TCR repertoire. Medicine (Baltimore) 95, e2839.

Hou, X., Wang, M., Lu, C., Xie, Q., Cui, G., Chen, J., Du, Y., Dai, Y., Diao, H., 2016c. Analysis of the repertoire features of TCR Beta chain CDR3 in human by high-throughput sequencing. Cell. Physiol. Biochem. 39, 651–667.

Hou, X., Yang, Y., Chen, J., Jia, H., Zeng, P., Lv, L., Lu, Y., Liu, X., Diao, H., 2019. TCRbeta repertoire of memory T cell reveals potential role for Escherichia coli in the pathogenesis of primary biliary cholangitis. Liver Int. 39, 956–966.

Im, C., Sapkota, Y., Moon, W., Kawashima, M., Nakamura, M., Tokunaga, K., Yasui, Y., 2018. Genome-wide haplotype association analysis of primary biliary cholangitis risk in Japanese. Sci. Rep. 8, 7806.

Jiang, X., Lian, M., Li, Y., Zhang, W., Wang, Q., Wei, Y., Zhang, J., Chen, W., Xiao, X., Miao, Q., et al., 2018. The immunobiology of mucosal-associated invariant T cell (MAIT) function in primary biliary cholangitis: regulation by cholic acid-induced Interleukin-7. J. Autoimmun. 90, 64–75.

Koutsoumpas, A.L., Smyk, D.S., Bogdanos, D.P., 2014. E. coli induced experimental model of primary biliary cirrhosis: at last. Int. J. Hepatol. 2014, 848373.

Larimore, K., McCormick, M.W., Robins, H.S., Greenberg, P.D., 2012. Shaping of human germline IgH repertoires revealed by deep sequencing. J. Immunol. 189, 3221–3230.

Lefranc, M.P., 2003. IMGT, the international ImMunoGeneTics database. Nucleic Acids Res. 31, 307–310.

Liaskou, E., Klemsdal, H.E., Holm, K., Kaveh, F., Hamm, D., Fear, J., Viken, M.K., Hov, J.R., Melum, E., Robins, H., et al., 2016. High-throughput T-cell receptor sequencing across chronic liver diseases reveals distinct disease-associated repertoires. Hepatology 63, 1608–1619.

Murugan, A., Mora, T., Walczak, A.M., Callan, C.J., 2012. Statistical inference of the generation probability of T-cell receptors from sequence repertoires. Proc. Natl. Acad. Sci. U. S. A. 109, 16161–16166.

Robins, H.S., Campregher, P.V., Srivastava, S.K., Wacher, A., Turtle, C.J., Kahsai, O., Riddell, S.R., Warren, E.H., Carlson, C.S., 2009. Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. Blood 114, 4099–4107.

Robins, H.S., Srivastava, S.K., Campregher, P.V., Turtle, C.J., Andriesen, J., Riddell, S.R., Carlson, C.S., Warren, E.H., 2010. Overlap and effective size of the human CD8+ T cell receptor repertoire. Sci. Transl. Med. 2, 47ra64.

Tanaka, A., Leung, P., Eric, G.M., 2019. Pathogen infections and primary biliary cholangitis. Clin. Exp. Immunol. 195, 25–34.

Tsai, S.L., Lai, M.Y., Chen, D.S., 1996. Analysis of rearranged T cell receptor (TCR) V beta transcripts in livers of primary biliary cirrhosis: preferential V beta usage suggests antigen-driven selection. Clin. Exp. Immunol. 103, 99–104.

Younossi, Z.M., Stepanova, M., Golabi, P., Epstein, R.S., Strauss, M.E., Nader, F., Racila, A., 2018. Factors associated with potential progressive course of primary biliary cholangitis: data from real-world US database. J. Clin. Gastroenterol [Epub ahead of print].

Yousfi, M.M., Giudicelli, V., Chaume, D., Lefranc, M.P., 2004. IMGT/JunctionAnalysis: the first tool for the analysis of the immunoglobulin and T cell receptor complex V-J and V-D-J JUNCTIONs. Bioinformatics 20 (Suppl 1), i379–85.

Zhang, W., Du, Y., Su, Z., Wang, C., Zeng, X., Zhang, R., Hong, X., Nie, C., Wu, J., Cao, H., et al., 2015. IMonitor: a robust pipeline for TCR and BCR repertoire analysis. Genetics 201, 459–472.

Zvyagin, I.V., Pogorelyy, M.V., Ivanova, M.E., Komech, E.A., Shugay, M., Bolotin, D.A., Shelenkov, A.A., Kurnosov, A.A., Staroverov, D.B., Chudakov, D.M., et al., 2014. Distinctive properties of identical twins' TCR repertoires revealed by high-throughput sequencing. Proc. Natl. Acad. Sci. U. S. A. 111, 5980–5985.