Human Immunology 80 (2019) 848–854

Contents lists available at ScienceDirect

Human Immunology

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

Deep targeted sequencing reveals the diversity of TRB-CDR3 repertoire in patients with preeclampsia

Changlong Guo[a,1], Ruikun Cai[a,1], Xiaofang Cao[a], Ying Yang[a], Qidi Wang[a], Runsheng He[a], Lisha An[a], Zuoqi Peng[a], Yequn Chen[f], Shuhua Ni[f], Xuerui Tan[f], Jian Han[e], Xin Liu[a,c], Li Lin[g], Song Yu[h], Xingyu Wang[a,c,*], Chunlin Wang[d,*], Xu Ma[a,b,*]

[a] *Department of Genetics, National Research Institute for Family Planning, Beijing 100081, China*
[b] *Graduate School of Peking Union Medical College, Beijing 100730, China*
[c] *Laboratory of Human Genetics, Beijing Hypertension League Institute, Beijing 100043, China*
[d] *Stanford Genome Technology Center, Stanford University, Palo Alto CA 94003, USA*
[e] *HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806, USA*
[f] *The First Affiliated Hospital, Medical College of Shantou University, Shantou, Guangdong 515041, China*
[g] *Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China*
[h] *Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing Maternal and Child Health Care Hospital, Beijing 100026, China*

ARTICLE INFO

Keywords:
Preeclampsia
Immunological characteristics
High-throughput sequencing
TRB-CDR3 repertoire
The pathogenesis of PE

ABSTRACT

Preeclampsia (PE) is one of the major causes of maternal and perinatal mortality worldwide. This study aimed to determine the immunological characteristics of PE patients and normal pregnancy at the T cell receptor beta-chain (TRB) level by using high-throughput sequencing. High-throughput sequencing was performed to analyze the expression of TRB-CDR3 in circulating T cells. T cells were isolated from 36 healthy pregnant women, 24 patients with severe PE, and 18 patients with moderate PE. Rearranged mRNA sequences were assigned to their germline V, D, and J counterparts, and translated into proper amino acids by the IMGT database. In general, PE samples had more TRB-CDR3 reads and types than those of normal pregnant woman in the circulation, but the mean number of TRB-CDR3 reads and unique TRB-CDR3 reads in severe group was lower than that in the moderate group. In PE patients, the V7_9 and V20_1 gene loci were more prevalent than in healthy pregnant women. In addition, 4 kinds of TRB-CDR3 peptides were found to be highly relevant to the pathogenesis of PE. Of them, peptides matched to herpes simplex virus antigen-specific T cells were much lower in PE samples.

1. Introduction

Preeclampsia (PE) is one of the major causes of maternal and perinatal mortality worldwide, affecting 2%–8% of the population worldwide [1–3]. Currently, there is no reliable predictive test for preeclampsia and no cure other than delivery is available [4]. Although the pathogenesis of PE is not fully understood, several studies have linked inflammation to cardinal features of this disorder. Most studies of women with PE have shown that there are increased levels of pro-inflammatory immune cells and cytokines [5], decreased levels of regulatory immune cells and cytokines [6,7], and/or the ratio of pro-inflammatory to anti-inflammatory immune cells and cytokines is increased [4,6,8–15]. Additionally, prior and prolonged exposure to paternal antigens in seminal fluid induces maternal tolerance to the semi-allogeneic fetus, protecting it from rejection, and facilitating successful implantation and placentation [16,17]. These findings strongly implicate an excessive reaction of the maternal immune system as a major contributor to the pathogenesis of PE.

The mammalian immune system has the ability to respond to almost any antigen to which it is exposed because of the incredible diversity of lymphocyte receptor molecules. As the most variable part, the complementarity determining region 3 (CDR3) sequence is the center of the antigen binding site where it plays a critical role in defining the affinity and specificity of T cell receptors [18,19]. The loss of diversity of an immune repertoire is associated with aging and implicated in various disease states [20–23]. However, the association of T cell repertoire

---

* Corresponding authors at: Graduate School of Peking Union Medical College, Department of Genetics, National Research Institute for Family Planning, Beijing 100081, China.

E-mail addresses: xingyuw@yahoo.com (X. Wang), chunlinw@outlook.com (C. Wang), genetic88@126.com (X. Ma).

[1] These authors contributed equally to the work.

https://doi.org/10.1016/j.humimm.2019.04.003
Received 21 January 2019; Received in revised form 3 April 2019; Accepted 6 April 2019

diversity and PE has not been determined yet.

Therefore, in this study, we applied the high-throughput sequencing method and amplicon-rescued multiplex PCR (ARM-PCR) to determine T cell receptor beta-chain (TRB)-CDR3 repertoire diversity of patients with PE and to examine CDR3 peptides that are relevant to PE.

2. Materials and methods

Previously, we have used successfully similar experimental methods and analytical process to reveal immunological characteristics of umbilical cord blood and psoriasis vulgaris [24,25]. For the research on TRB-CDR3 charcteristics of preeclampsia, a total of 40 healthy pregnant women, 30 women with severe PE, and 25 women with moderate PE were included in this study. PE was diagnosed according to ISSHP 2014 edition. Briefly, PE is defined as hypertension developing after 20 weeks'gestation with one or more of the following: proteinuria, maternal organ dysfunction (including renal, hepatic, hematological, or neurological complications), or fetal growth restriction [26]. Severe PE (SE) was defined as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg, with proteinuria ≥ 2.0 g in 24 h. Moderate PE (MI) was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg with proteinuria ≥ 0.3 g in 24 h. Age-matched pregnant women without any obstetric complications during the 38th week of gestation were included as healthy controls (HP). Pregnancy with chronic hypertension, chronic hypertension complicated with preeclampsia and pregnant women with congenital anomalies, an antenatal medical history, including hematopoietic cell transplantation, gene therapy, and a history of infection during gestation, were excluded from the study.

A volume of 8 ml of peripheral blood samples from participants were shipped to the laboratory and processed within 4 h after collection. Mononuclear cells were isolated by using Ficoll Paque Plus gradient cell separation. To preclude monocytes and macrophages, CD14-positive cells were discarded by CD14 + -labeled magnetic-activated cell sorting according to the manufacturer's instructions (Miltenyi Biotec GmbH, 130–050-201, Hilden, Germany). Separated cells were thawed in RNA Protec Reagent (Qiagen, 76106, Hilden, Germany) at − 80 °C until further processing. Total RNA was extracted according to the manufacturer's instructions (RNeasy mini kit, 74106, Hilden, Qiagen). The ARM-PCR-based amplification process was composed of two steps. In the first step, 1 μg total RNA was reversed to cDNA using the OneStep RT-PCR kit (679913, Hilden, Qiagen) and human T cell receptor beta-chain (HTBI, covers V and C genes) primers for TRB-CDR3 amplification according to the manufacturer's instructions (iRepertoire Inc., Huntsville, AL, USA). Each primer includes 3 regions as follows: common sequence tags located at the 5‘ external region, used for the binding and amplification of the second PCR process; a barcode tag located in the middle region, allowing donor identification; and a specific primer located at the 3‘ region, which can bind to the V and/or J genes and reverse transcript each CDR3 locus specifically. In the second step, 2-μl products from the first PCR step were used as templates for direct amplification of the whole CDR reads, with communal primers in the multiplex PCR system (Multiplex PCR Kit, 206143, Hilden, Qiagen) [27]. The target ARM-PCR products were separated by agarose gel electrophoresis and extracted using a gel extraction kit. The concentration and quality of each sample were determined with a qPCR kit (KAPA Biosystems, Wilmington, MA) and the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA). Ten samples with equal amounts were pooled together and mixed with Phix Control (Illumina, San Diego, CA) with the ratio of 3:1. Finally, 12.5-pM products were loaded and sequenced with the Illumina platform.

2.1. Data analysis

As previously described [24,25], raw data of CDR3 peptide reads, V, D, J, and C segment use for the TRB of each sample, were separated according to the sample's unique barcode sequence. Rearranged mRNA sequences were assigned to their germline V, D, and J counterparts, and translated into proper amino acids by the IMGT database. Target peptides were searched in the PDB database (http://www.wwpdb.org) and UniProtKB database (http://www.uniprot.org/) by the BLAST tool. Abundance and cumulative frequency of the CDR3 sequence were calculated. The Kruskal-Wallis test was used to assess differences between groups. Analysis was performed using SPSS 13.0 version [28], significant differences were defined by a P value less than 0.05.

2.2. Ethics

The study was approved by the ethics committee of the National Research Institute for Family Planning (record number: 201302) and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all individuals and/or guardians participating in the study.

2.3. Data availability statement

All data is available from the corresponding author upon request.

3. Results

Because of the low number of cells and poor RNA quality, we finally obtained TRB-CDR3 repertoire profiles from 36 normal pregnant women, 24 severe PE patients, and 18 moderate PE patients. The participant's age ($P = 0.239$), gravidity ($P = 0.446$), parity ($P = 0.628$) have no significant differences except gestation age (32.9 ± 0.77 (PE) vs 39.2 ± 0.21(HP), $P = 0.000$) between PE and healthy pregnancy, the detailed features of each group are shown in Table 1. Generally, 9.09×10^5 effective TRB-CDR3 reads were generated for each PE sample, and 8.49×10^5 reads were generated for each normal pregnant women ($P > 0.05$). Among patients with PE, the CDR3 reads were different in severe group (8.81×10^5) and moderate group (9.48×10^5, $P = 0.374$). Similar results were also observed in

Table 1
The clinical and immunological features of severe PE, moderate PE and normal pregnat in this study.

	Severe (No. = 24)			Moderate (No. = 18)			Control (No. = 36)		
	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median
Age (years)	24–41	31.39	31	24–39	30.79	31	26–40	30.48	30.5
Gravidity (times)	1–4	1.91	2	1–6	2.16	2	1–5	1.92	2
Parity (times)	0–2	0.375	0	0–1	0.16	0	0–1	0.22	0
Time of Blood Pressure Rising (weeks)	15–39	25.67	26	20–39	32.07	35	___	___	___
Time of Blood Sampling (weeks)	21–39	30.1	31	26–40	35.6	37	38–41	39.4	39
Systolic Pressure (mmHg)	160–220	174.67	170.00	140–150	145.74	146	100–120	114.17	110
Diastolic Pressure (mmHg)	90–150	108.63	110	80–110	95.05	95	60–84	73.31	70
CDR3 Reads (10^5)	2.44–13.30	8.81	8.19	5.93–14.97	9.48	9.84	0.3–14.6	8.49	8.93
Unique CDR3 Reads (10^4)	0.19–6.06	2.66	1.62	0.14–12.74	3.21	1.46	0.10–6.29	1.96	0.84

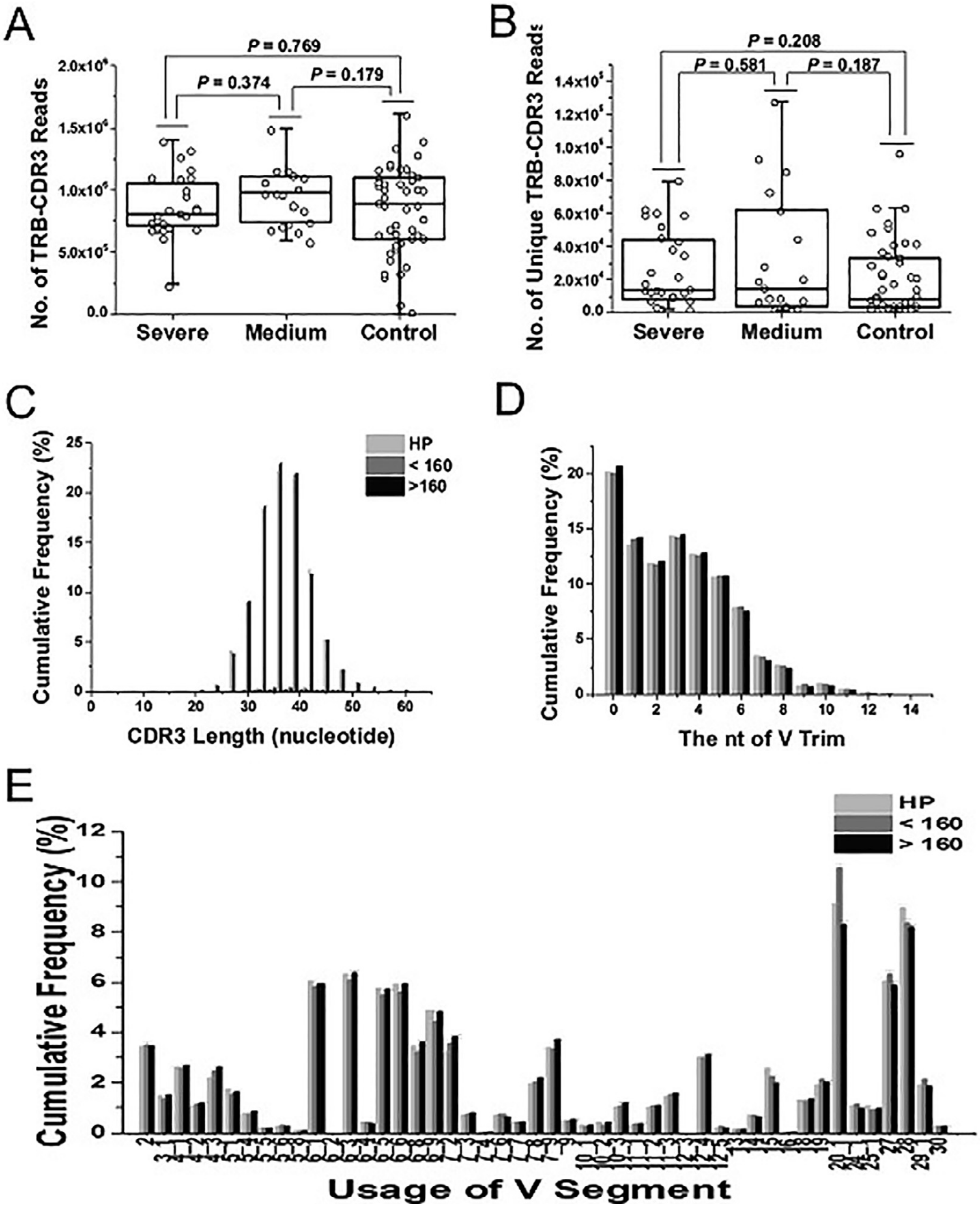

Fig. 1. TRB-CDR3 immunological characteristics of severe PE, moderate PE, and normal pregnancy (part A). A: Comparison of total TRB-CDR3 reads among the 3 groups; B: Comparison of unique TRB-CDR3 reads among the 3 groups; *C:* Comparison of CDR3 length among the 3 groups; *D:* Number of nucleic acids of V segment trimming in the 3 groups; *E:* Usage bias of the V segment in the 3 groups.

unique TRB-CDR3 reads (distinctive TRB-CDR3 peptide types): the number of each PE sample (2.90×10^4) was relative higher than that in healthy pregnant samples (1.96×10^4, $P > 0.05$), the mean number of unique TRB-CDR3 reads in severe group (2.66×10^4) was lower than that in the moderate group (3.21×10^4) ($P = 0.581$, Table 1, Fig. 1-A, -B). This finding suggested that PE samples had relative more TRB-CDR3 reads and distinctive TRB-CDR3 types in the circulation than in normal pregnant woman, though without significant difference.

The diversity of the TRB-CDR3 repertoire is encoded by recombined VDJ genes that are formed from sets of variable (V), diversity (D), and joining (J) genes. The resulting combinatorial diversity is expanded still further by junctional diversification. This diversification arises from exonuclease trimming of the recombining gene ends and from the essentially random addition of nucleotides between the recombining genes by terminal deoxynucleotidyl transferase enzyme [29,30]. Therefore, the cumulative frequencies of V, D, J, and C domain use and junctional diversity factors, including CDR3 length, V trimming, J trimming, and N addition from each sample, were calculated. We found that most of the CDR3 length of the 3 groups ranged from 21 to 54 nt, mainly residing in 36 nt (12aa), 39 nt (13aa), and 33 nt (11aa) (Fig. 1-C). The severe PE group had similar usage bias of the V gene in the moderate and normal pregnant groups except some gene locies, for example, the frequency use of V20_1gene loci was relatively higher in the moderate PE group (10.56% (MI) vs 8.3% (SE) and 9.14% (HP), $P = 0.080$. Fig. 1-E). With regard to D, J, and C gene usage bias analysis, the three groups showed similar patterns, including gene usage bias of D1_01 and D2_02 segments (Fig. 2-A), J2-7 and J2-1 segments (Fig. 2-B), and the C2_02 segment (Fig. 2-D). The highest cumulative frequency in 0 nt of V segment trim (Fig. 1-D), 4 nt of J segment trim (Fig. 2-C). In random addition of nucleotides (N addition), the three

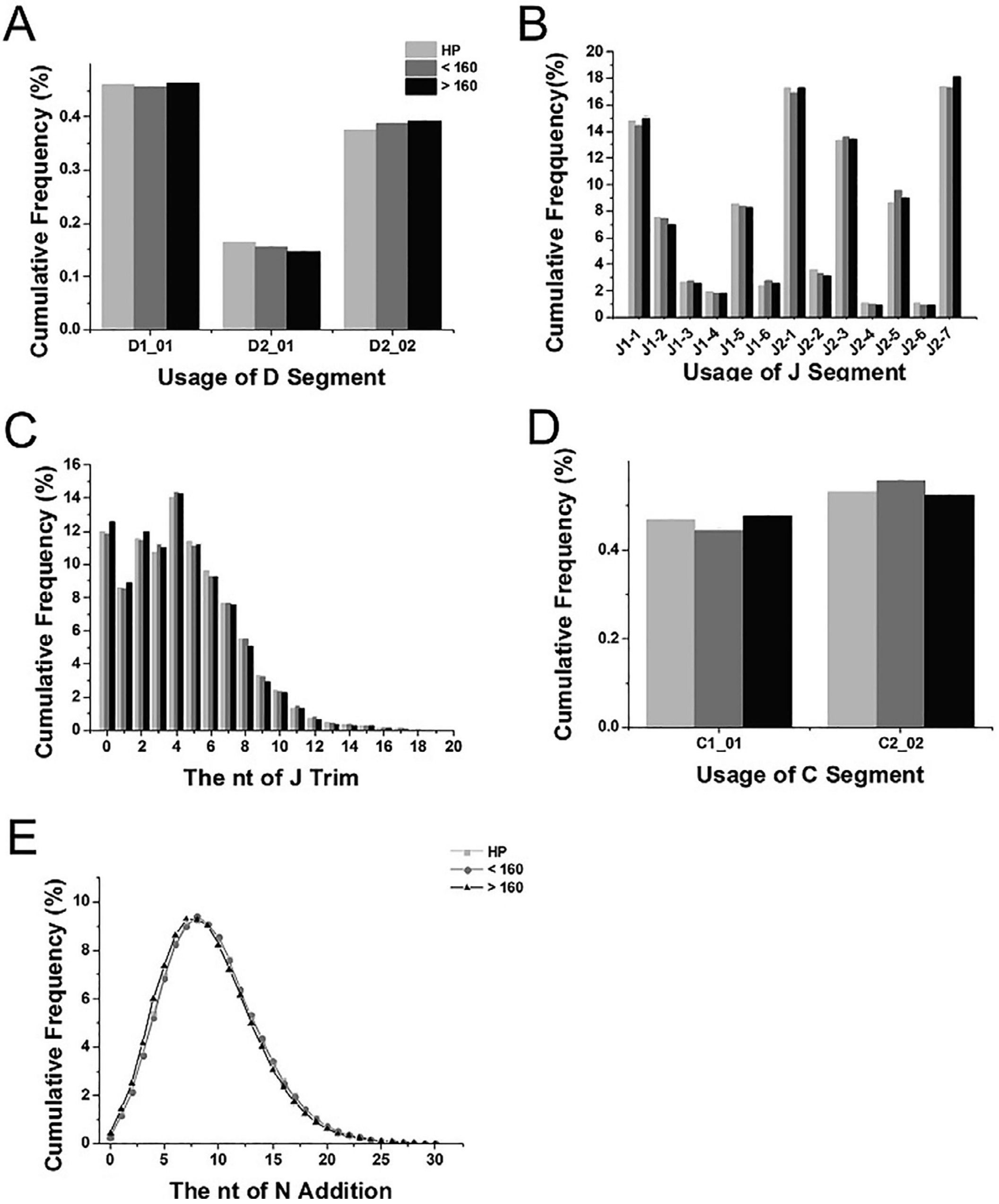

Fig. 2. TRB-CDR3 immunological characteristics of severe PE, moderate PE, and normal pregnancy (part B). *A:* Usage bias of the D segment in the 3 groups; *B:* Usage bias of the J segment in the 3 groups; *C:* Number of nucleic acids of J segment trimming in the 3 groups; *D:* Usage bias of the C segment in the 3 groups; *E:* The nt number of N additions in the 3 groups. Black line or column: features of severe PE samples; dark grey line or column: features of moderate PE samples; light gray line or column: features of healthy pregnant samples (control).

groups demonstrated Gaussian distribution and the highest frequency was 8 nt (Fig. 2-E), but there was no significant difference among the groups.

Because of continuous stimuli, every sample has contained obvious expanded clonotypes in the TRB-CDR3 repertoire, regardless of what group it was. An example of this situation is that in sample no. 10,376 (normal pregnancy), the number of clone copies of the peptide ASSLSPSEQF was 4.97×10^5, accounting for 44.72% of the total CDR3 reads (496800/1110849). Therefore, we wished to determine whether there were specific shared peptides that could induce PE. In the next step of analysis, we pooled the CDR3 peptides in each sample and searched for peptides that were relevant to PE. A total of 4 expression patterns composed of 40 distinctive TRB-CDR3 peptides were found to be abnormally expressed in patients with PE. Among them, pattern A (peptides ASRGTGELF, ASSRGGRETQY, and ASSHPGYEQY) was exclusively expressed in the severe PE group, with positive rates of 25%, 20.8%, and 20.8%, respectively. Pattern B (composed of 9 peptides) was expressed in the severe and moderate PE groups, but the expression level in the severe group was higher than that in the moderate group. Pattern C (composed of 13 peptides) was detected in the severe PE, moderate PE, and normal pregnant groups. However, the expression level of pattern C increased with the severity of PE (i.e., highest in the severe group and lowest in the normal pregnant group). The expression level of pattern D (composed of 15 peptides) decreased with the severity of PE (i.e., lowest in the severe PE group and highest in the normal pregnant group, Table 2). The detailed expression of each peptides was shown in supplemental data.

In the following analysis, we searched for these peptides in the PDB database and UniProtKB database. Inexpression pattern A, B and C, a few of these sequences were highly similar to T cell clones that were

Table 2
The TRB-CDR3 peptide sequences of PE relevant.

Pattern	Degree of change	Peptide seq	Shared cases in SE (n = 24)			Shared cases in MI (n = 18)			Shared cases in HP (n = 36)			Ratio 1 (SE/MI)	Ratio 2 (SE/HP)
			No.	Mean copies	Mean Fre.	No.	Mean copies	Mean Fre.	No.	Mean copies	Mean Fre.		
A	1	ASRGTGELF	6	8.33	9.96E-06	0	0.00	0	0	0.00	0	——	——
	2	ASSRGGRETQY	5	19.00	2.33E-05	0	0.00	0	0	0.00	0	——	——
	3	ASSHPGYEQY	5	26.00	3.08E-05	0	0.00	0	0	0.00	0	——	——
B	1	ASSGQGSSYEQY	7	444.57	0.000466	3	9.67	1.19E-05	0	0.00	0	39.19	——
	2	ASSFSGSGANVLT	6	13.71	7.86E-05	1	18.00	1.62E-05	0	0.00	0	11.34	——
	3	ASSERLNTEAF	5	50.80	6.18E-05	2	4.50	5.45E-06	0	0.00	0	4.85	——
	4	ASSFGSTYEQY	5	8.00	8.86E-06	1	2.00	3.37E-06	0	0.00	0	3.02	——
	5	ASSFPESYEQY	5	3.50	4.28E-06	1	2.00	1.8E-06	0	0.00	0	2.63	——
	6	ASSLAVSGNTIY	5	11.25	1.39E-05	2	4.00	4.6E-06	0	0.00	0	2.38	——
	7	ASSEFSGNQPQH	6	3635.50	0.004295	2	2245.50	0.002638	0	0.00	0	1.63	——
	8	ASSTDRNQPQH	5	20.00	2.39E-05	1	52.00	4.68E-05	0	0.00	0	0.78	——
	9	SARKQGSTSGPQH	5	1832.00	0.002136	3	2326.67	0.002734	0	0.00	0	0.51	——
C	1	ASSLADTQY	11	179.87	0.001171	9	86.00	7.88E-05	16	65.69	7.35E-05	14.87	15.93
	2	ASSFGSDTQY	6	85.50	0.000115	3	8.67	1.05E-05	1	6.00	5.64E-06	10.93	20.36
	3	ASSSSTDTQY	12	57.87	0.00036	9	121.78	9.93E-05	12	43.58	5.37E-05	3.63	6.71
	4	ASSENSPLH	5	34.75	4.64E-05	4	11.00	1.36E-05	1	11.00	1.16E-05	3.41	4.00
	5	ASSLNYGYT	10	48.80	5.05E-05	5	19.20	2.73E-05	9	10.00	1.71E-05	1.85	2.95
	6	ASSLRGNTEAF	10	74.77	6.28E-05	5	28.60	3.57E-05	13	11.77	2.29E-05	1.76	2.74
	7	ASSLSRDTQY	5	18.00	2.32E-05	4	14.50	1.46E-05	1	8.00	8.43E-06	1.59	2.75
	8	ASSSQGYEQY	11	24.17	3.78E-05	6	22.83	2.72E-05	10	12.70	2.58E-05	1.39	1.47
	9	ASSSQETQY	12	95.80	0.000117	11	90.82	9.79E-05	15	51.27	6.73E-05	1.20	1.74
	10	ASSGSMNTEAF	5	8.50	1.05E-05	2	7.50	8.97E-06	1	2.00	2.85E-06	1.17	3.69
	11	ASSQETQY	13	41.47	7.83E-05	10	84.10	8.26E-05	15	60.13	7.99E-05	0.95	0.98
	12	ASSLTANTEAF	10	54.67	6.63E-05	5	77.20	7.09E-05	9	19.00	2.64E-05	0.93	2.51
	13	ASSPSTDTQY	15	59.72	8.13E-05	8	88.63	0.000117	16	66.81	7.71E-05	0.69	1.05
D	1	ASSLGGNQPQH	10	43.31	4.16E-05	9	113.67	0.000172	15	40.87	0.000539	0.24	0.08
	2	ASSKINQPQH	6	3384.00	0.007069	5	23668.6	0.027473	11	36654.64	0.028945	0.26	0.24
	3	ASSSTDTQY	13	34.60	3.99E-05	9	140.89	0.000131	19	237.68	0.000218	0.31	0.18
	4	ASSLGLYNEQF	10	17.08	2.12E-05	4	38.50	5.02E-05	13	44.08	0.000107	0.42	0.20
	5	ASRDSNQPQH	10	27.80	3.16E-05	7	60.43	6.55E-05	11	124.73	0.000171	0.48	0.19
	6	ASSLGGTDTQY	12	16.92	1.86E-05	12	32.42	3.56E-05	18	141.39	0.000484	0.52	0.04
	7	ASSLGLNTEAF	11	36.23	3.82E-05	6	68.33	7.21E-05	15	44.67	7.45E-05	0.53	0.51
	8	ASSLAVNTEAF	10	18.60	2.3E-05	6	35.50	4.13E-05	9	80.33	7.19E-05	0.56	0.32
	9	ASSLDSNQPQH	11	48.50	5.1E-05	7	74.86	8.73E-05	17	53.18	0.000273	0.58	0.19
	10	ASSRSTDTQY	11	27.17	2.68E-05	8	39.88	4.26E-05	10	78.30	0.000141	0.63	0.19
	11	ASSLSYEQY	13	43.07	5.24E-05	11	66.82	6.73E-05	18	65.72	0.000164	0.78	0.32
	12	ASSLGPYEQY	10	14.83	1.65E-05	8	19.38	2E-05	13	22.08	4.89E-05	0.83	0.34
	13	ASSLQETQY	12	35.47	4.36E-05	10	53.10	5.18E-05	22	49.64	0.000106	0.84	0.41
	14	ASSLGSNQPQH	11	60.58	0.000133	9	168.33	0.000156	19	74.58	0.000172	0.86	0.78
	15	ASSLTGTDTQY	10	14.92	1.72E-05	5	8.40	1.07E-05	11	443.09	0.000412	1.61	0.04

Pattern A: expressed in severe PE only; *B:* expressed both in severe and moderate PE; C: up expressed in severe PE, compared with moderate PE and normal pregnant; *D:* down expressed in severe PE, compared with moderate PE and normal pregnant. *SE:* severe PE; *MI:* mild PE; *HP:* healthy pregnant; *No.:* numbers of sample which express certain peptide; *Mean copies:* the average clone copies of TRB-CDR3 peptide in positive samples; *Mean Fre.:* the average clone frequency of TRB-CDR3 peptide in positive samples; *Ratio 1 (SE/MI):* the ratio of clone frequency of TRB-CDR3 peptide in severe and mild PE patients, which is positive correlated with the severity of disease; *Ratio 2 (SE/HP):* the ratio of clone frequency of TRB-CDR3 peptide in severe patients and healthy pregnants, which is negative correlated with the severity of disease.

identified from patients with inflammation (supplemental table). However, most of the CDR3 peptides were present as part of TCR beta V sequences without further functional description (data not shown). However, in expression pattern D, 4 peptides were highly matched to the herpes simplex virus (HSV) antigen-specific T lymphocyte clone beta chain. These 4 peptides were ASSLGGTDTQY (11/11, 100% match, Sequence ID: AAB31869.1), ASSRSTDTQY (10/11, 91% match, Sequence ID: AAB31870.1), ASSLTGTDTQY (10/11, 91% match, Sequence ID: AAB31869.1), and ASSSTDTQY (9/11, 82% match, Sequence ID: AAB31870.1). With regard to ASSLGGTDTQY, the positive rates were 50% (12/24), 66.67% (12/18), and 50% (18/36) in severe PE, moderate PE patients, and normal pregnancy, with no significant difference among the groups. However, the average number clone copies in normal pregnancy women was 141.39, which was 8.36 times that in severe PE (16.92), and 4.36 times that in moderate PE (32.42), meanwhile, the mean frequency of ASSLGGTDTQY in normal pregnancy women was 25 times and 13 times than severe and moderate PE patients, respectively (Table 2).

To get the overall profile of PE, the same PE samples were divided into early-onset PE (< =34 week) and late-onset PE group (> 34 week) according to gestational age at diagnosis or delivery [31,32], and the data were analysized accordingly. However, the results showed different classified method did not affect the characteristics of PE, the mean CDR3 reads of early-onset PE was 8.53×10^5, which was lower than late-onset PE sample (9.83×10^5) but higher than HP samples (8.49×10^5); the similar trend was also observed in unique CDR3 reads, the mean unique CDR3 reads of early-onset PE was 2.62×10^4, which was lower than late-onset PE sample (3.26×10^4) but higher than HP samples (1.96×10^4) (Supplemental Table 2, Fig. 2). Other TRB-CDR3 characteristics, including the cumulative frequencies of V, D, J, and C domain use and junctional diversity factors, including CDR3 length, V trimming, J trimming, and N addition from each sample were the same as former result. In the analysis step, we pooled the CDR3 peptides of each sample and searched for shared peptides of each group.

Like severe/moderate group, a total of 4 expression patterns composed of 50 distinctive TRB-CDR3 peptides were found to be abnormally expressed among early- and late-onset PE. Among them, only one peptide (SAFGGSWTDTQY, pattern A) was exclusively expressed in early-onset PE group, with positive rates of 29.2%. Pattern B (composed of 17 peptides) was both expressed in the early- and late-onset PE groups, but the expression level in the early group was higher than that in the late-onset group. Pattern C (composed of 18 peptides) was detected in the early-, late-onset PE, and normal pregnant groups, however, the expression level of pattern C increased with the severity of PE (i.e., highest in the early-onset group and lowest in the normal pregnant group). The expression level of pattern D (composed of 14 peptides) decreased with the severity of PE (i.e., lowest in the early-onset PE group and highest in the normal pregnant group). The detailed expression of each peptide was shown in supplemental data (Supplemental Table 3, and 4). We searched the peptides in PDB database and UniProtKB database, but none of the peptides were matched 100% to known peptide sequence of the database.

4. Discussion

During the past decade, advanced methodologies in high-resolution adaptive immune repertoire analysis provide an essential clue to obtain deeper understanding of the ontogeny of our immune system at the individual level. In this study, we coupled high-throughput sequencing with ARM-PCR amplification to characterize the basic properties of TRB-CDR3 of patients with PE and to compare these properties with those in normal pregnant women. To the best of our knowledge, this is the first study to provide comprehensive profiles of the TRB-CDR3 repertoire in PE.

A highly adaptive immune system heavily relies on the existence of a diverse repertoire of T lymphocyte receptors, which are continuously shaped by exposure to exogenous and endogenous antigens [33]. In our study, we found almost every sample had obvious expanded clonotypes no matter in PE or healthy samples, meanwhile, though with no significant differences, PE samples had relative more TRB-CDR3 reads and types than normal pregnant samples in peripheral circulating blood. Given all of the samples showed no infection symptom during pregnancy, we inferred that excessive TRB-CDR3 reads and types maybe correspond to over-activated T cells, which ultimately participated in the pathological process of PE. In this study, we also demonstrated that PE had similar TRB-CDR3 immunological characteristics as those in normal pregnancy regarding CDR3 length, V trimming, J trimming, J gene, D gene, C gene usage, N addition, V gene use but not in V20_1 gene loci.

Finally, by comparing the TRB-CDR3 repertoire data of both PE groups and the normal pregnant group, we found 4 different expression pattern peptides were involved in the occurrence and development of PE. Pattern A was only expressed in severe PE. Pattern B was expressed in patients with PE, and the expression level in severe PE was higher than that in moderate PE. Pattern C was expressed in both types of PE and normal pregnancy, but the expression level increased with the severity of PE. Expression pattern D was expressed in both types of PE and normal pregnancy, but the expression level decreased with the severity of PE. Due to the lack of further information, we were still uncertain which peptides or peptide pattern is the major driving force in the process of PE, we favoured the assumption that some clones in pattern A, B, or C could be the culprit for development of PE, and that some of the clones in pattern D could protect against development of PE, moreover, maybe a combined action or ordinal interaction of these peptides induced the occurance of PE, and much more detailed assays should be done to verify this assumption.

To get the specified binding antigens or functions of the candidate peptides, we searched for these highly relevant CDR3 sequences in the IMGT and NCBI databases. Due to the lack of detailed data, most of sequences are present as part of TCR beta V sequences without further functional description. Only a few of these sequences are highly similar to T cell clones that were identified from patients with inflammation (see as in supplemental table). Given the complex immune responses, some possibilities may exist in the functions of those unknown CDR3 peptides: some peptides are PE related and aim at one or more pathogens responsible for PE process, with different binding abilities or binding site; some peptides binds to PE unrelated microbes, such as certain commensal bacteria; some peptides are just the result of sudden release of potent pro-inflammatory milieu induced by preeclampsia, with no binding targets. Another important finding in our study is that the number of HSV antigen-specific T lymphocytes was much higher in healthy pregnancy than in moderate and severe PE patients, and its expression level decreased with the severity of PE. HSV-1 is a typical human-restricted pathogen, which is carried by 50%–90% of the population worldwide, with higher frequencies in developing countries [6,26]. Infection with HSV triggers a robust response from the innate immune system until adaptive immunity is able to assist in clearing active infection. Our study gives the hint that an HSV infection-induced inflammatory reaction may contribute to the complexity of PE process. However, the association of HSV infection and PE is inconsistent among previous studies that used different detection methods. Vahdat et al. [34] and Haggerty et al. [35] reported that there was no association of HSV infection and PE by analyzing HSV immunoglobulin G antibody. However, Gibson and colleagues [36] showed that the risk of developing PE was increased in the presence of infection with HSV-2 viruses (OR 3.57, 95% CI 1.10–11.70) by detecting HSV DNA copies. So, it is important to take an appropriate method to ascertain HSV infection and PE morbidity in further research. However, HSV infection cannot be responsible for all occurrence of PE, based on our result, the positive rate of HSV antigen-specific T lymphocytes was from 50% to 66.7% in PE patients, the reason for negative HSV infection results in PE samples should be investigated in further research. Meanwhile, because of the limited information, we also cannot exclude the possibility that peptides without a functional description in patterns A, B, or C aimed at some PE-induced pathogen, more detailed assays need to be performed to test the function of these peptides in the following research.

To get a comprehensive profile of PE, we got the TRB-CDR3 repertoire based on early- and late-onset subtypes as well. Like data in severe/moderate PE, both CDR3 reads and unique CDR3 reads in late-onset PE was relative higher than early-onset PE, which was higher than normal pregnancy. In addition, other repertoire characteristics, including V, D, J, and C domain use frequencies, CDR3 length, V trimming, J trimming, and N addition showed same inclination, compared with severe/moderate PE subtypes. Later, we tried to search the shared peptides between severe/moderate PE and early/late-onset PE pattern, to our surprise, none peptide was shared in pattern A, B, and C, except pattern D. In pattern D, only 4 peptides (ASSSTDTQY, ASSLSYEQY, ASSLGPYEQY, ASSLQETQY) were both included in severe/moderate PE and early/late-onset PE pattern.

In conclusion, by using next-generation sequencing and multiplex PCR, we showed comprehensive profiles of TRB-CDR3 repertoires of severe PE, moderate PE, and normal pregnancy. PE samples had more TRB-CDR3 reads and types than those of normal pregnant woman in the circulation. Our study also shows that 4 types of TRB-CDR3 peptides are highly relevant to the occurrence of PE, and that clones of HSV antigen-specific T lymphocytes maybe involved in the pathogenesis of PE. The results of this study will greatly benefit pathological research and clinical treatment of PE in the future.

Acknowledgment

We thank all of the participants of the study for their contributions to the research.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Contribution

Contribution: C.L.G., Q.D.W., X.F.C., J.H., C.L.W., X.Y.W., R.S.H., and X.M. contributed to the experimental design and wrote the manuscript; C.L.G., R.K.C., and C.L.W. contributed to data analysis; Y.Y., X.L., L.L., S.Y., X.R.T., S.H.N., Y.Q.C., and L.S.A. contributed to enrolling patients in the study and provided patient-related data; X.Y.W., X.M., and Z.Q.P. conceptualized and supervised the research, and ensured compliance with regulatory requirements for enrolled patients.

Source of funding

The study are supported by grants 2016YFC1000300 and 2016YFC1000307 from the National Key Research and Development Program and the International Science & Technology Cooperation Program of China (item no. 0102012DFB30130).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.04.003.

References

[1] L. Duley, The global impact of pre-eclampsia and eclampsia, Semin. Perinatol. 33 (2009) 130.
[2] L. Say, D. Chou, A. Gemmill, et al., Global causes of maternal death: a WHO systematic analysis, Lancet Glob. Health 2 (2014) e323.
[3] E.A. Steegers, P. von Dadelszen, J.J. Duvekot, et al., Pre-eclampsia, Lancet 376 (2010) 631.
[4] A. Ohkuchi, C. Hirashima, K. Takahashi, et al., Prediction and prevention of hypertensive disorders of pregnancy, Hypertens. Res. 40 (2017) 5.
[5] J.H. Southcombe, C.W. Redman, I.L. Sargent, et al., Interleukin-1 family cytokines and their regulatory proteins in normal pregnancy and pre-eclampsia, Clin. Exp. Immunol. 181 (2015) 480.
[6] S. Sabour, Prediction and prevention of hypertensive disorders of pregnancy: a methodological mistake, Hypertens. Res. 40 (2017) 788.
[7] W. Visser, H.C. Wallenburg, Prediction and prevention of pregnancy-induced hypertensive disorders, Baillieres Best Pract. Res. Clin. Obstet. Gynaecol. 13 (1999) 131.
[8] K.R. Bounds, M.K. Newell-Rogers, B.M. Mitchell, Four Pathways Involving Innate Immunity in the Pathogenesis of Preeclampsia, Front. Cardiovasc. Med. 2 (2015) 20.
[9] B.C. Matson, K.M. Caron, Adrenomedullin and endocrine control of immune cells during pregnancy, Cell. Mol. Immunol. 11 (2014) 456.
[10] B. Fu, Z. Tian, H. Wei, TH17 cells in human recurrent pregnancy loss and pre-eclampsia, Cell. Mol. Immunol. 11 (2014) 564.
[11] P. Nancy, A. Erlebacher, T cell behavior at the maternal-fetal interface, Int. J. Dev. Biol. 58 (2014) 189.
[12] M.A. Moreno-Eutimio, J.M. Tovar-Rodriguez, K. Vargas-Avila, et al., Increased serum levels of inflammatory mediators and low frequency of regulatory T cells in the peripheral blood of preeclamptic Mexican women, Biomed. Res. Int. 2014 (2014) 413249.
[13] J. Valencia-Ortega, A. Zarate, R. Saucedo, et al., Clinical implications of basic research in preeclampsia: immunological tolerance, Ginecol. Obstet. Mex. 83 (2015) 505.
[14] M. Rahimzadeh, M. Norouzian, F. Arabpour, et al., Regulatory T-cells and preeclampsia: an overview of literature, Exp. Rev. Clin. Immunol. 12 (2016) 209.
[15] A. Ohkuchi, S. Matsubara, Response to Dr Sabour: 'Prediction and prevention of hypertensive disorders of pregnancy: a methodological mistake', Hypertens. Res. 40 (2017) 791.
[16] A.F. Saftlas, L. Rubenstein, K. Prater, et al., Cumulative exposure to paternal seminal fluid prior to conception and subsequent risk of preeclampsia, J. Reprod. Immunol. 101–102 (2014) 104.
[17] B. Ulkumen, D. Silfeler, K. Sofuoglu, et al., The incidence of preeclampsia in ICSI pregnancies, Pak. J. Med. Sci. 30 (2014) 101.
[18] W. Luo, W.T. He, Q. Wen, et al., Changes of TCR repertoire diversity in colorectal cancer after Erbitux (cetuximab) in combination with chemotherapy, Am. J. Cancer Res. 4 (2014) 924.
[19] Y. Li, S. Chen, L. Yang, et al., TRAV and TRBV repertoire, clonality and the proliferative history of umbilical cord blood T-cells, Transpl. Immunol. 18 (2007) 151.
[20] R.O. Emerson, J.M. Mathew, I.M. Konieczna, et al., Defining the alloreactive T cell repertoire using high-throughput sequencing of mixed lymphocyte reaction culture, PLoS One 9 (2014) e111943.
[21] S.M. Mahmoud, E.C. Paish, D.G. Powe, et al., Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer, J. Clin. Oncol. 29 (2011) 1949.
[22] S. Rusakiewicz, M. Semeraro, M. Sarabi, et al., Immune infiltrates are prognostic factors in localized gastrointestinal stromal tumors, Cancer Res. 73 (2013) 3499.
[23] W. Luo, W.J. Liao, Y.T. Huang, et al., Normalization of T cell receptor repertoire diversity in patients with advanced colorectal cancer who responded to chemotherapy, Cancer Sci. 102 (2011) 706.
[24] C. Guo, Q. Wang, X. Cao, et al., High-throughput sequencing reveals immunological characteristics of the TRB-/IgH-CDR3 region of umbilical cord blood, J. Pediatr. 176 (2016) 69.
[25] X. Cao, Q. Wa, Q. Wang, et al., High throughput sequencing reveals the diversity of TRB-CDR3 repertoire in patients with psoriasis vulgaris, Int. Immunopharmacol. 40 (2016) 487.
[26] A.L. Tranquilli, G. Dekker, L. Magee, et al., The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP, Pregnancy Hypertens. 4 (2014) 97.
[27] C. Wang, C.M. Sanders, Q. Yang, et al., High throughput sequencing reveals a complex pattern of dynamic interrelationships among human T cell subsets, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 1518.
[28] Y. Yang, C. Wang, Q. Yang, et al., Distinct mechanisms define murine B cell lineage immunoglobulin heavy chain (IgH) repertoires, Elife 4 (2015).
[29] S.V. Desiderio, G.D. Yancopoulos, M. Paskind, et al., Insertion of N regions into heavy-chain genes is correlated with expression of terminal deoxytransferase in B cells, Nature 311 (1984) 752.
[30] K.J. Jackson, M.J. Kidd, Y. Wang, et al., The shape of the lymphocyte receptor repertoire: lessons from the B cell receptor, Front. Immunol. 4 (2013) 263.
[31] T. Chaiworapongsa, P. Chaemsaithong, L. Yeo, et al., Pre-eclampsia part 1: current understanding of its pathophysiology, Nat. Rev. Nephrol. 10 (2014) 466.
[32] T. Chaiworapongsa, P. Chaemsaithong, S.J. Korzeniewski, et al., Pre-eclampsia part 2: prediction, prevention and management, Nat. Rev. Nephrol. 10 (2014) 531.
[33] G. Georgiou, G.C. Ippolito, J. Beausang, et al., The promise and challenge of high-throughput sequencing of the antibody repertoire, Nat. Biotechnol. 32 (2014) 158.
[34] K. Vahdat, M.R. Pourbehi, A. Ostovar, et al., Association of pathogen burden and hypertension: the Persian Gulf Healthy Heart Study, Am. J. Hypertens. 26 (2013) 1140.
[35] C.L. Haggerty, I. Panum, S.A. Uldum, et al., Chlamydia trachomatis infection may increase the risk of preeclampsia, Pregnancy Hypertens. 3 (2013) 28.
[36] C.S. Gibson, P.N. Goldwater, A.H. MacLennan, et al., Fetal exposure to herpesviruses may be associated with pregnancy-induced hypertensive disorders and preterm birth in a Caucasian population, BJOG 115 (2008) 492.