The Skewness of Alpha Beta T-cell Receptors in Peripheral Blood of the Patients with Type 1 Diabetes

J Zhou[*1], Y Jia[1], L Wang[2], C Kong[3], C Jin[1], X Wang[4]

ABSTRACT

Objective: To detect the skewness of TCR Vα and TCR Vβ of patients with Type 1 diabetes (T1D).

Methods: The heparinized venous blood was collected from ten patients with T1D. The peripheral blood lymphocytes (PBL) were isolated and used to extract mRNA. Reverse amplyfication was performed for cDNA synthesis. The skewness of TCR Vα and Vβ was detected with real-time florescence quantitative polymerase chain reaction (RQ-PCR) and analyzed by DNA melting-curve analysis technique, respectively.

Results: Among the TCR Vα genes, the skewness frequency rate (SFR) of Vα22 was 30%; both of Vα5 and Vα24 were 20%; the SFR of Vα28 was 10%, and which was the only gene showed restricted-clone. In all the Vβ genes, Vβ7 and Vβ17 were the the highest expression genes and their SFRs were both 60%. Vβ11 was near them with the SFR of 40%; the restricted clonal genes were Vβ18 and Vβ20, their SFRs were 10% and 20%, respectivley.

Conclusions: There are skewed genes in TCR Vα and TCR Vβ, which are probably relative to the onset of T1D.

Keywords: Peripheral blood, skewness frequency rate, TCR Vα, TCR Vβ, type 1 diabetes

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From: [1]Clinic Laboratory, [3]Nursing Department, [4]Department of Stomatology, Affiliated Hospital of Jining Medical College, Jining, Shandong Province, China and [2]Department of Pharmacology, the First People's Hospital of Jining City, Jining, Shandong Province, China.

Correspondence: Dr J Zhou, Clinic Laboratory, Affiliated Hospital of Jining Medical College, Jining 272029, Shandong Province, China. Tel: +86-537-2903223; +86-537-2903218, Fax: +86-537-2903223. E-mail: immunolife@126.com

INTRODUCTION

Type 1 diabetes (T1D) is one of the most prevalent autoimmune diseases in the world. To date, although there have been many experimental and clinical researches on it, the exact mechanisms still remain unclear (1). However, a common view that T-cells play an important role in the development of the disease has been proved by many researchers (2–5). As we known, the effects of T-cells depend on the identification of MHC which binding the peptides of auto-antigens, so the auto-respons of T-cells specific to this combination might make the T-cell receptor (TCR) changed, especially the genes locate in complementarity determining region 3 [CDR3] (6). Accordingly, it is possible to find out the immune mechanisms of T1D onset from the specific changes of TCR.

According to the different composition of the double chains, TCR is divided into two subpopulations: γδ TCR and αβ TCR. Because of the high percentage (95%), the researches on the relationship between the skewness of TCR and immune diseases usually focus on αβ TCR. To our knowledge, there are some reports concerning TCR Vβ, and few revolve the skewness of TCR Vα (7). In this study, using real-time florescence quantitative polymerase chain reaction (RQ-PCR) and DNA melting curve analysis technique, we simultaneously detected the skewness of TCR Vα and Vβ in the peripheral blood (PB) from the T1D patients, and hope to present a clue or idea for the future study on the onset mechanisms of T1D.

METHODS

Patients

Ten T1D patients and ten healthy volunteers were recruited. They all were not treated with immunomodulating drugs in the previous six months prior to the study, and were seronegative for the markers of hepatitis viruses, HIV and other pathogenic infections. The patients with tumours and immunological disorders were excluded. This study protocol was approved by the Hospital Ethics Committee.

Extraction of RNAs and synthesis of the first cDNA

The sense primer, anti-sense primer and specific primers for TCR Vα and TCR Vβ genes were previously described (8, 9) and synthesized by the Guangzhou Daangene Corporation of China. 5 mL of Heparinized venous blood were collected from each of the T1D patients, and peripheral blood lymphocytes (PBL) were isolated by Ficoll-Hypaque density centrifugation.

Using an Omega RNA extraction kit and according to the manufacturer's instructions, total RNA was extracted, and 1 μg total RNA was reverse transcribed with 250 pm olig (dT), 200 U Moloney murine leukaemia virus (M-MuLV) reverse transcriptase, and 2 μl of 10 mM dNTP mix (cDNA Synthesis Kit; MBI-Fermentas), in a total volume of 20 μl (six reactions for every sample). The cDNA was stored at -80 ˚C.

Skewness detection with RQ-PCR and melting-curve analysis technique

The cDNA products were continuously amplicated with RQ-PCR in a 20 μl volume with 10 μl 2 × Real-time PCR Master Mix (TOYOBO, JAPAN), which contained Taq-polymerase,

dNTPs, PCR buffers and SYBR green I. The final concentration of each primer was 0.3 μM. Subsequently, 1 μl liquid containing 10–50 ng reverse-transcribed total RNA was added to the reaction mixture as the PCR template. Reactions were performed in MJ Opticon 2 DNA engine and analysed with Opticon Monitor 3.0 software (Bio-rad, USA). The reactiong conditions was shown as following: pre-incubation at 94 °C for 3 min, 94 °C melting for 20 sec, primer annealing at 56 °C for 30 sec and extension at 72 °C for 30 sec. The above procedure was iterated 40 cycles for the whole amplification. Finally, the data obtained from RQ-PCR were further analysed with melting-curve analysis technique.

Calculation of skewness frenquency rates of TCR Vα and Vβ gene families

In order to evaluate the usage of every TCR gene respectively, the skewness frenquency rates (SFR) of TCR Vα and Vβ gene families were saperatly calculated with the following formula:

SFR = N1 / N0 × 100%.

N1, is the summary of certain a skewed gene family; N0, is the total of the corresponding genes which including the skewed and the non-skewed.

RESULTS

All the Vα gene families of the healthy objects exhibited multi-peaks. In T1D ptients, most of TCR Vα gene families of T1D patients exhibited multi-peaks; several genes showed oligo-peaks, single-peaks or low-level peaks. The different types of peaks respectively,

represented polyclone, oligoclone, monoclone and restricted-clone. The gene families with oligoclone, monoclone and restricted-clone were totally called skewed gene family. The genes exhibited oliogclone and monoclone were named predominant skewness (10). The details for the skewed gene families were as shown as in Table 1.

Table 1: All the skewed Vα gene families in PBL of 10 T1D patients

Patients	**Monoclone**	**Oligoclone**	**Restricted-clone**
1	Vα24	Vα14,Vα26	—
2	Vα4.1	Vα12, Vα20	—
3	—	Vα15, Vα24	—
4	Vα11	Vα17	Vα28
5	Vα13	Vα1.1, Vα22	—
6	—	Vα2, Vα6	—
7	Vα22	Vα4.2, Vα18	—
8	Vα6	Vα19, Vα23,	—
9	Vα22	Vα5, Vα10, Vα29	—
10	Vα32	Vα16, Vα27	—

Among the TCR Vα genes, the frequency of Vα22 was 30%; Vα5 and Vα24 were next to it with the SFRs of 20%. There was no skewness for some genes: Vα1.2, Vα3, Vα7, Vα8, Vα9, Vα21, Vα25, Vα30 and Vα31. The SFR of Vα28 was 10%, which was the only gene showed restricted-clone (Table 2).

Table 2: The skewness frequency rate of each Vα and Vβ gene family in PBL of ten T1D patients

TCR Vα	Times for each predominant gene	Skewness frequency rate (%)	TCRR Vβ	Times for each predominant gene	Skewness frequency rate (%)
1.1	1	10.0	1	3	30.0
1.2	0	0	2	2	20.0
2	1	10.0	3	0	0
3	0	0	4	0	0
4.1	1	10.0	5.1	1	10.0
4.2	1	10.0	5.2	0	0
5	1	10.0	6	2	20.0
6	2	20.0	7	6	60.0
7	0	0	8	0	0
8	0	0	9	1	10.0
9	0	0	10	0	0
10	1	10.0	11	4	40.0
11	1	10.0	12	0	0
12	1	10.0	13.1	1	10.0
13	1	10.0	13.2	1	10.0
14	1	10.0	14	0	0
15	1	10.0	15	0	0
16	1	10.0	16	0	0
17	1	10.0	17	6	60.0
18	1	10.0	18	2	20.0
19	1	10.0	19	1	10.0
20	1	10.0	20	3	30.0
21	0	0	21	2	20.0
22	3	30.0	22	1	10.0
23	1	10.0	23	1	10.0
24	2	20.0	24	2	20.0
25	0	0			
26	1	10.0			
27	1	10.0			
28	1	10.0			
29	1	10.0			
30	0	0			
31	0	0			
32	1	10.0			

TRAV: T-cell receptor alpha chain; TRBV: T-cell receptor beta chain; PBMC: peripheral blood mononuclear cell.

All the Vβ gene families of the healthy controls exhibited polyclone. In T1D ptients, most of TCR Vβ gene families of T1D patients exhibited polyclone, several genes showed oligoclone, monoclone or restricted-clone. Vβ7 and Vβ17 were the predominant usage genes with the highest SFRs, which were 60%; Vβ11 was next to them with the SFR of 40%; the SFRs of Vβ2, Vβ6, Vβ18, Vβ21 and Vβ24 were all 20%. However, there was no skewness for Vβ3, Vβ4, Vβ5.2, Vβ8, Vβ10, Vβ12, Vβ15 and Vβ16 in all the T1D patients. The restricted-clonal genes were Vβ18 and Vβ20; and their SFRs were 10% and 20%, respectivley (Table 3).

Table 3: All the skewed Vβ gene families in PBL of ten T1D patients

Patients	**Monoclone**	**Oligoclone**	**Restricted-clone**
1	Vβ6, Vβ7	Vβ8, Vβ13.2, Vβ17, Vβ21, Vβ24	Vβ18
2	Vβ7	Vβ11, Vβ17, Vβ21	—
3	Vβ11	Vβ9, Vβ17, Vβ19	—
4	—	Vβ2, Vβ17	—
5	Vβ11, Vβ17	Vβ1, Vβ7, Vβ23	—
6	Vβ7	Vβ11	Vβ20
7	Vβ6	Vβ18	—
8	Vβ5.1	Vβ1, Vβ1 3.1, Vβ24	Vβ20
9	—	Vβ1, Vβ7, Vβ22	—
10	Vβ7	Vβ2, Vβ17	—

According to the the SFR of the each gene family of Vα and Vβ, we respectively drew the histograms to further comprehensively analyse the skewness of TCR Vα and TCR Vβ of the T1D patients. The two grams were further put together back to back, and a figure like a

big key formed (Figure 1. A and B) side represented the predominant SFRs of TCR Vα and Vβ gene families, respectively. The prominent columns like the kits of the key; the higher the SFR of the Vα or Vβ gene family was, the higher the kit of the key would be.

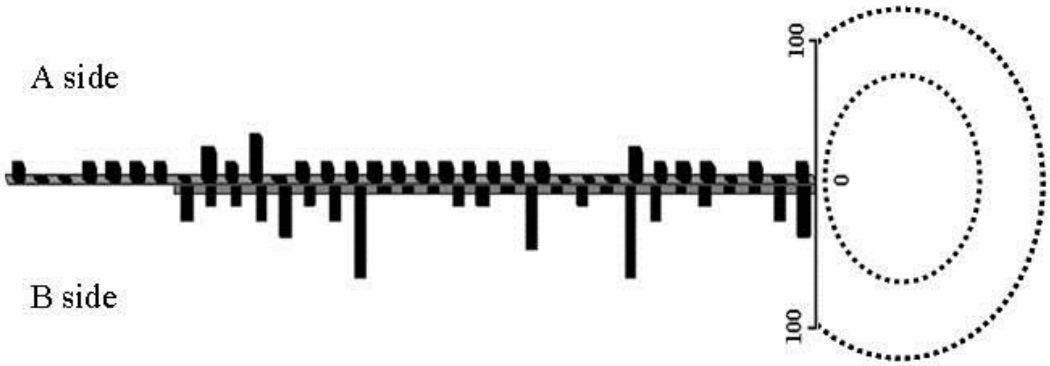

Figure 1 The key formed with the skewed TCR Vα and Vβ in PBMC of ten T1D patients

According to different skewness frequency rates of every TCR Vα and Vβ genes, two histograms were drawn out, respectively. Moreover, both were put together back to back. It was easy to see that the merged gram looked like a big key, which was due to the different frequency of the each gene family ofTCR. A and B side, respectively represents the skewed Vα and Vβ gene families. The prominent columns like the kits of the key, and the higher the frequency of the Vα and Vβ gene family was, the higher the kit of the key would be. To some extent, the key presented the total characterization of the skewness of Vα and Vβ of the ten T1D patients.

DISCUSSION

In the past thirty years, more and more scholars studied the associations between the TCR repertoires and tumour, infectious and autoimmune diseases. Among them, most of the reports focussed on the skewness of TCR Vβ, and few reports revolved that of TCR Vα (11–13). As to T1D, such a situation looks like more obvious (7). In an animal experiment, researchers found that TCR Vα5D-4 was the main gene in the mice model of T1D, the

invasion and clonal proliferation of T-cells with TCR Vα5D-4 was observed in insulin. According to these evidences, the scholars thought that TCR Vα5D-4 probably was the stimulative factor for T1D in mice (14). In another basic test, Du *et al* (15) found TCR Vα7 and Vα17 were the predominant expression genes. In this study, we directly detected the TCR Vα usage of PB of T1D patients, and found that, compared to the sole character of polyclone for the healthy donors, the usage of TCR Vα showed variety: except polyclone expression for most TCR Vα genes, there were several genes exhibited oligoclone, monoclone and restricted-clone. In all the T1D patients, Vα22 was the gene of highest SFR, Vα5 and Vα24 were next to it. Vα1.2, Vα3, Vα7, Vα8, Vα9, Vα21, Vα25, Vα30 and Vα31 were not predominantly used. These results were significantly different from those of the above animal experiments. This difference probably indicated that the onset mechanism for T1D was different between animal and human, and animal experiments could expose the mechanism of T1D onset, but the results could hardly truly reflect the skewed TCR repertoire of T1D patients.

In the studies on the skewness of TCR Vβ of the animals with T1D, Liu *et al* (16) found that, in spite of $CD4^+$ or $CD8^+$ T-cells, TCR–Vβ13S1A1 was identified as an allele of the TCR Vβ in the rat model with T1D. Further test showed that the spontaneous diabetes could be prevented though the vaccination of the antibody specific to TCR–Vβ13S1A1. In another study, Codina-Busqueta *et al* simultaneously detected the skewness of TCR Vβ in the peripheral blood mononuclear cells (PBMCs) of non-obese diabetic (NOD) mice and T1D patients, and found the monoclonaly expaned Vβ22 in both of them. These suggested that Vβ22 clone may have expanded or accumulated in situ by an autoantigen present in both

NOD mice and T1D patients (6). Interestingly, in the simultaneous study on NOD mice and pediatric patients with T1D, Marrero *et al* (17) found the common skewed TCR Vβ genes: TRBV1 (Vβ2), TRBV13-3 (Vβ8.1) and TRBV19 (Vβ6). Unfortunatly, this argument was not yet proved by others, including our studies on T1D patients. In the previous study (18), we found that TCR Vβ7 was the common predominant usage gene in two T1D patients; and moreover, the two Vβ7 genes shared the same amino acid sequences. In the present study, TCR Vβ7 was also determined as the most predominant usage gene in all the ten T1D patients. Besides, Vβ17 was identified as another skewed gene which SFR was equal to Vβ7.

These results were consistent with the report of Luppi (19), in which they found the frequency of circulating TCR Vβ7 and Vβ17 T-cells in PBMCs from T1D patients increased. Similarly, an increase of Vβ7 expression also reported in PBMCs of T1D patients in another study (20). In a study on two children with T1D, a marked overrepresentation of mRNA encoding TCR Vβ7 chain was observed (21). Based the studies on T1D patients, it was possible to drawn a conclusion that Vβ7 was the most predominant usage gene in T1D patients. However, this was denied by the Tzifis' study, in which they found Vβ4 was the most predominant gene family in the pediatric patients with T1D (22). This difference probably lays on different ages of the objects, or different techniques used to assay the skewness of TCR Vβ genes. Additionally, besides the common characters of TCR Vβ usage existing in the T1D patients, there were possible individualized properties between the different individuals with T1D (23,24).

According to Figure 1, we interestingly found that the merged histogram looked like a big key. The columns like the kits of the key, and the higher the SFR for each of the Vα and Vβ

gene family was, the higher the kit of the key would be. To some extent, the key represented the total characteristic of Vα and Vβ skewness of the ten T1D patients. As we known, the skewness of TCR (included TCR Vα and TCR Vβ) was specific to the associated antigen of T1D, so the key formed with the skewness frequency of each gene could be taken as the summary clonal changes of TCR Vα and Vβ. In another words, it should be a specific key to T1D. There is a China proverb says that ‘Open different locks with different keys’. In our opinions, an accurate ‘key’ specific to T1D will be drawn out through more studies in future, and with which the door hiding the secrets for the disease onset will be opened to a greater extent.

CONCLUSION

In this study, we found that Vα22, Vβ7 and Vβ17 were the predominant usage genes, while Vα28, Vβ18 and Vβ20 were the restrictedly clonal genes in T1D patients. However, the function of the skewness of TCR Vα and Vβ genes on the onset of T1D needs further and deep study in future.

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DECLARATION OF INTEREST

All the authors declare that there is no conflict of interest.

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