Article

Analysis of the CDR3 Length Repertoire and the Diversity of TCRα Chain in Human Peripheral Blood T Lymphocyts

Xinsheng Yao[1, 3, 4], Ying Diao[2, 3], Wanbang Sun[1], Junmin Luo[1], Ming Qin[1] and Xianying Tang[1]

Analysis of complementarity determining region 3 (CDR3) length of T lymphocyte receptors (TCRs) by immunoscope spectratyping technique has been used successfully to investigate the diversity of TCR in autoimmune diseases and infection diseases. In this study, we investigated the patterns of CDR3 length distribution for all 32 TCR AV gene families in human peripheral blood lymphocytes of four normal volunteers by the immunoscope spectratyping technique. It was found that PCR products exhibited an obscure band on 1.5% agarose gel electrophoresis. Each TCR AV family exhibited more than 8 bands on 6% sequencing gel electrophoresis. The CDR3 spectratyping of all TCR AV families showed a standard Gaussian distribution with different CDR3 length, and the expression frequency of CDR3 was similar among the gene families. Most of CDR3 in TCR AV family recombine in frame. However, some of the CDR3 showed out-of frame gene rearrangement. Additionally, we found that in some of TCR AV families there were 18 amino acid discrepancies between the longest CDR3 and shortest CDR3. These results may be helpful to further study the recombination mechanism of human TCR genes, the TCR CDR3 gene repertoire, and the repertoire drift in health people and disease state. *Cellular & Molecular Immunology*. 2007;4(3):215-220.

Key Words: TCR, genescan, CDR3, spectratyping, immunoscope

Introduction

Human peripheral T cell receptor (TCR) is membrane glycol-proteins, 95% of which are composed of α and β chain. Each chain is created by the random joining of variable (V), diversity (D), and joining (J) gene segments by recombination, thus providing the diversity of the TCR recognition spectrum (1). It has been reported that the TCR repertoire of variable gene segments in humans comprises more than 70 TCR AV (variable gene of TCRα chain) (2). These sequences were classified into 32 TCR AV families and 24 TCR BV families based on the nucleotide sequence similarity. CDR3 of TCRα chain is composed of the terminal of AV, the foreside of AJ and a nucleotide sequence inserted between them by terminal deoxynucleotidyl transferase (TdT); β chain of TCR is derived from rearrangement of the genes "BV-BD-BJ-BC" fragments. CDR3 of TCRβ chain is composed of the terminal of BV gene fragment, BD gene fragment, the foreside of BJ gene fragment, and a nucleotide sequence inserted between V-D and D-J by TdT. Different T cell clones have different sequences or lengths of CDR3. Sequence of CDR3 region determines T cell receptor structure and specificity, and one CDR3 sequence represents one T cell clone (3, 4), and most of T cell clones binding to the same MHC peptide complex have the same CDR3 length. Accordingly, determination of the frequency of a specific CDR3 sequence may present the replication level of a specific T cell clone and, thus, reflect the functional status of T cells.

Recently, the immunoscope spectratyping technique (5) approach for determining TCR CDR3 repertoire size has been proved suitable for detecting the poly-clonal and oligo-clonal expansion of T cells in tumors (6), transplantation (7), autoimmune diseases (8) and infectious diseases (4, 9). Some researchers reported the development of TCRβ chain CDR3 length repertoire of human T lymphocytes (10). However, it remains unknown about the detailed characteristics of CDR3 length repertoire in peripheral blood of healthy people. We studied the CDR3 spectratyping of TCRα chain in PBMCs of four normal volunteers. The results would provide the basic data for investigating TCR gene recombination, and CDR3 pedigree drift in disease state.

[1]Department of Immunology, Zunyi Medical College, Zunyi 563003, China;

[2]Center of Reproductive Medicine, Zunyi Medical College, Zunyi 563003, China;

[3]Xinsheng Yao and Ying Diao contributed equally to this work.

[4]Corresponding to: Dr. Xinsheng Yao, Department of Immunology, Zunyi Medical College, Zunyi 563003, Guizhou, China. E-mail: immunology @126.com

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Table 1. The primers of TCR AV family and TCR AC1-FAM

Family	Primer
AV 1-1	TCTGGTATGTGCAATACCCCAACC
AV 1-2	CTGAGGAAACCCTCTGTGCA
AV 2	GATGGAAGGTTTACAGCACAGCTC
AV 3	CACAGTGGAAGATTAAGAGTCACGC
AV 4-1	GGATTGCGCTGAAGGAAGAG
AV 4-2	AACAGAATGGCCTCTCTGGC
AV 5	TGAAGGTCACCTTTGATACCACCC
AV 6	AATCCGCCAACC TTG TCATCTCCG
AV 7	AACTGCACGTACCAGACATC
AV 8	ACCCTGAGTGTCCAGGAGGG
AV 9	CACTGCTGACC TTAACAAAGGCG
AV 10	TCCTGGTGACAGTAGTTACG
AV 11	AGGCTCAAAGCCTTCTCAGCAGGG
AV 12	TCCACCAGTTCCTTCAACTTCACC
AV 13	TTCATCAAAACCCTTGGGGACAGC
AV 14	CCCAG CAG GCAG ATGATTCTCG TT
AV 15	GGATAAACATCTGTCTCTGCG
AV 16	AAGGGAATCCTCTGACTGTG
AV 17	GATAGCCATACGTCCAGATG
AV 18	TGCCACTCTTAATACCAAGGAGGG
AV 19	ACACTGGCTGCAACAGCATC
AV 20	TTACAAACGAAGTGGCCTCC
AV 21	ACCCTGCTGAAGGTCCTACATTCC
AV 22	CTTGGAGAAAGGCTCAGTTC
AV 23	TG CCTCGCTGGATAAATCATCAGG
AV 24	TCCCAGCTCAGCGATTCAGCCTCC
AV 25	GTCCTGTCCTCTTGATAGCC
AV 26	AGCCCAGCCATGCAG GCATCTACC
AV 27	TTGATACCAAAGCCCGTCTC
AV 28	G AACATCACAGCCACCCAGACCGG
AV *29*	G CAAAGCTCCCTGTACCTTACGG
AV 30	TTTCTGCACAGCACAGCCCC
AV 31	AGCAAAAACTTCGGAGGCGG
AV 32	AAGGAG AG GACTTCACCACG
AC1	GCAGACAGACTTGTCACTGG

Materials and Methods

Blood samples

Peripheral blood samples were collected from 4 donors, who were negative of HBsAg and HIV antibody and there were no clinical or laboratory evidences of other infectious diseases and immunological disorders. Ten milliliters of blood were taken from the healthy donors and the peripheral blood mononuclear cells (PBMC) were obtained by Ficoll gradient centrifugation before total RNA extraction.

Primers and reagents

The primers were designed for TCR AV family as previously described with a little modification (Table 1) (3, 6, 9). Total RNA column extract kit (Omega) was purchased from Bio-Tek, cDNA synthesis kit was obtained from MBI-Fermentas, GeneScan-500-TAMRA (500 ROX) standard was obtained from Applied Bio-systems, and Formamide from Sigma.

Extraction of RNA and reverse transcription

As recommended by the manufacturer, total RNA was extracted from PBMC by Omega RNA extracted kit. Total RNA (1 μg) was reverse transcribed with 250 pm olig (dT), 200 U M-MuLV reverse transcriptase, 250 μM of each dNTP, in a total volume of 20 μl (six reaction of every sample). The cDNA was stored at -80^{o}C before used as the template for PCR amplification.

PCR amplification of TCRα chain

CDR3 size analysis within the TCRα chain was performed by a two-step PCR. The first-round PCR amplification reactions for each of 32 AV gene families were carried out in 50 μl of reaction mixtures containing 2 μl the forward AV primer and the reverse AC primer, 2.0 mM $MgCl_2$, 10 mM Tris-HCl, 200 mM dNTP and 1 μl cDNA. The program of PCR was at 95^{o}C for 5 min, one cycle; then 95^{o}C for 30 s; 60^{o}C for 30 s, 72^{o}C for 90 s, 35 cycles, and 72^{o}C for 10 min. The second-round PCR separate amplification reactions for human AV gene families were carried out in 30 μl of reaction mixtures containing 2 μl the forward AV primer and the reverse AC-FAM primer, 2.0 mM $MgCl_2$, 10 mM Tris-HCl, 200 mM dNTP, 4 μl of the first-round products as the template. The program of PCR was at 95^{o}C for 2 min, 60^{o}C for 2 min, 72^{o}C for 2 min, four cycles.

Analysis of CDR3 length by spectratyping (GeneScan)

The mixture containing 2 μl fluorescent PCR products, 2 μl formamide, 0.5 μl loading dye (25 mM EDTA, 50 ng/ml blue dextran) and 0.5 μl GeneScan-500 TAMRA dye-labeled size standards, was denatured at 95^{o}C for 2 min, and then was loaded onto the 6% acrylamide sequencing gel, run for 2 h on a 50-lane applied Bio-systems model 373A DNA sequencer. The data were analyzed by GeneScan software version 672.

Results

The patterns of CDR3 length distribution for all TCR AV family (products of PCR) were analyzed on a 1.5% agarose gel by ethidium bromide staining. We found PCR products of every TCR AV family had a blur band in four healthy donors (Figure 1).

When the fluorescent PCR products were analyzed on 6% acrylamide sequencing gel (GeneScan), the patterns of CDR3 length distribution for all the TCR AV family of four healthy donors showed Gaussian distribution; more than 8 bands of every family were observed (Figure 2). After the data were analyzed by GeneScan software version 672, the CDR3 of 32 TCR AV families showed the length repertoire and diversity

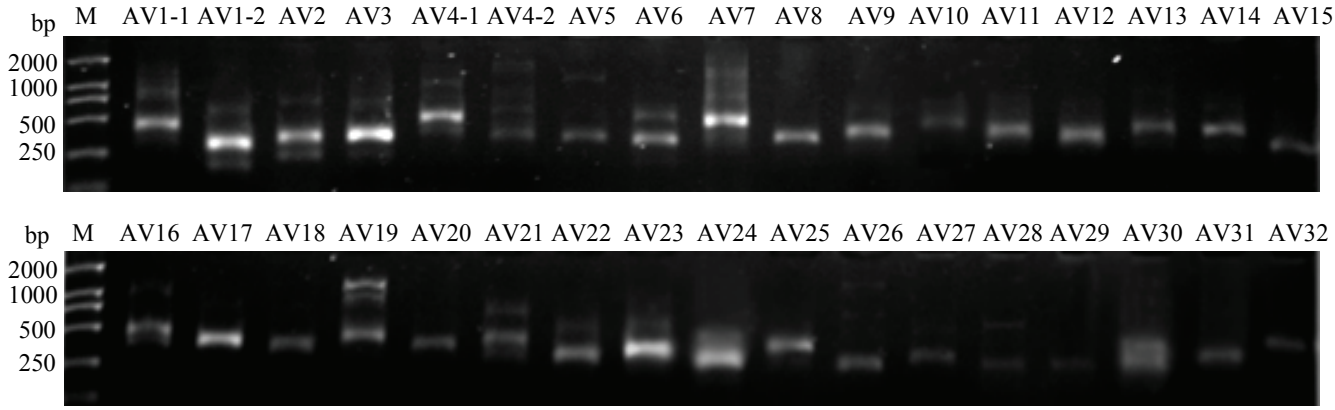

Figure 1. Analysis of TCR AV families in healthy donor. The PCR products of TCR AV families were analyzed on a 1.5% agarose gel by ethidium bromide staining. A blur band in the predicted position of products size was observed in all TCR AV family and some disorder bands were showed in some TCR AV family. The PCR products of one healthy donor were showed.

(Figure 3).

After analyzed the characteristics of the CDR3 spectratyping, we found most of CDR3 in TCR AV family recombined in frame, there were 3-bp intervals between two CDR3 products. However, some of the CDR3 showed out-of frame gene rearrangement in the four normal volunteers. It exhibited 1-2 bp or ≥ 6 bp discrepancy between two adjacent CDR3 PCR products. Furthermore, we found that there was 18-amino-acid discrepancy between the longest CDR3 and shortest CDR3 in some of TCR AV family (Table 2).

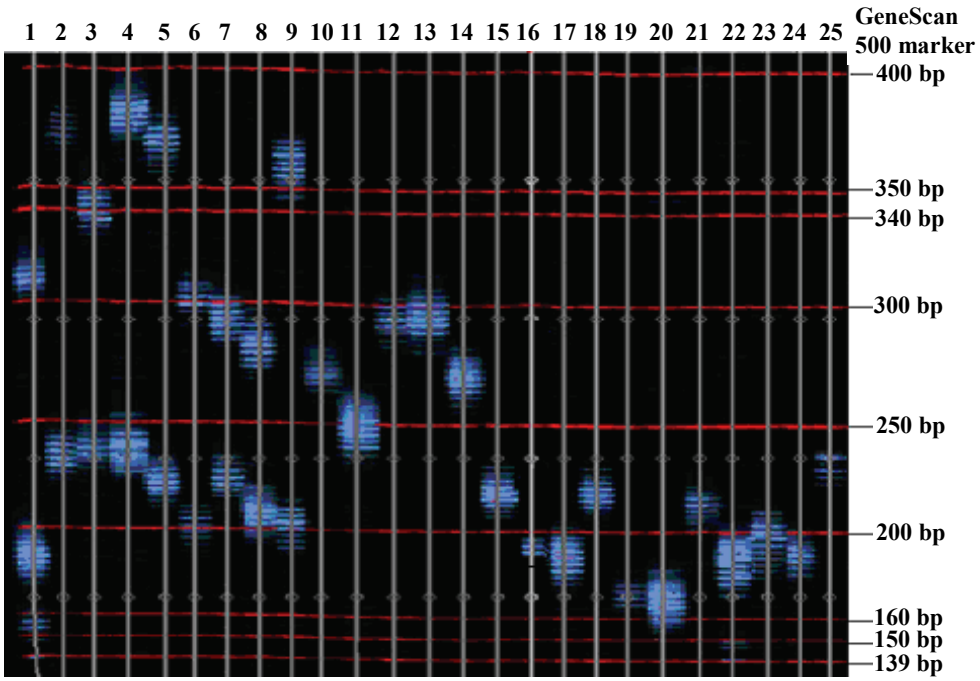

Figure 2. Analysis of the patterns of CDR3 length distribution for all the TCR AV family. The fluorescent RT-PCR products of TCR AV families and TCR AC (FAM-labeled) in the healthy control respectively analyzed on a 6% acrylamide sequencing gel (GeneScan). The red bands in the Lane were the GeneScan-500 marker, the blue bands were the products of TCR AV family. The polyclonal expansion T cells of TCR AV family showed Gaussian distribution, more than 8 bands in one family were observed. The PCR products of one healthy donor were shown. Lane 1, AV1-1 and AV1-2; Lane 2, AV2 and AV4-1; Lane 3, AV3 and AV7; Lane 4, AV4-2 and AV8; Lane 5, AV5 and AV16; Lane 6, AV16 and AV19; Lane 7, AV21 and AV9; Lane 8, AV12 and AV25; Lane9, AV15 and AV32; Lane 10, AV10; Lane 11,AV11; Lane 12,AV13; Lane 13, AV14; Lane14,AV17; Lane15, AV18; Lane 16, AV20; Lane 17, AV22; Lane 18, AV23; Lane 19, AV24; Lane 20, AV26; Lane 21, AV27; Lane 22, AV28; Lane 23, AV29; Lane 24, AV30; Lane 25, AV31.

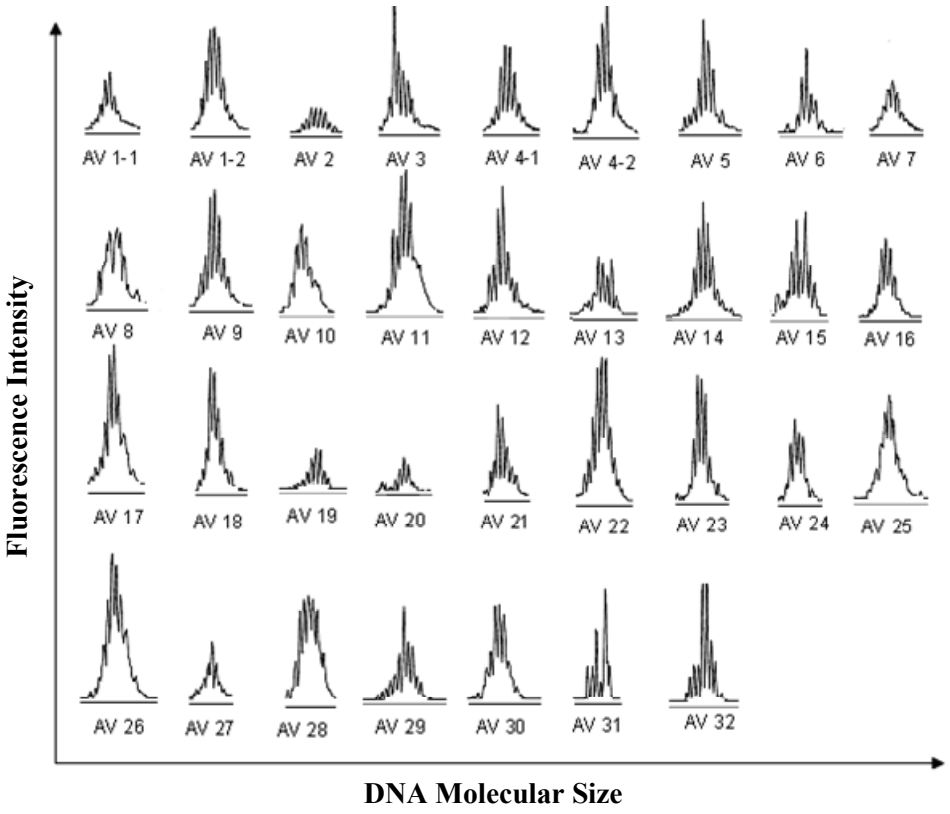

Figure 3. **The CDR3 length distribution of healthy donor.** Spectratyping of CDR3 sizes for all 32 TCR AV gene families (TCR AV, TCR AJ, TCR AC junction-size distribution profiles) in healthy was analysed. The x-axis of each plot corresponded to the size of nucleotides and the relative fluorescence intensity of the peaks was shown on the y axis. All the CDR3 of 32 TCR AV showed Gaussian distribution. The result of one healthy donor was showed.

Discussion

According to the classical clonal selection theory, TCR undergoes V(D)JC gene rearrangement in the thymus on the basis of their germline genes rearrangement, and matured T cells migrate to peripheral sites to form the responsive T cell reservoir. CDR3 region of TCR is the special molecular structure for representing the different population of T cells. Spectratyping of CDR3 of TCRα chain length repertoire and diversity is designed by the rules of TCR gene rearrangement. To date, CDR3 of TCR repertoire drift has been reported in some infectious diseases (HIV, viral hepatitis, measles, etc) (4, 9, 11), tumors (leukemia, cancer of colon, melanoma, etc) (6), transplantation (kidney and bone marrow transplantation, etc) (7, 12), autoimmune diseases (SLE, rheumatoid arthritis, etc) (8, 13). Although it had been reported the development of TCRβ chain CDR3 length repertoire of human T lymphocytes (10), these reports only noted that CDR3 of TCRα chain in normal people showed a Gaussian distribution, the characteristics of CDR3 length repertoire and diversity were not clear.

We researched the CDR3 of TCRα chain the length repertoire and diversity in PBMC of 4 normal donors. PCR products of CDR3 of the 32 TCR AV families exhibited an obscure band on 1.5% agarose gel electrophoretogram, which was due to many different products contained in each family. Each TCR AV family exhibited more than 8 bands on 6% sequencing gel electrophoretogram. These findings were consistent with previous reports, suggesting polyclonal proliferation of TCRαβ T cells was found in normal human.

Most studies involving spectratyping for studying CDR3 did not analyze its detailed sizes in various families, but reported the results with more than 8 bands in every family. We found that CDR3 of TCRα chain in 32 TCR AV families was high diversity with different length, and the expression frequency of CDR3 was similar among the families. Interestingly, there were some of TCR AV families with 18 amino acid discrepancy between the longest CDR3 and shortest CDR3 in different family. But in normal, there were just no more than 8 amino acid length discrepancy in the different CDR3 region. Some studies reported the shorter CDR3 related the CD4 single positive cells in thymus (14), the detailed differences and function of the longer or the shorter CDR3 group maybe need further research.

During TCR rearrangement in the thymus, 2/3 rearrangement develops out-of frame (AV-AJ recombination with not of 3 bp or 3 multiple bp inserted). No report is available

Table 2. Analysis of the characteristics of CDR3 of TCR AV families in peripheral blood of healthy donor by GeneScan technique

AV1-1	AV1-2	AV2	AV3	AV4-1	AV4-2	AV5	AV6	AV7	AV8	AV9	AV10	AV11	AV12	AV13	AV14	AV15
299	138	223	222	359	223	204	177	326	362	209	260	233	190	274	280	188
302	141	226	225	362	*225*	207	?	329	365	*210*	*261*	236	193	277	233	191
305	?	229	*226*	365	226	210	183	332	368	212	263	239	196	*278*	286	194
308	147	232	228	368	229	*212*	184	336	371	215	*264*	242	*197*	280	289	197
311	150	235	*229*	371	*230*	213	186	339	374	218	266	245	199	*282*	292	200
314	153	238	231	374	*231*	216	189	342	377	221	*267*	248	*201*	283	295	203
317	156	241	*232*	377	232	219	192	345	380	224	269	251	202	286	298	206
320	159	244	234	380	235	222	195	348	383	227	*270*	*253*	*204*	*288*	301	209
		247	*235*	383	238	225	198	351	386	230	272	254	205	289	*302*	212
	174	250	237	386	*239*	228	202	354	389	233	*273*	257	*207*	291	304	
	177	253	*238*	389	241	*230*	205	*355*	*391*		275		208	292	307	
	180	*254*	240		244	231	208	357	392		*276*		211	295	300	
	183	256	*241*		*245*	*233*	211	360	395		278		214	298		
	186		244		247	234	214		398		281		217	301		
	189		*245*		?	237								304		
	192		247		253											
	195		*248*		256											
	198		250													
	199															
	201															

AV16	AV17	AV18	AV19	AV20	AV21	AV22	AV23	AV24	AV25	AV26	AV27	AV28	AV29	AV30	AV31	AV32
356	254	207	287	182	282	164	182	154	267	151	193	150	179	175	223	349
359	257	210	290	185	285	167	185	157	270	154	196	153	182	178	226	*351*
362	*258*	213	293	188	288	170	188	*159*	273	157	199	156	185	*180*	229	352
365	*259*	216	296	191	291	172	191	160	276	*158*	202		188	181	232	355
368	260	219	299	194	294	173	*?*	163	279	160	205	162	191	184	235	358
371	263	222	302		297	176	197	166	282	163	208	165	194	187	238	361
374	266	225	305	215	300	179	200	169	285	166	211	168	197	190		364
377	269	*226*	*307*	218	303	*180*	203	172	288	169	214	171	200	*191*		367
380	272	228	308	221	306	182	206	175	291	172	217	174	203	193		370
383	275	231	311			185	207	178	*292*		*218*	177	206	196		373
386	*276*	234	*312*			188	209	181	294		220	180	209	199		
	278		314			191	212	184	297		223	183		202		
	281		317			194	215		300		226	186				
			320			197	218		303			189				
						200	221					192				
						203	224					195				
							227									
							230									

Most of CDR3 in TCR AV family recombined in frame. But some of the CDR3 showed out-of frame gene rearrangement, it exhibited 1-2 bp (the number were showed with underline and italic) or ≥ 6 bp (shown in the Table with "?" or "space") discrepancy between two adjacent CDR3 PCR products.

about out-of-frame/in-frame rearrangement during TCR transcription and expression at mRNA level of peripheral T cells. For the clonal selection theory, all the CDR3 gene recombination ought to be in frame in the mature T cells, but in our results, we found most of CDR3 in TCR AV family recombined in frame, however, some of the CDR3 showed out-of-frame gene rearrangement in the four normal volunteers. It exhibited 1 bp or 2 bp between two adjacent CDR3 PCR products, at the same time, some of the two adjacent CDR3 PCR products with 6 bp (or more than 6 bp) discrepancy. It means there is a default CDR3 group in the family. The mechanisms of this special CDR3 characteristics may be related to the individual germline gene or belong to the pseudo gene family (15).

TCR CDR3 protein is one of the most diversified and complex proteins in human body. Immunoscope spectratyping technique is a simple, sensitive and reliable method to analyze TCR. In terms of TCR CDR3 diversity, CDR3 of the same base fragment length may have different base types inserted in recombination, that is, CDR3 of the same family and of the same length may contain information for more than one protein. Our results maybe helpful for further study of human TCR gene recombination, TCR CDR3 high polymorphism of different normal people, and TCR CDR3 gene

repertoire drift in disease state.

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References

1. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. Nature. 1988;334:395-402.
2. Rowen L, Koop BF, Hood L. The complete 685-kilobase DNA sequence of the human β T cell receptor locus. Science. 1996; 272:1755-1762.
3. Gorski J, Yassai M, Zhu X, et al. Circulating T cell repertoire complexity in normal individuals and bone marrow recipients analyzed by CDR3 size spectratyping. Correlation with immune status. J Immunol. 1994;152:5109-5119.
4. Hohn H, Neukirch C, Freitag K, et al. Longitudinal analysis of the T-cell receptor (TCR)-VA and -VB repertoire in $CD8^+$ T cells from individuals immunized with recombinant hepatitis B surface antigen. Clin Exp Immunol. 2002;129:309-317.
5. Manfras BJ, Rudert WA, Trucco M, Boehm BO. Analysis of the α/β T-cell receptor repertoire by competitive and quantitative family-specific PCR with exogenous standards and high resolution fluorescence based CDR3 size imaging. J Immunol Methods. 1997;210:235-249.
6. Xin-Sheng Y, Zheng-Jun X, Li M, et al. Analysis of the CDR3 region of α/β T-cell receptors (TCRs) and TCR BD gene double-stranded recombination signal sequence breaks end in peripheral blood mononuclear cells of T-lineage acute lymphoblastic leukemia. Clin Lab Haematol. 2006;28:405-415.
7. Hirokawa M, Matsutani T, Saitoh H, et al. Distinct TCRAV and TCRBV repertoire and CDR3 sequence of T lymphocytes clonally expanded in blood and GVHD lesions after human allogeneic bone marrow transplantation. Bone Marrow Transplant. 2002;30: 915-923.
8. Cancrini C, Romiti ML, Finocchi A, et al. Post-natal ontogenesis of the T-cell receptor CD4 and CD8 Vβ repertoire and immune function in children with DiGeorge syndrome. J Clin Immunol. 2005;25:265-274.
9. Yao XS, Zhang GW, Ma L, et al. Analysis of the CDR3 length of TCR αβ T cells in the peripheral blood of patients with chronic hepatitis B. Hepatol Res. 2006;35:10-18.
10. Nishio J, Suzuki M, Nanki T, Miyasaka N, Kohsaka H. Development of TCRB CDR3 length repertoire of human T lymphocytes. Int Immunol. 2004;16:423-431.
11. Raaphorst FM, Schelonka RL, Rusnak J, Infante AJ, Teale JM. TCRBV CDR3 diversity of $CD4^+$ and $CD8^+$ T-lymphocytes in HIV-infected individuals. Hum Immunol. 2002;63:51-60.
12. Gorski J, Yassai M, Keever C, Flomenberg N. Analysis of reconstituting T cell receptor repertoires in bone marrow transplant recipients. Arch Immunol Ther Exp (Warsz). 1995;43: 93-97.
13. Kim G, Tanuma N, Matsumoto Y. Stage-dependent usage of TCRα chains with different CDR3 motifs by spinal cord T cells in autoimmune encephalomyelitis. J Neuroimmunol. 1999;96: 66-72.
14. Yassai M, Ammon K, Goverman J, Marrack P, Naumov Y, Gorski J. A molecular marker for thymocyte-positive selection: selection of CD4 single-positive thymocytes with shorter TCRB CDR3 during T cell development. J Immunol. 2002;168:3801-3807.
15. Nemazee D. Receptor editing in lymphocyte development and central tolerance. Nat Rev Immunol. 2006;6:728-740.