Original Article

Comparing TCR Beta Chain variable Gene Profile Skewness between Children with Tuberculosis and BCG-vaccinated Children

Jian-wei Zhou MD•[1], Cui Kong[1], Junmin Luo[3], Jinghe Cao[1], Yanxi Shi[2]

Abstract

Background: We compared the T cell antigen receptor (TCR-BV) gene families of peripheral blood mononuclear cells (PMBC) between children with tuberculosis (TB) and those inoculated with the Bacille Calmette Guerin (BCG) vaccine.

Methods: The total RNA was extracted from PMBC of 15 TB children, 15 BCG-vaccinated children and 15 healthy controls. The RNAs were reverse-transcribed and amplified by polymerase chain reaction (PCR). PCR products were separated on 1.5% agarose gel and analyzed with the Genescan technique.

Results: Some TCR-BV gene families in TB children and BCG-vaccinated children exhibited a blur band in the predicted position on 1.5% agarose gel, some showed a distinct or fainted band. In general, many shared predominant clonal TCR-BV gene families (Vβ2, Vβ16, Vβ21, Vβ22) and the restricted-expression families (Vβ14 and Vβ17). All the gene families of the control children only exhibited blur bands and polyclonal.

Conclusions: The skewed profile of TCR-BV gene families in TB children and BCG-vaccinated children are similar, which may probably explain the protective effects of BCG-vaccine against TB in children.

Keywords: Bacille Calmette Guerin, Skewed profile, tuberculosis, T cell antigen receptor

Cite the article as: Zhou JW, Kong C, Luo J, Cao J, Shi Y. Comparing TCR Beta Chain variable Gene Profile Skewness between Children with Tuberculosis and BCG-vaccinated Children. *Arch Iran Med*. 2013; **16(2):** 104 – 108.

Introduction

Tuberculosis (TB), a chronic disease which threatens human health, is caused by *Mycobacterium tuberculosis* (MTB). According to WHO estimates, approximately 9.4 million incident and 14 million prevalent cases of TB, with 1.6 million deaths have been attributed to this disease in 2009.[1] More importantly, children are more susceptible to MTB than adults, resulting in a higher incidence of disease and increased severity of pathogenesis.[2–4]

In the human body, the predominant immune response to MTB is T cell-mediated.[5–7] T cells recognize antigens by their T cell antigen receptor (TCR), a process that involves molecules of the human leukocyte antigen (HLA). Mature T cells express one of two types of TCR: a heterodimer of α and β chains or γ and δ chains. T cells expressing β receptors play an important role in immunization. High expansions of αβ T cells within the TCR repertoire have been shown to occur in various malignancies and immunological disorders, in addition to inflammatory and infectious diseases.[8] Repertoire diversity is further increased by the imprecise joining of the different gene segments that span the complementarity determining region 3 (CDR3).[9,10]

Although there are few reports regarding TCR CDR3 skewing in TB patients, no study has compared TCR CDR3 skewing between TB children and Bacille Calmette Guerin (BCG)-vaccinated healthy children. This study intended to compare the skewed spectratyping of these two groups, which would provide new ideas for prevention and treatment of children with TB.

Authors' affiliations: [1]Clinic Laboratory, The Affiliated Hospital of Jining Medical College, Jining, Shandong Province, China. [2]Tuberculosis Prevention and Control Institute of Jining City, Jining, Shandong Province, China. [3]Department of Immunology, Zunyi Medical College, Zunyi, Guizhou Province, China.
•**Corresponding author and reprints:** Jian-wei Zhou MD, Clinic Laboratory, The 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
Accepted for publication: 24 October 2012

Patients and Methods

Patients

We recruited 15 TB children (< 6 years) from the Affiliated Hospital of Jining Medical College and the Institute of Tuberculosis Prevention and Control of Jining City. Clinical diagnosis was made based on medical history, chest X-ray, tuberculin skin test (TST), sputum smear and mycobacteria culture. Fifteen BCG-vaccinated children (< 6 years) were recruited from the Department of Prevention and Protection of the Affiliated Hospital. BCG-vaccinated children had positive antibodies specific to MTB as assessed by the Elisapot Kit (MP Biomedicals Asia-Pacific Pte. Ltd.). Controls comprised 15 healthy children (< 6 years) who were negative for serum MTB-antibody. All subjects had not been treated with immunomodulating drugs in the previous six months prior to the study and were seronegative for markers of hepatitis viruses, HIV and other pathogenic infections. Excluded from the study were patients with tumors and immunological disorders. This study protocol was approved by the hospital Ethics Committee.

Extraction of RNAs and synthesis of the first cDNAs

The sense primer, anti-sense primer and specific primers for 24 TCR-BV genes families were previously described[11] and synthesized by the Guang-zhou Daangene Corporation of China. A total of 5 mL of blood was taken from TB children, BCG-vaccinated children and healthy controls. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient

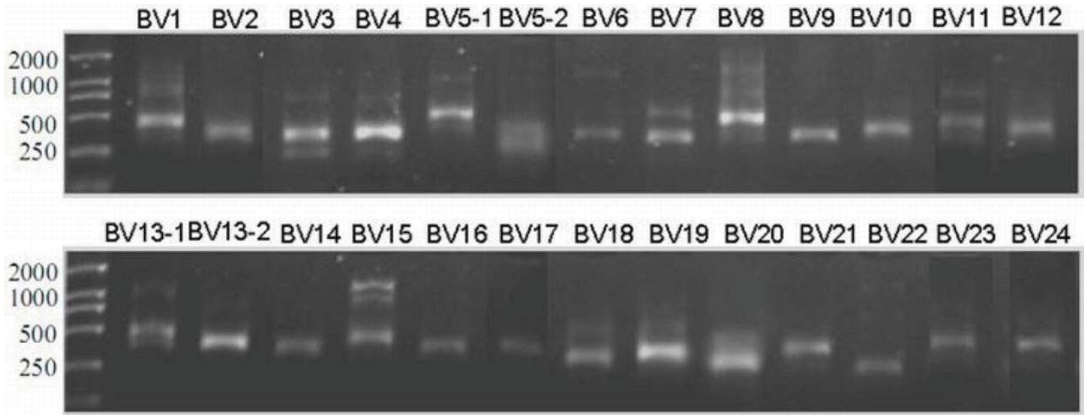

Figure 1. Analysis of TCR-BV families in tuberculosis-infected (TB) children. PCR products of the TCR-BV gene families were analyzed on a 1.5% agarose gel with ethidium bromide staining. A blur band and some abnormal bands in the predicted position were observed in most TCR-BV families. However, the Vβ17 gene family showed no bands. The PCR products of patient 1 are shown.

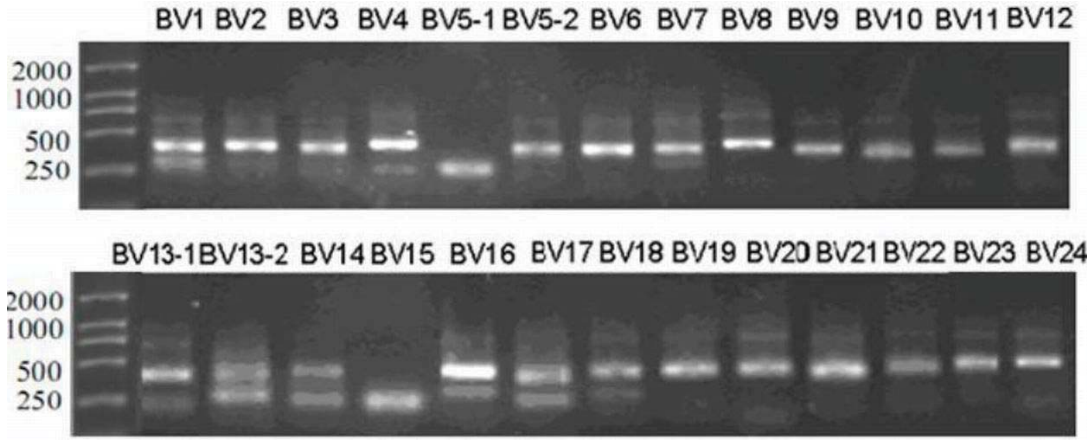

Figure 2. Analysis of TCR-BV families in BCG-vaccinated children. The PCR products of TCR-BV families were analyzed on a 1.5% agarose gel by ethidium bromide staining. A blur band and some abnormal bands in the predicted position were observed in each of the TCR-BV families. The PCR products of child 1 are shown.

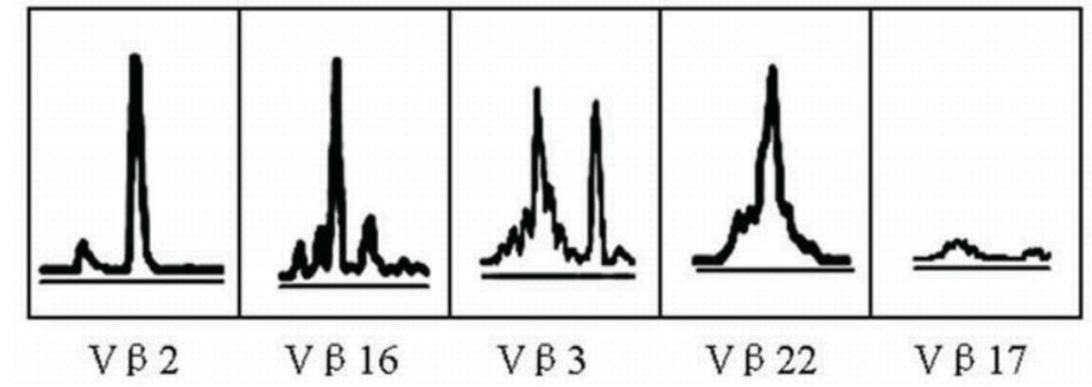

Figure 3. Skewed spectratyping of TCR beta chain gene families. The PCR products of TCR-BV families were analyzed by Genescan technology. Some of the gene families showed monoclonal (Vβ2, Vβ16), some showed oligoclonal (Vβ3, Vβ22), and others exhibited depressed clone (Vβ17). The skewing picture of TB patient 1 is shown.

centrifugation. Total RNA was extracted from PBMC (2 × 106/ sample) using an Omega RNA extraction kit according to the manufacturer's instructions. Total RNA (1 μg) was reverse transcribed with 250 pm olig (dT), 200 U Moloney murine leukemia 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.

Amplification of CDR3 cDNA

TCR CDR3 size analysis of each sample was performed by polymerase chain reaction (PCR) amplification and the reaction modified according to a report by Yao, et al.[11] The PCR for each of the 24 gene families were carried out in 40 μL mixtures that contained 2 μL sense primer and anti-sense primer, 2 μL MgCl2 (2.0 μM), 4 μL dNTP (10 mM), 5μL 10× buffer, 1.2 U Taq-polymerase, and 1μL cDNA template. The experimental conditions

Table 1. Spectratyping of TCR-BV of children with tuberculosis (TB).

Patients	Monoclonal gene families	Oligoclonal gene families	Low-level clonal gene families
1	Vβ2, Vβ16	Vβ3, Vβ22	Vβ17
2	Vβ21, Vβ20	Vβ13.1, Vβ14, Vβ17	Vβ24
3	Vβ2	Vβ18, Vβ22	—
4	Vβ21	Vβ2, Vβ18	Vβ14
5	Vβ2, Vβ21	Vβ22, Vβ13.1	—
6	Vβ21, Vβ22	Vβ2, Vβ20	Vβ17
7	Vβ16, Vβ2, Vβ20	Vβ3, Vβ18, Vβ22	Vβ14
8	Vβ21, Vβ22	Vβ13.1	Vβ20
9	Vβ2, Vβ16	Vβ22, Vβ21	—
10	Vβ13.1, Vβ21	Vβ2, Vβ6, Vβ20	Vβ3, Vβ14
11	Vβ2, Vβ16	Vβ21, Vβ14, Vβ6	Vβ17
12	Vβ2, Vβ21	Vβ18, Vβ13.1	Vβ23, Vβ17
13	Vβ16, VB22	Vβ2, Vβ21, Vβ23	Vβ13.2, Vβ14
14	Vβ13.1, Vβ21	Vβ1, Vβ5.1, Vβ7	—
15	Vβ2, Vβ5.1, Vβ16	Vβ6, Vβ21, Vβ22	Vβ1, Vβ14

Table 2. Spectratyping of TCR-BV of children vaccinated with BCG.

Children	Monoclonal gene families	Oligoclonal gene families	Low-level clonal gene families
1	Vβ21, Vβ22	Vβ1, Vβ20, Vβ23	—
2	Vβ2, Vβ16	Vβ20, Vβ22	Vβ17
3	Vβ2, Vβ21	Vβ22, Vβ20, Vβ24	Vβ17
4	Vβ21	Vβ20	Vβ14
5	Vβ2	Vβ22, Vβ21	Vβ14, Vβ17
6	Vβ20,Vβ21	Vβ2, Vβ12, Vβ22	—
7	Vβ20	Vβ21	Vβ14
8	Vβ16, Vβ22	Vβ2, Vβ18, Vβ21	—
9	Vβ22	Vβ16, Vβ13.1	—
10	—	Vβ2, Vβ20	—
11	Vβ21, Vβ22	Vβ16, Vβ13.1, Vβ18	Vβ17
12	Vβ16	Vβ1, Vβ12, Vβ20, Vβ22	Vβ14
13	Vβ20	Vβ2, Vβ21	—
14	Vβ16, Vβ21	Vβ6, Vβ20	Vβ15
15	Vβ16	Vβ2, Vβ18	—

Table 3. Comparison of skewed spectratyping of 24 TCR-BV families in two groups.

Vβ	Skewed gene families of childhood patients with tuberculosis (TB)		Skewed gene families of children vaccinated with BCG	
	Number	Skewing rate (%)	Number	Skewing rate (%)
1	1	13.3	2	13.3
2	9	60.0	8	53.3
3	3	20.0	3	20.0
4	0	0	0	0
5.1	2	13.3	0	0
5.2	0	0	0	0
6	3	20.0	1	6.7
7	1	6.7	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	2	13.3
13.1	6	40.0	2	13.3
13.2	1	6.7	0	0
14	7	46.7	4	26.7
15	0	0	1	6.7
16	6	40.0	7	46.7
17	5	33.3	4	26.7
18	4	26.7	3	20.0
19	0	0	0	9.0
20	5	33.3	10	66.7
21	12	80.0	10	66.7
22	9	60.0	9	60.0
23	2	13.3	1	6.7
24	1	6.7	1	6.7

were 94°C for 3 min, 94°C melting for 1 min, primer annealing at 56°C for 1 min, and 72°C for 1 min, 35 cycles; then extension at 72°C for 10 min. Finally, 8 μL PCR products were separated on a 1.5% agarose gel and stained with ethidium bromide.

Analysis of CDR3 length by Genescan

The mixture that contained 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 (ABI) dye-labeled size standards was denatured at 95°C for 2 min, then loaded onto a 6% acrylamide sequencing gel and allowed to run for 2 hours on a 50 lane applied Bio-systems model 373A DNA sequencer. Data were analyzed by Genescan software version 672.

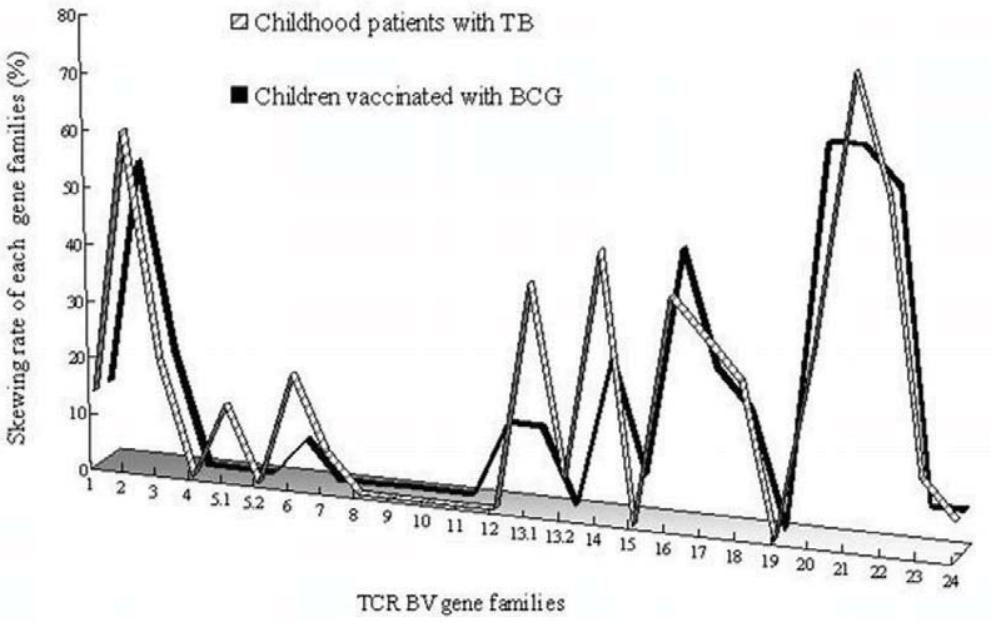

Figure 4. The profiles of TCR-BV spectratyping of the two groups which shows similarities between the two profiles of TB children and BCG-vaccinated children. The monoclonal gene families focused on Vβ2, Vβ16, Vβ21, and Vβ22, and the depressed expression families were Vβ14 and Vβ17.

Results

The results showed that each family of TCR-BV gene in healthy donors exhibited a blur band, while in TB children and BCG-vaccinated children, except for blur bands, several gene families showed distinct or fainted bands (Figures 1 and 2) in predicted positions. The melting curve derived by Genescan assay showed multi-peaks which represented polyclonal proliferation families, double-peaks that indicated the presence of oligoclonal proliferation families, single-peaks which were indicative of monoclonal proliferation families, and low-level peaks that represented depressed clonal proliferation families.[12,13] In all healthy subjects, the TCR-BV gene families were comprised of polyclonal proliferation, whereas some of the TCR-BV gene families in TB children exhibited not only polyclonal but also monoclonal, oligoclonal, and even depressed-clonal families (Figure 3). The same phenomenon was observed in BCG-vaccinated children. The monoclonal proliferation families consisted mainly of types Vβ2 (60.0%), Vβ16 (40.0%), Vβ21 (80.0%), and Vβ22 (60.0%). The key depressed clonal families were of Vβ14 (46.7%) and Vβ17 (33.3%) types (Tables 1 and 3). In BCG-vaccinated children, the monoclonal proliferation families were mainly Vβ2 (53.3%), Vβ16 (40.0%), Vβ21 (66.7%) and Vβ22 (66.7%), whereas the key clone depression families were Vβ14 and Vβ17. The skew rates were all 26.7% (Tables 2 and 3). However some families such as Vβ4, Vβ8, Vβ9, Vβ10, and Vβ11 were always polyclonal and did not show any skewness in any of the samples. Except for the common characters, the spectratyping skewness of many TCR-BV in both groups were individualized. For example Vβ5.1, Vβ7, Vβ13.2, and Vβ15 were monoclonal in certain gene families. In Figure 4, the TCR landscapes are plotted with 24 BV families on the *x*-axis and the reciprocal skewing rate on the *y*-axis.

Discussion

Following infection with MTB, T lymphocytes recognize the antigen presented by human leucocyte antigen (HLA), while the quantity and ratio of each T cell subpopulation reciprocally changes.[14,15] This process drives the immune effect to be beneficial to protect against pathogenic infections. TCR plays a critical role in recognizing the epitopes of the antigens handed by HLA-I or HLA-II. According to the composition of the two chains, there are two groups of TCRs. The αβ TCR subpopulation plays the more important role in the progress against MTB infection.[16] In this aspect, some studies have demonstrated that there are individualized skewed spectratypings in the T cell repertoire of TB patients.[17] There are few reports about the characteristics of TCR-BV gene families in TB children and a comparison of the TCR-BV gene families between TB children and BCG-vaccinated children has not been reported.

In this study, we used the Genescan technique to detect the spectratyping of the TCR beta chain in the peripheral blood cells of TB children and BCG-vaccinated children. We found that some TCR-BV gene families showed skewed profiles in both groups. Different persons exhibited different TCR-BV profiles. Even within the same group the profile of each child was significantly different. The profiles of TCR-BV families from healthy controls exhibited polyclonal. Some TCR-BV gene families from TB children and BCG-vaccinated children showed polyclonal, monoclonal, and oligoclonal. Some families had restricted clones, which was similar to a report that some TCR-BV gene families were deleted or absent.[18] It is possible that during the process of an immune response to the MTB antigen, some gene families became predominant, which has restricted or limited the expression of other gene families.[19,20]

The predominant proliferative gene families Vβ2, Vβ16, Vβ21,

and Vβ22 were seen in the TB and BCG-vaccinated children and might be a specific immune response to MTB. Our results differed from a study which reported that the predominant gene families were Vβ9 and Vβ12 according to QIAO D.[21] In some reports, the skewness of predominant and restricted TCR-BV gene families were totally called gene melting spectral patterns (GMSPs),[8,22,23] which is/are specific to a certain antigen. Generally, the T cells which TCR-BV contain such a GMSPs were taken as the specific T-cell clone to the immunogen. Numerous studies have proven that only the clonal proliferative T cells play important roles in the process of recognizing and removing antigens from bacteria, viruses and tumors.[22,24,25]

Some reports have proven that BCG could stimulate T cells to produce an immune response.[26,27] In this study, we found a similarity in the TCR-BV gene profile between TB children and BCG-vaccinated children, which has further suggested a molecular mechanism for T cell mediated cell immunity for BCG in protecting the human body against MTB infection. However, the T cell subpopulation needs additional study in TB children and BCG-vaccinated children. Although the effects of BCG on the predominant TCR-BV gene families is clear,[28,29] the effects on the limited gene families have not been explored. Finally the BCG vaccine is effective in protecting children against TB, but not adults, thus this mechanism also needs additional investigation.

Acknowledgments

All of authors are grateful of the fifteen patients, the fifteen healthy volunteers and medical personnel for supporting this study. The work was supported by the Nature Science Foundation (No. ZR2012HL29), the High School Science and Technology Plan Program (No. J11LF18), the Population and Family Planning Commission (No. [2011]13), the Development Plan Project of Jining Science and Technology Bureau (No. [2011] 57), and the Young Researcher Fund of Jining Medical University (No. [2011]) of Shandong Province of China.

References

1. WHO. WHO Report 2011: Tuberculosis Control. Available from: URL: http://www.who. int/tb/publications/global_reprot/ en/ index. html [Accessed October 2011].
2. Carrol ED, Clark JE, Cant AJ. Non-pulmonary tuberculosis. *Peadiatr Respir Rev*. 2001; **2:** 113 – 119.
3. Marais BJ, Rabie H, Cotton MF. TB and HIV in children-advances in prevention and management. *Paediatr Respir Rev*. 2011; **12:** 39 – 45.
4. Walls T, Shingadia D. Global epidemiology of pediatric tuberculosis. *J Infect*. 2004; **48:** 13 – 22.
5. Collins HL, Kaufmann SH. The many faces of host responses to tuberculosis. *Immunology*. 2001; **103:** 1 – 9.
6. Flynn JL, Chan J. Immunology of tuberculosis. *Annu Rev Immunol*. 2001; **19:** 93.
7. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor-neutralizing agent. *N Engl J Med*. 2001; **345:** 1098 – 1014.
8. Zhou J, Ma R, Luo R, He X, Sun W, Tang W, et al. Primary exploration of molecular and spectratyping features of CDR3 of TCR β chain in the peripheral blood and tissue of patients with colorectal carcinoma. *Cancer Epidemiology*. 2010; **34:** 733-740.
9. Assaf C, Hummel M, Dippel E, Oerdt S, Müller HH, Anagnostopoulos I, et al. High detection rate of T-cell receptor beta chain rearrangements in T2 cell lymphoid proliferations by family specific polymerase chain reaction in combination with the GeneScan technique and DNA sequencing. *Blood*. 2000; **96:** 640 – 646.
10. Dongzhi Cen, Yangqiu Li, Shaohua Chen. Distribution and clonality of peripheral blood TCR Vα subfamily T cells in patients with acute promyelocytic leukemia. *Chinese-German Journal of Clinical Oncology*. 2007; **6:** 591 – 593.
11. Xin-Sheng Yao, Zheng-Jun X, Li M, Wan-Bang S, Wei-Yang Z, Qian W, et al. Analysis of the CDR3 region of alpha/ beta 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 Haem*. 2006; **28:** 405 – 415.
12. Xin-Sheng Yao, Ying-Diao, Wan-Bang Sun, Junmin Luo, Ming Qin, Xianying Tang. Analysis of the characteristics of CDR3 polymorphism and length distribution of TCR alpha chain in peripheral blood of normal human. *Cell Mol Immunol*. 2007; **4:** 215 – 220.
13. Yao XS, Zhang GW, Ma L, Wen Q, Hou JL, Meng MJ, et al. Analysis of the CDR3 length of TCR alpha/beta T cells in the peripheral blood of patients with chronic hepatitis B. *Hepatol Res*. 2006; **35:** 10 – 18.
14. Avgustin B, Kotnik V, Skoberne M. CD69 expression on CD4+ T lymphocytes after in vitro stimulation with tuberculosis is an indicator of immune sensitization against mycobacterium tuberculosis antigens. *Clin Diagn Lab Immunol*. 2005; **12:** 101 – 106.
15. Pène J, Chevalier S, Preisser L, Vénéreau E, Guilleux MH, Ghannam S, et al. Chronically inflame human tissues are infiltrated by highly differentiated Th17 lymphocytes. *J Immunol*. 2008; **180:** 7423 – 7430.
16. Akbulut HH, Deveci F, Celik I, Ilhan F, Turgut T. Investigation of T cell receptors in the peripheral blood of patients with active pulmonary tuberculosis. *Indian J Med Microbiol*. 2009; **27:** 40 – 43.
17. Glenn T, Cornelius K, Hanni H, Zehbe I, Hitzler WE, Neukirch C, et al. Highly focused T cell responses in latent human pulmonary Mycobacterium tuberculosis infection. *J Immunol*. 2005; **174:** 2174 – 2184.
18. Wallace ME, Bryden M, Cose SC, Coles RM, Schumacher TN, Brooks A, et al. Junctional biases in the naïve TCR repertoire control the CTL response to an immunodominant determinant of HSV-1. *Immunity*. 2000; **12:** 547 – 556.
19. Sun TK, Maki T, Koh T, Sun ZY, Dave VP, Kappes DJ, et al. Distinctive CDR3 Heterodimeric ectodomain topologies maximize antigen-triggered activation of T cell receptros. *J Immunol*. 2010; **185:** 2951 – 2959.
20. Xu C, Wan C, Wang L, Yang HJ, Tang Y, Liu WP. Diagnostic significance of TCR gene clonal rearrangement analysis in early mycosis fungoides. *Chin J Cancer*. 2011; **30:** 264 – 272.
21. Qiao D, Li L, Guo J, Lao S, Zhang X, Zhang J, et al. Mycobacterium tuberculosis Culture Filtrate Protein 10-specific effector/memory CD4+ and CD8+ T Cells in tubercular pleural fluid, with biased usage of T cell receptor V(beta) chains. *Infect Immun*. 2011; **79:** 3358 – 3365.
22. Jian-wei Zhou, Rui Ma, Wen-tai Tang, Rong Luo, Jun-min Luo, Xin-sheng Yao. Primary exploration of the third complementarity determining region spectratyping and molecular features of T cell receptor alpha chain in the peripheral blood and tissue of patients with colorectal carcinoma. *ACTA Medica Mediterr*. 2011; **27:** 97 – 104.
23. Yang JZ, Li MW, Wang JG, Lu HF, Yao XS, He JQ, et al. Rapid detection of clonal expansion of T-cell receptor-beta gene in patients with HBV using the real- time PCR with DNA melting curve analysis. *Hepatol Res*. 2010; **40:** 407 – 414.
24. Dong L, Li P, Oenema T, McClurkan CL, Koelle DM. Public TCR use by herpes simplex virus-2-specific human CD8 CTLs. *J Immunol*. 2010; **184:** 3063 – 3071.
25. Ren GL, Chen JP, Jia MM, Kou ZC, Liu S, Ma PF, et al. T cell receptor diversity of CD8+ T lymphocytes and its association with viral load in individuals with HIV-1 infection. *Zhonghua Yu Fang Yi Xue Za Zhi*. 2009; **43:** 404 – 408.
26. Keyser A, Troudt JM, Taylor JL, Izzo AA. BCG sub-strains induce variable protection against virulent pulmonary Mycobacterium tuberculosis infection, with the capacity to drive Th2 immunity. *Vaccine*. 2011; **29:** 9308 – 9315.
27. Siddiqui N, Price S, Hope J. BCG vaccination of neonatal calves: potential roles for innate immune cells in the induction of protective immunity. *Comp Immunol Microbiol Infect Dis*. 2012; **35:** 219 – 226.
28. Ferrara J, Reddy P, Paczesny S. Immunotherapy through T-cell receptor gene transfer induces severe graft-versus-host disease. *Immunotherapy*. 2010; **2:** 791 – 794.
29. Linnemann C, Schumacher TN, Bendle GM. T-cell receptor gene therapy: critical parameters for clinical success. *J Invest Dermatol*. 2011; **131:** 1806 – 1816.