Try to Apply T Cell Receptor Repertoire Diversity Detection for Acquired Immune Deficiency Syndrome Research of Traditional Chinese Medicine

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Abstract. A new approach will be carried out to make clear the mechanism of promotion immune system function with TCM treatment on AIDS and provide a parameter of clinical therapeutic effects. DNA was extracted with periphery blood from forty AIDS patients before and after treatment, and multiple primers PCR technique and capillary electrophoresis were used to detect T cell receptor beta repertoire diversity. Meanwhile, seven control subjects were measured repeatedly to define the threshold. Sets of typical patterns for positive and negative were shown respectively from the scanning map angle. Moreover, positive detection rates of the forty AIDS patients were calculated for different T cell receptor gene segments. To some degree, this study is suggestive of it how TCM treatment effects on AIDS. However, the reliability needs further verification.

Introduction

Acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV), has become a major public health problem in China. Since the first Chinese HIV-infected patient was revealed in 1985, some Chinese medicine practitioners (CMPs) began to use traditional Chinese medicine (TCM) on acquired immunodeficiency syndrome (AIDS). Successful treatment with TCM can reduce viral load, increase the level of CD4+ T cells, and promote immunity reconstruction, but the mechanism remains poorly revealed[1]. TCM-treatment considers the body as a whole, so finding a method to comprehensively evaluate efficacy maybe is an urgent problem to be solved. Gene rearrangement diversity within T cell receptor (TCR) repertoire is a qualitative feature of T cell responses that may be associated with control of viral loads and improvement the level of T cell activation, but there are limited data to inform our understanding of how the TCR repertoire may be affected by TCM.

In this study, multiple primers were designed, which involved most T cell receptor beta (TCR-β) gene rearrangement segments. Capillary electrophoresis promises distinct progress in sensitivity and specificity[2]. Gene-scanning technology is an accurate scanning analysis approach. The principle is DNA amplification through polymerase chain reaction (PCR) by fluorescence labeling primer, then fluorescent signal recognition from gene with capillary electrophoresis, and finally data analysis using software. GeneMapper ID-X software is a rapid and high efficient assay tool for data analysis of this study. Scanning map from the software not only shows peaks clearly, but also gets many peak parameters about products such as size, area, and height[2].

Here we detected gene rearrangement repeatedly diversity on healthy subjects who had not either underlying medical condition or recent illness to define the difference threshold. Moreover, we compared AIDS patients’ TCR repertoire diversity by measurement of TCR-β variable region respectively before and after treatment with TCM. Maybe data in this study promises reference value, at least in part, for clinical efficacy of TCM on AIDS.
Materials and Methods

Study Subjects
Forty AIDS patients from endemic area in Henan province enrolled in this study, aged 18-45, including twenty-three males and seventeen females detected before and one year after treatment with traditional Chinese medicine. Twenty-four individuals infected HIV by blood, eleven patients by sex and seven persons’ unknown. All patients were diagnosed with guidelines to AIDS formulated by Chinese medical association[3]. The control subjects included seven age-matched healthy subjects who had neither underlying medical condition nor recent illness.

Ethical Consideration
Ethical approval was obtained from the Institutional Review Board of the First Affiliated Hospital of Henan University of TCM. Written informed consent was obtained from each subject before recruited in the study.

Isolation DNA from Peripheral Blood
20 μl protease K (Qiagen)were added to a 1.5ml Eppendorf (EP) tube, following 200 μl sample and 200 μl buffer AL (Qiagen). Trembled the tube by vortex briefly to complete lysis, incubated at 56°C for 10min, then added 200 μl 100% ethanol and mixed fully. Transferred the mixture to QIAamp Mini spin-column (Qiagen) which then centrifuged at 6000g for 1min. Following, washed the column with buffer AW1 and AW2 (Qiagen) respectively. At last, Transferred the column to a new EP tube and added 200 μl ddH2O to elute DNA by centrifuging.

PCR by Multiple Fluorescence Primers
Multiple primers were designed to cover all functional Vβ, Dβ and Jβ gene segments being suitable for amplification in multiplex PCR reactions. The PCR reactions for TCR-β gene are performed in 3 tubes (A, B and C) using forward and reverse primer sets. All reverse primers were labeled by fluorescent dye 6-FAM. Details about primer sets were shown bellow (Table.1). The total PCR reaction volume of each other was 20 μl, including 10 μl Master-Mix (TOYOBO), 2 μl Primer-Mix, and DNA template and ddH2O add up to 8 μl. PCR reaction was done on thermal cycler system(Bio-Rad C1000), and program was shown following: (i) 96°C for 10min to melt; (ii) 40 cycles of 96°C for 30s, 60°C for 30s and 72°C for 30s to amplify; (iii) 72°C for 30min to complete extension.

<table>
<thead>
<tr>
<th>Detection subject</th>
<th>Primer set name</th>
<th>Segment set</th>
<th>Size(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCR-β(Vβ-Jβ)</td>
<td>TCR-β Tube A</td>
<td>23Vβ+9Jβ</td>
<td>240-285</td>
</tr>
<tr>
<td>TCR-β Tube B</td>
<td>23Vβ+4Jβ</td>
<td>240-285</td>
<td></td>
</tr>
<tr>
<td>TCR-β(Dβ-Jβ)</td>
<td>TCR-β Tube C</td>
<td>Dβ1+ Dβ2+13Jβ</td>
<td>170-210; 285-325</td>
</tr>
</tbody>
</table>

Measurement of T Cell Receptor Diversity
The diversity of TCR-β CDR3 region was determined by capillary electrophoresis on ABI 3500Dx genetic analyzer, using the PCR products mixture of 1 μl PCR products, 9 μl Hi-Di formamide and 0.2 μl GS-LIZ500 size standard, which was melted at 96°C for 10min, then incubated on ice for 10min. According to fluorescence intensity (FI), Some products was properly diluted to avoid incorrect results because of huge peak.

Data Analysis
GeneMapperID-X software was used for data analysis, by calculating automatically product size, height, area and so on. Firstly, repeatedly detected control samples, respectively sum areas of targeted sizes of each detected segments, then calculated difference (D) between before and after treatment,
and finally, averaged all difference of gene segments. The mean difference was fewer than 10000 (D <10000), so the difference (D=10000) was defined as threshold. Judgment standard following: patterns whose difference was fewer than 10000 were defined negative (D <10000); inversely, defined positive (D ≥10000).

**Calculation the Positive Detection Rate**

Statistically analyze the percentage of positive pattern in the detected patients for every gene rearrangement segments. Furthermore, calculate the positive percentage for TCR-β.

**Results**

**Typical peak patterns** Although transformation of diversity in humans was ever-changing, it was easy to find typical patterns. Graphs below showed typically positive and negative peak patterns of one sample for five gene rearrangement segments at detection range respectively. Left part of each maps displayed the individual before treatment with TCM (Pre-TCM). Moreover, maps of a typical sample after treatment with TCM (Post-TCM) were displayed on each right parts. The peak difference of positive patterns was distinct and obvious between Pre-TCM and Post-TCM. Nevertheless, there was not significantly different between them in typically negative patterns (Fig1, 2).

![Figure 1](image1.png)

**Figure 1.** Typically positive peaks for TCR beta of one sample in the comparison of pre-TCM and post-TCM.

![Figure 2](image2.png)

**Figure 2.** Typically negative peaks for TCR beta of one sample in the comparison of pre-TCM and post-TCM.
Detection rate of positive peak patterns

The mean difference (D=10000) from control subjects was defined as threshold. Fewer than 10000 were defined negative (D < 10000); on the contrary, defined positive (D ≥ 10000). Comparison of the difference in control subjects, 21 of 40 patient samples were defined positive patterns (53%) for TCR-β Tube A, 28 positive for TCR-β Tube B (70%), 39 positive for TCR-β Tube C (98%). The summing positive detection rate were 85% for TCR-β (Table 2).

Table 2. Detection rate of typical peak patterns on AIDS patients (n=40).

<table>
<thead>
<tr>
<th>Group</th>
<th>TCR-β</th>
<th></th>
<th>TCR-β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tube A</td>
<td>Tube B</td>
<td>Tube C</td>
</tr>
<tr>
<td>Positive</td>
<td>21</td>
<td>28</td>
<td>39</td>
</tr>
<tr>
<td>Negative</td>
<td>19</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Positive  Rate</td>
<td>53%</td>
<td>70%</td>
<td>98%</td>
</tr>
</tbody>
</table>

Discussion

T cell receptor (TCR) repertoire diversity plays a critical role in the function of immune system at aspects of resisting cancer, infectious diseases. There are high numbers of αβ T-cells (about 60%-70% in peripheral blood) and a small population of γδ T-cells (0.5-15% in peripheral blood in humans [4]). αβ T-cells mainly reflect almost CD4+ T and CD8+ T cells response. The TCR repertoire is highly diverse, consisting of an estimated 10^15 potential sequences, which recent studies suggest may be even higher [5]. Diversity comes from differences in the V gene segments. They are generated by a recombinational process of variable (V) and junctional (J), and in the case of the TCR β chain, also diversity (D) gene segment rearrangement [6]. Analysis of TCR gene rearrangement is of practical value in estimating antigen recognition capacity and the magnitude of immune response.

Some Chinese medicine practitioners (CMPs) began to initiate TCM for treatment on AIDS, since 1980s when the first HIV-infected patient was diagnosed in China. By now, previous studies have found the improvement of health related quality of life [7]. A highly amount of reports reveal that TCM treatment can reduce viral load in plasma, increase the level of CD4+ T cell, and promote immunity reconstruction [2]. But, comparison of antiretroviral therapy (ART), detection the level of both viral loads and CD4+ T cells are of less advantage. Furthermore, it is currently unclear that how TCM treatment effects on promotion immunity reconstruction.

As a result of blood TCR diversity decreases during the course of HIV infection, the level of antigen recognition capacity reduces correspondingly [8]. Maybe this provides powerful evidence, if it is proved the impaction on TCR diversity by TCM treatment on AIDS. So far, there is still a major gap in measurement TCR diversity of HIV-infected patients in China, which is an excellent way for elucidation the mechanism of TCM. Many technological assays are used to TCR diversity detection, such as multiplex PCR, PCR-SSCP, high-throughput sequencing [9]. There exists certain defect in above methods, because of either low distinguish ability or large operation difficulty.

A variety of methodologies analyzes the TCR repertoire, for example Simpson’s diversity index or calculation to determine entropy [10]. In this study, multiple primers were designed, including 23Vβ, 13 Jβ and 2Dβ, which involved most rearrangement segments. Reverse primers were labeled by 6-FAM. Capillary electrophoresis promises distinct progress in sensitivity and specificity [4]. Additionally, this analysis method is conveniently mastered. In data analysis, A method summation to determine sizes, considered immune system function in whole that is happen to have the same view of TCM. The results displayed that a variety of peaks in individuals. Peaks in control subject samples were showed typical Gauss distribution [4]. The mean difference value was fewer than 10000 between two repeatedly calculation. Obviously, the function immune system in humans was impacted in terms of large factors, resulting that the difference was not zero, in spite of repeatedly calculation in control subjects. Although transformation of diversity in humans was ever-changing, it was easy to find typically positive or negative patterns as shown in above results. This was newly an exploratory study.
for application of the technical approach embracing the concept of TCR repertoire diversity into research of AIDS with TCM treatment, due to little report in China. The results of calculation positive detection rate, including 53% positive patterns for TCR-β Tube A, 70% positive for TCR-β Tube B, and 98% positive for TCR-β Tube C, were suggestive of change in diversity pre-TCM and post-TCM. Nevertheless, owing to little reference, it is currently unclear that whether or not reliable the data is to elucidate the mechanism of TCM treatment on AIDS. In the near future, we intend to detect more and more samples to define the variation trend. Additionally, establishing a cohort research to long-term observation, analyzing the correlation to the level of CD4+T cells and viral load, and discussing the consistency with symptoms are the future main work.

In summary, this study shows that TCR repertoire diversity changes on AIDS patients after treatment with TCM, including some typically positive patterns. We tried a new approach in making clear the mechanism of TCM treatment on AIDS. However, further evidence for the reliability needs validation.

Acknowledgement

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