Study of difference of peripheral blood lymphocytes immunophenotyping and NK cells on connective tissue disease combined with interstitial lung disease.

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Abstract

Objective: To investigate the clinical value of peripheral blood lymphocytes immunophenotyping and Natural Killer cells (NK) in the judgment of the types of Connective Tissue Disease Combined with Interstitial Lung Disease (CTD-ILD).

Methods: 262 CTD-ILD patients and 50 healthy volunteers in our hospital from January 2010 to December 2015 were enrolled into the study. There were 164 patients with CTD-ILD and 98 patients with ILD. The clinical symptoms and High Resolution CT (HRCT) of all patients were observed and compared between the two groups. The serum levels of CD3⁺, CD4⁺, CD8⁺, CD19⁺, CD56⁺ T cells were detected by flow cytometry and compared among the three groups.

Results: There were no difference of the clinical symptoms and the characteristics of HRCT between the two patients groups (P>0.05). The serum level of CD3⁺-CD8⁺ T cells of patients in the CTD-ILD group was higher than the control group and ILD group (P<0.05); and the serum level of CD3⁻CD56⁺ T cells and the ratio of CD4⁺/CD8⁺ in the CTD-ILD group was lower than the control group and ILD group (P<0.05). But there was no difference of the peripheral blood lymphocytes immunophenotyping between the ILD group and control group (P>0.05).

Conclusion: The immunophenotype of peripheral blood lymphocytes could be helpful to judge the types of connective tissue disease combined with interstitial lung disease.

Keywords: Interstitial lung disease, Connective tissue disease, Immunocyte, Immune function, Natural killer cell.

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Introduction

Interstitial Lung Disease (ILD) is generic term of lung diseases of different immune pathological process caused by multiple pathogenic factors, the onset mechanism of ILD is still unclear [1,2]. Connective Tissue Disease (CTD) belongs to injury disease of body caused by abnormal autoimmune system, of which, ILD is one of common CTD complications, which will induce failure of kidney function even death easily [3,4]. Early ILD prediction is vital of improving CTD curative effects and prognosis [5]. The onset of CTD-ILD is increasingly gradually, it has no typical clinical indications. So the diagnosis is difficult. The onset rate, pathological type and treatment effects of different CTD with ILD have relatively great differences. Therefore, it is difficult to judge whether CTD-ILD caused by infection or immune injury [6,7]. This study provides theoretical basis for clinical treatment by detecting difference changes of peripheral blood lymph cells and NK cell level of CTD-ILD patients and non-CTD ILD patients.

Materials and Methods

Clinical data

This study had enrolled 262 CTD-ILD patients and 50 healthy volunteers in internal respiratory department and IUC of State Key Laboratory of Respiratory Disease, Guangzhou Institute of Respiratory Health, the First Affiliated Hospital of Guangzhou Medical University from January 2010 to December 2015. All patients were given HRCT examination and X-ray. Complains contain cough, short of breath, chest fullness, dyspnea and so on. There were 118 male patients and 144 female patients. The age was from 21 to 76 y old. The average age was 45.27 ± 10.62 y old. According to diagnostic criteria of MCTD diagnostic and treatment guide issued by CRA in 2011 [8], 164 patients belong to CTD-ILD type, of which, there were 72 RA, 35 SLE, 16 primary SS, 11 multiple myositis, 9 mixed connective tissue diseases, 7 JDM, 7 scleroderma, 5 overlap syndrome and 2 vasculitis. In addition, there were 98 patients as non-CTD with ILD, including 24 secondary infections, 14
drugs, 12 GER, 48 patients without clear reasons. Furthermore, this study selected 50 healthy volunteers with health examination in outpatients department of our hospital as the control group. Comparison of general clinical data of patients in two groups, there were no statistical differences. It had comparability, P<0.05 (Tables 1 and 2).

Table 1. Analysis of general data of admitted subjects.

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients number (case)</th>
<th>Average age (years old)</th>
<th>Sex (case)</th>
<th>Average course (y)</th>
<th>Glucocorticoid use (case)</th>
<th>Respiratory frequency min (times/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The control group</td>
<td>50</td>
<td>44.73 ± 10.86</td>
<td>Male 23, Female 27</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CTD-ILD</td>
<td>164</td>
<td>44.29 ± 9.49</td>
<td>Male 76, Female 88</td>
<td>1.96 ± 2.77</td>
<td>11 (6.71%)</td>
<td>21.86 ± 2.73</td>
</tr>
<tr>
<td>ILD</td>
<td>98</td>
<td>45.81 ± 10.05</td>
<td>Male 42, Female 56</td>
<td>2.15 ± 2.08</td>
<td>7 (7.14%)</td>
<td>22.07 ± 3.15</td>
</tr>
</tbody>
</table>

Table 2. Comparison of main symptoms of admitted patients (n, %).

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients number</th>
<th>Cough (75.0)</th>
<th>Short breath (72.56)</th>
<th>Chest pain (28.68)</th>
<th>Dyspnea (52.31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTD-ILD</td>
<td>164</td>
<td>123</td>
<td>119</td>
<td>47</td>
<td>52</td>
</tr>
<tr>
<td>ILD</td>
<td>98</td>
<td>72</td>
<td>74</td>
<td>28</td>
<td>33</td>
</tr>
</tbody>
</table>

Inclusive criteria: first, all patients were given chest HRCT within three days after admitted hospital; second, patients who met ILD criteria; third, patients or their families signed informed consent form.

Exclusive criteria: first, patients who cannot meet inclusive criteria above; second, patients accompanied with other blood system, alimentary system and malignant tumor; third, patients with emotional disorder; fourth, patients with poor compliance so on.

**Instruments and reagents**

S3e flow cytometer was bought from Japanese Bio-Rad company; FITC-CD45, RD1-CD4, ECD-CD8 and PE-CD3 from American Beckman Kurt limited company; hemolysin Opti-Lyse No-Wash Lysing Solution from French Immunotech company.

**Observation indexes**

This study collected 2 ml peripheral vein blood of all subjects, it put into EDTA anticoagulant tube. Then collecting 100 μl anticoagulant blood into EP tube, 20 μl same-type, ITC-CD45, RD1-CD4, ECD-CD8 and PE-CD3 fluorescently-labeled antibody were added, then blending. They were given incubation at 37°C without light for 10 min, then adding 500 μl hemolysin, blending, giving incubation at 37°C without light for 10 min. 500 μl PBS buffer solution was added, given centrifugation. Supernatant was discarded. 500 μl PBS buffer solution was added, then blended, detected on computer laboratory.

**Statistical management**

This study used SPSS 21.0 statistical software to do t-test and $\chi^2$ test between two groups. Measurement data used t-test to do data comparison between two groups. Enumeration data $\chi^2$ test or Fisher’s exact test. P<0.05, there were statistical differences.

**Results**

**Comparison of CT iconography features of patients in two groups**

High resolution CT iconography examination results of patients in two groups show that manifestations of ground glass opacity, grid opacity, tractive bronchiectasia, consolidation opacity, pleural thickening or few pleural effusion are similar generally, there were no statistical differences (P<0.05, Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients number</th>
<th>Ground glass opacity (42.07)</th>
<th>Grid opacity (40.85)</th>
<th>Tractive bronchiectasia (35.98)</th>
<th>Consolidation opacity (66.46)</th>
<th>Pleural thickening (21.95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTD-ILD</td>
<td>164</td>
<td>69</td>
<td>67</td>
<td>59</td>
<td>109</td>
<td>36</td>
</tr>
<tr>
<td>ILD</td>
<td>98</td>
<td>35</td>
<td>46</td>
<td>28</td>
<td>71</td>
<td>17</td>
</tr>
</tbody>
</table>

**Immune typing comparison of peripheral lymph cells of patients in two groups**

Compared with the normal control group and ILD group, CD3⁺CD8⁺ T of patients in CTD-ILD increase obviously, CD3⁻CD56⁺ cell number and CD4⁺/CD8⁺ ratio decrease obviously, there were statistical differences (P<0.05). T cell of different types in peripheral blood of patients in ILD group...
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Compared with the control group, there were no statistical differences (P>0.05, Table 4).

Table 4. Immune typing comparison of peripheral lymph cells of patients in two groups (± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>CD3$^+$</th>
<th>CD3$^+$CD4$^+$</th>
<th>CD3$^+$CD8$^+$</th>
<th>CD3 CD19$^+$</th>
<th>CD3 CD56$^+$</th>
<th>CD4$^+$/CD8$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>The control group</td>
<td>64.27 ± 4.91</td>
<td>35.72 ± 2.79</td>
<td>24.45 ± 1.17</td>
<td>12.96 ± 2.29</td>
<td>16.24 ± 3.78</td>
<td>1.52 ± 0.18</td>
</tr>
<tr>
<td>CTD-ILD</td>
<td>68.33 ± 4.26</td>
<td>36.03 ± 3.14</td>
<td>30.56 ± 2.91abc</td>
<td>13.16 ± 1.97</td>
<td>10.69 ± 5.26abc</td>
<td>1.19 ± 0.22abc</td>
</tr>
<tr>
<td>ILD</td>
<td>66.82 ± 6.37</td>
<td>36.19 ± 2.96</td>
<td>25.82 ± 3.40</td>
<td>14.77 ± 2.51</td>
<td>14.73 ± 4.35</td>
<td>1.43 ± 0.21</td>
</tr>
</tbody>
</table>

Note: abc compared with the control group, P<0.05; compared with ILD group, P<0.05.

Discussion

CTD is the common autoimmune system diseases in clinic at present, it mainly injuries connective tissue and vessels of whole body, such as ILD [9]. CTD-ILD occurs in female patients. This study selects 262 patients in respiratory department of our hospital randomly. The female patients accounts for 54.96%. Since 70s last century, CTD-ILD has attracted each nations widely gradually, but its mechanism is still unclear [10]. Because of different CTD primary disease, treatment is vital important for selecting proper treatment methods.

This study explores pathogenesis of different ILD patients by comparing peripheral blood lymph cell typing of CTD-ILD patients, ILD patients and normal subjects. The study results show that, comparing with the normal control group and ILD group, CD3$^+$CD8$^+$T cells of patients in CTD-ILD group increase obviously, CD4$^+$/CD8$^+$ decrease obviously, there are statistical differences (P<0.05). It shows CD8$^+$T as regulatory T cell group with independent function, activated obviously to participate the immune injury of lung in active period of CTD. Mature T cells in peripheral blood of body are mainly CD3T cells. CD4T cells belong to assistant T cells. CD8T cells are mainly inhibited T cells and NK T cells. After activating CD3T cells and CD4T cells, which will participate cellular immune activity of body. Besides, CD3-CD19$^+$ belongs to feature markers of B lymph cytomembrane, which reflects immune function of liquid in patients. CD3$^+$/CD8$^+$ level of CDT-ILD patients’ increase. CD4$^+$/CD8$^+$ ratio increase, it shows that cellular immune function of CTD-ILD patients is disorder. Proper immune intervention for patients may induce ideal treatment effects.

NK cells are the first defensive line of immune system of body, it only participates natural immune response, also can activate proliferation and activation of T lymph cells, participate acquired immune response by secreting multiple cellular factors [13,14]. CD56 is the typical molecular markers of NK surface [15]. Results of this study show that comparing with the normal control group and ILD group, CD3-CD56$^+$ cells of patients in CTD-ILD group decrease obviously, there are statistical differences (P<0.05). Various T lymph cells of peripheral blood of patients in ILD group compared with the control group, there are no statistical differences (P>0.05). It shows immune state of CTD-ILD patients is abnormal furtherly. But for ILD patients, immune state is stable. It is uncertain to take the immune intervention. Examining pathogenesis furtherly, such as fungal infection, bacterial infection, viral infection and so on, so we can treat according to symptoms.

In conclusion, detecting changes of peripheral blood lymph immune typing and natural killer cells level of patients with ILD have significant clinical guidance for judging CTD and ILD whether have immune injury, and which will provide experimental basis for treatment methods selection in clinic.

References


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