Original Article

The Frequency, Activation, and Coreceptor Expression of Lymphocytes in Human Immunodeficiency Virus/Hepatitis C Virus Co-Infected Patients in China

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SUMMARY: The purpose of this study was to characterize the frequency, activation, and coreceptor expression of lymphocytes in Chinese human immunodeficiency virus (HIV)/hepatitis C virus (HCV) co-infected patients, and to study the impact of HCV on immune status and disease progression of HIV infection. Flow cytometry was used to analyze the numbers of T cells and NK cells, the level of activation and the coreceptors of T lymphocytes. The chronic liver diseases associated with HCV have become one of the major causes of death in HIV/HCV co-infected patients. With disease progression, co-infected patients expressed lower numbers of CD4 T-cells and NK cells, and higher activation levels and coreceptor expression of T lymphocytes. Compared to the counts of HIV mono-infected patients, the NK cell counts of co-infected patients were significantly lower in the asymptomatic HIV-infected and AIDS groups, and the levels of HLA-DR and CXCR4 were significantly elevated in the AIDS group. The viral load of HIV and HCV in the co-infected group increased gradually with the progression of disease. With disease progression, the immune status of HIV/HCV co-infected patients decreased gradually, and the HIV viral load increased. HCV appears to accelerate the natural course of the HIV disease by damaging the innate immune function and aggravating the levels of activating markers and coreceptors on T lymphocytes in co-infected patients.

INTRODUCTION

Due to similar risk factors for exposure, human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are often found concurrently. According to a study investigation performed in 2002, approximately 56.9% of HIV-infected patients were co-infected with HCV in different areas of China (1). In addition, HIV/HCV co-infection has emerged as a cause of higher HCV virus loads, increased rates of hospitalization and mortality, and more rapid progression of liver fibrosis compared to infection with HCV only (2). However, the alteration in the immune function of HIV/HCV co-infected individuals during different progression stages has rarely been addressed. As a result, the impact of HCV on the immune function of HIV-infected individuals is still under debate. Some studies suggest that HCV does not participate in the progression of HIV infection (3-5). By contrast, other studies report that HCV elicits the progression of HIV infection (2,6,7). The chronic liver diseases associated with HCV have become one of the major causes of death in HIV/HCV co-infected patients (8). In addition, though highly active antiretroviral therapy (HAART) is capable of effectively controlling virus levels of HIV-infected individuals, the liver dysfunctions that develop due to HCV and the side effects associated with certain antiretroviral regimens make the therapeutic strategies of HIV more complicated. Treatment of HIV/HCV co-infection has thus become one of the bottlenecks in HIV treatments in China. There is a pressing need to define the natural course of HIV/HCV co-infection and the impacts of HCV on the progression of the HIV disease.

This study uses flow cytometry to determine the absolute lymphocyte counts, including CD4 T-cells and NK cells, as well as the levels of activation markers (HLA-DR and CD38) and coreceptors (CCR5 and CXCR4) on T lymphocytes in HIV mono-infected and HIV/HCV co-infected individuals during different stages of disease. Cross-sectional comparisons were made among 110 HIV/HCV co-infected patients and 101 HIV mono-infected patients.

METHODS

Study subjects and specimens: A total of 211 HAART-naive HIV carriers were recruited from Liao Ning, Ji Lin, He Nan, Yun Nan provinces and the autonomous region of Xin Jiang in China. None of these individuals had been on IFN-α treatment within 6 months of study participation. The patients were divided into two groups according to whether they were co-infected with HCV or not. The HIV/HCV co-infected group consisted of 110 patients (59 males and 51 females; mean age, 40 years; range, 22-60 years). The HIV mono-infected group consisted of 101 patients (54 males and 47 females; mean age, 38 years; range, 18-67 years). According to the duration of infection time and CD4 T-cell absolute counts, each group was further stratified into three groups: (i) slow progression (SP) group (asymptomatic HIV infection for at least 10 years, stable CD4-T cell count ≥ 500/μl); (ii) asymptomatic HIV-infected group (CD4 T-cell counts ranging from 200/μl to 500/μl and without AIDS symptoms), and (iii) AIDS group (CD4 T-cell count < 200/μl or showing indications of AIDS). According to these criteria, there were 15 SP patients, 78 asymptomatic HIV patients,
and 17 AIDS patients in the HIV/HCV co-infected group. In the HIV mono-infected group, there were 10 SP patients, 77 asymptomatic HIV patients, and 14 AIDS patients. This study also included 56 healthy age-matched HIV−, HCV− control patients without any history of immune system disease (31 male and 25 females; mean age, 39). Only HCV-infected patients with serum HCV antibodies were eligible to participate. All patients lacked any other chronic hepatitis diseases.

Monoclonal antibodies (mAbs): mAbs were all obtained from Becton Dickinson (San Jose, Calif., USA): CD4 FITC, CD8 FITC, CD38 PE, CCR5 PE, CXCR4 PE, CD3 PerCP, and HLA-DR PerCP. Trucount tubes were also obtained from Becton Dickinson: TriTEST: CD4 FITC/CD8 PE/CD3 PerCP and CD3 FITC/CD16+56 PE/CD45 PerCP.

Absolute counts of T lymphocytes and NK cells: Absolute counts of T cells and NK cells were enumerated in 50 µl freshly obtained EDTA-anticoagulated whole blood using 20 µl directly labeled mAbs (TriTEST: CD4 FITC/CD8 PE/CD3 PerCP, CD3 FITC/CD16+56 PE/CD45 PerCP) according to the “lyse-no-wash” procedure. All samples were acquired with a four-color FACSCalibur (Becton Dickinson) and analyzed using MultiSET software.

Expressions of activation markers and coreceptors on T lymphocytes: One hundred microliters whole blood was incubated with CD4+ or CD8-FITC mAb, CD38-, CCR5- or CXCR4-PE mAb, HLA-DR- or CD3- PerCP mAb and their isotype controls for 30 min at room temperature in the dark. Erythrocytes were then lysed by incubation with a 3-ml lysing solution for 8 min at room temperature in the dark, and were washed twice with phosphate-buffered saline (PBS). The stained cells were fixed with 200 µl 1% paraformaldehyde and were analyzed on FACSCalibur using the CELLQuest software.

Viral load: Plasma viral load was determined with RTPCR detecting HIV-1 RNA (Roche Amplicor Monitor Standard Assay; Roche Diagnostics, Branchburg, N.J., USA)

Flow-cytometric analysis: Samples were analyzed using a four-color FACSCalibur equipped with argon and 488-nm diode lasers. The lymphocyte population in peripheral blood was defined by gating based on forward and side scattering. CD4 and CD8 T-cells were also defined by gating on the CD3+CD4+ lymphocyte population and the CD3+CD8+ lymphocyte population, respectively. Positive staining for each marker was determined by comparing them to the corresponding isotype-matched negative controls. The levels of HLA-DR, CD38, CCR5, and CXCR4 were established by gating on CD3+CD4+ and CD3+CD8+ lymphocytes populations as well.

Statistical analysis: Analyses were performed using Statistical Package for Social Sciences (SPSS, Inc., Chicago, Ill., USA) version 13.0. Means of different groups were compared using one-way ANOVA. Correlations were calculated with Spearman’s correlation coefficients. Statistical significance was set at P < 0.05.

RESULTS

Alterations of immune parameters of HIV/HCV co-infected patients in different disease stages: (i) Alterations of T lymphocytes and NK cells: The CD4 T-cell absolute counts of HIV/HCV co-infected patients showed significant decrease. The AIDS group exhibited the largest decrease in cell count, followed by the asymptomatic HIV-infected and SP groups, respectively (P < 0.01). With the exception of the SP group, the CD4 T-cell counts in the asymptomatic HIV-infected and AIDS groups were significantly lower than healthy controls (P < 0.01). The counts of CD8 T-cells in all infected groups were significantly higher than in healthy controls (P < 0.01), and significantly lower in the AIDS group than in the asymptomatic HIV-infected and SP groups. The counts of NK cells followed the same pattern (P < 0.05). The counts of NK cells in the asymptomatic HIV-infected and AIDS groups were significantly reduced compared to that in healthy controls, but not in the SP group (P > 0.01) (Table 1).

(ii) Levels of activating markers and coreceptors on T lymphocytes: The mean levels of activating markers (HLA-DR and CD38) in different co-infected groups increased in the order of SP, asymptomatic HIV-infected, and AIDS groups, and were significant higher than in healthy controls (P < 0.01). Furthermore, the CD8 and CD38 levels (P < 0.05) as well as CD4/HLA-DR and CD8/HLA-DR levels were significantly higher in the AIDS group than in the SP and asymptomatic HIV-infected groups (P < 0.01). The levels of CD3/CCR5 and CD4/CCR5 were notably higher in asymptomatic HIV-infected and AIDS groups than in healthy controls (P < 0.05). Mean levels of CD3/CXCR4 were also significantly higher in the AIDS group than in the asymptomatic HIV-infected and SP groups and the healthy controls (P < 0.01) (Table 1).

Comparison of immune parameters between HIV/HCV co-infected and HIV mono-infected groups: (i) Alterations of T lymphocytes and NK cells: Compared to the HIV mono-infected group, the counts of NK cells of the HIV/HCV co-infected group were considerably lower (P < 0.05). The counts of NK cells in the AIDS and asymptomatic HIV-infected groups of HIV/HCV co-infected patients were significantly lower than those of HIV mono-infected patients (P < 0.05). The mean count of CD4 T-cells in the AIDS group of co-infected patients was also lower than that of HIV mono-infected group, but the difference did not reach statistical significance. No statistical differences in the counts of CD4 T-cells and NK cells in the SP group were detected between mono- and co-infected groups (Table 1).

(ii) Levels of activating markers and coreceptors on lymphocytes: Overall, the levels of CD4/CD38, CD3/CCR5, CD3/CXCR4, and CD4/CXCR4 of co-infected groups were significantly higher than those of HIV mono-infected patients (P < 0.05). The levels of CD4/HLA-DR, CD8/HLA-DR, CD4/ CXCR4, and CD3/CXCR4 in the AIDS group of co-infected patients were significantly higher than those of HIV mono-infected patients (P < 0.01). The levels of CD4/CD38, CD8/ CD38, and CD4/CXCR4 in the asymptomatic HIV-infected group of co-infected patients were also significantly higher than that of the HIV mono-infected patients (P < 0.05). There were no statistical differences in the levels of activation markers and coreceptors on T lymphocytes between the SP groups of mono- and co-infected groups (Table 1).

Virus load of HIV/HCV co-infected patients: The HIV virus load of HIV/HCV co-infected patients was significantly different at different disease progressions; namely, those of the asymptomatic HIV-infected and AIDS groups were statistically higher than that of the SP group (P < 0.01). There was no statistical difference between the co-infected group and the HIV mono-infected group. No significant alteration in the HCV virus load was detected for any of the disease stages (Table 2).

Associated factors of NK cells and activation, coreceptor expression of T lymphocytes in HIV/HCV co-infected patients: The counts of NK cells correlated positively with the
Table 1. Numbers of T lymphocytes and NK cells and activation, coreceptor expression of T lymphocytes in different disease stages among HIV+HCV+, HIV+HCV−, and control patients

<table>
<thead>
<tr>
<th>Index</th>
<th>HIV+HCV+</th>
<th>HIV+HCV−</th>
<th>AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n = 110)</td>
<td>SP (n = 15)</td>
<td>HIV (n = 78)</td>
</tr>
<tr>
<td>CD4</td>
<td>397.65 ± 220.33</td>
<td>684.94 ± 147.06</td>
<td>352.15 ± 82.37</td>
</tr>
<tr>
<td>CD8</td>
<td>1,117.26 ± 621.83</td>
<td>1,225.94 ± 526.56</td>
<td>1,182.96 ± 704.05</td>
</tr>
<tr>
<td>NK</td>
<td>243.32 ± 238.23</td>
<td>373.96 ± 284.54</td>
<td>186.05 ± 137.01</td>
</tr>
<tr>
<td>HLA-DR/CD4</td>
<td>20.98 ± 14.99</td>
<td>15.58 ± 12.04</td>
<td>18.25 ± 11.73</td>
</tr>
<tr>
<td>HLA-DR/CD8</td>
<td>45.13 ± 19.31</td>
<td>40.95 ± 17.50</td>
<td>43.27 ± 19.82</td>
</tr>
<tr>
<td>CD38/CD4 (log10 copies/mL)</td>
<td>74.58 ± 12.53</td>
<td>74.06 ± 12.06</td>
<td>74.74 ± 11.37</td>
</tr>
<tr>
<td>CD38/CD8</td>
<td>84.37 ± 12.06</td>
<td>79.51 ± 14.05</td>
<td>85.46 ± 9.16</td>
</tr>
<tr>
<td>CCR5/CD8</td>
<td>42.31 ± 17.60</td>
<td>29.0 ± 15.08</td>
<td>41.34 ± 15.92</td>
</tr>
<tr>
<td>CXCR4/CD4</td>
<td>73.06 ± 18.48</td>
<td>71.41 ± 17.34</td>
<td>72.41 ± 20.23</td>
</tr>
<tr>
<td>CXCR4/CD8</td>
<td>63.64 ± 28.90</td>
<td>60.25 ± 22.51</td>
<td>62.68 ± 30.94</td>
</tr>
</tbody>
</table>

P < 0.05, P < 0.01 (comparing to control).

Table 2. Comparison of viral load in different disease stages of HIV+HCV+ and HIV+HCV− patients

<table>
<thead>
<tr>
<th>Virus load (log10 copies/mL)</th>
<th>HIV+HCV+</th>
<th>HIV+HCV−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n = 101)</td>
<td>SP (n = 10)</td>
</tr>
<tr>
<td>HIV-VL</td>
<td>4.13 ± 0.89</td>
<td>3.71 ± 0.67</td>
</tr>
<tr>
<td>HCV-VL</td>
<td>5.63 ± 1.00</td>
<td>5.49 ± 2.00</td>
</tr>
</tbody>
</table>

P < 0.01 (comparing to SP).

Table 3. Associated factors of NK cells and activation, coreceptor expression of T lymphocytes in HIV/HCV co-infected patients

<table>
<thead>
<tr>
<th>Activation or coreceptor expression on T cells</th>
<th>CD4+ T cell count</th>
<th>HIV-viral load</th>
<th>HCV-viral load</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK cells</td>
<td>0.509</td>
<td>-0.238</td>
<td>0.026</td>
</tr>
<tr>
<td>HLA-DR+/CD4</td>
<td>-0.446</td>
<td>0.352</td>
<td>0.016</td>
</tr>
<tr>
<td>CD38+/CD4</td>
<td>-0.233</td>
<td>0.259</td>
<td>0.097</td>
</tr>
<tr>
<td>HLA-DR+/CD8</td>
<td>-0.329</td>
<td>0.243</td>
<td>0.078</td>
</tr>
<tr>
<td>CCR5/CD4</td>
<td>-0.556</td>
<td>0.388</td>
<td>0.025</td>
</tr>
<tr>
<td>CCR5/CD8</td>
<td>-0.319</td>
<td>0.046</td>
<td>-0.202</td>
</tr>
<tr>
<td>CXCR4/CD4</td>
<td>-0.011</td>
<td>-0.003</td>
<td>0.023</td>
</tr>
</tbody>
</table>

P < 0.01 (comparing to SP).

CD4 T-cell absolute counts (P < 0.01) and inversely with the HIV virus load (P < 0.01), but did not correlate with the HCV virus load. An inverse correlation between the levels of CCR5 on CD4 T-cells and the HCV virus load was also observed (P < 0.05) (Table 3).

**DISCUSSION**

Since HIV and HCV share similar risk factors for transmission, co-infection with both is a common occurrence, especially among drug abusers, homosexuals, and hemophilic patients (9, 10). In Europe and the United States, approximately 30% of HIV-infected individuals are co-infected with HCV (11). In China, the rate of co-infection is even higher, at 56.9% (1). Studies have shown that morbidity and mortality rates from HIV/HCV co-infected patients have been rising (2, 12-14). As a result of the high rates of HIV/HCV co-infection worldwide and the severe consequences that come with it, there is a pressing need for more research regarding HIV/
HCV co-infection. It has been indicated that HIV may accelerate HCV liver damage in HIV/HCV co-infected individuals (2), but whether HCV can accelerate HIV-associated immunodeficiency and disease progression to AIDS is still poorly characterized. Thus far, there has been no report on this issue in China. Therefore, a comprehensive characterization of immune status, including T and NK lymphocyte counts in addition to activation markers and coreceptor levels on T lymphocytes involved in the immunopathogenesis of HIV/HCV co-infection, is crucial to gain insight into this co-infection.

T lymphocytes and NK cells play important antiviral roles through innate and adaptive immunity. The chronic activation of the immune system is an important feature regarding the spread of virus and the progression of disease. HLA-DR and CD38 are markers of activation that increase with augmented levels of cell activation (15). The chemokine factor receptors (CCR5 and CXCR4) are the coreceptors that facilitate HIV virus entry into target cells; their densities may influence the susceptibility of target cells to the HIV virus, and consequently HIV disease progression (16). It has been reported that in HIV infection, the absolute counts of CD4 T-cells and NK cells decrease gradually while the immune system remains in an activated state. The decline of CD4+ T cell counts is inversely correlated with viral load and directly correlated with the activation levels (17,18). Interestingly, the activated CD4+ T cells are more susceptible to the HIV virus because of the elevation in the CCR5 level and the more robust HIV virus replication in CD4+ T cells (16). In HCV infection, up to 85% of acute HCV patients may develop chronic hepatitis, lasting viremia and hepatocellular carcinoma (19,20). Acute HCV patients also display impaired immune functions, evidenced by decreased NK cell counts and increased levels of T lymphocyte activation and CCR5 expression (20). In contrast to HIV infection, the correlation between HCV viral load and HCV disease progression has not been observed (21), which suggests that immunoreactions against HCV, not the HCV virus itself, are responsible for contributing to liver pathogenesis. However, the alteration of immune status during different disease stages associated with the combined impact of HIV and HCV virus is still poorly understood.

To evaluate the relationship between dynamic alterations of immune parameters and disease stages in HIV/HCV co-infected patients, we analyzed the counts of T and NK lymphocytes and the levels of activation markers and coreceptors on T lymphocytes by comparing 110 HIV/HCV co-infected patients divided into SP, asymptomatic HIV-infected, and AIDS groups according to CD4 T-cell counts and NK cells decrease gradually while the immune system remains in an activated state. The decline of CD4+ T cell counts is inversely correlated with viral load and directly correlated with the activation levels (17,18). Interestingly, the activated CD4+ T cells are more susceptible to the HIV virus because of the elevation in the CCR5 level and the more robust HIV virus replication in CD4+ T cells (16). In HCV infection, up to 85% of acute HCV patients may develop chronic hepatitis, lasting viremia and hepatocellular carcinoma (19,20). Acute HCV patients also display impaired immune functions, evidenced by decreased NK cell counts and increased levels of T lymphocyte activation and CCR5 expression (20). In contrast to HIV infection, the correlation between HCV viral load and HCV disease progression has not been observed (21), which suggests that immunoreactions against HCV, not the HCV virus itself, are responsible for contributing to liver pathogenesis. However, the alteration of immune status during different disease stages associated with the combined impact of HIV and HCV virus is still poorly understood.

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To evaluate the relationship between dynamic alterations of immune parameters and disease stages in HIV/HCV co-infected patients, we compared the data of HIV/HCV co-infection to that of HIV mono-infection. We found that, regarding CD4 T-cell damage, co-infected patients tended to display lower CD4 T-cell counts at the advanced stage compared to HIV mono-infected patients. Concerning the innate immunity damage, co-infected patients in the asymptomatic HIV-infected and AIDS groups showed significantly lower NK counts compared to HIV mono-infected patients. Finally, pertaining to activation and coreceptor expression, the levels of CD4/HLA-DR and CD8/HLA-DR in the AIDS group and CD4/CD38 and CD8/CD38 in the asymptomatic HIV-infected group of co-infected patients were significantly higher than those of HIV mono-infected patients, while the mean frequency of CD3/CCR5 and the levels of CXCR4 on CD3 T-cells and CD4 T-cells in the AIDS group of co-infected patients was higher than those of the AIDS group of the HIV mono-infected patients. Our data indicates that, in the initial stage of co-infection when the immune function has not been seriously destroyed, the impact of the HCV on the immune status of HIV-infected individuals and HIV disease progression is not obvious. However, in the advanced stage when the immune system is very fragile, HCV co-infection facilitates the activation of T cells, the rise of coreceptor levels, and the further damage of the innate immune system, thereby increasing the susceptibility of CD4 T-cells to the HIV virus and accelerating the disease progression.

In this study, we divided the HIV/HCV co-infection patients into SP patients, asymptomatic HIV-infected patients, and AIDS patients to study the relationship between the immune parameters and disease progression. We further compared the immune parameters between HIV mono-infected and HIV/HCV co-infected individuals in the same disease-progression stages to analyze whether HCV infection would impact HIV progression, and found that HCV may accelerate the natural course of the HIV disease by damaging the innate immune function and aggravating the levels of activating markers and coreceptors on T lymphocytes. But as a cross-sectional observation, the limitation of this study was that the difference in the means among different disease stages may not fully reflect the time-series changes with the disease progression, and the SP date might reflect background advantageous host factors. In the future longitudinal studies should be used to investigate the correlation between immune responses and disease progression and may further validate or confirm the findings in this study. Another potential limitation is the relatively low absolute correlation coefficients between 0.233 and 0.556. Generally speaking, a high absolute correlation coefficient such as 0.8 can imply a stronger correlation of the two variables, while some of the absolute
correlation coefficients were less than 0.3 in our study. But the sample size in this study was relatively large (>200), which can partly offset the deficiency of relatively low absolute correlation coefficients and can to some extent reflect statistical relevance between frequency, activation and coreceptor expression of lymphocytes on the one hand and CD4+ T cell count and viral loads on the other.

In conclusion, our results suggest that HIV/HCV co-infection may accelerate the progress of HIV infection by destroying innate immune function and facilitating the activation of the immune system and the rise of coreceptor levels. At the advanced stage of HIV/HCV co-infection, treatments that are capable of maintaining the immune function, including HAART, should be utilized in order to inhibit virus replication and slow down the development of disease progression.

ACKNOWLEDGMENTS

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