Analysis of bronchoalveolar lavage fluid in patients with chronic hepatitis C before and after treatment with interferon alpha

Shinji Yamaguchi, Keishi Kubo, Keisaku Fujimoto, Takayuki Honda, Morie Sekiguchi, Takeshi Sodeyama

Abstract

Background – Previous studies have shown that patients with idiopathic pulmonary fibrosis (IPF) were more likely to be seropositive for hepatitis C virus (HCV) than normal controls, and that patients with chronic hepatitis C treated with interferon alpha (IFN-α) sometimes developed pulmonary fibrosis. The possibility that HCV infection and/or treatment with IFN-α are involved in the pathogenesis of pulmonary fibrosis or alveolitis was investigated.

Methods – A prospective non-randomised study was performed in 13 healthy controls and in patients with chronic hepatitis C before (n = 13) and after (n = 10) treatment with IFN-α. Bronchoalveolar lavage (BAL) fluid cell counts, ratios and T cell subsets, and the concentrations of interleukin(IL)-1β, tumour necrosis factor(TNF)-α, and hepatocyte growth factor (HGF) were measured.

Results – Lymphocyte counts in the BAL fluid were significantly increased in both groups of patients (median (range) values: before treatment, 36.8 (1.5–226.0); after treatment, 16.2 (4.5–97.6)) compared with the normal controls (5.3 (0.5–32.3)). In the pretreatment group the activated T cell (HLA-Dr positive) count was also increased (51 (40–74)) compared with that in the normal controls (27 (4–52)), but after treatment it was decreased (40 (0–76)) compared with the pretreatment count. Administration of IFN-α did not affect these parameters. IL-1β, TNF-α, and HGF were not detected.

Conclusions – These findings suggest that HCV infection is associated with increased counts of lymphocytes and neutrophils in BAL fluid and that treatment with IFN-α appears to alter lymphocyte surface markers.

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Keywords: chronic hepatitis C, interferon-α, alveolitis, T cell subsets.

Some workers have reported that viral infection may be important in the pathogenesis of idiopathic pulmonary fibrosis (IPF).1–4 Hepatitis C virus (HCV), an RNA virus first discovered by Choo and coworkers in 1989,5 causes non-A, non-B hepatitis and fibrotic changes in the liver.6 Ueda et al have recently reported that Japanese patients with IPF are more likely to be seropositive for HCV than normal controls, suggesting that HCV infection may play a part in the pathogenesis of IPF.7 Melicori et al also reported the same trend in Italian patients.8 However, Irving et al9 have suggested that, in the UK, HCV infection is no more prevalent in patients with IPF than in the general population. We have reported that HCV infection might be a trigger of active alveolitis which can lead to pulmonary fibrosis.10 Since patients with chronic hepatitis C treated with interferon alpha (IFN-α) sometimes develop pulmonary fibrosis,11–14 there has been speculation that IFN-α treatment leads to pulmonary fibrosis.

In order to investigate the possibility that HCV infection and/or treatment with IFN-α is involved in the pathogenesis of pulmonary fibrosis or alveolitis, bronchoalveolar lavage (BAL) fluid was obtained from normal volunteers and from patients with chronic hepatitis C infection both before and after treatment with IFN-α. Differential cell counts and T cell surface markers in the BAL fluid were analysed. We also measured BAL fluid levels of interleukin(IL)-1β as a fibroblast growth factor, tumour necrosis factor (TNF)-α as an index of inflammation, and hepatocyte growth factor (HGF) as an epithelial growth factor.

Methods

SUBJECTS

Patients with chronic hepatitis C were studied before (n = 13; 10 men, median age 60 (range 31–64 years)) and after (n = 10; seven men, median age 59 (range 37–63 years)) treatment with IFN-α and compared with 13 normal volunteers (eight men, median age 53 years (range 24–67)). We excluded from the study any patients who had received a course of antiviral or immunosuppressive therapy during the previous six months, those who had a positive titre for autoantibodies to detect collagen diseases, and those with leucopenia (<3000/μl) and thrombocytopenia (<80 000/μl). No patients had a family history of IPF, respiratory symptoms, or radiographic changes suggesting pulmonary fibrosis on chest radiography, computed tomographic (CT) scanning, and gallium scintigraphy.

Subjects were defined as smokers (S) if they currently smoked cigarettes, ex-smokers (ES)
if they had quit smoking more than six months earlier, or non-smokers (NS) if they had never smoked. More information on the subjects is shown in table 1.

A diagnosis of chronic hepatitis C required a history of liver dysfunction of six months or longer with abnormal serum levels of glutamine oxaloacetic transaminase (sGOT) and glutamic pyruvic transaminase (sGPT), positive serum HCV antibody determined with a second generation enzyme-linked immunosorbent assay (ELISA) kit (Immuchek-HCV Ab, Kokusai, Kobe, Japan), and biopsy evidence of chronic inflammation of the liver without evidence of cirrhosis. The histological findings for the liver in the pretreatment group included chronic persistent hepatitis (CPH) in five patients, chronic active hepatitis 2A (CAH2A) in four, and CAH2B in four. The post-treatment group included chronic persistent hepatitis (CPH) in five patients, chronic active hepatitis 2A (CAH2A) in four, and CAH2B in four. Three patients in the pretreatment group did not agree to undergo a second bronchoscopy. Those who had received a Shosaikoto (a Chinese medicine) for the treatment of chronic hepatitis C in Japan an increased frequency of interstitial pneumonitis has been observed following treatment with Shosaikoto for chronic hepatitis C (unpublished data).

The study was performed according to the criteria set out in the Helsinki Declaration, and free and informed consent was obtained from all subjects.

Table 1 Clinical and physiological characteristics of the groups studied

<table>
<thead>
<tr>
<th></th>
<th>No. (M,F)</th>
<th>Age (years)</th>
<th>Smoking (S, ES, NS)</th>
<th>VC (%)</th>
<th>FEV₁ (%)</th>
<th>TLC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13(8,5)</td>
<td>53(24-67)</td>
<td>(4,9)</td>
<td>107.5(96.9-142.7)</td>
<td>82.8(71-97.6)</td>
<td>110.0(81.2-143.0)</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>13(10,3)</td>
<td>60(31-64)</td>
<td>(5,3,5)</td>
<td>96.6(78.6-129.0)</td>
<td>82.6(75-94.0)</td>
<td>96.4(72.0-126.1)</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>10(7,3)</td>
<td>59(37-63)</td>
<td>(5,3,2)</td>
<td>116.0(86.6-139.0)</td>
<td>79.8(68-91.9)</td>
<td>103.0(71.6-140.9)</td>
</tr>
</tbody>
</table>

Values are median (ranges). S = smoker; ES = ex-smoker; NS = non-smoker; VC = vital capacity; FEV₁ = forced expiratory volume in one second; TLC = carbon monoxide transfer factor.

Statistical analysis: Values in the text, tables and figures are expressed as medians and ranges. The Mann-Whitney non-parametric test was used for comparison between the groups and the Kruskal-Wallis test was used for comparison of the medians of all three groups. A p value of less than 0.05 was considered significant.

Results

Clinical effects of IFN-α
In the pretreatment group sGOT and sGPT levels were 54–377 U/l (median 145 U/l) and 102–487 U/l (median 160 U/l), respectively. After treatment the levels of sGOT and sGPT were both significantly lower (p<0.01) at 20–168 U/l (median 58 U/l) and 12–176 U/l (median 81 U/l), respectively.

Pulmonary function tests
The pulmonary function test parameters were within normal limits in all patients and controls (table 1) and pulmonary function was not affected by treatment with IFN-α.

Cell concentrations in BAL fluid
Cell concentrations in the BAL fluid from normal subjects and from patients before and after...
Table 2  Cell concentrations in BAL fluid

<table>
<thead>
<tr>
<th></th>
<th>Total cells</th>
<th>Macrophages (×10³/ml)</th>
<th>Lymphocytes (×10³/ml)</th>
<th>Neutrophils (×10³/ml)</th>
<th>Eosinophils (×10³/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59 (25–808)</td>
<td>56.3 (22.5–771.6)</td>
<td>3.3 (0.5–32.3)</td>
<td>0.5 (0–5.2)</td>
<td>0.00 (0–0.16)</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>160 (42–372)</td>
<td>94.0 (89.0–97.0)</td>
<td>4.5 (2.0–10.0)</td>
<td>1.0 (0–2.0)</td>
<td>0.00 (0–0.3)</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>90 (43–177)</td>
<td>76.0 (38.0–93.0)</td>
<td>19.8 (13.5–61.0)</td>
<td>0.5 (0–8.0)</td>
<td>0.17 (0–6.0)</td>
</tr>
</tbody>
</table>

Values are median (ranges).

* p<0.05, ² p<0.01, ³ p<0.001 for control versus pretreatment group.

There was no significant difference between pretreatment and post-treatment groups.

Figure 1  Percentage of lymphocyte surface markers in the bronchoalveolar lavage fluid from normal healthy volunteers (control, (Ο), n=13) and from patients with chronic hepatitis C before (pretreatment group, (●), n=13) and after (post-treatment group (Φ), n=10) treatment with IFN-α. Vertical lines show median values.

The number of lymphocytes with HLA-Dr (range 40–74%), a marker of early T cell activation, was significantly higher than in the normal controls. However, the CD4/CD8 ratio

Treatment with IFN-α are shown in table 2. Lymphocyte and eosinophil counts were significantly higher in both groups compared with the normal volunteers.

Surface Marker Analysis of Lymphocytes

The results of surface marker analysis of lymphocytes are outlined in fig 1. In the normal controls the percentage medians and ranges of CD2, CD3, CD4, CD8 and HLA-Dr were 67 (15–90), 53 (6–90), 39 (8–62), 11 (2–41), and 27 (4–52), respectively. CD2, CD3, CD4, CD8, HLA-Dr and CD4/8 were significantly different between the three groups. In the pretreatment group an increase was seen in T lymphocytes (CD2, 78±98%; CD3, 54±94%) and in the CD4 helper subset (67 (23–81%). The number of lymphocytes with HLA-Dr (range 40–74%), a marker of early T cell activation, was significantly higher than in the normal controls. However, the CD4/CD8 ratio
(range 0.09–1.15) was almost the same as that in the controls. The number of B lymphocytes (CD20, median and range 1 (0–6)% was not increased. On the other hand, after treatment the levels of CD2 (4–98%), CD3 (4–75%), and CD4 (32 (3–82)% subsets were normal. The CD8 subset (6 (0–16)) was significantly decreased and the CD4/CD8 ratio (2.2–30) was significantly increased compared with the normal controls.

CYTOKINE LEVELS IN BAL FLUID

The IL-1β, TNF-α, and HGF levels were all less than the lower limits for detection. The albumin levels in the BAL fluid did not differ significantly between the three groups.

Discussion

In this study we have analysed the BAL fluid in patients with chronic hepatitis C before and after treatment with IFN-α to investigate the possibility that HCV infection and/or IFN-α treatment are involved in the pathogenesis of pulmonary fibrosis or alveolitis.

Patients with chronic hepatitis C had significantly increased concentrations of lymphocytes, eosinophils and neutrophils in their BAL fluid, although the total cell counts did not differ from those in the normal volunteers.

The lymphocytes were identified as T cells (CD2+ and CD3+) and the T helper/inducer subset of cells (CD4+). The number of lymphocytes expressing HLA-Dr (the expression of class II major histocompatibility complex antigens on T lymphocytes), a marker of early T cell activation, was also increased. The numbers of T suppressor/cytotoxic cells (CD8+) and B lymphocytes (CD20+) were not increased. The CD4/CD8 ratio was almost the same as that in the normal volunteers. The CD4/CD8 ratio (3.75 (0.89)) found in our normal controls is higher than the control values of 1.4–2.7 reported in other laboratories.

There is no clear explanation for this, which is a consistent finding in studies in our unit. In our unit the CD4/CD8 ratio in patients with sarcoidosis is higher (6.8 (1.2)).

The levels of IL-1β, TNF-α, and HGF were all less than the detection limit. This is in contrast with studies in progressive IPF and adult/acute respiratory distress syndrome (ARDS) in which increased levels of IL-1β, TNF-α, and HGF were reported in the BAL fluid.

We believe that smoking had little effect on the cell populations in the BAL fluid since the number of current smokers was similar in both groups and the cell population profiles of ex-smokers were almost identical to those of non-smokers.

On the other hand, after treatment the concentrations of lymphocytes and eosinophils were similar to those before treatment although almost all lymphocyte surface marker values were similar to those in the normal volunteers and, surprisingly, the HLA-Dr value was within the normal range.

Bronchoalveolar lavage is a useful method for investigating both the pathogenesis and the diagnosis and management of pulmonary diseases, particularly interstitial lung diseases.16 17 20 The percentage of lymphocytes is increased in sarcoidosis and hypersensitivity pneumonitis, under which conditions lymphocytic alveolitis is exhibited. Laviolette7 and Merchant et al2 determined the distribution of cells in the BAL fluid of normal subjects and reported that a concentration of lymphocytes above 14% should be considered abnormal. According to this criterion, the patients in both groups in the present study showed a significant increase in the percentage of lymphocytes in the BAL fluid. We did not perform lung biopsies so the histological presence of pneumonitis was not determined, but we speculate that HCV infection has the potential to induce lymphocytic alveolitis and fibrotic changes in the lung. It seems likely that the increase in HLA-Dr+ lymphocytes in the pretreatment group was activated by HCV and that the antiviral activity of the administration of IFN-α can decrease the percentage of HLA-Dr+ lymphocytes.

In the pretreatment group the high count of eosinophils in the BAL fluid may be of importance in the pathogenesis of the disease.10 After treatment with IFN-α the eosinophil count was similar to that before treatment. Eosinophils have a potent armamentarium of highly cytotoxic products that may be involved in the development of tissue damage.25–27 It has recently been suggested that the eosinophilia in the BAL fluid may, in fact, be a marker of progressive lung disease in patients with IPF.28 The increase in the eosinophil count in the BAL fluid seen in this study, although lower than in the IFP study, may nonetheless suggest a role for eosinophils in the development of alveolitis and/or pulmonary fibrosis. IFN-α did not affect the level of eosinophils in the BAL fluid.

The part played by IFN-α in the pathogenesis of IPF is unknown. Pneumonitis associated with IFN-α has been reported in case reports on the treatment of malignant diseases with anticancer drugs37 and recently in the treatment of chronic hepatitis C.11–14 In general, drug induced pneumonitis can be kept under control by stopping the drug treatment and/or administering corticosteroids. However, pneumonitis associated with IFN-α treatment may have several forms such as an allergic reaction or cytotoxicity, and the conditions associated with treatment are not always reversible when the drug therapy is discontinued, as has been reported for IFN-α neurotoxicity.40

In summary, we have demonstrated significantly increased levels of lymphocytes, mainly active T cells, and eosinophils in the BAL fluid of patients with chronic hepatitis C, and have found evidence that IFN-α treatment appears to alter lymphocyte surface markers. HCV infection might be a trigger of lymphocytic alveolitis. Treatment with IFN-α appears to be beneficial for chronic hepatitis C and has no adverse effects on pulmonary abnormalities.
BAL fluid analysis in patients with hepatitis C treated with IFN-α

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