

Short papers

Bronchoalveolar lavage fluid findings in patients with chronic hepatitis C virus infection

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Abstract

Background – Hepatitis C virus (HCV) infection has recently been incriminated as an aetiological agent in idiopathic pulmonary fibrosis. This study was performed to determine the cellularity and lymphocyte phenotypes of bronchoalveolar lavage (BAL) fluid in patients with chronic hepatitis C.

Methods – BAL fluid and lavage lymphocyte subsets from 13 patients (10 men) with active chronic hepatitis C, diagnosed by sustained elevated serum glutamic pyruvic transaminase and typical histological findings in the liver, were analysed. Lavage findings in these patients were compared with those from 13 healthy volunteers (eight men) as controls.

Results – There was no difference in total cell counts in lavage fluid between the two groups. Lavage lymphocyte and eosinophil numbers were increased in patients with chronic hepatitis C. Surface marker analysis of the lymphocyte populations showed increases in CD2, CD3, CD4, and HLA-DR. CD4/CD8 ratios were not different.

Conclusions – The numbers of lymphocytes and eosinophils in BAL fluid are increased in patients with chronic hepatitis C. These findings suggest that HCV infection may trigger alveolitis.

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Keywords: bronchoalveolar lavage, hepatitis C virus, idiopathic pulmonary fibrosis, alveolitis.

ever, Irving and coworkers⁶ have suggested that HCV infection is no more prevalent in patients with IPF than in the general population in the UK.

To elucidate whether HCV infection is related to the pathogenesis of IPF, we investigated the cellularity and lymphocyte phenotypes of bronchoalveolar lavage (BAL) fluid, and performed pulmonary function tests in patients with chronic hepatitis C.

Methods

SUBJECTS

Thirteen patients (10 men) of median age 57 years (range 31-64) with chronic hepatitis C proven by the typical histological findings observed within the previous six months and the presence of sustained elevated serum levels of glutamic pyruvic transaminase (GPT) for at least six months were studied. All patients were positive for antibody to HCV in serum and negative for hepatitis B surface antigen. They were treated with interferon after this study. The following patients were excluded from the study: those who had received a course of antiviral or immunosuppressive therapy which may lead to alveolitis within six months; those who had a positive titre for autoantibodies to collagen diseases; those who exhibited leucopenia (<3000/ml) and thrombopenia (<80 000/ml). None of the patients had a familial history of IPF and none had pulmonary fibrosis as determined from symptoms, physical signs, and radiographic findings. Thirteen healthy volunteers (eight men) of median age 49 years (range 24-67) acted as controls.

Subjects in both groups were defined as smokers if they currently smoked cigarettes and ex-smokers if they had not smoked within the preceding six months.

This study was performed according to the criteria of the Helsinki Declaration and free and informed consent was obtained from all patients and normal volunteers.

BRONCHOALVEOLAR LAVAGE (BAL)

Subcutaneous injections of atropine (0.5 mg) and pethidine hydrochloride (0.5 mg/kg) were given. The oral pharynx and upper airway was anaesthetised with 2% lignocaine (lidocaine).

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Idiopathic pulmonary fibrosis (IPF) is characterised by inflammation and fibrosis of the pulmonary interstitium and peripheral air spaces.¹ The aetiology of IPF remains unknown. One interesting and important possible cause is viral infection.² Hepatitis C virus (HCV), the RNA virus discovered in 1989,³ is the cause of non-A, non-B hepatitis. One of the pathological manifestations of HCV is fibrotic changes in the liver.⁴ Ueda *et al*⁵ have reported an unusually high prevalence of serum antibodies to HCV in Japanese patients with IPF, and speculated that HCV may play an important part in the pathogenesis of IPF. How-

Table 1 Mean (SE) demographic and physiological characteristics of the study population

	Number (men)	Age (range) (years)	Smoking (S, ES)	VC (% predicted)	FEV ₁ /FVC (%)	TLCO (% predicted)
HCV	13 (10)	57 (3) (31–64)	5, 3	99.3 (15.6)	82.6 (5.3)	95.7 (16.3)
Control	13 (8)	49 (4) (24–67)	4, 0			

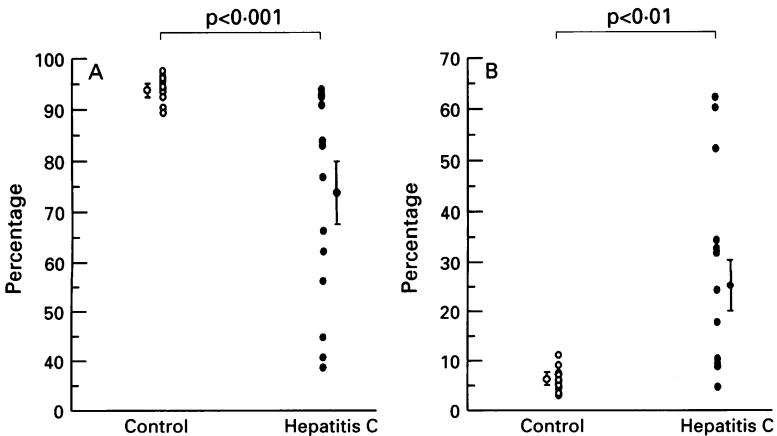
HCV=chronic hepatitis C; VC=vital capacity; FEV₁/FVC=forced expiratory volume in one second; TLCO=diffusing capacity for carbon monoxide; S=current smoker; ES=ex-smoker.

Table 2 Mean (SE) surface marker analysis of lymphocytes from BAL fluid

	CD2 (%)	CD20 (%)	CD3 (%)	CD4 (%)	CD8 (%)	HLA-DR (%)	CD4/CD8 (ratio)
HCV (n=13)	90.5 (1.8)*	1.7 (0.6)	79.8 (3.1)*	57.8 (5.1)*	22.7 (4.1)	54.8 (3.2)*	5.09 (1.62)
Control (n=13)	61.3 (6.9)	1.6 (0.3)	51.8 (7.4)	36.8 (4.9)	16.2 (3.4)	24.7 (4.4)	3.75 (0.89)

HCV=chronic hepatitis C.
*p<0.05 between the two groups.

A fibreoptic bronchoscope (Olympus BF 1T, ED 6 mm, Olympus Co, Tokyo, Japan) was wedged in the middle lobe segmental bronchus. Three 50 ml aliquots of sterile normal saline warmed to 37°C were instilled into the lung and each aliquot was then removed by gentle suction. The BAL fluid was filtered through gauze. One small aliquot was used for counting total cell numbers. Another aliquot was spun in a cytometer at 500 rpm for five minutes and stained with the May-Grünwald-Giemsa stain to identify cell populations. Five hundred cells, excluding epithelial cells, were identified per slide to establish differential cell counts and counts were expressed as percentages and total numbers of dividing cell types. The rest of the BAL fluid was centrifuged at 300 g for 10 minutes at 4°C and the supernatant removed. The BAL fluid pellets were analysed for lymphocyte subsets. The cell subsets were analysed by flow cytometry using CD2, CD3, CD4, CD8, CD20, and HLA-DR monoclonal antibodies⁷ (Becton Dickinson Co, Mountain View, California, USA).



Percentages of (A) macrophages and (B) lymphocytes in bronchoalveolar lavage fluid in normal healthy volunteers (n=13) and patients with chronic hepatitis C (n=13). Vertical lines and bars show mean values and SE, respectively, in each group.

PULMONARY FUNCTION TESTS

Spirometric tests were performed for all patients with chronic hepatitis C using a water spirometer (Gadart Exprograph, Godart-Statham, Bilthoven, Holland). Vital capacity (VC) and percentage forced vital capacity expired in one second (FEV₁/FVC) were calculated. Carbon monoxide diffusion capacity (TLCO) was measured by the single breath method (Pulmorecorder, model R1551S, Anima, Tokyo, Japan). VC and TLCO were expressed as a percentage of the predicted values.

STATISTICAL ANALYSIS

Values in the text, tables, and figure are expressed as means (SE). For statistical analysis Wilcoxon's non-parametric rank sum test was used for comparison between the groups and Spearman's rank correlation test for testing the relationship between variables. A p value of <0.05 was considered significant for all tests.

Results

The demographic and pulmonary function test data of patients with chronic hepatitis C (HCV group) and normal healthy volunteers are summarised in table 1. The HCV group had normal pulmonary function in terms of %VC, FEV₁%, and TLCO values.

Cell concentrations and surface marker analysis of lymphocytes from BAL fluid are summarised in the figure and table 2. The total cell counts in the BAL fluid from the HCV and control groups were not significantly different (146.8 (27.1) and 147.5 (59.3) × 10³/ml, respectively). However, lymphocyte counts and percentages were significantly higher in the HCV group (49.8 (8.4) × 10³/ml; 24.4 (96.2)%) than in the controls (7.0 (2.4) × 10³/ml; 5.2 (0.7)%; p<0.05). Eosinophil counts and percentages were also significantly higher in the HCV group (1.89 (0.84) × 10³/ml; 2.0 (1.2)%) than in the controls (0.03 (0.01) × 10³/ml; 0.1 (0.0)%; p<0.05). Neutrophil counts were not significantly different in the HCV group (1.1 (0.4) × 10³/ml) and controls (1.5 (0.5) × 10³/ml). Surface marker analysis of lymphocytes demonstrated significant increases in CD2, CD3, CD4, and HLA-DR. The markers CD20 and CD8 were not increased in the HCV group. The CD4/CD8 ratio was comparable, being 5.09 (1.62) in the HCV group and 3.75 (0.89) in the controls. There was no significant correlation between serum GPT levels and the total or percentages of lymphocytes in the BAL fluid (data not shown).

Discussion

This study has shown that patients with chronic hepatitis C had a significantly increased total number and percentage of lymphocytes and eosinophils in the BAL fluid, although total cell counts did not differ from those of the normal individuals. Furthermore, surface marker analysis of lymphocytes in the BAL fluid from patients with chronic hepatitis C showed increases in CD2, CD3, CD4, and

HLA-DR. 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 populations of ex-smokers were almost identical to those who had never smoked.⁸

The increased lymphocyte number in patients with chronic hepatitis C was identified as T cells (CD2+ and CD3+) and the T helper/inducer subset of T cells (CD4+). The number of lymphocytes expressing HLA-DR, 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 showed a tendency to be higher in patients with chronic hepatitis C compared with controls. There have been no previous studies of lymphocyte subsets in BAL fluid or peripheral blood lymphocytes in patients with chronic hepatitis C. However, the findings of our study are consistent with the work of Pham *et al*⁹ who showed higher CD4/CD8 ratios in liver-derived lymphocytes from patients with chronic hepatitis B. The CD4/CD8 ratio of 3.75 (0.89) found in our normal controls is higher than the control values of 1.4–2.7 in other laboratories.⁸ There is no clear explanation for this which is a consistent finding in the control studies in our unit.

The findings of increased eosinophils may be of importance in the pathogenesis of chronic hepatitis C. Eosinophils contain highly cytotoxic products which may be involved in tissue damage.¹⁰ Peterson *et al*¹¹ reported that eosinophilia (10.7 (2.7)%) in the BAL fluid predicted a deterioration in vital capacity in patients with IPF. The increase in eosinophils in the BAL fluid in this study (1.99 (1.22)%), although lower than in the IPF study, may nonetheless suggest a role for eosinophils in the development of alveolitis and/or pulmonary fibrosis in patients with chronic hepatitis C.

The relationship of HCV infection to IPF has been described in two studies.^{5,6} Ueda *et al*⁵ found that 19 of 66 (28.8%) Japanese patients with IPF were positive for serum antibodies to HCV using the screening ELISA method (first generation). This percentage of positive findings was significantly higher than that in 9464 age matched control subjects (3.66%), and the authors speculated that HCV infection might play an important part in the pathogenesis of IPF. By contrast, Irving *et al*⁶ reported that only one of 62 patients with IPF was positive for HCV by second generation anti-HCV ELISA. The discrepancy between the two studies may be due to three factors. Firstly, different methods were used for determining the serological reaction to HCV; secondly, raised IgG levels have been shown

to be a cause of false positive anti-c100 results¹² and the differing IgG profiles in the studies by Ueda and Irving may account for some of the discrepancy in anti-HCV results; and thirdly, there may be geographical differences in the pathogenesis of IPF that could be due to differences between the genotypes of HCV virus^{13,14} which influence the clinical course of hepatitis C and the response to interferon therapy. We have no data from viral cultures of BAL fluid or lung tissue in patients with IPF to support the hypothesis of HCV infection triggering IPF, but Geist and Hunninghake² have emphasised the possibility of latent viral infection in the development of pulmonary fibrosis, and IPF in particular.

In summary, we have shown significantly increased levels of lymphocytes, mainly active T cells, and eosinophils in the BAL fluid of patients with chronic hepatitis C and no clinical evidence of lung disease, which suggests that HCV infection might be a trigger of active alveolitis which can lead to pulmonary fibrosis.

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