The purpose of this study was to determine the phenotype profiles of immune effector cells and the concentrations of immunoglobulins in the lower respiratory tract of nonsmoking patients with alcoholic liver cirrhosis (ALC). Nine nonsmoking patients with liver biopsy-proved ALC (grade B or C cirrhosis in Child’s classification), free of clinical pulmonary symptoms, and with normal chest roentgenogram were included in the study. The control group included 12 healthy nonsmokers. Each patient had fiberoptic bronchoscopy with bronchoalveolar lavage (BAL). The number of T cells and of lymphocyte subpopulations was determined by immunofluorescence studies using monoclonal antibodies that were specific for CD3, CD4, and CD8 markers. Patients with ALC exhibited a dramatically increased percentage of CD8+ cells in BAL that induced a low CD4/CD8 ratio (0.96 ± 0.15 vs 1.8 ± 0.12 in healthy controls). Further characterization of lymphocyte subsets’ dual immunofluorescence analysis demonstrated that most of the CD8+ alveolar lymphocytes had a phenotype of cytotoxic cells (CD8+ CD11b+; 48 percent ± 13 in ALC vs 10 percent ± 5 in controls). ALC was associated with an appreciable alveolar-capillary “leak” as demonstrated by a significant increase in BAL fluid albumin. In addition, the concentrations of immunoglobulins in BAL fluid were significantly greater in ALC than in controls. However, the relative (to albumin) coefficient of excretion of IgG, A, and M in and α1-macroglobulin BAL fluid was not significantly different between controls and ALC. Our results indicate that increased proportions of CB8+ and especially of CD8+ CD11b+ cells are a common feature in the lower respiratory tract of nonsmoking patients with ALC. These changes may be of potential functional importance in the regulation of the local pulmonary immune response in ALC.

(Chest 1992; 101:468-73)

ALC = alcoholic liver cirrhosis; BAL = bronchoalveolar lavage; FITC = fluorescein isothiocyanate; HBSS = Hanks balanced salt solution; IRMA = immunoradiometric assay; NK = natural killer; PBS = phosphate-buffered saline solution; RCE = relative coefficient of excretion

In the last decade, several lines of evidence suggested that long-term alcohol intake and alcoholic liver cirrhosis (ALC) might be associated with an imbalance of the immune regulatory system. In this context, several immune abnormalities have been demonstrated in patients with ALC. Hypergammaglobulinemia, with elevations of IgA, IgM, and IgG, which is thought to be related to increased production, is a usual finding in patients with ALC. There is convincing evidence that development and expression of cell-mediated immunity are depressed in ALC. Patients with ALC have reduced skin test reactivity to ubiquitous antigens. Peripheral blood T cells, lymphocyte proliferation, suppressor cell function, and natural killer cell function are all diminished in ALC. However, wide variations were observed according to the studies suggesting that patient selection might be heterogenous since most of the investigations were reported without details about clinical status.

Although extensive phenotypical and functional studies have allowed the characterization of a number of immune defects in their peripheral blood, no information is available about the local immune defense system in the lower respiratory tract of patients with ALC. It is known that the determination of lymphocyte phenotypes in peripheral blood does not provide information on the situation in specific organs. The fact that lymphocytes have the capacity to recirculate from blood to lymph and back to blood supports the concept that lymphocyte recirculation might be important in the segregation of lymphocytes with distinct functions in the different lymphoid organs of the body. For example, comparison of T cells obtained from bronchoalveolar lavage (BAL) to those circulating in peripheral blood has revealed large differences in various lung disorders.

Therefore, because patients with ALC experience an increased risk of lung infection, we initiated this study to...
Table 1—Characteristics of the 12 Nonsmoking Patients with Alcoholic Liver Cirrhosis*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, yr</th>
<th>Child's Classification</th>
<th>Albumin, g/100 ml (3.5-4.4)</th>
<th>IgA, g/100 ml (0.9-4.5)</th>
<th>IgG, g/100 ml (0.9-2)</th>
<th>IgM, g/100 ml (0.6-2.5)</th>
<th>Bilirubin, g/ml (0.2-1.2)</th>
<th>SGOT, IU/l (7-40)</th>
<th>Alkaline Phosphatase, IU/l (50-180)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>B</td>
<td>2.9</td>
<td>5.6</td>
<td>2.6</td>
<td>3.5</td>
<td>0.5</td>
<td>140</td>
<td>144</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>B</td>
<td>2.9</td>
<td>5.8</td>
<td>2.6</td>
<td>6.3</td>
<td>2.5</td>
<td>146</td>
<td>133</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>B</td>
<td>3.4</td>
<td>3.7</td>
<td>1.5</td>
<td>2.4</td>
<td>1.5</td>
<td>129</td>
<td>77</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>B</td>
<td>3.4</td>
<td>5.3</td>
<td>1.9</td>
<td>8.7</td>
<td>2.5</td>
<td>146</td>
<td>133</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>C</td>
<td>3.1</td>
<td>4.2</td>
<td>1.2</td>
<td>2.1</td>
<td>1.7</td>
<td>161</td>
<td>303</td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>C</td>
<td>3.5</td>
<td>3.5</td>
<td>0.9</td>
<td>1.1</td>
<td>0.9</td>
<td>148</td>
<td>126</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td>C</td>
<td>4.5</td>
<td>3.7</td>
<td>1.5</td>
<td>2.2</td>
<td>0.8</td>
<td>167</td>
<td>150</td>
</tr>
<tr>
<td>8</td>
<td>36</td>
<td>C</td>
<td>3.6</td>
<td>5.4</td>
<td>1.4</td>
<td>1.2</td>
<td>0.7</td>
<td>220</td>
<td>120</td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>C</td>
<td>3.9</td>
<td>2.1</td>
<td>1</td>
<td>1.6</td>
<td>1.4</td>
<td>136</td>
<td>184</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are normal values.

characterize lymphocyte phenotype profiles in the BAL and peripheral blood from nonsmoking patients with ALC using cell surface marker phenotyping by monoclonal antibodies. In addition, using highly sensitive techniques to analyze the secretions of plasma proteins in external body fluids, we measured the concentrations of albumin, IgG, IgA, IgM and α1-macroglobulin in serum and in BAL fluid.

Methods

Patients

Nine nonsmoking patients with biopsy-proven alcoholic cirrhosis (grade B or C cirrhosis in Child's classification) were included in the study (Table 1). They were six men and three women, with a mean age of 51 ± 3 years. The following criteria were used for inclusion in the study: (1) presence of at least one major sign of cirrhosis (jaundice, ascites, or gastrointestinal hemorrhage); (2) absence of infection; and (3) absence of surgery, antibiotherapy, or corticosteroid therapy for one month before hospital admission. None of them had signs and symptoms of acute alcoholic intoxication. Patients with ALC had a greater than 80-g daily intake of alcohol for more than ten years. All the patients had not ingested alcohol for at least one week before these studies were performed and blood alcohol levels were zero at the time of the study. All were nonsmokers, had normal chest roentgenograms, and did not present evidence of lung infection three months before evaluation. None had signs of severe malnutrition. All gave informed consent for the procedure. Results were compared with those obtained from 12 age-matched healthy nonsmokers.

Bronchoalveolar Lavage

Bronchoalveolar lavage was performed after premedication with atropine under local anesthesia with lidocaine (lidocaine) using a wedged fiberoptic bronchoscope (model BF-B3; Olympus Corp of America, New Hyde Park, NY) and 250 ml of sterile saline solution was applied in five 50-ml aliquots with immediate gentle vacuum aspiration after each aliquot, as previously described. The aspirated fluid was collected into siliconized jugs and immediately transported on ice to the laboratory. BAL was filtered through several layers of sterile surgical gauze and the cells were separated from the fluid by low-speed centrifugation (10 min, 800 g). After three washings, the cells were resuspended in 10 ml of Hank's balanced salt solution (HBSS) and evaluated for their total number using a hemocytometer. A differential cell count (made from a total of 300 cells) was accomplished by morphologic criteria in cytocentrifuged smears stained with Wright-Giemsa. The viability of BAL cells as assessed by trypan blue dye exclusion was consistently greater than 90 percent in each experiment.

Identification of Lung and Blood Lymphocyte Subpopulations

The BAL cell pellet was resuspended and the cells were washed twice in HBSS and finally resuspended at 5.10^6/ml in RPMI 1640 (Eurobio). BAL T lymphocytes or peripheral T cells were identified by indirect immunofluorescence. Briefly, 200 μl of cell suspension was first incubated with 5 μg/L of unlabeled antibody (anti-CD3 and anti-CD4 from Ortho Pharmaceutical, Raritan NJ; anti-CD6 and anti-CD11b from Becton Dickinson, Meylan, France) for 30 min at 4°C. After three washings with HBSS, cells were incubated with fluorescein isothiocyanate (FITC) labeled (Fab/2) fragment of antimouse IgG antibody (Dynatech Paris, France) for 30 min at 4°C and were washed twice with HBSS. The cells were resuspended in RPMI 1640 and examined using a fluorescence microscope equipped with phase contrast optics (Olympus BHS, Tokyo, Japan). The percentage of fluorescein-labeled lymphocytes was calculated after counting a minimum of 200 lymphocytes per slide.

Cell subpopulations were additionally characterized using the same method by their reactivity with anti-CD4 (Ortho Pharmaceutical, Raritan, NJ) and anti-CD8 (Becton Dickinson, Meylan, France). CD11b has been proposed to distinguish among CD8+ lymphocytes with suppressor activities (CD8+ CD11b+) from those with cytotoxic activities (CD8+ CD11b−). To simultaneously determine the coexpression of CD8 and CD11b markers, a double staining technique was performed by using monoclonal antibodies conjugated to either FITC (CD8) or to the BD1 phycoerythrin derivative (CD11b) obtained from Becton Dickinson. BAL cells were incubated with 5 μl of labeled antibodies for 30 min at 4°C washed three times with phosphate-buffered saline solution (PBS) at 4°C. The number of positive cells was determined by using fluorescence microscopy.

The surface phenotype of peripheral blood lymphocytes was determined using the procedure described above on blood mononuclear cells isolated by Ficoll/Hyphaque sedimentation. All results were expressed as the percentage of total lymphocytes.

Protein Assay

An immunoradiometric assay (IRMA) was used for measurement of the proteins in BAL. This assay previously described in detail, provides a sensitivity in the range of ng/ml and was performed on nonconcentrated BAL fluid. All BAL samples were diluted in 20 percent goat serum in PBS, at a pH of 7.4. Serum levels of the different proteins were determined by immunonephelometry. Results are expressed in absolute concentrations in BAL (milligrams per liter) and in terms of the relative coefficient of excretion (RCE), which expresses the secretion rate of each protein relative to that of the entirely plasma-derived albumin as follows: 

RCE = [(protein BAL/protein serum)/(albumin BAL/albumin serum)]

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Table 2—Distribution of Bronchoalveolar Cells*

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls (n = 12)</th>
<th>Patients with Alcoholic Liver Cirrhosis (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovered fluid, ml</td>
<td>138 ± 12</td>
<td>124 ± 6</td>
</tr>
<tr>
<td>Total cell number, 10⁶/ml</td>
<td>18.4 ± 2.3</td>
<td>21 ± 4.2</td>
</tr>
<tr>
<td>Alveolar macrophages, %</td>
<td>91.1 ± 2.2</td>
<td>84.3 ± 1.8†</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>9.9 ± 1.5</td>
<td>16.1 ± 2.1†</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>0.5 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>0.06 ± 0.06</td>
<td>0</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SEM.
†Significantly different from healthy controls (p<0.05).

Statistical Analysis

When applicable, data were expressed as mean ± standard error of the mean (SEM). Because most of the data were nonparametric, results were compared using the Mann-Whitney U test, Wilcoxon signed rank test, and Spearman rank coefficient. Only p values less than 0.05 were considered significant.

RESULTS

Bronchoscopic Findings

Fiberoptic bronchoscopy showed normal airways in all subjects without evidence of bronchial infection or inflammatory disease. There was no significant difference in percentage return of lavage fluid between patients and controls.

Number and Types of Bronchoalveolar Cells

Results of BAL total and differential cell count are summarized in Table 2. Total recovery cell yield did not significantly differ among patients and controls. Evaluation of the BAL cell differential of the patients with ALC demonstrated a slight but significant increased proportion of lymphocytes. Alveolar macrophage proportions were thus altered accordingly, and were lower than normal in the ALC group. The mean proportion of neutrophils and of eosinophils was not different between patients with ALC and controls.

Characterization of Lung and Blood Lymphocyte Subpopulations

Identification of lung lymphocyte subpopulations showed that BAL lymphocytosis was predominantly composed of T lymphocytes (Table 3). Analysis of T-cell subsets pointed out that most of the T cells in patients with ALC expressed the CD8 suppressor/cytotoxic phenotype, with a marked reversal of the CD4/CD8 ratio (0.96 ± 0.15 vs 1.8 ± 0.12 in healthy nonsmokers, p<0.05). The percentage of BAL CD8+ cells in ALC did not differ according to the Child's score (B or C) or to biologic abnormalities (eg, as judged by albumin, IgA, IgM, IgG, bilirubin, SGOT, and alkaline phosphatase). In addition there was no correlation between percentage of BAL CD8+ cells and the number of inflammatory cells (macrophages or neutrophils) in BAL. Among BAL T cells from patients with ALC, the percentage of cells coexpressing CD8 and CD11b determinants was markedly decreased, whereas the percentage of CD8+ CD11b+ cells was dramatically increased (p<0.001, Table 3).

The percentage of T cells in peripheral blood of patients did not significantly differ from the proportion of T cells in normal subjects. Similarly, no differences were found in the proportions of peripheral blood CD4+ or CD8+ T cells compared with normal controls (Table 3). Thus, the percentage of CD8+ T cells was increased in BAL fluid of patients with ALC compared with the blood values. In addition, the percentage of blood CD8+ CD11b+ and CD8+ CD11b+ cells was similar between patients with ALC and controls.

Concentrations of Proteins in BAL Fluid

Absolute protein concentrations in BAL in ALC are shown in Table 4. ALC was associated with an appreciable alveolar-capillary "leak," as evidenced by a significant increase in BAL fluid albumin. In addition, all immunoglobulin concentrations in BAL were increased. Interestingly, α-M concentrations, the molecular weight of which is near that of IgM, were not different between patients and controls. The secretion rates of the proteins relative to albumin (RCE) were not significantly different from those observed in healthy controls (Fig 1).

DISCUSSION

The purpose of this study was to gain some insight...
into the immune system of the lower respiratory tract in ALC through evaluation of BAL T lymphocytes with monoclonal antibodies and of BAL fluid immunoglobulin. To eliminate confounding factors that could have independent effects, we studied nonsmoking patients. Our results demonstrate that patients with ALC have a selective increase in the number of CD8+ T lymphocytes in the lower respiratory tract which is not associated with similar abnormalities in the blood.

The mechanisms responsible for the shift in the CD4/CD8 balance in the lower respiratory tract in ALC is unclear. One cannot exclude that a subclinical infection with viruses or Pneumocystis carinii might have resulted in the abnormalities observed herein. However, our patients with ALC were asymptomatic, had clear chest roentgenograms, and cytologic examination of BAL cells and bacteriologic analysis of BAL fluid did not demonstrate evidence of pulmonary infection. Our group and others previously demonstrated that nonalcoholic patients with primary biliary cirrhosis exhibited an increased percentage of CD4+ T cells in BAL. These findings suggest that expansion of CD8+ T cells in ALC was not caused by either the underlying cirrhotic process or its biologic consequences since the patients were similar with regard to Child's classification and biologic associated serum abnormalities. In addition, it is unlikely that the abnormalities found in patients with ALC are due to the effect of malnutrition since our patients did not exhibit signs of severe malnutrition. Moreover, in a previous study of patients with Crohn's disease and who demonstrated various degrees of malnutrition, BAL studies showed an expansion of CD4+ T lymphocytes.

Our results are in agreement with those obtained by immunohistologic study of liver biopsy specimens. Si and coworkers demonstrated that the T lymphocytes accumulating in hepatic tissues of patients with alcoholic liver disease consisted mostly of CD8+ suppressor/cytotoxic cells. However, one cannot exclude that this local relative hepatic increment in suppressor/cytotoxic T cells might be something unique for hepatic inflammatory processes in general which may activate CD8+ cells and attract them to sites within liver lesions. Indeed, CD8+ T cell increment has been reported also in chronic active hepatitis and primary biliary cirrhosis.

CD8 is a surface protein bound on cytotoxic and suppressor T cells. A local excess of suppressor/cytotoxic cells might result either from nonspecific activation of the immune system by mononuclear cells or from activation by cytokins produced within the lung or may reflect an increase in natural killer (NK) cells that may bear more than one phenotype. Using

![Figure 1](http://journal.publications.chestnet.org/pdaccess.ashx?url=/data/journals/chest/21639/)

**Figure 1.** Relative (to albumin) coefficient of excretion (RCE) of immunoglobulins in the bronchoalveolar lavage (BAL) of healthy nonsmokers and of nonsmoking patients with alcoholic liver cirrhosis (ALC). Results are expressed as mean ± SEM.
Thus, the was although barrier, plasma of ALC and complete with we of similar tion ulin. Evaluation of BAL fluid proteins demonstrated an appreciable alveolar-capillary “leak” as evidenced by a significant increase in lavage fluid albumin. Because of the efficiency of the epithelium barrier in the lower respiratory tract of healthy subjects, concentrations of plasma proteins of medium molecular weight are much lower in the epithelial lining fluid than in the plasma. In addition, because of the molecular-size affected seepage of plasma proteins across the epithelium barrier, IgM (900,000 daltons) concentrations in BAL fluid are usually very low. The present study demonstrated that ALC was associated with an increased movement of molecules such as albumin and immunoglobulins from the blood to the epithelial surface of the lower respiratory tract. Increased albumin and immunoglobulin concentrations in BAL are usually due to a pronounced leakage of the alveolar capillary barrier altered by inflammatory processes. However, although the RCE of IgG, IgA, and IgM was normal in ALC, one cannot exclude that a local immunoglobulin secretion in the lower respiratory tract might account, at least in part, for the increased concentration of BAL immunoglobulins. In this context, it is of importance that the BAL α2M concentration in ALC was not significantly different from that of controls. The fact that the molecular weight of IgM and α2M is similar suggests that IgM might be locally secreted. Thus, an increased production of immunoglobulins in the lung of patients with ALC might contribute to the increased concentrations of immunoglobulins both in BAL and in serum, as was reported in interstitial lung disease.

It is of particular interest to point out that similar observations have been found in the intestinal mucosa of alcoholics. Bjarnason and coworkers clearly demonstrated that alcohol abuse was associated with significant changes in intestinal permeability leading to the concept of the leaky gut of alcoholism. Moreover, although densities of immunoglobulin-producing cells in the jejunal lamina propria of alcoholic patients were normal, these patients exhibited an increased permeability to plasma proteins such as albumin and immunoglobulins.

In conclusion, our results demonstrated that ALC is associated with profound imbalance of the immune respiratory T cells. Although mechanisms responsible for local immune disturbances are unknown, expansion of CD8+ 11b− cytotoxic cells might be of crucial importance in the defense mechanisms of the lower respiratory tract in patients with ALC.

ACKNOWLEDGMENT: Nadine Duhautois and Véronique Lequeux provided technical assistance.

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