Interleukin-8 and leukotriene B4 in bronchoalveolar lavage fluid from HIV-infected patients with bacterial pneumonia

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Human immunodeficiency virus (HIV)-infected patients are at increased risk of contracting bacterial infections, mainly pneumonia. Despite this, little is known about immunopathogenic mechanisms in HIV-related bacterial pneumonia. This paper investigates the presence of the neutrophil chemotactic mediators, interleukin-8 (IL-8) and leukotriene B4 (LTB4), in bronchoalveolar lavage (BAL) fluid from 27 HIV-infected patients with bacterial pneumonia. Significantly elevated levels of IL-8 were found in BAL fluid of patients with bacterial pneumonia [529 pg ml⁻¹ (296–1161 pg ml⁻¹)] compared to matched patients with Pneumocystis carinii pneumonia (PCP) [59 pg ml⁻¹ (42–254 pg ml⁻¹)] and healthy controls [58 pg ml⁻¹ (37–82 pg ml⁻¹)]. Levels of LTB4 were not elevated during bacterial pneumonia when compared to PCP patients and healthy controls. Furthermore, a positive correlation was found between IL-8 levels in BAL fluid and relative BAL neutrophilia (r=0.60, P=0.001) in bacterial pneumonia. In conclusion, elevated IL-8 levels in BAL fluid were found in patients suffering from bacterial pneumonia, which may account for the influx of neutrophils to the lung, whereas LTB4 appears not to be an important chemotactic factor in this setting.

Introduction

Although infection with Pneumocystis carinii remains the most frequent cause of severe pulmonary disease in patients infected with human immunodeficiency virus (HIV), it has also been recognized that these patients are at increased risk for contracting bacterial pneumonia (1-4). Despite this, immunopathogenic mechanisms have received little attention.

Increased levels of interleukin-8 (IL-8) in bronchoalveolar lavage (BAL) fluid have been reported in a number of pulmonary diseases characterized by neutrophil inflammation. These include bacterial pneumonia, adult respiratory distress syndrome (ARDS) (5,6), cystic fibrosis (7) and chronic inflammatory airway disease (8). Interestingly, a positive correlation between BAL levels of IL-8 and the total number of neutrophils in BAL fluid has been demonstrated in ARDS (9).

HIV-infected patients are capable of initiating an IL-8 response to infection despite their general immune dysregulation. In AIDS-associated P. carinii pneumonia (PCP), the present authors have recently established a correlation between IL-8 levels, BAL neutrophilia and arterial oxygen tension (PaO₂) (10), and have demonstrated that elevated IL-8 levels confer a poor prognosis (11). Leukotriene B4 (LTB4) is a potent mediator of neutrophil chemotaxis in vitro (12), and may play a role in the lung injury of bacterial pneumonia in non-HIV patients (13).

The authors' hypothesis is that IL-8 and LTB4 play a role in recruiting neutrophils in pulmonary disease. However, the beneficial effects of this response may also initiate and drive an inflammatory response, which may have detrimental effects.
To the authors' knowledge, no reports have been published addressing levels of cytokines and neutrophilia in BAL fluid from HIV-positive patients with bacterial pneumonia. The aim of this study was to measure IL-8 and LTB₄ in BAL fluid, and study a possible correlation between these mediators and neutrophils in BAL fluid.

**Materials and Methods**

**PATIENTS**

A total of 448 fibre-optic bronchoscopic procedures were performed in 287 HIV-positive patients with pulmonary symptoms in the period from January 1989 to December 1993. Fifty-three of these patients had bacterial pneumonia, 27 of whom had one sole pathogen identified both by microscopy and culture of BAL fluid. Clinical and laboratory data were collected prospectively. None of the patients had received treatment with corticosteroids.

In order to compare possible differences in degree of pulmonary inflammation between HIV-infected patients suffering from bacterial and *P. carinii* pneumonia, 10 HIV-positive patients with *P. carinii* pneumonia were selected and matched for age, CD4 count and PaO₂ level. Ten medical students had bronchoscopy performed to act as healthy controls. Informed consent was obtained from both patients and volunteers. The study was conducted in accordance with the guidelines of the local Danish Ethics Committee.

**BRONCHOSCOPY**

Bronchoscopy was performed using local anaesthesia, as described previously (10). Briefly, 60 ml aliquots of saline were instilled either in the right middle lobe (diffuse infiltrates) or at the site of localized infiltration. This was repeated up to a maximum of 300 ml. On average, 60-80% of the instilled volume was recovered. Bronchoalveolar lavage fluid was divided into samples for microbiology, pathology, cell differentials and cytokines (IL-8, LTB₄).

**IL-8 AND LTB₄ ANALYSIS**

After centrifugation of BAL fluid, the supernatant was stored immediately at -20°C. Analysis for IL-8 was done using a commercially available ELISA kit (Research & Diagnostic System, Minneapolis, U.S.A.). The manufacturer's protocol was followed. Minimal detectable amount of IL-8 was 18·6 pg ml⁻¹. Undetectable levels were regarded as 0 pg ml⁻¹.

Analysis of levels of LTB₄ in BAL fluid was performed as described previously (10). Briefly, precipitation was done in 70% ethanol (16 h, 5°C), and after centrifugation, lipids were extracted over a C-18 Sep-pak column (Waters Associates, Milford, MA, U.S.A.). Extract was applied to a dual-pump, high-pressure, liquid chromatograph (HPLC, Beckman Instruments, Fullerton, CA, U.S.A.) with a Ultrasphere C₁₈ reverse-phase column (Beckman), and the eicosanoids were separated. After collection of relevant fractions, lipids were extracted over a C-18 Sep-pak column, and radioimmunoassay for LTB₄ was performed as recommended by the manufacturer (Advanced Magnetics, Cambridge, MA, U.S.A.).

**STATISTICS**

Results are expressed as median values and 95% confidence intervals (95% CI). Differences between groups were evaluated using the Mann-Whitney test. The Spearman rho test was used to test for correlations between baseline values and mediators in the BAL fluid. *P<0·05* was regarded as statistically significant.

**Results**

The most frequently isolated organisms were *Streptococcus pneumoniae* (12), *Haemophilus influenzae* (6) and *Staphylococcus aureus* (5). Less frequent were *Branhamella catarrhalis* (2), *Klebsiella pneumoniae* (1) and *Actinetobacter* (1). As shown in Table 1, no significant difference was found in PaO₂, CD4 counts or IL-8 between these groups. Eleven (40%) of the patients had received prophylactic treatment with either trimethoprim/sulphamethoxazole (three patients) or inhaled pentamidine (eight patients), and 10 patients received zidovudine when the pneumonia developed.

Substantially elevated levels of IL-8 in BAL fluid were found in patients with bacterial pneumonia compared to healthy controls.
Table 1. Correlates of respiratory distress, death and inflammatory markers in human immunodeficiency virus (HIV)-positive patients with bacterial pneumonia or Pneumocystis carinii pneumonia (PCP) and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>CD4 cell count (10^6 l^-1)</th>
<th>IL-8 (pg ml^-1)</th>
<th>Neutrophil (%)</th>
<th>PO2 (kPa)</th>
</tr>
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<tbody>
<tr>
<td>Streptococcus pneumonia</td>
<td>12 0.045 (0.000-0.351)</td>
<td>443.5 (18.6-1741)</td>
<td>13 (1-95)</td>
<td>9.6 (7.3-13.2)</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>6 0.001 (0.000-0.096)</td>
<td>121.5 (296-2423)</td>
<td>66 (2-94)</td>
<td>10.2 (7.8-11.4)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5 0.060 (0.016-0.162)</td>
<td>972.0 (443-2003)</td>
<td>26 (6-58)</td>
<td>11.0 (7.3-12.7)</td>
</tr>
<tr>
<td>Other bacteria</td>
<td>4 0.013 (0.000-0.432)</td>
<td>269.5 (118-1984)</td>
<td>5.5 (1-63)</td>
<td>9.3 (6.7-10.9)</td>
</tr>
<tr>
<td>Bacteria combined</td>
<td>27 0.040 (0.000-0.432)</td>
<td>529 (296-1161)</td>
<td>26 (1-95)</td>
<td>10.4 (6.7-13.2)</td>
</tr>
<tr>
<td>Pneumocystis carinii</td>
<td>10 0.013 (0.000-0.060)</td>
<td>57 (42-254)</td>
<td>—</td>
<td>10.1 (9.7-11.2)</td>
</tr>
<tr>
<td>Control</td>
<td>10 —</td>
<td>58.5 (29-86)</td>
<td>13 (1-95)</td>
<td>14.4 (13.1-16.5)</td>
</tr>
</tbody>
</table>

Values are medians (95% CI).

As mentioned above and shown in Table 1, there was no significant difference in IL-8 in BAL fluid among different groups of patients with bacterial pneumonia. A positive correlation between the relative amount of neutrophils and IL-8 in BAL fluid existed (r=0.60, P=0.001), as shown in Fig. 1. In contrast, levels of LTB4 were the same in patients with bacterial pneumonia [21.7 pg ml^-1 (14.0-39.9 pg ml^-1)] and healthy controls [22.6 pg ml^-1 (18.7-32.0 pg ml^-1), P=0.64].

This study found no statistically significant correlation between amount of neutrophils in BAL fluid and PaO2 (r=0.35, n=25, P=0.09).

Ten patients with P. carinii were matched with pneumonia patients on age, CD4 count and PaO2 in order to compare BAL IL-8 levels in these patients and patients with bacterial pneumonia. Patients with bacterial pneumonia had significantly higher IL-8 levels compared to patients with PCP (579 pg ml^-1 (796-1161 pg ml^-1) vs. 57 pg ml^-1 (42-254 pg ml^-1), P<0.05).

![Fig. 1. Correlation between neutrophils and interleukin-8 (IL-8) in bronchoalveolar lavage (BAL) fluid in human immunodeficiency virus (HIV)-positive patients with bacterial pneumonia. r=0.596.](image-url)
Discussion

This study has identified IL-8 as a potential chemoattractant for neutrophils in HIV-infected patients with bacterial pneumonia. Interleukin-8 levels were substantially higher than those found in HIV-infected patients suffering from PCP, and in healthy controls. Furthermore, the levels of IL-8 correlated with the percentage of neutrophils in the BAL fluid. These findings agree with findings published previously in smaller series of patients (14).

In this study, IL-8 levels in BAL fluid reached levels comparable with those found in immunocompetent patients suffering from bacterial pneumonia (5), regardless of the fact that the CD4 cell count was low and thus the specific immunological apparatus of the study patients was substantially compromised. This suggests that HIV-infected patients have an intact ability to recruit neutrophils independent of impairments in the immune function.

Interleukin-8 levels in the HIV-infected patients with bacterial pneumonia were higher than IL-8 levels in matched patients with PCP; patients were matched for demographics and clinical severity (including PaO2). In PCP patients with more advanced respiratory impairment, IL-8 levels in BAL fluid are substantially higher (10,11).

It was found that in HIV-infected patients with bacterial pneumonia, the degree of inflammation failed to correlate with clinical severity, although the parameters tended to do so (r=0.35, P=0.09). This is in contrast to previous studies of patients with bacterial pneumonia (5). Furthermore, IL-8 levels in BAL fluid may correlate with markers of clinical severity and may predict the prognosis in a variety of patient groups, e.g. HIV-infected patients suffering from PCP (10,11), and immunocompetent patients suffering from ARDS (1) and cystic fibrosis (7). In these situations, IL-8 is believed to cause impairment of the pulmonary function by causing influx of neutrophils to the alveolar milieu. One possible explanation for these discrepancies may be that the advanced HIV infection from which most of the study patients were suffering allowed for a rapid multiplication of the bacteria, and thus the quantum of bacteria in itself (and not the host reaction) was the primary factor in causing decreased arterial oxygen tension. Further studies should be carried out to determine if the bacterial load in HIV-infected patients suffering from bacterial pneumonia is higher than what is seen in immunocompetent patients.

The levels of LTB4 in BAL fluid from patients suffering from bacterial pneumonia were not different from LTB4 levels in PCP and in healthy controls. This agrees with previous findings in other patient categories (13). Thus, LTB4 appears not to be an important chemotactic factor for the influx of neutrophils in the lung. Experimental studies have found that IL-8 is the primary chemotactic factor for neutrophils in the lungs (6,8,9,14,15).

In conclusion, elevated levels of IL-8 were found in HIV-infected patients suffering from bacterial pneumonia, which presumably causes influx of neutrophils. The pathogenic mechanism allowing for the development of bacterial pneumonia and which causes clinical symptoms should be further studied.

References

7. Dean TP, Dail Y, Shute JK, Church MK, Warner JO. Interleukin-8 concentrations are elevated in bronchoalveolar lavage, sputum, and sera of


