Alveolar Damage in AIDS-Related Pneumocystis carinii Pneumonia*

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Objective: Pneumocystis carinii pneumonia is the most common and serious of the pulmonary complications of AIDS. Despite this, many basic aspects in the pathogenesis of HIV-associated P carinii pneumonia are unknown. We therefore undertook a light and electron microscopic study of transbronchial biopsy specimens to compare pathologic features of P carinii pneumonia and other HIV-related lung diseases.

Design and patients: Thirty-seven consecutive HIV-infected patients undergoing a diagnostic bronchoscopy.

Results: P carinii pneumonia was characterized by an increase in inflammation, edema, exudate, fibrosis, type II pneumocyte proliferation, and cellular infiltration of the alveolar wall when compared with other lung diseases (all p<0.05). Electron microscopy showed apposition of the trophozoite to the type I pneumocyte. Erosion of type I pneumocytes was observed in 13 of 15 patients with P carinii pneumonia, whereas none without P carinii pneumonia had this finding (p<0.05). Erosion of the type II pneumocyte was not observed.

Conclusion: Inflammation, interstitial fibrosis, and alveolar epithelial erosion are characteristic features of P carinii pneumonia. The changes may form the pathologic basis for the respiratory failure seen in patients with P carinii pneumonia. Electron microscopy did not show any diagnostic advantage over conventional light microscopy using routine stains.

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Key words: AIDS; electron microscopy; histopathology; Pneumocystis carinii pneumonia

Abbreviations: CMV=cytomegalovirus; TBB=transbronchial biopsy

Pneumocystis carinii pneumonia is the most common and serious of the opportunistic infections among patients infected with HIV.1-3 Despite this, many basic aspects of P carinii and the interactions between host and organisms in HIV-infected patients remain unknown.

Previous studies suggest that the P carinii organism requires attachment or close apposition to the alveolar epithelium to carry out its life cycle, but that these events do not involve fusion between the cell wall and alveolar epithelium or tissue invasion.4-7 The close attachment is assumed to cause degeneration and, subsequently, erosion of the type I pneumocyte, thereby disrupting epithelial integrity and exposing the underlying basement membrane to the alveolar space.5,6,8

Clinically, inflammation in the P carinii-infected lung plays a significantly pathophysiologic role.9,10 This is underscored by the finding that adjunctive corticosteroid therapy improves survival among AIDS patients with P carinii pneumonia,11-14 and that levels of several inflammatory mediators are elevated and associated with outcome.15-18 Furthermore, several atypical pathologic features, including formation of cysts, granulomas, and vasculitis, are recognized with increasing frequency. These findings may be caused by P carinii pneumonia due to an ongoing inflammatory process in the infected lung.10

We therefore undertook this study to investigate the host-organism relationship as it appears in human HIV-related P carinii pneumonia. Consecutive HIV-infected patients undergoing fiberoptic bronchoscopy were included in the study. Pathologic features in transbronchial biopsy (TBB) specimens compared by light and electron microscopy are reported.
MATERIALS AND METHODS

Subjects

A total of 112 fiberoptic bronchoscopic procedures were performed on 95 HIV-infected patients with pulmonary symptoms from January 1990 to January 1991. Bronchoscopic is the primary choice of workup if tracheal suction findings are noninformative. There were 50 (45%) episodes of P carinii pneumonia, 19 (17%) episodes of bacterial pneumonia, four (4%) episodes of cytomegalovirus (CMV) pneumonitis, three (3%) episodes of pulmonary Kaposi’s sarcoma, and one case of pulmonary tuberculosis. Thirty-five (31%) procedures did not provide a definitive diagnosis. BAL was always performed and in 90 (80%) cases, TBB specimens were taken. Biopsy specimens were processed for electron microscopy only if sufficient tissue was available after providing material for routine pathologic evaluation. Only patients with material processed for electron microscopy were included in the study. Clinical data were collected prospectively. Laboratory values, including CD4 T-cell count and arterial blood gases, were gathered <24 h before bronchoscopy. For comparison, patients with P carinii pneumonia were regarded as one group, and all other patients were regarded as another group.

Analysis of BAL Fluid and Biopsy Specimens

Bronchoscopic was done under local anesthesia as previously described. BAL and TBB were performed as previously described. BAL fluid was centrifuged at 800 g for 10 min and smears were prepared from the sediment. The smears were stained with May–Griinwald-Giemsa, Papanicolaou, Grocott methenamine-silver, and a monoclonal immunoperoxidase stain against P carinii antigens (DAKO-Pneumocystis, M 775; DAKO Denmark; Glostrup) in order to identify P carinii. Bacteriologic examination of BAL included Gram and Ziehl-Neelsen staining and cultures for bacteria, mycobacteria, virus, and fungi. Differential cell counts were determined by counting 200 cells in a representative area of the May–Griinwald-Giernsa slide containing a monolayer of cells.

Biopsy specimens were immediately fixed in 4% buffered formalin. For light microscopy, biopsy specimens were embedded in paraffin. Microscopic sections were prepared and stained with hematolin-eosin, van Gieson-Hansen, periodic acid-Schiff, Grocott methenamine-silver, an immunoperoxidase stain against P carinii antigens, and a monoclonal immunoperoxidase stain against CMV (DAKO-Cytomegalovirus, M0757; DAKO, Denmark). For electron microscopy, biopsy specimens were transferred to 2.5% glutaraldehyde/0.1 mol/L cacodylate. After 2 h, the specimens were postfixed in 1% osmium tetroxide for 2 h, dehydrated, and embedded in epoxy resin (Epon). Ultrathin sections were cut on an ultratome (LKB; Bromma, Sweden) and stained with uranyl acetate-lead citrate. The sections were examined in an electron microscope (jeol JEM 100 or a JEM 1010; Garden City, UK).

Histopathology

Biopsy specimens were considered satisfactory for evaluation if they contained more than 10 well-preserved alveolar lumina. Specimens were evaluated prospectively without knowledge of the patient’s clinical status using a semiquantitative method as previously described. Briefly, biopsy specimens were evaluated for the following features and graded on a scale from 0 to 3: amount of P carinii, inflammation, edema, type II pneumocyte proliferation, fibrosis, interstitial neutrophils, interstitial lymphocytes, and interstitial plasma cells. Further, alveolar exudate, fibrin deposits, hyaline membrane formation, and cell types were graded similarly in the alveolar lumen.

Electron microscopic examination focused on possible detection of P carinii, detection of alveolar epithelial damage, presence of inflammatory cells, and fibrosis of the alveolar wall.

Statistics

All values are expressed as median and range. Groups were compared using the Mann-Whitney test. Differences in histopathology scores were tested by the Fisher Exact Test. p<0.05 was considered to be statistically significant.

RESULTS

Subjects

Thirty-seven patients were included in the study. Of these, 15 (41%) had P carinii detected by conventional staining methods. Of the remaining 22 patients, 5 (14%) had bacterial pneumonia, 3 (8%) had CMV pneumonitis, and one (3%) had pulmonary Kaposi’s sarcoma. In 13 (35%) cases, a definitive diagnosis was not established. Statistical analysis for differences in clinical parameters for each group did not reveal a statistically significant difference between the patients entering the electron microscopy study and patients who were not included. None of the P carinii pneumonia patients had clinical or microbiological evidence of coexisting pulmonary disease at the time of diagnosis. Short-term mortality was low, as only one patient (suffering from P carinii pneumonia) died within the first month. Baseline characteristics are shown in Table 1.

Light Microscopy

P carinii was detected in 14 of 15 BAL fluid smears and in 13 of 15 biopsy specimens from patients known to have P carinii pneumonia. All specimens were positive for P carinii in at least one of the two specimens.

The amount of P carinii in the biopsy specimen varied from a few scattered organisms in a single alveolar lumen to numerous organisms filling almost

<table>
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<tr>
<th>Table 1—Patient Characteristics*</th>
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<tr>
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<tr>
<td><strong>P carinii Pneumonia</strong></td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>CD4 count, mm−3</td>
</tr>
<tr>
<td>PaO2, mm Hg</td>
</tr>
<tr>
<td>PaCO2, mm Hg</td>
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<tr>
<td>Gender, female/male</td>
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*All values, except gender, are median and range.

1 Denotes p value <0.05 (Mann-Whitney).
all lumina. On an arbitrary semiquantitative scale from 0 (none) to 3 (numerous), the median score was 1.5 (range, 0 to 3).

Differential cell counts in BAL fluid did not differ statistically between the two groups. For P. carinii pneumonia, the differential cell count was as follows: macrophages, 45% (range, 13 to 92); lymphocytes, 31% (2 to 86); and neutrophils, 10% (1 to 71). These counts compared with 75% (3 to 98), 13% (1 to 62), and 5% (0 to 95) for the remaining patients.

An assessment of the inflammatory reaction in the biopsy specimen showed that patients with P. carinii pneumonia had significantly higher scores of inflammation, edema, type II pneumocyte proliferation, alveolar exudate, and fibrosis than patients without P. carinii pneumonia (all p<0.05) (Fig 1 and Table 2). Further, patients with P. carinii pneumonia had significantly higher scores for interstitial neutrophils and lymphocytes (p<0.05), whereas there were no differences in cell types in the alveolar lumen (Table 2). There was no difference in fibrin deposit or formation of hyaline membranes between the two groups.

**Electron Microscopy**

P. carinii was present in 12 of 15 biopsy specimens. Of the three specimens negative for P. carinii by electron microscopy, two were diagnosed by BAL fluid smear only, whereas the last one was positive in both BAL fluid and TBB by light microscopy.

Both cysts and trophozoites were present in the alveolar lumen. Trophozoites varied in size and shape. In general, they were poor in organelles. However, a nucleus was almost always identified, as well as mitochondria and endoplasmic reticulum (Fig 2A). Cysts were seen in both a complete and a collapsed, empty form. The complete form often contained intracytic bodies and organelles similar to the findings in trophozoites. Collapsed cysts appeared devoid of these features (Fig 2B). In the cytoplasm, dense bodies resembling secretory granules were observed (Fig 2C). The trophozoite and the cyst were enclosed by a cell wall to which the plasma membrane was closely apposed. The cell wall was extensively folded, giving rise to small projections or buds, which in cross-section appeared circular. Inside the buds, the plasma membrane could be seen (Fig 2A). These buds were often a considerable distance from the cell wall, and the ratio between the diameter of the circular form and the length of the rare longitudinal form was approximately 1:2 to 3 (Fig 2F). Trophozoites were much more abundant in the alveolar space than cysts (Fig 2E).

### Table 2—Histopathologic Scores in TBB Specimens Assessed by Light Microscopy*

<table>
<thead>
<tr>
<th></th>
<th>P. carinii Pneumonia</th>
<th>Other</th>
<th>p Value</th>
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<tbody>
<tr>
<td>Intervillous</td>
<td>1 (0-3)</td>
<td>0 (0-2)</td>
<td>0.02</td>
</tr>
<tr>
<td>Inflammation</td>
<td>1 (0-3)</td>
<td>0 (0-1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Edema</td>
<td>1 (0-3)</td>
<td>0 (0-1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Type II pneumocyte proliferation</td>
<td>1 (0-3)</td>
<td>0 (0-3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>1 (0-3)</td>
<td>0 (0-2)</td>
<td>0.02</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>1 (0-3)</td>
<td>0 (0-2)</td>
<td>0.002</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1 (0-2)</td>
<td>0 (0-2)</td>
<td>0.02</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>1 (0-2)</td>
<td>0 (0-1)</td>
<td>NS</td>
</tr>
<tr>
<td>Alveolar lumen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar exudate</td>
<td>1 (0-3)</td>
<td>0 (0-0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Fibrin</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>NS</td>
</tr>
<tr>
<td>Hyaline membranes</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>NS</td>
</tr>
<tr>
<td>Macrophages</td>
<td>1 (0-3)</td>
<td>1 (0-2)</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>NS</td>
</tr>
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*All values are expressed as median (range). NS = not significant, Fisher’s Exact Test.

**Figure 1.** IA: classic foamy P. carinii exudate (EX) in the alveolar lumen of a TBB specimen. The alveolar wall shows slight inflammation with cellular infiltration (thin arrows) and fibrosis (F). The alveolar lining is characterized by type II pneumocyte proliferation (broad arrows) (hematoxylin-eosin, original magnification ×250). IB: TBB specimen from a patient without P. carinii. The alveolar wall and lining appear normal (thin arrows). The alveolar lumen contains scattered macrophages (broad arrows) (hematoxylin-eosin, original ×250).
Figure 2. 2A: electron micrograph of two trophozoites near a type II pneumocyte (II), but not apposing it. Note budding of the cell wall (open arrows), as well as a nucleus (N) and rough endoplasmatic reticulum (solid arrow). Within the cell wall, the slightly denser plasma membrane may be discerned. 2B: *P. carinii* cysts (PC) containing intracystic bodies (arrows). Below the intact cysts are two empty and collapsed cysts (E). Remaining structures are human derived. 2C: trophozoites (TR) intimately apposing a type I pneumocyte (I) and one another. Below the type I pneumocyte is the basement membrane (BM). Note the nucleus (N) and dense bodies (arrows) in the trophozoite, presumably representing secretory granules. 2D: loss of type I pneumocyte integrity. On the left side, trophozoites (TR) remain apposed to the type I pneumocyte (thin arrows), whereas the basement membrane (hm) is left denuded to the right (thick arrows). Note numerous dense bodies in the trophozoite cytoplasm. 2E: alveolar lumen (A) packed with predominantly *P. carinii* trophozoites. 2F: budding (arrows) of *P. carinii* trophozoites (TR). Note the predominance of circular cross-sections and their distance from the cell wall (arrows). In each photo, bar indicates 0.5 μm.
Trophozoites were closely apposed to the type I pneumocyte, whereas this was never observed for cysts (Fig 2C). None of the specimens showed tissue invasion by P carinii organisms, nor was any fusion between host membranes and the parasite cell wall demonstrated. Apposition between type II pneumocytes and P carinii organisms was not observed.

Erosion of Alveolar Lining

In 13 of the 15 patients with P carinii pneumonia, we were able to detect alveolar epithelial erosions, ie, loss of type I pneumocytes, whereas none of the remaining patients (without P carinii) in the study showed any evidence of alveolar epithelial erosion (p<0.05) (Fig 2D). Interestingly, two of the three biopsy specimens negative for P carinii by electron microscopy, but positive by light microscopy, showed erosion of the alveolar epithelial lining.

Inflammation, Fibrosis, and Phagocytosis

By electron microscopy, inflammation was considered to be present if any inflammatory cell was noted in either the interstitium or the alveolar lumen. Evaluated by this method, inflammation was independent of infectious etiology as most biopsy specimens in both groups showed signs of inflammation. Similarly, the presence of collagen in the interstitium was considered an indicator of fibrosis. As is the case with inflammation, fibrosis was a nonspecific event as approximately two thirds of all patients had evidence of fibrosis.

Alveolar macrophages were studied to detect whether P carinii organisms were phagocytized by this cell type. Although alveolar macrophages were often present in the alveolar lumen, lysosomes containing phagocytized P carinii material could not be positively identified.

Discussion

To our knowledge, this study is the first to report both light and electron microscopic findings of pulmonary disease in a large group of HIV-infected patients. We present information on pathologic findings in TBB specimens, in particular, that P carinii apposes the type I pneumocyte and that apposition may be followed by erosion of this cell type. Furthermore, the presence of inflammation and interstitial fibrosis is a characteristic feature of P carinii pneumonia.

The histopathologic state of P carinii pneumonia is characterized by an eosinophilic foamy exudate completely filling the alveolar lumen.20 Travis et al19 found both inflammation and fibrosis to be commonly associated with P carinii pneumonia, and Nash and Fligiel21 noted a prevalence of diffuse alveolar damage. Studies comparing the histopathologic state of HIV-related P carinii pneumonia with other HIV-related pulmonary complications have not been published (to our knowledge). In the present study, population, inflammation, fibrosis, edema, type II cell proliferation, and cellular infiltration, were more abundant in P carinii pneumonia than in other HIV-associated lung diseases. It is conceivable that interstitial edema and cellular infiltration of the alveolar wall may form the basis for the compromised oxygenation characteristically seen in this disease.22,23 An interesting and novel finding was that P carinii patients exhibited signs of increased fibrosis compared with patients not having P carinii pneumonia. A marker of collagen formation, the aminopropeptide of type III procollagen, has been found to predict a poor outcome from an episode of P carinii pneumonia.18 Furthermore, in patients dying of P carinii pneumonia, aminopropeptide of type III procollagen levels increased markedly in the days preceding death.15 At autopsy, we have noticed that some of these patients show excessive pulmonary fibrosis. The development of fibrosis, and a possible role of corticosteroids in preventing this, should be further studied.

It should be recognized that differences in the progression of the underlying HIV infection may account for the increased inflammation and fibrosis found in patients with P carinii pneumonia but rarely in patients without P carinii pneumonia. Based on the lower CD4 T-cells counts, the HIV infection may have progressed further in patients with P carinii pneumonia. However, as this pneumonia rarely occurs in patients with CD4 counts >200/µL, the lower cell count may reflect the susceptibility to P carinii infection in HIV-infected patients.24,25 Further, most patients without P carinii pneumonia had encountered an AIDS-defining illness at the time of bronchoscopy, which also is an indicator of severe immunosuppression. Therefore, the presence of inflammation, cellular infiltration, and fibrosis appears to be directly caused by P carinii pneumonia and not the underlying HIV infection.

P carinii is believed to exert part of its pathogenetic effects through induction of erosion of the type I pneumocyte.26 The exact mechanism of this erosion is unclear. It is possible that erosion is caused by physical contact, ie, friction, release of toxic substances from the P carinii trophozoite, or by release of proteases, toxic oxygen radicals, and other mediators generated by invading neutrophils and activated alveolar macrophages. Owing to the cross-sectional nature of the study, we were generally unable to determine if P carinii organisms were
attached to the type I cells prior to degeneration, as
the basement membrane mostly was left exposed to
the alveolar lumen. However, we assume this to have
been the case, since areas with exposed basement
membranes and areas with organisms attached to
type I cells were identified in the same specimen.
Integrity loss of the alveolar epithelium disturbs the
exchange of water, gases, and macromolecules be-
tween the alveolar lumen and the interstitium, thus
giving rise to alveolar edema, which may further
compromise oxygenation.

Surrounding the \textit{P. carinii} organisms are numerous
projections or buds. The nature and purpose of these
projections have been debated. Early reports sug-
gested that they are structures of motility, \textit{i.e.}, filo-
podia or pseudopodia,\textsuperscript{27} or an anchoring mecha-
nism.\textsuperscript{7} A recent study regarded the projections as
foldings of the cell wall.\textsuperscript{28} There appear to be
differences in the morphologic features of the pro-
jections in rats and humans, since human \textit{P. carinii}
projections have short and wide projections, whereas
rat \textit{P. carinii} projections are slender and long. We
found no evidence to support that they are filopodia
or pseudopodia, since (1) the plasma membrane
often can be identified within the buds, indicating
that it is an evagination of the organism, (2) longitudi-
nal sections are rarely found, and (3) supportive
fibers are not found within these structures. In our
opinion, the buds represent true budding of the cell
wall, which may provide a mechanism for rapid
turnover of surface antigens.

Of the three biopsy specimens negative for \textit{P.
carinii} by electron microscopy, two were also nega-
tive using conventional stains and monoclonal anti-
bodies, although the BAL fluid smears were positive
by the same methods. Lack of \textit{P. carinii} in a biopsy
specimen may be due to sampling error. Two of the
negative specimens by electron microscopy actually
had type I pneumocyte necrosis despite the lack of
\textit{P. carinii} organisms, suggesting that the biopsy speci-
men was taken from a site of recent active infection.
Although electron microscopy is time consuming and
not necessary for the diagnosis of \textit{P. carinii} pneu-
omia, it does provide valuable information for the
researcher, which otherwise would be impossible to
obtain.

In conclusion, our findings emphasize that HIV-
related \textit{P. carinii} pneumonia, as compared with other
HIV-related pulmonary diseases, is associated with
alveolar damage characterized by increased inflam-
mation, interstitial fibrosis, and cellular infiltration.
The alveolar damage likely results in the impairment
of lung function during \textit{P. carinii} pneumonia. The
ultrastructural basis appears to be the alveolar epi-
thelial erosion associated with the intimate apposition
between the \textit{P. carinii} trophozoite and the type I pneumocyte.

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