In this study of 28 immunocompromised patients, it was found that
Pneumocystis carinii pneumonia could be easily and reliably diagnosed
by examination of routinely prepared, Papanicolaou-stained cellular
samples obtained by bronchoalveolar lavage, bronchial brushing, and
bronchial washing. The distinctive intra-alveolar exudate of pneumo-
cystosis observed in lung biopsy specimens was readily discernible in
all of the cellular samples that demonstrated P. carinii by special
stains. The exudate was not present in any of the P. carinii-negative
samples. Routinely prepared, Papanicolaou-stained cellular sample
can be relied upon for the rapid diagnosis of P. carinii pneumonia.

Infection with Pneumocystis carinii accounts for a large proportion of the
rapidly progressive and often fatal pneumonias occurring in a variety of
immunocompromised patients [1,2]. Recently, additional attention has
been focused on pneumocystosis as a major complication of the ac-
quired immune deficiency syndrome (AIDS) [3]. The diagnosis of pneu-
cystosis rests on the microscopic demonstration of the protozoa in
closed or open lung biopsy specimens, or in cellular samples obtained
primarily by bronchoalveolar lavage, endobronchial brushing and wash-
ing, and transthoracic fine needle aspiration. In such specimens, the P.
carinii cysts are commonly detected by employing the methenamine
silver nitrate stain [4] or one of its modifications [5,6], the Gram-Wiegart
stain [7], or the toluidine blue O stain [8]. The Giemsa, Wright, and
Wright-Giemsa stains [9,10] demonstrate not only the cysts, but also the
sporozoite and trophozoite forms. Most recently, Ghali and associates
[11] used direct immunofluorescence to demonstrate the cyst forms.
With the exception of the reports of Pintozzi et al [12] and Greaves and
Strigle [13], the detection of P. carinii in Papanicolaou-stained cellular
samples has received little attention. In this report, we give an account of
the diagnostic usefulness of this preparation.

PATIENTS AND METHODS

All specimens submitted to the Cytopathology Laboratory over the last 16
months from immunosuppressed patients in whom pneumocystosis was
suspected were reviewed. Such suspicion was based on abnormal chest
roentgenograms, a history of dyspnea or fever or both, or positive findings
on gallium lung scanning. Forty-eight specimens (21 bronchoalveolar la-
vages, 12 bronchial washings, eight bronchial brushings, five sputum
samples, and two transtracheal aspirates) were received from 28 patients
(Table I). The clinical course and follow-up of each patient were recorded
by chart review and, in three patients, by review of necropsy protocols.
TABLE I  Underlying Disorders of 28 Immunocompromised Patients

<table>
<thead>
<tr>
<th>Diagnosis/Clinical Condition</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquired immune deficiency syndrome</td>
<td>14</td>
</tr>
<tr>
<td>Lymphoma/leukemia</td>
<td>6</td>
</tr>
<tr>
<td>Renal/cardiac transplantation</td>
<td>2</td>
</tr>
<tr>
<td>Primary immunodeficiency disorders</td>
<td>2</td>
</tr>
<tr>
<td>Wegener's granulomatosis</td>
<td>2</td>
</tr>
<tr>
<td>Progressive systemic sclerosis</td>
<td>1</td>
</tr>
<tr>
<td>Adenocarcinoma (colon)</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 1. Detailed view of the granular exudate. The outlines of the cyst walls of P. carinii are clearly discernible (Papanicolaou stain; original magnification X 500).

Each cellular sample had been smeared and stained according to the standard Papanicolaou method; additional smears from each specimen were stained with either methenamine silver [4] or toluidine blue O [8]. Each Papanicolaou-stained sample was also examined by fluorescent microscopy [11]. Two observers independently recorded the cytologic features of each smear and the presence or absence of P. carinii cysts. Lung biopsy specimens concurrently obtained from 22 of the 28 patients were also examined by the methenamine silver stain for the presence of P. carinii cysts.

RESULTS

The cyst forms of P. carinii were detected in 14 Papanicolaou-stained cellular samples (five bronchoalveolar lavages, five bronchial washings, three bronchial brushings, and one sputum sample) obtained from nine of the 28 patients. In each case, silver methenamine and toluidine blue O stains as well as direct fluorescence examination confirmed the presence of the cysts. P. carinii was also present in the lung biopsy specimens from seven of the nine patients (biopsy was not performed in the other two patients).

Each smear contained irregularly shaped clumps of granular exudate that contained a definite honeycomb architecture due to the subtle outlines of the enmeshed cysts (Figure 1). The presence of the cysts were corroborated by direct fluorescence microscopy and the special stains. This material, which varied in amount from case to case, was most readily found in the samples obtained from the patients with AIDS (six of the nine patients), and was identical to that characteristically observed filling the alveoli of lung biopsy specimens of patients with pneumocystosis.

The cellular samples from 19 of the 28 patients did not show P. carinii. Lung biopsy specimens obtained from 15 of these 19 patients similarly showed no abnormalities. Ten of the patients with negative cytologic results did not receive treatment at any time for P. carinii pneumonia; all subsequently improved clinically.

COMMENTS

Almost without exception, P. carinii infects the immunocompromised host [14]. As Burke [15] stated, the single most valuable aid in the diagnosis of P. carinii infection is a high index of suspicion; however, demonstration of the organism remains at present the only unequivocal diagnostic evidence of infection. In our experience, the amorphous granular exudate we have described in cytologic specimens stained by the Papanicolaou method is specific for P. carinii infection. The formation of fairly well-defined clumps of exudate with its internal "honeycomb" structure is quite distinctive and unlike exudate we have seen in cellular samples from the respiratory tract from patients with other conditions. Our experience is shared by Greaves and Strigle [13] who were similarly impressed by the specificity of the cytologic features. Clumps of inflammatory exudate are often found in cellular samples obtained from patients with pneumonia due to other causes; however, in such cases, the exudate does not have the granularity of architecture as just described in patients with pneumocystosis. Examination of this exudate by fluorescence microscopy or special stains confirmed the presence of the cysts in all cases.

A careful search with special stains did not reveal cysts in those cellular samples in which the distinctive exudate was absent, and the corresponding lung biopsy specimens also did not demonstrate the cyst forms. Although false-negative results were not obtained in this study, it is well to remember that such results may occur as the characteristic intra-alveolar exudate is often not observed in patients with pneumocystosis [16].

A variety of histologic stains may be used to demonstrate P. carinii. Many of these stains often require special
expertise in either their preparation or their interpretation. Although their diagnostic efficacy, when properly performed and interpreted, is unquestioned, these special stains are not as easily employed for the rapid screening of cytologic specimens. In contrast, Papanicolaou-stained smears are easily and quickly prepared in a routine manner and an unequivocal diagnosis may be rapidly established. In view of the increasing numbers of immunocompromised patients encountered in clinical practice and the usefulness of bronchoalveolar lavage [17, 18] in the diagnosis of pulmonary infiltrates in such patients, the use of routinely stained Papanicolaou preparations may prove to be of great value in identifying those patients with P. carinii pneumonia.

REFERENCES