Role of macrophages in tuberculous peritonitis: longitudinal follow-up of 16 continuous ambulatory peritoneal dialysis patients

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Abstract
Objective: Mycobacterium tuberculosis is an intracellular pathogen susceptible to macrophage action, which also has an important role in peritoneal defense. To explore the peritoneal host defense mechanism and inflammatory process, we longitudinally followed up 16 cases of continuous ambulatory peritoneal dialysis complicated by tuberculous peritonitis over the past 9 years in Prince of Wales Hospital.

Methods: Serial peritoneal fluid cell population was monitored in 16 end-stage renal disease patients with tuberculous peritonitis.

Results: The mean age of the participants was 53 ± 15 years with a mean peritoneal dialysis duration of 34 months. Peritoneal fluid lymphocytosis was not evident and their population contributed to 10% ± 7%, 10% ± 6%, and 9% ± 8% of all leukocytes on day 1, 10, and 20 after peritonitis, respectively. Of the 16 patients, six had either failed to resume peritoneal dialysis or died of tuberculous peritonitis, and they were defined as the failure group. Compared with the success group, referring to those who could pursue peritoneal dialysis, the peritoneal fluid macrophage cell count percentage was lower in the failure group. Twenty days after tuberculous peritonitis, the peritoneal fluid macrophage cell count proportion in success and failure groups was 45% ± 11% and 10% ± 10%, respectively (p=0.021). However, polymorphonuclear leukocytes were more abundant in failure groups as early as 3 days after peritonitis, 93% ± 3% versus 44% ± 12% in the success group (p=0.003).

Conclusion: Data in this study support the hypothesis that macrophages represent an important defense mechanism of the peritoneal cavity against mycobacterial infection and possibly the major peritoneal inflammatory process.

Key words: Leukocytes, Macrophage activation, Mycobacterium tuberculosis, Peritoneal dialysis/continuous ambulatory, Phagocytosis

中文摘要
目的：研究结核病的临床治疗和预防，患者对结核性腹膜炎的长期随访。

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INTRODUCTION
Peritoneal inflammation after continuous ambulatory peritoneal dialysis (CAPD) peritonitis is a tightly orchestrated sequence of leukocyte activation and recruitment (1). Inflammation has a pivotal role in modulating the function and possibly structure of the peritoneal membrane (2). In view of the close link between peritoneal inflammation and membrane function in the peritoneal dialysis population, it is important to understand this inflammatory process.

Resident peritoneal macrophages represent one of the major differentiated cells of the mononuclear phagocyte system, and have been thought to represent the first line of defense of the peritoneal cavity by virtue of the phagocytosis and bactericidal capacity (3,4). *Mycobacterium tuberculosis*, the causative agent of tuberculous peritonitis, is an intracellular pathogen that resides predominantly within macrophages, which are, in turn, the first line of defense against this pathogen (5, 6). Tuberculous peritonitis in CAPD patients, therefore, provides a unique opportunity to investigate the roles of mononuclear phagocytes in the immune response and host defense of the peritoneal cavity. Analysis of the cellular characteristics of peritoneal fluid can provide valuable information about this disease entity and possibly peritoneal inflammation as a whole.

METHODS

Patient selection
From January 1994 through December 2001, 16 consecutive patients with CAPD complicated by tuberculous peritonitis were recruited for monitoring. Clinical data were collected, including demographic details, coexisting medical problems, clinical course, and patient and membrane survival information as of April 1, 2002.

Cellular composition of peritoneal fluid was serially monitored every 3 to 5 days throughout the course of tuberculous peritonitis. An aliquot of the effluent was recovered, after a dwell time of at least 4 hours, for determination of total white cell count and differential count. Morphologic identification of the various cell types was performed after centrifuging and staining the peritoneal fluid sample, within 3 to 5 hours of storage in sample tubes containing ethylenediaminetetraacetic acid. The cell count profiles were taken as a crude surrogate of cell recruitment. No immunophenotypic and cytochemical analyses were performed.

All patients with a diagnosis of tuberculosis peritonitis received a standard combination treatment protocol including isoniazid, rifampicin, pyrazinamide, and a fourth agent (either ethambutol or streptomycin). The policy of removing the Tenckhoff catheter (7) was at the discretion of physicians; all removals were followed by attempted reinsertion.

Patients were divided into two groups based on the clinical outcome. Success group referred to those who could be maintained on or resumed peritoneal dialysis after temporary cessation of CAPD. The failure group consisted of patients who died of tuberculous peritonitis and those for whom reimplantation of Tenckhoff catheters failed, both of which serve as markers of severe peritoneal inflammation.

Statistics
Data analysis was performed by using SPSS for Windows version 9.0 (SPSS Inc., Chicago, IL). Results were expressed as mean ± standard deviation unless otherwise stated. Data were compared by chi-square test, Fisher’s exact test, or Mann-Whitney U test where appropriate. A two-tailed p value of less than 0.05 was taken as statistical significance.

RESULTS

Clinical data
Among the patients undergoing CAPD, 16 cases of tuberculous peritonitis were identified. There were eight men and eight women, with a mean age of 53 ± 15 years.
Macrophages in tuberculous peritonitis

(range, 31-72 years). On average, they had received peritoneal dialysis for 34 months before they developed tuberculous peritonitis.

In seven (44%) cases, Tenckhoff catheters were removed before completion of the therapy. Subsequent attempted reinsertion of peritoneal dialysis catheters in three of them was abandoned because of dense peritoneal adhesion. Another three patients failed to pursue peritoneal dialysis simply because they died of severe tuberculous peritonitis episodes. These six cases were regarded as failure group.

For the remaining 10 cases who were maintained on peritoneal dialysis (success group), two of them required additional 2-L peritoneal exchanges subsequently.

**Peritoneal cell counts and outcomes**

The effluent leukocytes from CAPD patients increased after tuberculous peritonitis. The overall white blood cell differentiation upon presentation was 63% polymorphonuclear leukocytes (range, 13%-95%), 10% lymphocytes (range, 0%-25%), 28% mononuclear macrophages (range, 0%-68%), and 2% eosinophils (range, 0%-11%). In general, polymorphs predominated in the peritoneal fluid compared with the lymphocyte population throughout the clinical course (Fig. 1). The percentages of lymphocytes were 10% ± 7%, 10% ± 6%, and 9% ± 8% of all leukocytes on day 1, 10, and 20 after peritonitis, respectively.

Table 1 describes the demographic characteristics and clinical features between the two groups of patients with different outcomes. No difference was noted between the baseline characteristics among the success (n = 10) and failure groups (n = 6), apart from increased likelihood of Tenckhoff catheter removal in the failure group. Otherwise, the difference in outcome was not related to the comorbidity, duration of peritoneal dialysis, or previous CAPD peritonitis episodes.

The proportion of mononuclear macrophage cells of all leukocytes tended to be lower in the failure group (Fig. 2), and so did the absolute concentration of macrophages (not shown). The difference in the macrophage peritoneal

<p>| Table 1. Demographic characteristics of the success and failure groups. |
|-----------------------------------------------|------------------|-----------------|---------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Success (n = 10)</th>
<th>Failure (n = 6)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>5:5</td>
<td>3:3</td>
<td>1.00*</td>
</tr>
<tr>
<td>Age, years</td>
<td>56 ± 15</td>
<td>52 ± 16</td>
<td>0.61†</td>
</tr>
<tr>
<td>Duration of peritoneal dialysis, months</td>
<td>38 ± 42</td>
<td>28 ± 34</td>
<td>0.79‡</td>
</tr>
<tr>
<td>Episode(s) of previous CAPD peritonitis</td>
<td>1.1 ± 1.3</td>
<td>1.7 ± 0.4</td>
<td>0.06†</td>
</tr>
<tr>
<td>Coexisting diseases (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (30)</td>
<td>3 (50)</td>
<td>0.61*</td>
</tr>
<tr>
<td>Corticosteroid therapy</td>
<td>1 (10)</td>
<td>1 (17)</td>
<td>1.00*</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>1 (10)</td>
<td>0</td>
<td>1.00*</td>
</tr>
<tr>
<td>Hepatitis B surface antigen</td>
<td>2 (20)</td>
<td>2 (33)</td>
<td>0.60*</td>
</tr>
<tr>
<td>Malignancy</td>
<td>3 (30)</td>
<td>0</td>
<td>0.25*</td>
</tr>
<tr>
<td>Hemoglobin level, g/dL</td>
<td>8.6 ± 2.9</td>
<td>8.4 ± 1.7</td>
<td>0.88†</td>
</tr>
<tr>
<td>Concurrent tuberculosis (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>1 (10)</td>
<td>3 (50)</td>
<td>0.12*</td>
</tr>
<tr>
<td>Extrapulmonary</td>
<td>3 (30)</td>
<td>2 (33)</td>
<td>1.00*</td>
</tr>
<tr>
<td>Diagnosed cases in life (%)</td>
<td>10 (100)</td>
<td>5 (83)</td>
<td>0.38‡</td>
</tr>
<tr>
<td>Treatment within 6 weeks (%)</td>
<td>6 (60)</td>
<td>3 (50)</td>
<td>1.00*</td>
</tr>
<tr>
<td>Tenckhoff catheter removal required (%)</td>
<td>2 (20)</td>
<td>5 (83)</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

*Fisher’s exact test.  
†Student’s t test.  
‡Mann-Whitney U test.
population between the success and failure groups reached statistical significance only by day 20 of peritonitis. Mononuclear macrophages sampled at day 20 contributed to $45 \pm 11\%$ of all peritoneal fluid leukocytes in the success group and $10\% \pm 10\%$ in failure group respectively ($p=0.021$). A greater degree of peritoneal polymorph dominance occurred in the failure group than the success group (Fig. 3), but there was only a significant difference at Day 3 of tuberculous peritonitis, with $93\% \pm 3\%$ in the former versus $44\% \pm 12\%$ in the latter group ($p=0.003$).

**DISCUSSION**

This study confirms earlier findings (7-11) that peritoneal fluid lymphocytosis is not characteristic of tuberculous peritonitis, as opposed to what had once been thought to be true (12,13). More importantly, findings of the patients in this study with tuberculous peritonitis on dialysis therapy facilitate us to explore the role of mononuclear phagocytes in the immune response and host defense of the peritoneal cavity.

Significant recent advances have been made in understanding the mechanisms by which local peritoneal defense mechanisms and cell-mediated immune response prevent and eradicate peritoneal infection. It is now recognized that all three main cell systems operate in the peritoneal cavity, namely, macrophages, lymphocytes, and mesothelium, acting against invasion of microorganism (14). Among them, macrophages provide the first-line host defense via phagocytic activity and production of inflammatory cytokines.

Instead of performing sophisticated in vivo or in vitro experiments as have been done by previous investigators (15-17), we made an effort to correlate the peritoneal leukocyte profiles and measurable clinical outcomes. In such case, the clinical outcome(s) examined needs to be of biologic and practical relevance. Clearly, mortality from peritonitis and intraperitoneal adhesion precluding resumption of CAPD are well-recognized sequelae of the inflammatory process with clinical bearing. It was therefore reasonably valid to use them as tools to define the failure group in this study. Because we have demonstrated that lower macrophage population predated subsequent patient death and/or peritoneal failure from tuberculous peritonitis, it would suggest a link between macrophages and inflammation. Aside from resident macrophages (4,15,16,18) inside or within the peritoneum membrane, macrophages from delayed recruitment from systemic circulation are implicated in this study.

How can a role for polymorphonuclear cells then be reconciled with the lack of the association with peritoneal integrity or patient survival in this study? Evidence has long existed that activated macrophages, rather than neutrophils, mediate the key intracellular mycobacterial killing mechanisms (5,19). In other words, the defense mechanism in the failure group from this study was presumably upregulated, but only via an ineffective pathway, as characterized by an influx and activation of neutrophils at the site of infection. The polymorphonuclear immune response (20) is therefore incapable of eradicating intracellular mycobacteria and poorly targeted, leading to indiscriminate tissue (peritoneal) damage.

One criticism of our reporting leukocyte cell counts alone is that the qualitative state of each leukocyte class could not be addressed. The ability of different macrophage phenotypes to produce cytokines, besides chemotactic and phagocytic capacity (17), is thus ignored.

Although we evaluated the effect of macrophage recruitment on adverse outcomes (patient mortality and peritoneal failure), it may be important (21) to correlate...
the macrophage defense with subsequent peritoneal membrane function (permeability and ultrafiltration volume) after recovery from tuberculous peritonitis.

REFERENCES


