Abnormalities of Cellular Immunity in Uremic Patients Undergoing Continuous Ambulatory Peritoneal Dialysis (CAPD)

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Introduction

Continuous ambulatory peritoneal dialysis (CAPD) is an alternative replacement therapy for patients with chronic renal failure. The most serious complications are peritonitis and fibrosis, including failure of the dialysis technique. When germs enter peritoneum, cells of the immune system act in defence and trigger tissue injury. The immune system is composed of two intercommunicated cellular and molecular compartments, those components are identified and isolated by flow cytometry, the staining of proteins specific for each cell group with fluorescent monoclonal antibodies called CD markers.

It is well established that chronic renal failure exhibit peripheral blood lymphopenia, which is accompanied by a decreased delayed hypersensitivity response to a variety of antigens, decreased lymphocyte proliferative response, when stimulated by different antigens, and decreased production of immunoglobulins by B cells to specific stimuli. The clinical relevance of altered lymphocyte function is not well understood. At the same time, infections are the second leading cause of death in hemodialysis patients and peritonitis is the primary complication in CAPD patients.

As we may realize the role of lymphocytes in host immunity for CAPD patients is just beginning to be understood. In order to clarify the abnormalities of cellular immune responses in uremic patients undergoing CAPD, we studied as immunological parameters lymphocytes subsets counts in comparison with normal subjects.

Patients and Methods

The study included 37 CAPD patients (21 female, 16 male, age: 66,88 ± 13,48 M ± SD) and 45 normal individuals (28 female, 17 male, age: 35,8 ± 10,8 M ± SD) who served as our control group. Primary causes of chronic renal failure were diabetes mellitus (17), hypertensive nephropathy (6), glomerulonephritis (9), polycystic kidney disease (1), others (4).

Lymphocyte subsets (CD2+, CD3+, CD3+/4+, CD3+/8+, CD19+, CD3-/16+56+, CD4/CD8 ratio) were quantitated using monoclonal antibodies (Immunotech, Coulter) and flow cytometric analysis (table 1).

<table>
<thead>
<tr>
<th>Cluster Differentiation</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>CD2+</td>
<td>T lymphocytes, thymocytes</td>
</tr>
<tr>
<td>CD3+</td>
<td>Mature T cells</td>
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<tr>
<td>CD3+/4+</td>
<td>T helper cells</td>
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<tr>
<td>CD3+/8+</td>
<td>T suppressor/cytotoxic cells</td>
</tr>
<tr>
<td>CD19</td>
<td>B lymphocytes</td>
</tr>
<tr>
<td>CD3-/16+56+</td>
<td>NK cells</td>
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Briefly, 20 µL of the appropriate monoclonal antibody was incubated with 100 µL of blood sample for 20 min in the dark. The samples were then lysed by ImmunoPrep reagent system (Beckman Coulter Company) and analyzed in the flow cytometer. (Epics Elite ESP, Coulter)

Student’s t-test was performed to test differences between groups (SPSS vs 10).

Results

Table 2 shows immunophenotypes of patients on CAPD. CAPD patients showed increased natural killer cells than controls (15,22±9,49 vs 10,13±4,10, p=NS). CD4/CD8 ratio levels were higher in CAPD patients compared with controls (2,11±1,42 vs 2,01±0,74, p=NS). CAPD patients showed lower lymphocyte subpopulations compared with controls and especially CD3, CD3+/4+, CD19+ were lower than healthy subjects (p=NS).

Discussion

The immune system is composed of cells and molecules vigilantly defending and maintaining the homeostasis of the host. Functionally, the system has two branches: natural (innate, unspecific) immunity and specific (acquired) immunity. Phagocytic cells manage innate immunity. Acquired immunity has two branches: humoral immunity managed by B cells, which secrete antibodies or immunoglobulins and cellular immunity managed by T cells, CD4+ and CD8+.

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Cells of the innate immune system (neutrophils, monocytes, eosinophils and dendritic cells) start and amplify the immune response by phagocytosis of germs and antigens, presenting them to T helper cells from the specific immunity system. The T cells determine the kind of specific immunity that will fight the antigen: humoral or cellular. The CD8 cells are divided into cytotoxics and suppressors and the Natural killer cells (NK) are functionally from the innate system, but they are essential for inducing cellular immune responses. Specific cellular immunity, which is mediated by T cells, and defects offering these lymphocytes underlie the most severe immune deficiencies. Because antibody production requires intact T cell function, most T cell defects lead to combined (cellular and humoral) immune deficiency. Lymphocytes normally comprise 20-40% of peripheral blood leukocytes. Of these about 70-80% are T lymphocytes and 10-20% are B lymphocytes. T cells can further be classified into cytotoxic T, helper T and suppressor T cells. Helper T and suppressor T cells serve as immunoregulatory cells of the immune response. Altered numbers of immune cells contribute to immunologic abnormalities, depressed erythropoiesis, increased infection rates and poor outcome. Early detection of immunologic disturbances may initiate early clinical intervention, resulting in more effective treatment with peritoneal dialysis. Repeated determinations of T lymphocyte counts seems to be helpful in the early diagnosis of such disturbances. When comparing the percentages of peripheral blood T cells from CAPD patients with normal controls, we found no significant differences. The CD4/CD8 ratio levels were higher in CAPD patients compared with controls (p=NS). These findings have been verified by other researchers, which found expansion of CD14+CD16+ cells in CAPD patients, along with high levels of factors that stimulate monocyte and granulocyte production. Peritoneal dialysis appears to contribute to state of chronic activation of the immune system resulting in a localized chronic inflammatory response. The causes and clinical consequences of this chronic activation remains unknown. These results may explain the increased vulnerability to infections in CAPD patients compared with healthy subjects. Additionally increased NK may reflect chronic sterile or infectious inflammatory response.

## References


### Table 2. Immunophenotypes of patients on CAPD and controls

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>WBC</th>
<th>LYM</th>
<th>CD2</th>
<th>CD3</th>
<th>CD3/4</th>
<th>CD3/8</th>
<th>CD19</th>
<th>NK</th>
<th>NK like</th>
<th>CD4/CD8</th>
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<tbody>
<tr>
<td>CAPD</td>
<td>37</td>
<td>8377</td>
<td>22,9</td>
<td>839</td>
<td>68,0</td>
<td>41</td>
<td>24,2</td>
<td>7,2</td>
<td>15,22</td>
<td>2,7</td>
<td>2,11</td>
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<td></td>
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<tr>
<td></td>
<td>2404</td>
<td></td>
<td>8,55</td>
<td>5,5</td>
<td>15,35</td>
<td>7,8</td>
<td>9,5</td>
<td>4,35</td>
<td>9,5</td>
<td>1,9</td>
<td>1,42</td>
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<td>control</td>
<td>45</td>
<td>7151±1387</td>
<td>31,75</td>
<td>80,15±4,66</td>
<td>73,7±5,8</td>
<td>46,3±6,64</td>
<td>24,35±6,44</td>
<td>12,3±3,44</td>
<td>10,13</td>
<td>3,14±4,1</td>
<td>2,0±2,54</td>
</tr>
</tbody>
</table>

**Notes:**
- CD4/CD8 ratio levels were higher in CAPD patients compared with controls.
- Increased secretion of IL-2 by lymphocytes found in these patients.
- Expanded NK found in CAPD patients, although not significant, accord with reports.
- Expansion of NK that we observed, although not significant, may reflect chronic sterile or infectious inflammatory response.


