Abdormalities of Cellular Immunity in Uremic Patients Undergoing Continous Ambulatory Peritoneal Dialysis (CAPD)

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Introduction

Continous 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 techique [1]. When germs enter peritoneum, cells of the immune system act in defence and trigger tissue enjury. The immune system is composed of two intercommunicated cellular and molecular compartments[2], 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[3].

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 [4,5], decreased lymphocyte proliferative response, when stimulated by different antigens [6,7] and decreased production of immunoglobulins by B cells to specific stimuli [8]. The clinical relevance of altered lymphocyte function is not well understood. At the same time, infections are the second leading course of death in hemodialysis [9] patients and peritonitis is the primary complication in CAPD patients [10,11].

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 abdormalities 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 falure 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 1. Monoclonal antibodies used for lymphocyte analysis

Cluster Differentiation	Specificity
CD2+	T lymphocytes, thymocytes
CD3+	Mature T cells
CD3+/4+	T helper cells
CD3+/8+	T suppressor/cytotoxic cells
CD19	B lymphocytes
CD3-/16+56+	NK cells

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 subpopoulations comparing 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 maintaing the homeostasis of the host. Functionally, the system has two branches: natural (innate, unspecific) immunity and specific (aquired) immunity. Phagocytic cells manage innate immunity. Aquired 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+[4].

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Table 2. Immunophenotypes of patients on CAPD and controls

	N	WBC	LYM	CD2	CD3	CD3/4	CD3/8	CD19	NK	NK like	CD4/CD8
CAPD	37	8377 ± 2404	22,9 ± 8,55	83,9 ± 5,5	68,0 ± 15,35	41 ± 7,8	24,2 ± 9,5	7,2 ± 4,35	15,22 ± 9,5	2,7 ± 1,9	2,11 ± 1,42
control	45	7151±1387	31,75 ±7,3	80,15 ±4,66	73,7 ±5,8	45,2 ±6,65	24,35 ±6,44	12,3 ±3,44	10,13 ± 4,1	3,14 ± 2,54	2,0 ± 0,72

Cells of the innate immune system (neutrophils, monocytes, eosinophils and dendritic cells) start and aplify 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[2] . 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[2].

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[12] and poor outcome[13]. Early detection of immunologic distrurbances 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 [14,15]. On the other hand, an increase in the percentage of activated T cells in the peripheral blood of CAPD patients as compared to normal controls, have been reported by other authors[16]. The reasons for this considerably variability are unknown. As has been reffered, the proportion of CDs subsets counts varies with age[17]. In CAPD patients it is possible that the decrease ih these lymphocyte subsets has a role in the decreased secretion of IL-2 by lymphocytes found in these patients[18].

Natural killer cells are derived from bone marrow. They play a role in the defence agaist infection.[19]. Their cytotoxic function is bone marrow and cytokine dependent[20]. The expansion of NK that we observed, although not significant, accords with reports [21,22], 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

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