Comparison of Immunophenotypes in the Blood of Patients on Continuous Ambulatory Peritoneal Dialysis, Asymptomatic and with Peritonitis

Peritonitis is a complication of continuous ambulatory peritoneal dialysis (CAPD) that often causes fibrosis. Understanding the role played by the immune system is required if we are to understand the mechanisms of defense and tissue lesion triggered by germs.

We compared the characteristics of the blood immunophenotypes of patients on CAPD, with and without peritonitis. This descriptive, prospective study was carried out in the dialysis unit of San José University Hospital, a tertiary care institution in Popayán, Colombia.

In blood samples from 46 patients on CAPD (26 with peritonitis and 20 without peritonitis), we used flow cytometry to measure cytokine production at the single-cell level. The diagnosis of peritonitis was made by standard clinical and laboratory criteria. We noted general clinical characteristics of the patients; percentages of lymphocytes, monocytes, neutrophils, and eosinophils; and cell counts of lymphocytes (CD4 cells, CD8 cells) and their subsets [B1, B2, and natural killer (NK) cells]. We also determined the CD4:CD8 ratio.

We found significant differences in the levels of serum albumin (p < 0.001), the percentages of lymphocytes (p < 0.04) and neutrophils (p < 0.04), and the counts of B1 and B2 cells, especially in patients whose CD4:CD8 ratio was below 1.2. Those patients also had more intense CD4 lymphopenia, more CD8 cells (principally T-suppressor cells), and more expansion of NK cells.

In patients on CAPD, an important immune activation and rise in the percentage of B1 cells occurs that increases with peritonitis. Among the general clinical characteristics, albumin was the only one to show a statistically significant difference between patients with and without peritonitis. An important CD4 lymphopenia occurred in patients with a CD4:CD8 ratio below 1.2.

Key words
Immunophenotype, CAPD peritonitis, CD4:CD8 ratio, B1 cells

Introduction
One solution for patients with kidney failure is continuous ambulatory peritoneal dialysis (CAPD), whose most serious complications are peritonitis and fibrosis, inducing failure of the dialysis technique (1,2). When germs enter the peritoneum, cells of the immune system act in defense and trigger tissue injury (3). The immune system is composed of two intercommunicated cellular and molecular compartments (3—5); 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 (6,7)

Immune system organization
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+ (5).

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 (TH) CD4+ cells from the specific immunity system. The T˚cells determine the kind of specific immunity that will fight the antigen: humoral or cellular (4,5). The B˚cells are CD19+, and two subsets exist: B1 cells [CD5+, fewer than 10% in peritoneum and 5% in blood; they produce immunoglobulin˚M (IgM) and auto-antibodies], and B2 cells that produce the other classes of immunoglobulins. The CD8 cells are divided into cytotoxics and suppressors, differentiated because the cytotoxics are CD28+. Natural killer cells (NKs) are functionally from the innate system, but they are essential for inducing cellular immune responses (4—6).

The objective of the present project was to observe the presence and activation of the various populations of blood immune cells by flow cytometry in patients on CAPD with and without peritonitis.

Patients and methods
Blood samples from 46˚patients on CAPD [26˚with peritonitis (group˚B) and 20˚without peritonitis (group˚A)] were taken in the morning during the first change of dialysis fluid. Patients were in the CAPD program of the San Jos Kidney Diseases Unit, Department of Internal Medicine of the Health Sciences Faculty, University of Cauca, and the dialysis program of RTS Cauca in Popay n, Colombia. Dialysis consisted of 4˚daily exchanges of Dianeal (Baxter Healthcare Corporation, Deerfield, IL, U.S.A.).

The criteria for a diagnosis of peritonitis was (A) cloudy dialysis fluid or abdominal pain or fever; (B) >100 leucocytes/mm3 in the fluid, with at least 50% neutrophils; and (C) detection of microbes by Gram stain or culture of the dialysis fluid. We exposed 50˚μL blood to 20˚μL of each of the following fluorescent monoclonal antibodies [two or three antibodies per tube: fluorescein isothiocyanate conjugate (FITC), phycoerythrin (PE), fluorescent chlorophilic protein of 35˚kDa from Glenodinium species (PerCP), human immune-response D-related antigen (HLA-DR)]; anti-CD45 FITC/CD14 PE, CD4 FITC/CD3/PerCP/HLA-DR PE, CD8 FITC/CD28 PE, CD5 FITC/CD19 PE, CD3 FITC/CD56 PE (Becton—Dickinson, San Jos , CA, U.S.A.). After incubation in the dark at 4˚C for 30˚minutes, 2˚mL red-cell lysing solution (FACS: Becton—Dickinson) was added. After incubation at 37˚C with 5% CO2 for 10˚minutes, the cells were centrifuged at 1500 rpm for 10˚minutes. After the supernatant was removed, the cell pellet was washed twice in phosphate-buffered saline (PBS). Finally, the pellet was diluted in 0.5˚mL phosphate-buffered saline (PBS) and analyzed in a FACSCalibur flow cytometer using the CellQuest software (Becton—Dickinson).

Statistical analysis
Analysis of the data was performed using STATA˚6.0 at the clinical epidemiology unit (INCLEN) of the Faculty of Health Sciences, University of Cauca. The data are provided as median and dispersion measures (range, standard deviation, and percentages).

The data are kept in the clinical records and protected in fulfillment of the ethical principles of research. The data generated from the project will be useful for improving the health care of our patients on CAPD.

Results
Table˚I shows the characteristics of both groups of patients. The mean ages and general profiles were similar. The groups contained more women than men. The only parameter that was statistically significantly different was serum albumin.

CD4:CD8 ratio. The thinking behind that decision was that the CD4:CD8 ratio indicates either a decrease of CD4+ cells or an expansion in CD8+ cells, and the observations of our lab associate a lower ratio with the presence of chronic viruses such as those of the herpes group. The division was interesting, because the lower-ratio patients showed intense CD4 lymphopenia, while having more cytotoxic cells (CD8 and NK).

High expression of HLA-DR, which was more intense during peritonitis, was seen on T˚cells in CAPD patients (Tables˚II and III; group˚A: 22.4%; group˚B: 27.3%; healthy controls: >8%).

The percentage of CD8+CD28— cells (T-suppressor cells) was high (<1.1) in all groups.
Expansion of NK cells was seen in 55% of patients of group A and in 73% of patients group B. That expansion was more intense in patients with a low CD4:CD8 ratio and peritonitis (low-ratio patients in group A: 40%; low-ratio patients in group B: 78%).

In both groups, total B cells were normal, but the percentage of B1 cells was high. The difference was more intense in the peritonitis group (Tables II and III).

### Discussion

The intense CD4 lymphopenia in patients with a lower CD4:CD8 ratio deserves further research to look for its cause and clinical meaning. It could mean cellular immunodeficiency that could be complicated with opportunistic infections and tumors.

Asymptomatic patients on CAPD showed important expression of HLA-DR on T lymphocytes, which indicates immune activation. Those data accord
with reports of immune activation in asymptomatic patients on CAPD, which is more intense during peritonitis. Davies et al (8) saw increased chemiluminescence on monocytes of asymptomatic patients on CAPD, especially on peritoneal macrophages; high expression of HLA-DR on T˚cells and monocytes; and high expression of interleukin-2 receptor (IL-2R) and immunoglobulin˚G (IgG) receptor. In another report, Lai et al (2) found a significant rise in neutrophil numbers (400-fold) in dialysis fluid even 3˚weeks after clinical resolution of peritonitis together with increased numbers of dead mesothelial cells and high production of pro-inflammatory cytokines and transforming growth factorβ (TGFβ). In parallel work in our laboratory, we found increased expression of CD69, HLA-DR, and pro-inflammatory cytokines in asymptomatic patients on CAPD, with an even greater rise during peritonitis. In sum, important elevation is seen in the numbers of immune activation markers in patients on CAPD, and the numbers increase even more during peritonitis (9,10).

It is important to recognize two groups of patients according to CD4:CD8 ratio, because the lower-ratio group is different. Patients in that group have more CD4 lymphopenia, especially during peritonitis; more CD8 cells, especially the suppressor type; and more NK cells. Those findings suggest a need for more research and care about the CD4:CD8 ratio in clinical CAPD settings such as transplant (11).

The expansion of NK and CD8 cells accords with reports from Saionji and Osaka (12) and Saionji et al (13), who found expansion of CD14+CD16+ cells in CAPD patients, along with high levels of factors that stimulate monocyte and granulocyte production [macrophage colony-stimulating factor (M-CSF) and granulocyte colony-stimulating factor (G-CSF)].

Elevated levels of B1 cells in CAPD patients are enigmatic still, because of their greater rise during peritonitis. The obvious suggestion is that they produce IgM, the first immunoglobulin to peak after microbes gain entrance.

Conclusions
The present study, and the literature, found important immune activation and increased secretion of cytokines in asymptomatic CAPD patients. The activation increases during peritonitis. Furthermore, we saw evidence of two different groups of patients according to the CD4:CD8 ratio. A lower ratio is associated with more CD4 lymphopenia and more activity of cytotoxic cells such as NK and CD8+CD28— cells. All of those findings suggest new biologic and clinical research to explore the causes and influences of the immune system in the evolution and complications of patients on CAPD for example, tissue degeneration (atherosclerosis, depression, and even aging, among others) (14,15).

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Immunophenotypes in CAPD


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Cytokines and Peritonitis in Continuous Ambulatory Peritoneal Dialysis: Immunodeviation and Immunodeficiency

Cytokines are soluble mediators of the immune system, which regulate the immune response to antigenic stimuli. In continuous ambulatory peritoneal dialysis (CAPD) patients with peritonitis, an inflammatory process occurs, but the patterns of cytokine secretion have not yet been clarified. We compared the characteristics of the intracellular production of cytokines and looked for the immunophenotypes TH1, TH2, and TH0 in CAPD patients with and without peritonitis.

Our descriptive, prospective study was carried out in the dialysis unit of the San Jos University Hospital, a tertiary health care center in Popayan, southwest Colombia.

We obtained 28 peripheral blood samples from CAPD patients (8 with peritonitis and 20 without peritonitis) and processed them by flow cytometry for intracellular detection of cytokines. The peritonitis diagnosis was made based on established clinical and laboratory criteria. We measured the general clinical characteristics and percentage of intracellular production of interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-12 (IL-12), tumor necrosis factor α (TNFα), interleukin-4 (IL-4), and interferon-γ (INF-γ) in T lymphocytes.

Diabetic nephropathy and chronic glomerulonephritis were the most frequent primary pathologies in both groups of patients. The patients on CAPD without peritonitis expressed high levels of CD69 and the pro-inflammatory cytokines IL-1, IL-6, IL-12, TNFα, and IL-4, indicating in vivo immune activation similar to the group with peritonitis. In the group without peritonitis, 95% of samples displayed immunodeviation TH2. Just 5% of samples approached TH0, producing IFN-γ. After mitogen activation, 45% of the samples stayed in TH2; 55% approached TH0.

Patients with peritonitis produced high levels of IL-4 and little IFN-γ, which indicates immunodeviation TH2 in 75% of samples; 25% approached TH0. When cells were stimulated by ionomicin and phorbol 12-myristate 13-acetate (PMA), more IFN-γ appeared and high levels of IL-4 persisted in 75% of the samples, which looked like intent to correct the TH2 immunodeviation toward TH0.

Patients on CAPD with and without peritonitis showed immune activation per se and high production of pro-inflammatory cytokines accompanied by a strong pattern of cytokine TH2 and a deficiency of IFN-γ production, suggesting heavy immunodeviation TH2 and immunodeficiency TH1 (owing to the deficit of IFN-γ). Finally, with in vitro immune stimulation, the TH2 pattern tried to approach TH0.

Key words
Cytokines, TH1/TH2/TH0, immunoregulation, flow cytometry

Introduction
Continuous ambulatory peritoneal dialysis (CAPD) is a therapy that replaces the kidney function of patients with chronic renal insufficiency. The therapy is frequently complicated in some patients with repeated peritonitis, inducing peritoneal fibrosis and irreversible loss of the dialysis capacity of peritoneum (1,2). Cytokines or interleukins (ILs) are proteins secreted during inflammation to modulate the immune...
response. In that way, cytokines influence healing and complications of infections (such as sepsis and tissue fibrosis). Studying the behavior of cytokines is essential to understanding the mechanisms of defense and complications in the war against germs (3—6).

Cytokines are produced by many cell types. The cytokines secreted by monocytes are called monokines, and the ones secreted by lymphocytes are called lymphokines. Monokines are pro-inflammatory and anti-inflammatory (7,8), and the differences in the production of cytokines by T-helper lymphocytes (TH CD4+) determine at least 3 different subsets of T-lymphocytes called TH1, TH2, and TH0 helper cells (9—1).

The TH1 lymphocytes are heavy producers of interferon-γ (IFN-γ) and IL-2. The TH2 subset produces IL-4, IL-5, IL-10, and IL-13. The TH0 group secretes the entire range of TH1 and TH2 cytokines. The TH lymphocyte subsets are important in the immunoregulation of the response to germs and antigens, and they influence the evolution of infectious and immunologic diseases (10—13). The TH1 cells induce cellular immunity, granuloma formation, and the delayed type of hypersensitivity. The TH2 subset favors production of the various immunoglobulin classes that shape or help humoral immunity.

An inflammatory process occurs in patients on CAPD with peritonitis, but the secretion patterns of cytokines has not yet been clarified (2,3). The objective of the present study was to observe the intracellular production of the pro-inflammatory monokines IL-1, IL-6, IL-12, and tumor necrosis factor-α (TNF-α), and the lymphokines IFN-γ and IL-4 in peripheral blood of patients on CAPD with and without peritonitis.

Patients and methods

Patients

Peripheral blood samples were obtained from 20 CAPD patients without peritonitis (group A) and 8 CAPD patients who fulfilled at least two of the following criteria for a diagnosis of peritonitis (group B): (A) cloudy dialysate or abdominal pain or fever; (B) leukocyte count in peritoneal liquid greater than 100 cells/mm³, with at least 50% neutrophils; (C) presence of micro-organisms by Gram stain or culture of dialysis liquid.

The patients came from the dialysis unit of San Jos University Hospital in Popayán, or the referral center in the department of Cauca, southwest Colombia. All received 4 daily exchanges of Dianaeal (Baxter Healthcare Corporation, Deerfield, IL, U.S.A.). The blood samples were taken during the years 2000—2001.

All participants signed an informed consent to participate in the study. The data were collected using an instrument that gathers sociodemographic and clinical information along with laboratory results. We decided to divide both groups of CAPD patients (with and without peritonitis) into two subgroups, depending on the value of the CD4:CD8 ratio (set point, 1.2), considering that low CD4:CD8 ratios are caused by a reduction of T CD4+ cells, and a predominance of T CD8+ cells, as happens in infection by the human immunodeficiency virus (HIV) and as has been observed in our laboratory in other chronic virus infections such as cytomegalovirus, herpesvirus, and hepatitis B and C.

Design

Intracellular cytokines were detected by standard flow cytometry (13—16).

Cells were cultivated for 6 hours with and without mitogenic stimulation by phorbol 12-myristate 13-acetate (PMA), ionomicin, and an inhibitor of the Golgi apparatus (Brefeldin A: Sigma, St. Louis, MO, U.S.A.; or GolgiStop: Becton—Dickinson, San Jos, CA, U.S.A.), which allows the intracellular accumulation of proteins. The proteins are then detected by fluorescent monoclonal antibodies, introduced after permeabilization of the cellular membrane with saponin (Cytofix/Cytoperm: Becton—Dickinson) (17).

For intracellular staining, we used the following anti-cytokine antibodies conjugated with fluorescent substances [fluorescein isothiocyanate conjugate (FITC)/phycoerythrin (PE)/fluorescent chlorophyll protein of 35 kDa from Glenodinium species (PerCP)]: anti-IL-1 FITC [mouse immunoglobulin’G1 (IgG1), clone 364-3B3-14]’/ IL-6 PE (rat IgG1, clone MQ2-1A5)’/ CD3 PerCP, IL-12 FITC (mouse IgG1, clone C11.5)’/TNFα PE (mouse IgG1, clone Mab11)’/CD3 PerCP, and IL-4 FITC (rat IgG2b, clone BVD4-1D11)’/ IFN-γ PE (rat IgG1, clone XMG1.2)’/ CD3 PerCP (Pharmineng, San Diego, CA, U.S.A.). To confirm cellular activation, we used anti-CD14 PE (mouse IgG2b, clone MfP9), which identifies monocytes by surface staining, and anti-CD69 (mouse IgG1, clone L78), an early marker of immunologic activation (Becton—Dickinson), in intracellular
labeling. For identification of T-cell subsets and to obtain the CD4:CD8 ratio, we used anti-CD8 FITC (IgG1, clone SK1) and CD4 PE (mouse IgG1, clone SK3). The antibodies for negative fluorescence control were R35-95 (rat IgG, 2APE) and MOPC-21 (mouse IgG1) FITC (Pharmingen).

**Procedure**

Culture and cellular activation were processed in two parallel sets of tubes (with activation and without activation). Whole blood (100 µL) was dropped into the respective 12×75 mm polypropylene tubes, together with 100 µL RPMI-1640 complete cell culture medium [containing 2 mmol/L L-glutamine, 250 U/mL penicillin G, 250 µg streptomycin (Gibco BRL, Rockville, MD, U.S.A.), 50 mmol/L of mercaptoethanol, and 10% bovine fetal serum]. Next, 10 µL Brefeldin A (stock solution, 1 mg/mL) was added to all tubes. To the activated tubes, we also added 10 µL ionomycin (stock solution, 1 µg/mL) and 20 µL phorbol 12-myristate 13-acetate (PMA: stock solution, 25 ng/mL). The tubes were then incubated for 6 hours at 35°C in humidity and 5% CO₂.

**Lysis of red cells**

After incubation, 2 mL solution for lysing red blood cells (FACS: Becton—Dickinson) was added to all tubes. Incubation then continued for 10 minutes at 37°C. Afterward, the cells were centrifuged at 1500 rpm for 10 minutes, and the supernatant was removed. The cells were washed twice with 1 mL phosphate-buffered saline (PBS) and centrifuged for 10 minutes.

**Blockade of Fc receptors**

Soon after the second washing, the cell pellets were incubated with 50 µL polyvalent human immunoglobulin'G (Sandoglobulin: Novartis—Pharma AG, Basle, Switzerland) for 20 minutes in the dark at 4°C to block the Fc receptors and avoid unspecific binding of the antibodies that would later be used to dye the cellular surface and the intracellular cytokines.

**Surface staining**

Antibodies (anti-CD3 PerCP, anti-CD4 PE, anti-CD4 PE, anti-CD8 FITC, and anti-CD14: Becton—Dickinson) were added (20 µL) to the corresponding tubes and incubated for 30 minutes at 4°C in the dark. The cells were then washed once with PBS and centrifuged.
in vivo or per se, that was increased with the mitogenic stimuli, indicating success in the activation in vitro.

In group A patients, we observed high levels of production of pro-inflammatory cytokines (IL-1, IL-6, IL-12, TNFα) that exceeded 89% of cells, and little IFN-γ (15.7% of cells), indicating that 75% of those patients had immunodeviation TH2; 25% approached TH0. After stimulation in vitro (Figure 2), cells from group A showed persistent production of pro-inflammatory cytokines and IL-4 at elevated levels, with a slight increase in the production of IFN-γ in 55% of the patients (average: 36.4%). That finding indicates a tendency to correct the immunodeviation TH2 (45% of the patients) toward TH0 (55% of the patients). In group B patients, the production of pro-inflammatory cytokines remained about the same, with the exception of a slight reduction in TNFα. The IL-4 remained high, and better production of IFN-γ was observed, which indicates a tendency to correct the immunodeviation TH2 toward TH0 in 75% of patients.

**Discussion**

The present study of CAPD patients with and without peritonitis made several important findings concerning the production of cytokines. Patients on CAPD without peritonitis presented intense immunologic activation per se, expressing in vivo the early activation marker CD69 that has also been seen in other studies (18). CAPD patients presented immunodeviation TH2; just 5% approached TH0, producing IFN-γ.

In group B patients, we observed high levels of production of pro-inflammatory cytokines and IL-4 (exceeding 89% of cells) and little IFN-γ (15.7% of cells), indicating that 75% of those patients had immunodeviation TH2; 25% approached TH0. After stimulation in vitro (Figure 2), cells from group A showed persistent production of pro-inflammatory cytokines and IL-4 at elevated levels, with a slight increase in the production of IFN-γ in 55% of the patients (average: 36.4%). That finding indicates a tendency to correct the immunodeviation TH2 (45% of the patients) toward TH0 (55% of the patients). In group B patients, the production of pro-inflammatory cytokines remained about the same, with the exception of a slight reduction in TNFα. The IL-4 remained high, and better production of IFN-γ was observed, which indicates a tendency to correct the immunodeviation TH2 toward TH0 in 75% of patients.

**Table I**

Characteristics of the continuous ambulatory peritoneal dialysis patients with peritonitis (n = 8)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N (%)</th>
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<tbody>
<tr>
<td>Mean age (years)</td>
<td>45.5</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Females</td>
<td>6 (75)</td>
</tr>
<tr>
<td>Provenance</td>
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</tr>
<tr>
<td>Urban</td>
<td>19 (73.1)</td>
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<td>Rural</td>
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<tr>
<td>Renal disease</td>
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</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>Other glomerulopathies</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>Previous mean episodes of peritonitis</td>
<td>0.62</td>
</tr>
<tr>
<td>Mean serum albumin (g/dL)</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* The data for the 20 asymptomatic patients is similar (Not shown).
The level of the marker was not different between infected and non-infected patients.

The group without peritonitis secreted many pro-inflammatory cytokines and IL-4, which confirms data described by other groups (2,19—22) showing important immune activation in blood and peritoneal fluid of CAPD patients with and without peritonitis, with great production of pro-inflammatory and anti-inflammatory cytokines [IL-10 (22) and transforming growth factor $\beta$ (TGF$\beta$) (2,23)]. In the group with peritonitis, the findings of activation and secretion of cytokines were similar, but the level of IFN production was a little greater.

All the CAPD patients displayed immunodeviation TH2, indicating that the patients have an immunodeficiency of the cellular type, because they are incapable to firing up TH1 cells. That immunodeviation was recently described by Yokohama et al (24) in asymptomatic CAPD patients, whose IFN-$\gamma$ values were similar to those found in the present study. It is necessary to better define the immunodeviation TH2, owing to reports of high levels of TGF$\beta$ and IL-10 in CAPD patients. Immunosuppression and genesis of fibrosis is associated with TGF$\beta$, as with other cytokines. Also, IL-10 and TGF$\beta$ are anti-inflammatory cytokines, and leaders in the TH3 lymphocyte subset recently described as inducers of immune tolerance.

**Conclusions**

Patients on CAPD without and with peritonitis showed immune activation *per se*, and production of pro-inflammatory cytokines, accompanied by a pattern of cytokine TH2 and IFN-$\gamma$ deficiency. Patients with peritonitis secrete a little more IFN-$\gamma$ and show immunostimulation, too. The immunostimulation tries to

<table>
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<tr>
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<th>Peritonitis</th>
<th>No peritonitis</th>
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<tbody>
<tr>
<td></td>
<td>CD4:CD8&gt;1.2</td>
<td>CD4:CD8&lt;1.2</td>
</tr>
<tr>
<td><strong>No activation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH1</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>TH0</td>
<td>16.7%</td>
<td>50.00%</td>
</tr>
<tr>
<td>TH2</td>
<td>83.3%</td>
<td>50.00%</td>
</tr>
<tr>
<td><strong>Activation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH1</td>
<td>0.00%</td>
<td>40.00%</td>
</tr>
<tr>
<td>TH0</td>
<td>66.67%</td>
<td>100.00%</td>
</tr>
<tr>
<td>TH2</td>
<td>33.33%</td>
<td>0.00%</td>
</tr>
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</table>

**TABLE II** Comparison of the patterns T-helper$^1$/T-helper$^2$/T-helper$^0$ (TH1/TH2/TH0) in patients on continuous ambulatory peritoneal dialysis with and without peritonitis.

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Figure 2: The upper square shows TH2 cells stained with interleukin-4 phycoerythrin (IL-4 PE) antibody and no interferon-$\gamma$ (IFN-$\gamma$)–fluorescein isothiocyanate conjugate (FITC) in non-stimulated cells of a patient on continuous ambulatory peritoneal dialysis with peritonitis. The lower square shows cells stained mainly with IL-4 and some IFN-$\gamma$ after cell stimulation. Data was obtained in a FACS Calibur flow cytometer (Becton-Dickinson, Mountain View, CA, U.S.A.).
correct the immunodeviation from TH2 to TH0; however, it is far from getting TH1 cells.

Acknowledgment
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Immunodeviation and Immunodeficiency in CAPD