Peritonitis in Continuous Ambulatory Peritoneal Dialysis: Cytokines in Peritoneal Fluid and Blood

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Cytokines are soluble mediators of the immune system that regulate the response to antigens and microorganisms. In patients on continuous ambulatory peritoneal dialysis (CAPD) who have peritonitis, an inflammatory process exists that must be understood if susceptibility to, and the mechanisms of, complications such as fibrosis and others are to be understood. To that end, we studied 9 CAPD patients with peritonitis. The case series was conducted in Popayán, Colombia, at the RTS Cauca dialysis unit and the University of Cauca hospital, a tertiary health care facility.

Peritonitis was diagnosed by standard clinical and laboratory criteria. Using flow cytometry, we measured the percentage production of intracellular cytokines [interleukin-1α (IL-1α), IL-6, IL-12, tumor necrosis factorα (TNFα), IL-4, and interferon-γ (IFN-γ)] in T lymphocytes from blood and peritoneal fluid.

Among the studied patients, all (100%) produced high levels of IL-1, IL-6, TNFα, IL-12, and IL-4 in both fluids (blood: 89% ± 6.3% of cells; peritoneal fluid: 81.6% ± 10.1% of cells). In blood, 25% of patients produced IFN-γ (mean: 15.7% of cells), showing that 75% of patients had the TH2 pattern, and 25% were close to TH0. In peritoneal fluid, 34% of patients produced IFN-γ spontaneously (mean: 24.5% of cells), indicating that 66% of patients were TH2, and 34% were close to TH0. After stimulation, expression of cytokines, including IFN-γ (39% of T cells), was increased, and high production of IL-4 indicated that 25% of patients were TH2, and 75% were TH0. In peritoneal fluid, production of cytokines, including IFN-γ, was increased, with high production of IL-4, indicating switching from TH2 (34% of patients) to TH0 (66% of patients). Of the studied patients, 35% had a CD4:CD8 ratio <1.1 in blood, and also produced IL-12 (94.5% of cells) and IFN-γ (30% of cells), as compared with patients in whom the CD4:CD8 ratio was >1.2.

Patients on CAPD who have peritonitis produce large amounts of pro-inflammatory and TH2 cytokines. More IFN-γ is produced in peritoneal fluid than in blood, which suggests more inflammation. Immunodeviation TH2 is seen in blood and peritoneal fluid of CAPD patients with peritonitis. Patients with a CD4:CD8 ratio <1.1 produce more IFN-γ and IL-12, and are more able to switch from TH2 to TH0.

Key words
Peritonitis, cytokines, TH0/TH1, peritoneal fluid, blood

Introduction
Continuous ambulatory peritoneal dialysis (CAPD) is a therapy for patients with kidney failure. The most frequent complication of CAPD is recurrent peritonitis, leading to fibrosis and irreversible loss of the dialysis ability (1). Interleukins (ILs) or cytokines are communication signals of the immune system that are produced during inflammation. Interleukins are important in immune regulation, homeostasis, and tissue repair (2—4). Excess or deficit of ILs causes undesirable consequences such as sepsis, chronic inflammation, fibrosis, and failure to clear infections (2—5). Investigating cytokines is basic to understanding bodily defense in infections and complications (4,5).

The cytokines secreted by monocytes are called monokines. According to their biologic action, the monokines are divided into pro-inflammatory IL-1,
IL-6, IL-8, tumor necrosis factor α (TNFα), IL-12, interferon-α (IFN-α) and anti-inflammatory IL-10 and transforming growth factor β (TGFβ) (4,5). The cytokines produced by lymphocytes are called lymphokines.

Several subsets of T-helper lymphocytes [TH cells (CD4+)] exist. They are differentiated by their cytokine profiles (6,7): TH1 lymphocytes make interferon-γ (IFN-γ) and IL-2; TH2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13; and TH0 cells secrete TH1 and TH2 cytokines at the same time (8). The TH subsets regulate the response to micro-organisms and influence immunologic and infectious diseases (6,7). The TH1 cells direct cellular immunity and delayed-type hypersensitivity. The TH2 cells help B cells to secrete the various classes of antibodies in humoral immunity. The system is cross-regulating in that TH1 cells support their own development, while inhibiting TH2 activity. Conversely, TH2 cells inhibit TH1 responses while helping themselves. Defective cytokine production therefore causes undesirable consequences (9,10).

The present project used flow cytometry (11) to explore the production of pro-inflammatory cytokines IL-1α, IL-6, IL-12, and TNFα, and the lymphokines IFN-γ and IL-4, in cells from the peritoneal fluid and blood of CAPD patients with peritonitis.

Patients and methods
Samples of blood and peritoneal fluid from 9 CAPD patients with peritonitis were taken in the morning at the first change of dialysis fluid. Of the 9 patients, 6 (65%) were women, and 3 (35%) were men. The median age was 50.26 ± 14.85 years. The patients were from the CAPD program of Nephrologic San Jos Kidney Diseases unit in the department of Internal Medicine of the Health Sciences Faculty, University of Cauca, and from the dialysis program RTS Cauca in Popayán, Colombia. Patients used four daily exchanges of Dianeal (Baxter Healthcare Corporation, Deerfield, IL, U.S.A.). The criteria for a diagnosis of peritonitis were (A) cloudy dialysis fluid or abdominal pain or fever; (B) more than 100 leukocytes/mm³, with at least 50% neutrophils in peritoneal fluid; and (C) micro-organisms detected by Gram stain or culture of the fluid. Samples for analysis were grown in a 6-hour cell culture. Intracellular cytokines were detected using a FACScalibur flow cytometer (11) with CellQuest software (Becton—Dickinson, San José, CA, U.S.A.).

Intracellular detection of cytokines by flow cytometry

FUNDAMENTALS OF THE TECHNIQUE
Peritoneal cells were concentrated in a centrifuge at 1500 rpm for 10’ minutes. Blood cells and peritoneal cells were placed in culture for 6 hours with and without polyclonal stimulation by mitogens [phorbol 12-myristate 13-acetate (PMA) and ionomicin], and were exposed to Brefeldin’A (Sigma, St. Louis, MO, U.S.A.) or GolgiStop (Becton—Dickinson) to inhibit the Golgi system, enhancing intracellular concentration of proteins for detection by fluorescent monoclonal antibodies introduced after cell permeabilization with saponin (Cytofix/Cytoperm: Becton—Dickinson).

The following fluorescent anti-cytokine antibodies were used [fluorescein isothiocyanate conjugate (FITC)/phycoerythrin (PE)/fluorescent chlorophilic protein of 35 kDa from Glenodinium species (PerCP)]: anti—IL-α FITC mouse immunoglobulin G1 (IgG1), clone 364-3B3-14); IL-6 PE (rat IgG1, clone MQ2-13A5)/CD3 PerCp, anti—IL-12 FITC (mouse IgG1, clone C11.5)/TNFα PE (mouse IgG1, clone Mab11)/CD3 PerCP; and IL-4 FITC (rat IgG2b, clone BV4-1D11)/IFN-γ PE (rat IgG1, clone XMG1.2)/CD3 (Pharmingen, San Diego, CA, U.S.A.). To probe cell activation, we used anti-CD14 FITC (mouse IgG2b, clone MF9) that labels monocytes by surface staining and anti-CD69 PE (mouse IgG1, clone L78), which indicates early activation (Becton—Dickinson) in intracellular staining. Anti-CD8 FITC (IgG1, clone SK1) and anti-CD4 PE (mouse IgG1, clone SK3) were used for identification of T-cell subsets and to determine the CD4:CD8 ratio. Negative fluorescence control was performed using the monoclonal antibodies R35-95 (rat IgG, clone 2APE) and MOPC-21 (mouse IgG1) FITC (Pharmingen).

CELL CULTURE AND STIMULATION
Two files of tubes were used: with and without activation. Blood or peritoneal cell suspension (100 µL) was placed in 12×74 polypropylene tubes with 100 µL RPMI-1640 complete medium [supplemented with 2 mmol/L L-glutamine, 250 U/mL penicillin G, 250 µg streptomycin (Gibco BRL, Rockville, MD, U.S.A.), 50 mmol/L mercaptoethanol, and 10% bovine fetal serum]. Afterward, 10 µL Brefeldin’A was added (stock solution, 1 mg/mL); and, after that, cell activation was done after adding 10 µL ionomicin (stock
solution, 1 μg/mL) and 20 μL PMA (stock solution, 25 ng/mL). The samples were then placed in a cell culture cabinet at 37°C in a 5% CO₂ atmosphere for 6 hours.

LYSING RED CELLS
After incubation, 2 mL red-cell lysing solution (FACS: Becton—Dickinson) was added to each tube. The tubes were then incubated again for 10 minutes at 37°C. Afterward, the cells were washed twice, 1 mL phosphate buffered saline (PBS) solution was added, and centrifugation took place at 1500 rpm for 10 and 5 minutes.

BLOCKADE OF FC RECEPTORS
After the supernatant was removed, the cell pellets were incubated in 50 μL human IgG for 20 minutes at 4°C in darkness (to block Fc receptors), thus avoiding unspecific binding of fluorescent antibodies.

SURFACE STAINING
To the respective tubes, 20 μL anti-CD3 FITC, CD8 FITC, CD4 PE, and anti-CD14 FITC were added, with incubation for 30 minutes at 4°C in the dark, and a single wash with PBS.

CELL PERMEABILIZATION
After centrifuging, 250 μL permeabilization solution [saponin (Cytofix/Cytoperm: Becton—Dickinson)] was added to each tube. The cells were then incubated for 10 minutes at 4°C. Afterward, the cells were washed twice with 1 mL Perm/Wash (Becton—Dickinson) and centrifuged for 5 minutes at 1500 rpm. Finally, the supernatant was removed.

INTRACELLULAR STAINING
Cells were diluted in 100 μL Perm/Wash, and 20 μL anti-cytokine antibodies were added to the respective tubes, which were then incubated 40 minutes at 4°C in the dark. Afterward, the cells were washed once with 2 mL PBS, and centrifuged at 1500 rpm for 5 minutes. The supernatant was then removed, and the pellet was suspended in 500 μL 1% paraformaldehyde (PFA) solution for fixing the antibodies. The cells were then analyzed in the FACScalibur flow cytometer.

STATISTICAL ANALYSIS
The results were analyzed using STATA® 6.0 in the Clinical Epidemiology unit (INCLEN) of the Faculty of Health Sciences, University of Cauca. Median and dispersion measures (range, standard deviation, and percentages) were obtained.

The information acquired is held confidentially in the clinical records, in fulfillment of the ethical principles of research. The data generated from the project will be useful for improving the health care of our CAPD patients.

Results
Tables I and II show cytokine production, the CD4:CD8 ratio, and CD69 expression in peritoneal fluid and blood of CAPD patients with peritonitis. The values of the CD4:CD8 ratio covered a broad range, but each patient had a similar ratio in both sample types.

With regard to the CD4:CD8 ratio, we identified two different groups of patients. In group A, the ratio was >1.1 (65% of patients; mean in blood: 2.24; range in blood: 1.9—5; mean in peritoneal fluid: 2.4; range in peritoneal fluid: 1.2—4.6); and, in group B, the ratio was <1.1 (35% of patients; mean in blood: 0.69; range in blood: 0.2—4.6); mean in peritoneal fluid: 0.66; range in peritoneal fluid: 0.4—1.0).

Without stimulation, all patients (100%) produced high levels of IL-1α, IL-6, IL-12, TNFα, and IL-4 in both samples (general mean: 89.2%±6.3% of cells in blood, and 81.6%±10.1% in peritoneal fluid; Tables I and II), which suggests immune activation in vivo.

With stimulation, 25% of patients had few cells producing IFN-γ in blood (mean: 15.7%; range: 1%—48% of cells); 34% of patients expressed a little more IFN-γ in peritoneal fluid (mean: 24.5%; range: 0%—94% of cells; Tables I and II), which suggests immune activation in vivo.

Patients with a CD4:CD8 ratio <1.1 (group B) produced more IL-12 and IFN-γ in blood and peritoneal fluid than did those with a ratio >1.2 (group A). Values in group B for IL-12 were 94.5% (mean) and 92%—94% of cells (range), as compared with group A (mean: 70.57%; range: 1%—100% of cells). Production of IFN-γ in group B was 30% (mean) and 10%—50% of cells (range); in group A, with stimulation, IFN-γ increased in 75% of patients (mean: 39% of cells), and IL-4 levels and pro-inflammatory cytokines remained high.

In unstimulated monocytes and lymphocytes, all patients (100%) had high expression of CD69 (mean: 70% of cells; range: 60%—80%) which indicates immune activation in vivo. After stimulation, 100%
### TABLE I Intracellular cytokine production by CD4:CD8 ratio in the peritoneal fluid of 9 patients on continuous ambulatory peritoneal dialysis who had peritonitis

<table>
<thead>
<tr>
<th>CD4:CD8 ratio</th>
<th>&gt;1.1 (= 66% of patients)</th>
<th>&lt;1.1 (= 34% of patients)</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean: 2.47) (range: 1.2—4.6)</td>
<td>(mean: 0.66) (range: 0.4—1.0)</td>
<td>(mean: 1.82) (range: 0.4—4.6)</td>
</tr>
<tr>
<td>No activation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td>84%</td>
<td>65%</td>
<td>72.55%</td>
</tr>
<tr>
<td>IL-6</td>
<td>87.42%</td>
<td>85%</td>
<td>77.44%</td>
</tr>
<tr>
<td>TNFα</td>
<td>84.57%</td>
<td>93%</td>
<td>75.55%</td>
</tr>
<tr>
<td>IL-12</td>
<td>70.57%</td>
<td>99.5%</td>
<td>97.22%</td>
</tr>
<tr>
<td>IL-4</td>
<td>96.57%</td>
<td>47%</td>
<td>24.55%</td>
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<tr>
<td>IFN-γ</td>
<td>18.14%</td>
<td>50%</td>
<td>70%</td>
</tr>
<tr>
<td>Activated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td>78.28%</td>
<td>70.5%</td>
<td>76.44%</td>
</tr>
<tr>
<td>IL-6</td>
<td>70.71%</td>
<td>85%</td>
<td>74.88%</td>
</tr>
<tr>
<td>TNFα</td>
<td>77.42%</td>
<td>92%</td>
<td>80.66%</td>
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<td>IL-4</td>
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<td>82.55%</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>37.85%</td>
<td>70%</td>
<td>45%</td>
</tr>
<tr>
<td>Expression of CD69 in patients</td>
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<td></td>
</tr>
<tr>
<td>No activation</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>TH1</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>TH0</td>
<td>14.28%</td>
<td>66.00%</td>
<td>34.00%</td>
</tr>
<tr>
<td>TH2</td>
<td>71.42%</td>
<td>34.00%</td>
<td>66.00%</td>
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<td>TH1</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>TH0</td>
<td>66.66%</td>
<td>66.66%</td>
<td>66.66%</td>
</tr>
<tr>
<td>TH2</td>
<td>33.33%</td>
<td>33.33%</td>
<td>33.33%</td>
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</tbody>
</table>

### TABLE II CD4:CD8 ratio and intracellular cytokine production in blood of 9 patients on continuous ambulatory peritoneal dialysis with peritonitis

<table>
<thead>
<tr>
<th>CD4:CD8 ratio</th>
<th>&gt;1.1 (= 64% of patients)</th>
<th>&lt;1 (= 34% of patients)</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean: 2.24) (range: 1—5)</td>
<td>(mean: 0.69) (range: 0.3—1.2)</td>
<td>(mean: 1.75) (range: 0.3—5.2)</td>
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<tr>
<td>No activation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td>97.83%</td>
<td>84.5%</td>
<td>94.5%</td>
</tr>
<tr>
<td>IL-6</td>
<td>89.33%</td>
<td>84.5%</td>
<td>88.1%</td>
</tr>
<tr>
<td>TNFα</td>
<td>90%</td>
<td>90%</td>
<td>90%</td>
</tr>
<tr>
<td>IL-12</td>
<td>74%</td>
<td>94.5%</td>
<td>89.2%</td>
</tr>
<tr>
<td>IL-4</td>
<td>93.5%</td>
<td>98%</td>
<td>94.6%</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>11%</td>
<td>30%</td>
<td>15.75%</td>
</tr>
<tr>
<td>Activated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td>97%</td>
<td>84.5%</td>
<td>93.8%</td>
</tr>
<tr>
<td>IL-6</td>
<td>92%</td>
<td>79.5%</td>
<td>88.8%</td>
</tr>
<tr>
<td>TNFα</td>
<td>75.16%</td>
<td>89%</td>
<td>89.75%</td>
</tr>
<tr>
<td>IL-4</td>
<td>99%</td>
<td>99%</td>
<td>99.25%</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>37.16%</td>
<td>45%</td>
<td>39.12%</td>
</tr>
<tr>
<td>No activation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH1</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>TH0</td>
<td>16.6%</td>
<td>50.00%</td>
<td>25.00%</td>
</tr>
<tr>
<td>TH2</td>
<td>83.3%</td>
<td>50.00%</td>
<td>75.00%</td>
</tr>
<tr>
<td>TH1</td>
<td>0.00%</td>
<td>40.00%</td>
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<tr>
<td>TH0</td>
<td>66.66%</td>
<td>100.00%</td>
<td>75.00%</td>
</tr>
<tr>
<td>TH2</td>
<td>33.33%</td>
<td>0.00%</td>
<td>25.00%</td>
</tr>
</tbody>
</table>
Cytokines in CAPD Peritonitis

of monocytes and lymphocytes expressed CD69, indicating successful stimulation in vitro.

Discussion
The present study in CAPD patients with peritonitis found these facts:

1. A large percentage of the immune cells in the blood and peritoneal fluid of CAPD patients with peritonitis are activated in vivo (they express CD69). Furthermore, they produce a lot of cytokines without stimulation.

2. A deficit of IFN-γ exists in the blood and peritoneal fluid, especially when the patient's CD4:CD8 ratio is >1.1. Production improves a little after stimulation. Those findings suggest that CAPD patients with peritonitis have cellular immunodeficiency, because they show TH2 immunodeviation (excessive IL-4 and lack of IFN-γ) and are therefore unable to produce a TH1 response.

3. The CD4:CD8 ratio is important in CAPD patients with peritonitis, because it divides patients into two groups that differ in secretion of IL-12 and IFN-γ. Patients with a ratio <1.1 produce more cytokines in vivo and are better able to switch from TH2 to TH0 with stimulation.

Those data, together with the scanty literature about peritonitis in CAPD immunology suggest certain hypotheses.

Immune activation
Expression of CD69 in blood and peritoneal fluid does not differentiate infected from noninfected CAPD patients, because several reports showed immune activation in CAPD patients without evidence of infection (9). For example, in 1989, Davies et al (9) found increased chemiluminescence of blood monocytes in CAPD patients (more in peritoneal cells), and high expression of HLA-dr on T cells. In parallel work in our laboratory, we found activation demonstrated as high levels of HLA-dr and CD69 in the blood of 26 CAPD patients without peritonitis. (Production was even more intense in hemodialysis.) That production complements the increase of neutrophils in the peritoneum (400-fold) during the 3 weeks after the resolution of peritonitis, and the increase in cell death and production of pro-inflammatory cytokine TGFβ [always elevated noninfected CAPD patients with higher levels in peritonitis (1)]. Also, Lu et al saw increased IL-10 and IL-2R during peritonitis, while IFN-γ and IL-2 were just detectable in cases of peritonitis by Staphylococcus aureus and S. epidermidis (13).

Other cytokines that were seen by Saionji et al (8) and Saionji and Osaka (14) to be elevated in patients on hemodialysis and CAPD without infection were monocyte and granulocyte colony-stimulating factor (M-CSF, G-CSF), together with expansion of CD14+CD16+ cells as compared with normal controls. Also, Takahashi found high expression of mRNA of TNFα, IL-1β, and IL-6 in blood cells of noninfected patients on CAPD and hemodialysis (10).

The production of pro-inflammatory cytokines is associated with reports about production in vivo of the anti-inflammatory cytokines TGFβ (15) and IL-10 (13) in infected and noninfected CAPD patients, which suggests a need for new studies that globally explore the relationship between cytokines and other factors in CAPD patients.

Immunodeviation TH2 and deficit of IFN-γ: cellular immunodeficiency?
The present study introduces the TH1/TH2/TH0 concept (6,7) in CAPD with peritonitis. A strong TH2 pattern is seen in vivo and as a tendency to switch to TH0 with stimulation, all of which indicate a functional T-cell immunodeficit. In a parallel study in our lab, the same behavior of cytokines was seen in asymptomatic CAPD patients. The same immunodeviation was also reported by Yokoyama (16) in asymptomatic CAPD patients. The TH2 converts to TH3 (17), because reports exist of increased TGFβ and IL-10 in CAPD patients. [Those cytokines are characteristic of a new subset of lymphocytes (TH3), which are immunosuppressors and inducers of tolerance (18).]

Is the CD4:CD8 ratio in CAPD patients with peritonitis useful?
Other data from the present project enable the division of CAPD patients into two groups according to whether the CD4:CD8 ratio is closer to the normal value of 1.5
(group 'A' and group 'B'). Those groups showed differences in the secretion of IL-12 and IFN-γ, which suggests a need for new studies looking at the reason for, and the meaning of, that ratio. The observations of our lab associate the low CD4:CD8 ratio to an expansion of CD8 cells and a reduction of CD4 cells, such as is seen in chronic virus infections such as infection with the human immunodeficiency virus (HIV).

Other unanswered questions include these: What is the clinical meaning of the various ratios in fibrosis and other complications? Is the CD4:CD8 ratio important in regard to transplantation? Is it possible to regulate the immune system toward TH1? Could such regulation reduce peritonitis and fibrosis?

Conclusions
1. Patients on CAPD with peritonitis produce a large amount of pro-inflammatory and TH2 cytokines.
2. More IFN-γ is produced in peritoneal fluid, which suggests more intense inflammation in the peritoneum.
3. Immunodeviation TH2 is seen in blood and peritoneal fluid of CAPD patients with peritonitis.
4. Patients with a CD4:CD8 ratio < 1.1 produce more IFN-γ and IL-12, and are more able to switch from TH2 to TH0.

Acknowledgment
Our thanks go to the CAPD patients in the programs that participated in this project. The work was financially supported by the Vicerectory of Investigations of the University of Cauca and the Kidney Unit Nephrologic San José, Popayán, Colombia.

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19 Marin GH, Menna ME, Saba S, et al. Flow cyto-


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Immunophenotyping by Flow Cytometry of Peritoneal Fluid of Patients with Peritonitis on Continuous Ambulatory Peritoneal Dialysis

Our project identified, by flow cytometry, the immunophenotypes and activation state of the immune cells in the peritoneal dialysis fluid from patients with peritonitis on continuous ambulatory peritoneal dialysis. The results showed that all kinds of cells of the immune system were present in the peritoneal fluid in percentages and activation states similar to those seen in blood. Also, two subgroups of patients were noted, according to CD4:CD8 ratio. Patients whose ratio was <1.1 had more expansion of CD8 and NK cells, and a higher percentage of B1 cells in both fluids than were seen in healthy people.

Key words
Immunophenotype, CD4:CD8 ratio, flow cytometry, peritonitis

Introduction
Continuous ambulatory peritoneal dialysis (CAPD) is a therapy technique for patients with kidney failure. The most frequent complication of CAPD is recurrent peritonitis, which induces fibrosis and irreversible damage of the dialytic capacity of the membrane (1—5). Cells of the immune system act to defend against microorganisms in the peritoneum and trigger complications.

The immune system is a group of cells and molecules that are organized and that communicate for vigilance, defense, and homeostasis of the host.

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The objective of the present project was to identify, by flow cytometry, the presence and activity of the various populations of immune cells in the peritoneal fluid in CAPD patients with peritonitis (9,10).

**Patients and methods**

Samples of peritoneal fluid from 26 CAPD patients with peritonitis were taken at the first change of dialysis fluid. Patients were from the CAPD program of the Nephrological San José Kidney Diseases unit, department of Internal Medicine of the Health Sciences Faculty, University of Cauca, and the dialysis program of RTS Cauca in Popayán, Colombia. Dialysis therapy was performed using four daily exchanges of Dianeal (Baxter Healthcare Corporation, Deerfield, IL, U.S.A.). The criteria for diagnosing peritonitis were (A) cloudy dialysis fluid or abdominal pain or fever; (B) more than 100 leukocytes/mm³, with at least 50% neutrophils in the fluid; and (C) detection of microorganisms by Gram stain or culture of the dialysis fluid.

Samples were centrifuged for cell concentration and staining with 20 μL of these fluorescent monoclonal antibodies [fluorescein isothiocyanate conjugate (FITC)/phycoerythrin (PE)/fluorescent chlorophilic protein of 35 kDa from Glenodinium species (PerCP)]: 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 José, 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 in a 5% CO₂ atmosphere for 10 minutes, the cells were centrifuged at 1500 rpm for 10 minutes. The supernatant was then removed in a vacuum, and the cell pellet was washed twice in phosphate-buffered saline (PBS). Finally, the pellets were resuspended in 0.5 mL PBS and read in a FACScalibur flow cytometer with CellQuest software (Becton—Dickinson).

**Statistical analysis**

The data were analyzed using STATA™6.0 at the Clinical Epidemiology unit (INCLEN) of the Health Sciences Faculty, University of Cauca. Data are provided as median and dispersion measures (range, standard deviation, and percentages).

Information is kept confidential in the clinical records, in fulfillment of the ethical principles of research. The data generated from the project will be useful for improving the health care of our CAPD patients.

**Results**

Tables I and II show our data.

1. Peritoneal fluid and blood showed the same percentages of eosinophils, neutrophils, monocytes, and lymphocytes.
2. The values of the CD4:CD8 ratio ranged broadly, but were similar in peritoneal fluid and blood for each individual patient.
3. Two groups of patients were identified according to CD4:CD8 ratio: group A (64% of patients) had a ratio > 1.2 (mean: 2.47; range: 1.2—5.0). Group B (36% of patients) had a ratio < 1.1 (mean: 0.66; range: 0.4—1.1).
4. In group A patients, the number of CD4+ cells in the peritoneal fluid and blood was higher (mean in peritoneal fluid: 376 cells/mm³; range: 15—119 cells/mm³) than in patients from group B (mean in peritoneal fluid: 81 cells/mm³; range: 18—234 cells/mm³, p < 0.005, Mantel-Haenszel).
5. Compared to the patients in group A, the group B patients had a higher number of cytotoxic CD8 cells (126 cells/mm³ vs 80.2 cells/mm³) and more expansion of NK cells (67% vs 50%). Expansion of NK cells was defined as CD56+ cells > 300/mm³. (See Table II.)
6. All patients (100%) had strong expression of HLA-DR on T cells (42.9% of cells) in blood, indicating immune activation as compared with healthy volunteers (<8%).
7. All patients (100%) showed high percentages of B1 lymphocytes in blood (40% of cells) as compared with healthy volunteers.

**Discussion**

Our study provides evidence that, in the peritoneal fluid of CAPD patients with peritonitis, all of the immunologic cells are present in the same percentages in which they appear in blood. The cells are also activated in both blood and peritoneal fluid, which has been reported several times (2—4). For example, Lai et al (2) found increased numbers of neutrophils in the peritoneal fluid (400-fold) until 3 weeks after clinical resolution of peritonitis, along
with increased numbers of dead mesothelial cells and production of pro-inflammatory cytokines and transforming growth factor β (TGFβ). Also, in 1989, Davies et al (4) found immune activation in asymptomatic CAPD patients: they showed more intense chemiluminescence of blood monocytes than did healthy controls, and the result was even more intense in peritoneal macrophages. Those researchers also saw high expression of HLA-DR on T lymphocytes (32% in peritoneum and 13.8% in blood of CAPD patients without peritonitis as compared with healthy controls at 11.3%) and monocytes (76% in peritoneum and 46% in blood), plus high expression of other activation markers such as IL-2 receptor, IgG receptors, and RFD7. In parallel work in our lab, we saw important expression of HLA-DR (22.4% of cells) and CD69 (70% of cells) in 26 CAPD patients without peritonitis; however, that result was lower than the expression of HLA-DR found in the present work in patients with peritonitis (42.92% of cells).

Grzegorzewska and Leander (11) followed the immunophenotypes of asymptomatic CAPD patients over time and saw an increase in lymphocytes and in the CD4:CD8 ratio during the first months of therapy. However, after some time, those cells decreased and NK cells increased. The present study also shows increased NK and B1 cells. Moreover, the CD4:CD8 ratio differentiated two subpopulations of patients: patients with a ratio < 1.1 had more cytotoxic cells such as CD8 and NK cells. That finding could have importance in various clinical settings for example, transplantation (12). In clinical observations in our laboratory, low CD4:CD8 ratio is associated with viral infections such as cytomegalovirus (CMV) and human immunodeficiency virus (HIV).

Normally, B1 cells are found in high percentages in human and mouse peritoneum, but in blood they are low. The present study found high percentages of B1 cells in the blood of CAPD patients with peritonitis, an important new basic finding about that type of cell.

**TABLE I** Immunophenotypes in peritoneal fluid of 26 patients with peritonitis on continuous ambulatory peritoneal dialysis

<table>
<thead>
<tr>
<th></th>
<th>CD4:CD8 &gt; 1.2 (64% of patients)</th>
<th>CD4:CD8 &lt; 1.1 (36% of patients)</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>CD4:CD8</td>
<td>2.47</td>
<td>1.2—4.6</td>
<td>0.66</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>8.40%</td>
<td>2%—25%</td>
<td>9.20%</td>
</tr>
<tr>
<td>Monocytes</td>
<td>4.90%</td>
<td>2%—1%</td>
<td>6.80%</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>57.20%</td>
<td>6%—92%</td>
<td>65%</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>3.80%</td>
<td>1%—20%</td>
<td>3.00%</td>
</tr>
<tr>
<td>TCD4+ cells/mm³</td>
<td>376</td>
<td>15—119</td>
<td>81</td>
</tr>
<tr>
<td>TCD8+ cells/mm³</td>
<td>80</td>
<td>5—333</td>
<td>126</td>
</tr>
<tr>
<td>T’cells CD8+CD28+</td>
<td>47.70%</td>
<td>24%—82%</td>
<td>53.60%</td>
</tr>
<tr>
<td>T’cells CD8+CD28—</td>
<td>41.80%</td>
<td>18%—75%</td>
<td>38.50%</td>
</tr>
<tr>
<td>T active HLA-DR+</td>
<td>43%</td>
<td>4%—77%</td>
<td>39%</td>
</tr>
<tr>
<td>B1 cells CD19+CD5+</td>
<td>39.10%</td>
<td>10%—77%</td>
<td>42.00%</td>
</tr>
<tr>
<td>NK cells (expansion)</td>
<td>Yes: 50%</td>
<td>Yes: 67%</td>
<td>Yes: 56%</td>
</tr>
</tbody>
</table>

**TABLE II** Immunophenotypes in blood of 26 patients with peritonitis on continuous ambulatory peritoneal dialysis

<table>
<thead>
<tr>
<th></th>
<th>CD4:CD8 &gt; 1.2 (64% of patients)</th>
<th>CD4:CD8 &lt; 1.1 (36% of patients)</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>CD4:CD8</td>
<td>2.24</td>
<td>1—5</td>
<td>0.69</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>13.8%</td>
<td>3%—36%</td>
<td>12.5%</td>
</tr>
<tr>
<td>Monocytes</td>
<td>5.7%</td>
<td>1%—10%</td>
<td>6.5%</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>64%</td>
<td>25%—81%</td>
<td>68.2%</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>6.9%</td>
<td>1%—42%</td>
<td>4.6%</td>
</tr>
<tr>
<td>CD4+ cells/mm³</td>
<td>504.2</td>
<td>335—978</td>
<td>248</td>
</tr>
</tbody>
</table>
Conclusions
1. An important immune activation occurs in the peritoneum of CAPD patients with peritonitis, with the presence of eosinophils, neutrophils, monocytes, TCD4, TCD8, B1 and B2 lymphocytes, and expansion of NK cells.
2. Two subpopulations of patients exist: one with low and the other with normal or high CD4:CD8 ratio.
3. Patients with a lower CD4:CD8 ratio have lower numbers of lymphocytes, which suggests a more intense immunodeficit.

Acknowledgment
Our thanks go to the CAPD patients in the programs that participated in this project. The work was financially supported by the Vicerectory of Investigations of the University of Cauca and the kidney unit, Nephrologic San Jos, Popayn, Colombia.

References

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