RESEARCH ARTICLE

Effects of different peritoneal dialysis fluids on the TH1/TH2 balance

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ABSTRACT. *Background.* Peritoneal dialysis (PD) is associated with a depression of T cell function, as suggested by the impaired production of cytokines by Th cells collected from PD patients. Although treatment biocompatibility could be implicated in this immune dysfunction, it has been poorly investigated, thus far. Therefore, we undertook a study aiming to analyze the effects of different peritoneal dialysis fluids on the Th1/Th2 balance in PD patients. *Methods.* Twenty three patients on continuous ambulatory peritoneal dialysis (CAPD) were evaluated. Seven patients were on CAPD with icodextrin solution (ICO-PD), seven with glucose and lactate/bicarbonate-buffered solution (LAC/BIC-PD), and nine with glucose and lactate-buffered solution (LAC-PD). The Th1/Th2 balance was evaluated by measuring IFN-γ (Th1 subset) and IL-4 (Th2 subset), both in circulating and peritoneum-derived Th lymphocytes unstimulated or stimulated by phytohemoagglutinin (PHA). Moreover inflammatory, nutritional and dialysis-related parameters were recorded. Eight normal subjects comprised the control group (CON). *Results.* Circulating T cells: IFN-γ was significantly lower in the LAC-PD group (p<0.05) compared to the ICO-PD and LAC/BIC-PD groups. The IFN-γ/IL-4-producing cell ratio was significantly lower in PD patients than in CON. Peritoneal T cells: after 24-h PHA stimulation, IFN-γ increased in all patients, but the rise was less pronounced in the LAC-PD group (p<0.05) than in the other two PD groups. The Th1/Th2 ratio was significantly lower in the LAC-PD group when compared both to LAC/BIC-PD and ICO-PD groups. In addition, the LAC-PD group presented a significantly higher rate of peritoneal infections compared to the other PD groups. *Conclusions.* CAPD with lactate-buffered peritoneal fluid has deleterious effects on the Th1 cell subset, while the use of more biocompatible fluids, bicarbonate-buffered and icodextrin, is associated with a more physiologically representative Th1/Th2 balance and a reduced peritonitis rate.

Key words: peritoneal dialysis, peritoneal fluids, Th1/Th2 imbalance, icodextrin, biocompatibility

Patients with end-stage renal disease (ESRD) present a state of immunodeficiency that renders them liable to infections and malignancies, and unresponsive to vaccination [1]. This immunodeficiency is mainly the result of an altered function of various types of immune cells, including polymorphonuclear leukocytes, monocytes, natural killer cells and T lymphocytes [2]. CD4+ T-helper (Th) cells are central regulators of both humoral and cellular immune responses. Naïve Th cells can differentiate into distinct subsets, the most prominent of which are T-helper 1 (Th1), Th2, Th-17, and T-regulatory cells (Treg). Th1-type cells secrete IFN-γ, IL-2 and TNF-α, and are involved in cell-mediated immune responses, which include, for example, macrophage activation and delayed immune response. Th2-type cells secrete IL-4, IL-5, IL-10 and IL-13, which mainly regulate humoral-mediated immunity through the stimulation of B-cells producing antibodies [3, 4].

Recently, another subset of T-helper cells, the Th17 cells, has been characterized by the production of IL-17; in addition, they are also able to produce IL-21 and IL-22 [5]. Th17 cells are important for the clearance of a variety of pathogens, and it has been postulated that the primary function of Th17 cells is to clear pathogens that are not adequately handled by Th1 or Th2 cells [6]. Finally, Treg cells are specialized for regulating different aspects of immunity. These cells are defined on the basis of the expression of specific markers, such as CD25 and the transcription factor FoxP3+, and they control the immune responses of effector T-cells to auto- and allo-antigens [7, 8]. They have

been demonstrated to prevent organ-specific auto-immune diseases and to control anti-tumour, anti-viral and immune responses to allo-antigens in the setting of organ transplantation [9, 10].

In previous studies, we have shown that pre-dialysis uremia and hemodialysis are associated with a dysfunction of Th cells, resulting in a defective Th1 response to mitogenic stimulation and a functional prevalence of Th2 over Th1 [11]. It has been claimed that PD is more biocompatible than hemodialysis (HD), because it avoids the pathogenic effects resulting from the contact of blood with artificial membranes and the backfiltration from dialysate. However, PD seems to be associated with a depression of T cell function, as suggested by the impaired *in vitro* production of cytokines by Th cells collected from PD patients [12]. In addition, in a previous study we have demonstrated that peripheral blood mononuclear cells (PBMC) from PD patients *in vitro*, release abnormally high amounts of IL-6 and B2-microglobulin, comparable to those released by PBMC drawn from patients on HD [13]. These observations suggest that in PD patients an immune cell dysfunction occurs that may be relevant, not only for the ensuing risk of systemic and peritoneal infections, but also for the implications that peritoneal infections have on the feasibility and efficacy of PD treatment. In fact, PD efficacy depends strictly on the integrity and preservation of the peritoneal membrane, and infectious peritonitis is the most common cause of PD failure. In peritoneal effluent, different cell populations have been detected, such as macrophages, polymorphonuclear neutrophils and T cells, both Th1 and Th2 subtypes. These play a pivotal role in defending the peritoneum from infections [14]. It has been suggested that different Th1/Th2 subsets are associated with different outcomes of peritonitis. In particular, a prevalence of Th1 in peritoneal effluent correlates with a favorable outcome, possibly because IFN-γ enhances macrophage activity, offering a better defense from infectious agents [15].

These observations emphasize the importance of Th cells in the regulation of the immune response in patients on PD, raising the question of the role of PD modalities and the type of PD fluids (PDFs) on the differentiation and function of these cells. Bicarbonate-buffered and the more recent, glucose-free (amino acid and icodextrin) PDFs, have been introduced in clinical practice to improve biocompatibility and tolerability, and to preserve the structural and functional integrity of the peritoneum. In fact, *in vitro* and *in vivo* studies, both in animals and in patients, suggest that these PDFs are more biocompatible than conventional lactate-buffered PDFs [16], as they cause less peritoneal fibrosis and mesothelial cell dysfunction, and they prolong the preservation of peritoneum integrity [17, 18].

However, these observations have not been confirmed in larger randomized trials [19] and, until now, there has been no evidence concerning the impact of the use of new PDFs on firm clinical outcomes, such as mortality rate [20].

The potential effects and benefits of different PDFs remain under investigation. For this reason we performed the present study, focusing on a particular aspect of biocompatibility, *i.e.* the effects of PD solutions on the differentiation of circulating and peritoneal T cells into Th1 and Th2 subsets.

DONORS AND METHODS

Patients and treatments

We performed a multicenter observational study. Out of a total of 41 patients undergoing PD, 29 were under continuous ambulatory peritoneal dialysis (CAPD) and 12 on automatic peritoneal dialysis (APD). Because of the small number of APD patients and to minimize inter-individual variability, we focused our study only on patients undergoing CAPD for at least six months, and who had presented clinical stability during the previous three months. Patients with evidence of peritonitis or any acute or chronic infection, acute, intercurrent illnesses and those receiving immunosuppressive drugs were excluded. Out of the 29 CAPD patients, two were excluded because of recent episodes of peritonitis, one for pneumonia, one for rheumatoid arthritis under immunosuppressive treatment, while two patients refused consent to participate in the study. Thus, twenty three patients (10 males, 13 females, age 64.2±12.7 years) remained eligible for the study. Among these 23 CAPD patients, seven used icodextrin solution (Extraneal, Baxter Healthcare, ICO-PD group), seven glucose and lactate-bicarbonate buffered solution (Physioneal, Baxter Healthcare, LAC/BIC-PD group) and nine glucose and lactate-buffered solution (Dianeal, Baxter Healthcare, LAC-PD group). In the ICO-group, one daily icodextrin exchange was scheduled associated with exchanges with low glucose concentration (1.36%) bicarbonate-buffered solutions. The choice and management of individual treatments were the responsibility of the prescribing physicians. Medical history, clinical data, body mass index (BMI), incidence of diabetes and peritonitis, peritoneal transport characteristics, daily glucose load, routine monthly biochemistry data, dialysis prescription and drug therapy were analyzed. Residual renal function (RRF) was calculated as follows: (creatinine clearance mL/min+urea clearance mL/min)/2. Peritoneal transport was categorized by the peritoneal equilibration test (PET) using a 2.27% glucose solution and Twardowski's classification. The average daily glucose load for each patient was evaluated as the product of volume and glucose concentration for all daily exchanges, as previously described [21]. C-reactive protein (CRP) serum levels were measured by nephelometry (normal laboratory values 0-0.6 mg/dL), while serum albumin was evaluated using a routine laboratory method (normal laboratory values 3.7-5.7 g/dL).

Eight, healthy, age-matched individuals were included as the control group (four males, four females, age 66.7±13.5 years). The study was conducted in accordance with the Declaration of Helsinki, and all patients provided written, informed consent. The study was approved by the local Ethics Committee.

Cell collection and culture

Peritoneal fluid drawn from PD patients was collected after an overnight, 10-hour dwell time for the CAPD fluid. Immediately after drainage, the peritoneal fluid was ice-cooled and peritoneal fluid cells were isolated by gentle centrifugation (1,000 rpm, 15 minutes, 10°C) of 1,600-2,300 mL of the effluent. Cell pellets were washed twice with phosphate-buffered saline, and peritoneal mononuclear cells (PMC) were isolated by a

Table 1
Clinical characteristics of the patients studied.

	LAC-PD	LAC/BIC-PD	ICO-PD	CON
N	9	7	7	8
Male/female	4/5	3/4	3/4	4/4
Age, years	63.9±12.7	62.4±9.8	67.1±9.4	66.7±13.5
Dialysis age, months	23.9±15.7	26.8±17.7	22.6±16.5	-
Primary renal disease Chronic glomerulonephritis, number	1	2	2	-
Chronic pyelonephritis, number	1	0	0	-
Polycystic kidney disease, number	2	1	0	-
Obstructive uropathy, number	1	0	1	-
Hypertension, number	1	2	1	0
BMI, kg/m^2	29.8±3.8	29.3±2.7	28.4±3.6	23.4±1.7[@]
Diabetic patients, number (%)	2 (22)	2 (28)	2 (28)	0
Peritonitis, episode number/year	0.73±0.64 [§]	0.13±0.16	0.09±0.16	-
Albumin, g/dL	3.16±0.42	2.92±0.24	3.09±0.38	4.05± 0.14[°]
CRP, mg/dL	1.42±0.88	1.1±0.63	1.17±0.84	0.20±0.15[°]
RRF, mL/min	3.59±0.81	2.91±1.3	3.21±1.1	-
Daily glucose load, g/day	147.9±24.9	151.7±34.3	74.6±11.8*	-
KT/V	2.0±0.45	2.0±0.57	2.1±0.62	-
PET, D/P creatinine	0.64±0.25	0.72±0.12	0.69±0.19	-

PD: peritoneal dialysis; BMI: body mass index; CRP: C-reactive protein; RRF: residual renal function; PET: peritoneal equilibration test; KT/V: delivered dialysis dose.
[§] $p<0.05$ *vs* LAC/BIC-PD and ICO-PD; * $p<0.001$ *vs* LAC-PD and LAC/BIC-PD.
[@] $p<0.05$ *vs* LAC-PD and LAC/BIC-PD; [°] $p<0.05$ *vs* LAC-PD, LAC/BIC-PD and ICO-PD.

gradient centrifugation method using a lymphocyte separation medium (Mediatech Inc., Herndon, VA, USA). The average lymphocyte fractions in the PMC harvested from the three groups were similar: 31%, 33 % and 29% in LAC-PD, LAC/BIC-PD and ICO-PD, respectively. PMC viability, determined by the tryptan blue dye exclusion test, was always greater than 95%. Blood samples were collected from both healthy subjects and PD patients after the overnight exchange. Samples were drawn from a peripheral vein using a 20-gauge needle and applying gentle aspiration to minimize shear stress. PBMC were isolated from heparinized blood using the same gradient centrifugation method described in our previous paper [13].

The average lymphocyte and monocyte fractions were 76% (range 74-79%) and 24% (range 22-27%) respectively, and were similar in the three groups: 79%, 77% and 76%, respectively. The PBMC viability determined using the tryptan blue dye exclusion test was always greater than 95%.

Enzyme-linked immunosorbent spot (ELISPOT) assay

Th1 and Th2 cell functional subtypes were quantified by measuring the percentage of IFN-γ- and IL-4-producing cells using an ELISPOT assay, as previously described [22]. Briefly, 96-well plates (Polyfiltronics, Rockland, MA, USA) were coated overnight at 4°C with 100 μL of capture monoclonal antibodies (MoAb) specific for IFN-γ (clone 2G1, 5 μg/mL, Endogen, Woburn, MA, USA) in phosphate-buffered saline (PBS). Plates were

washed and blocked for 2 hours at room temperature in 10% FCS PBS, then 2×10^5 PBMC were resuspended in 100 μL of RPMI. Unstimulated or phytohemoagglutinin (PHA)-stimulated (15 μL/mL) plates were incubated for 48 hours at 37°C and then washed. Wells were coated with 100 μL IFN-γ MoAb (clone B133.5, 1 μg/mL, Endogen, Woburn, MA, USA) for 2 hours at 37°C. After washing, all wells were coated with 100 μL of HRP-conjugated streptavidin (Endogen, Woburn, MA,USA), incubated for 30 min, washed again and developed using a 3-amino-9-ethylcarbazole (Sigma-Aldrich, St. Louis, Mo, USA) filtered solution, obtained by diluting 1 mg into 30 mL of 0.1 mol/L sodium acetate buffer mixed with 15 mL of H_2O_2. IL-4-producing cells were measured using a commercial ELISPOT assay (Quantikine, R&D System, Minneapolis, USA), performed on microtiter plate. Spots were counted with an automated ELISA-Spot Assay Video Analysis System (A-EL-VIS, Hannover, Germany). Results were expressed as the number of spots/2×10^5 seeded cells.

Statistics

Data are expressed as mean±standard deviation. Statistical analysis was performed using analysis of variance for repeated measures (ANOVA), and Bonferroni's multiple comparisons test. The null hypothesis was rejected when the *P*-value was less than 0.05. All analyses were performed using the statistical package Stata 8.0 (Stata Corporation. College Station, Texas 77845 USA, 2003).
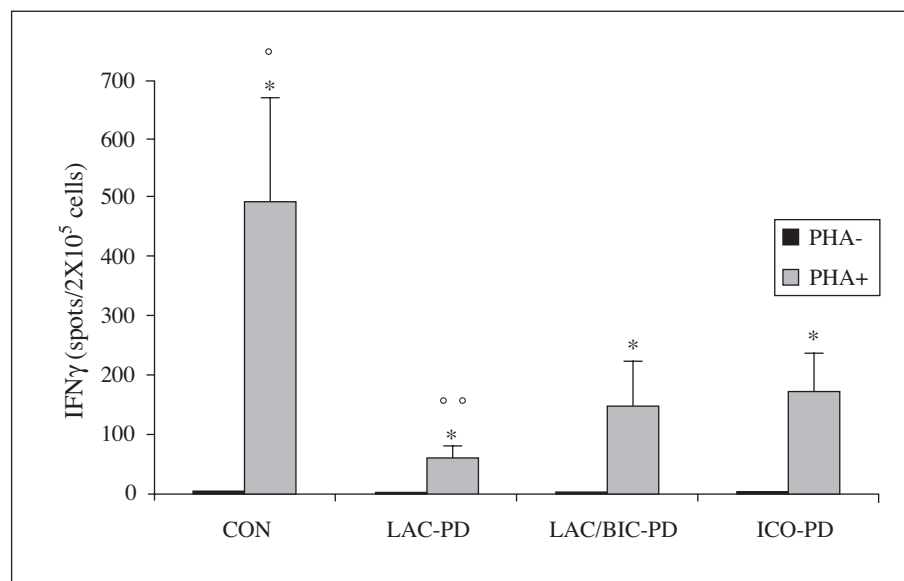
Figure 1
Frequency of IFN-γ-producing, circulating PBMC in unstimulated (PHA-) and stimulated (PHA+) cultures for normal subjects (CON) and for patients with ESRD maintained on continuous ambulatory peritoneal dialysis (CAPD). Seven patients were on CAPD with icodextrin (ICO-PD), seven with glucose and lactate/bicarbonate-buffered solution (LAC/BIC-PD), and nine with glucose and lactate-buffered solution (LAC-PD). * p<0.01 *vs* unstimulated PHA; ° p<0.01 *vs* all PD groups; °° p<0.05 *vs* ICO-PD and LAC/BIC-PD all PD groups.

RESULTS

Patients

The characteristics of the patients studied are summarized in *table 1*. They did not differ as regards age and gender, and the prevalence of diabetes was quite similar among the PD patients. BMI was very similar in all the PD patients. Time on PD, dialysis adequacy (expressed as weekly KT/V), and peritoneal transport characteristics were also similar. ICO-PD patients presented a significantly lower daily glucose load (48.6±10.7 g/day) when compared to both LAC/BIC-PD and LAC-PD (151.7±34.3 g/day and 147.9±24.9 g/day, p<0.001 *versus* ICO-PD).

Albumin levels were not significantly different between the three groups of PD patients (LAC-PD 3.16±0.42, LAC/BIC 2.92±0.24, ICO-PD 3.09±0.38 g/dL, p=0.67), although they were significantly lower in PD patients when compared to healthy subjects (4.05±0.14 g/dL, p<0.05 *versus* all PD patient groups). At the same time, serum CRP levels were not dissimilar among the patients (LAC-PD: 1.42±0.88, LAC/BIC 1.1±0.63, ICO-PD 1.17±0.84 mg/dL, p=0.66), whereas they were significantly higher in PD patients as compared to the control group (0.20±0.15 mg/dL, p<0.05 *versus* all PD patients).

Interestingly, the peritonitis rate in the LAC-PD group was significantly higher than in the ICO-PD and LAC/BIC-PD groups (0.73±0.64 *versus* 0.09±0.16 and 0.13±0.16 peritonitis/year, p<0.05, respectively).

Circulating PBMC

IFN-γ-producing cells
The number of unstimulated IFNγ-producing cells was similar in all groups *(figure 1)*. The number of PHA-stimulated, IFNγ-producing cells for all PD groups was significantly lower (ICO-PD: 174±71.5 spots/2×10^5 cells; LAC/BIC-PD: 146±82.8 spots/2×10^5 cells; LAC-PD: 58.3±26.4 spots/2×10^5 cells) when compared to CON (496±189 spots/2×10^5 cells; p<0.01). Moreover, IFNγ-producing cell numbers were significantly lower in the LAC-PD group (p<0.05) when compared to both ICO-PD and LAC/BIC-PD groups *(figure 1)*.

IL-4-producing cells
As shown in *figure 2*, the number of unstimulated, IL-4-producing cells was significantly higher in LAC-PD patients (p<0.05) than in the other groups. After PHA stimulation, no significant difference in IL-4-producing cells was observed between patients on PD and CON,

Table 2
Th1/Th2 (IFN-γ/IL-4) ratio in cultured, unstimulated (PHA-) and stimulated (PHA+) PBMC ,harvested from control subjects (CON) and patients undergoing CAPD involving three different methods (ICO-PD, LAC/BIC-PD, LAC-PD).

	Systemic PBMC		**Peritoneal MC**	
	IFN-γ/IL-4 (PHA-)	**IFN-γ/IL-4 (PHA+)**	**IFN-γ/IL-4 (PHA-)**	**IFN-γ/IL-4 (PHA+)**
CON	1.06±0.21	1.56±1.28	-	-
ICO-PD	0.62±0.13*	0.67±0.21*	1.07±0.40	0.92±0.52
LAC/BIC-PD	0.45±0.15*	0.60±0.28*	0.86±0.92	0.91±0.47
LAC-PD	0.18±0.09* °°	0.35±0.21*°°	0.63±0.36°	0.25±0.28°°

PBMC: peripheral blood mononuclear cells; MC: mononuclear cells.
* p<0.05 *vs* CON; ° p<0.05 *vs* ICO-PD; °° p<0.05 *vs* ICO-PD and LAC/BIC-PD.
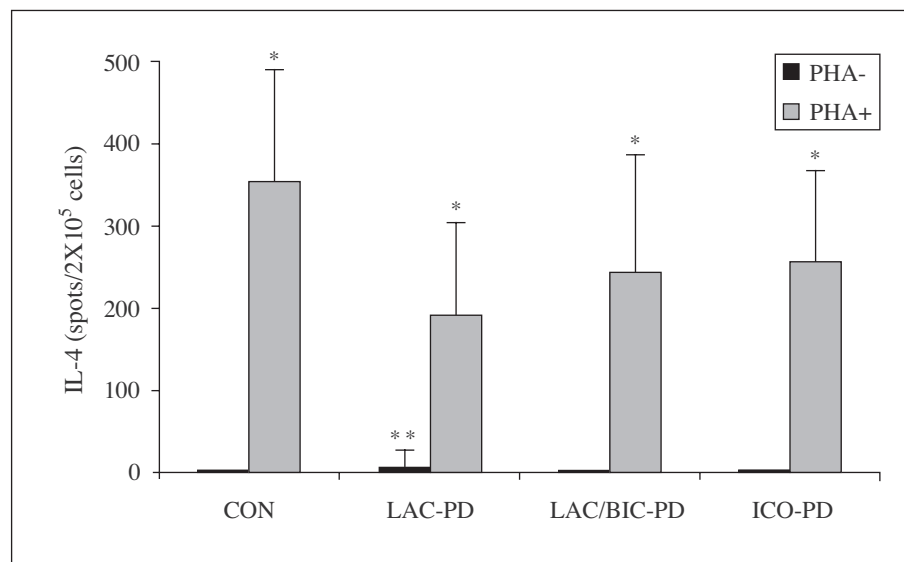
Figure 2
Frequency of IL-4-producing, circulating PBMC in unstimulated (PHA-) and stimulated (PHA+) cultures for normal subjects (CON) and for patients with ESRD maintained on continuous ambulatory peritoneal dialysis (CAPD). Seven patients were on CAPD with icodextrin (ICO-PD), seven with glucose and lactate/bicarbonate-buffered solution (LAC/BIC-PD), and nine with glucose and lactate-buffered solution (LAC-PD).
[*] $p < 0.01$ *vs* unstimulated PHA; [**] $p < 0.05$ *vs* other PD groups and CON.

independently of the peritoneal solution used (ICO-PD: 256 ± 128 spots/2×10^5 cells; LAC/BIC-PD: 242 ± 157 spots/2×10^5 cells; LAC-PD: 190 ± 132 spots/2×10^5 cells; CON 318.0 ± 182 spots/2×10^5 cells).

Th1/Th2 balance
The IFN-γ/IL-4-producing cell ratio, both in basal conditions and after mitogen stimulation, was significantly lower in the PBMC of PD patients compared to those of healthy controls ($p < 0.05$) *(table 2)*. This immune dysfunction was significantly accentuated in lactate-buffered PDF when compared to icodextrin and lactate-bicarbonate solutions ($p < 0.05$).

Peritoneal mononuclear cells

IFN-γ-producing cells
The number of unstimulated, IFN-γ-producing cells was similar in all groups *(figure 3)*. When cells were stimulated with PHA, IFN-γ-producing cells significantly increased in all PD groups (ICO-PD: 11.7 ± 7.5 spots/2×10^5 cells; LAC/BIC-PD: 9.5 ± 6.1 spots/2×10^5 cells; LAC-PD: 2.8 ± 0.43 spots/2×10^5 cells), but this increase was significantly lower in LAC-PD ($p < 0.05$ *versus* ICO-PD and LAC/BIC-PD).

IL-4-producing cells
As shown in *figure 4*, the number of IL-4-producing cells, both unstimulated and after mitogenic stimulation, was similar in all groups. After PHA stimulation, the number of IL-4-producing cells significantly increased in all PD groups (ICO-PD: 12.9 ± 10.7 spots/2×10^5 cells; LAC/BIC-PD: 10.4 ± 11.9 spots/2×10^5 cells; LAC-PD: 11.2 ± 2.6 spots/2×10^5 cells).

Th1/Th2 balance
The IFN-γ/IL-4-producing cell ratio is shown in *table 2*. As in PBMC, in PMC we also found a Th1/Th2 imbalance with Th2 polarization. The basal Th1/Th2 ratio was significantly lower in the LAC-PD group (0.63 ± 0.26; $p < 0.05$)

than in ICO-PD group (1.07 ± 0.4). After PHA stimulation, the IFN-γ/IL-4-producing cell ratio was significantly lower in the LAC-PD group (0.25 ± 0.28; $p < 0.05$) when compared to both LAC/BIC-PD (0.91 ± 0.47) and ICO-PD groups (0.92 ± 0.52).

DISCUSSION

The Th1/Th2 paradigm has evolved to provide a unifying model for a rational interpretation of T cell activity in different settings, including the response to infection, autoimmune diseases and immune deficiency. Peritonitis is a major problem in patients on PD, in whom the risk of infection is increased as a result of defective cell immunity. Recent studies suggest that, in patients on PD, susceptibility to peritonitis is due to an altered differentiation of T cells into Th1 and Th2 subsets, and clinical resolution depends on the development of a cell-mediated (Th1) immune response. In particular, local IL-12 and IL-18 production is part of a protective, early immune response to PD-related peritonitis. In fact, high IL-12 and IL-18 levels in PD effluent during the early phases of peritonitis correlated with a predominant, Th1 immune response and favorable outcome [23].

The present study, based on ELISPOT analysis, provides new information on this issue. In fact, we can extend to patients on PD the notion that renal disease is associated with a suppression of IFNγ production by circulating PBMC, similar to that which we have observed in patients on regular hemodialysis treatment. We previously reported that PBMC from uremic patients on regular hemodialysis treatment spontaneously produce abnormally high amounts of the Th1 cytokine IL-12 [11]. These phenomena may be causally related to the prevalence of Th2 lymphocytes, and suggest that the default of Th1 cells results from their continued stimulation, eventually causing functional exhaustion [13, 24]. It is possible that the same mechanism occurs in patients undergoing PD, since we have
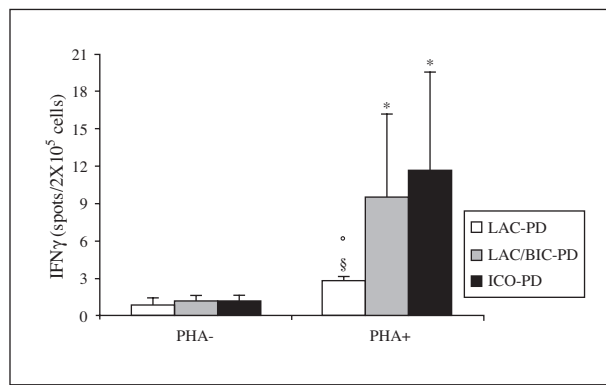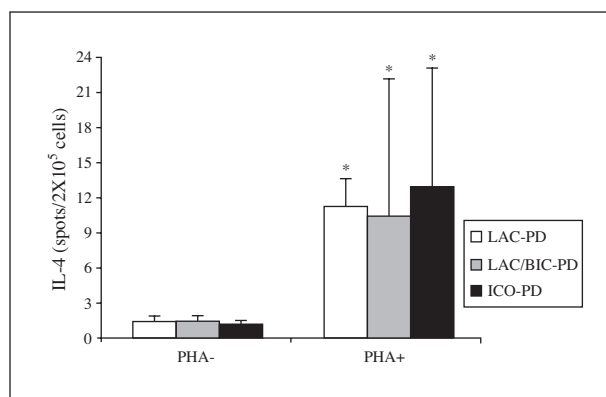
Figure 3
Frequency of IFN-γ-producing peritoneal mononuclear cells in unstimulated (PHA-) and stimulated (PHA+) cultures for patients with ESRD maintained on continuous ambulatory peritoneal dialysis (CAPD). Seven patients were on CAPD with icodextrin (ICO-PD), seven with glucose and lactate/bicarbonate-buffered solution (LAC/BIC-PD), and nine with glucose and lactate-buffered solution (LAC-PD).
* $p<0.01$ *vs* unstimulated PHA (ICO-PD and BIC-PD); § $p<0.05$ *vs* unstimulated PHA (LAC-PD); ° $p<0.05$ *vs* ICO-PD and LAC/BIC-PD.

demonstrated that mononuclear cells, drawn from both systemic circulation and peritoneal effluent, presented a Th2 polarization. Moreover, in the current study, the degree of immune dysfunction was variable, being more significant in lactate-buffered PDF when compared to icodextrin and bicarbonate solutions. Of particular interest is the unresponsiveness of peritoneal Th1 cells, exposed to lactate, to the powerful stimulation with PHA and the resulting overwhelming prevalence of Th2 cells, a dysfunction that could well explain the higher peritonitis rate found in LAC-treated patients. In fact, there is solid evidence that Th1 cells are the effectors of the immune defense against infective agents, while Th2 cells are tolerogenic, so that a prevalence of Th2 over Th1 impairs the immune response. The imbalance between Th cell subsets generated by lactate-buffered solution adds new evidence for the bioincompatibility of this solution. The use of PDFs is associated with a progressive loss of peritoneal membrane function, due to damage of mesenchymal cells, matrix

Figure 4
Frequency of IL-4-producing peritoneal mononuclear cells in unstimulated (PHA-) and stimulated (PHA+) cultures for patients with ESRD maintained on continuous ambulatory peritoneal dialysis (CAPD). Seven patients were on CAPD with icodextrin (ICO-PD), seven with glucose and lactate/bicarbonate -buffered solution (LAC/BIC-PD), and nine with glucose and lactate-buffered solution (LAC-PD).
* $p<0.01$ *vs* unstimulated PHA.

deposition and fibrosis [25]. *In vitro* studies have demonstrated that exposure to conventional lactate-buffered PDFs reduces mesenchymal cell and leukocyte survival, and impairs macrophage function [26]. Nevertheless, different studies in CAPD patients have proved the presence of chronic, sterile inflammation at the level of the peritoneum [27]. Overall, these data suggest that local inflammatory effects are strictly dependent on CAPD treatment *per se*.

The harmful factors potentially involved in this process include low pH, elevated concentrations of lactate, high osmolality and the presence of glucose degradation products (GDPs) [28]. GDPs are produced during the heat sterilization process and can induce the formation of advanced glycation end products (AGEs). The clinical consequences of AGE accumulation are not well known, but it has been implicated in dialysis-related amyloidosis, atherosclerosis and peritoneal membrane deterioration in PD [29]. These observations have led to the development of new, more biocompatible PDFs by decreasing acidity and replacing lactate buffer and glucose. Bicarbonate is a more physiologically compatible buffer than lactate, and it is now widely used for HD. So, lactate/bicarbonate or bicarbonate-buffered PDFs have been developed, which result in greater proliferation of mesenchymal cells and improvement of leukocyte functions as compared to lactate-buffered PDFs [30]. Moreover, the replacement of lactate with bicarbonate is related to a reduced formation of GDPs and AGEs [31].

More recently, glucose-free PDFs have been introduced into clinical practice.

Icodextrin is a high molecular weight, iso-osmolar glucose polymer, developed as an alternative osmotic agent for PD. It is necessary to underline that the presence of an osmotic agent in a PD fluid is mandatory, since osmosis is the driving force of ultrafiltration, the physical process that allows the removal of extracellular volume excess in PD patients. Glucose is the most widely used and studied osmotic agent, but its use is hampered by a number of side effects, such as diabetes, obesity and AGE production [32]. It was for this reason that icodextrin was developed. This is used mainly to treat diabetic patients and those with reduced ultrafiltration capacity, who need hyperosmotic glucose PD fluid to achieve an adequate volemic state [33, 34].

The results of many studies are now available which demonstrate that the use of icodextrin solutions allows higher ultrafiltration volumes, low glucose exposure and a more lasting preservation of residual renal function [35-37]. Different studies have also reported a better preservation of membrane function and mesenchymal cell survival in the course of icodextrin treatment [38]. However, the specific aspects of icodextrin as regards biocompatibility are not clear, since discordant results have been reported. The reduced production of vascular endothelial growth factor and pro-collagen III N-terminal peptide by mesothelial cells, as well as the reduced formation of GDP and AGEs in patients treated with icodextrin, instead of conventional PDFs, has been advanced as evidence of the biocompatibility of this treatment [39, 40]. On the other hand, some authors have reported the cytotoxicity of icodextrin and increased IL-6 and TNF levels in the effluents of patients receiving this treatment, suggesting the presence of an enhanced peritoneal inflammatory response [41, 42]. Actually, whether this increased

production of inflammatory cytokines is the expression of an enhanced capacity of the host defense or merely a marker of irritation and inflammation of the peritoneum, is still matter of debate [43].

A recent *in vitro* study on the effects of different PDFs on the cytokine production by PBMC, drawn from healthy subjects, showed that the use of icodextrin is associated with a more physiological PBMC function. In particular, the concentrations of IL-6 and TNF released by PBMC, cultured in lactate-buffered PDFs, were significantly lower when compared to the control medium. Conversely, PBMC incubated with icodextrin solution produced higher IL-6 and TNF concentrations, which were not significantly different from the control medium. Thus, the authors concluded that PBMC exposure to icodextrin results in fewer adverse effects on cytokine release when compared to glucose-containing lactate-buffered PDFs, which are mainly hypertonic solutions, of which icodextrin represents the most frequently used alternative [44].

Despite the large number of reported studies, no previous investigations have focused attention on the specific effects of icodextrin on T cell function and differentiation towards Th1 and Th2 subtypes. In the present study, we provide initial evidence that more biocompatible PDFs, such as bicarbonate-buffered and icodextrin solutions, improve Th cell function when compared to conventional lactate-buffered solutions, leading to a more physiological Th1/Th2 balance. In particular, systemic and peritoneal lymphocytes, harvested from patients treated with conventional lactate-buffered PDFs, have shown a significantly lower number of IFN-γ-producing cells when compared to these new solutions, indicating a major Th1 inhibition and a decreased ability to respond to antigenic stimuli. The reasons for these different effects on Th cell function and differentiation remain unknown. As discussed above, the use of new solutions, *i.e.* bicarbonate-buffered or glucose-free, is related to improved leukocyte recruitment and function, which is probably due to lower lactate concentrations, a more physiological pH and a lower content of GDPs and AGEs [45]. The clinical impact of reduced Th1 responses is not well known, thus far. We have demonstrated that LAC-PD patients, characterized by a more pronounced Th1/Th2 imbalance, experienced a significantly higher peritonitis rate compared to LAC/BIC-PD and ICO-PD groups, which presented a more physiological Th1/Th2 cytokine profile. These findings could have important clinical consequences, since the occurrence of peritonitis is the most important factor for determining PD treatment and duration [46].

Finally, attention should also be focused on the possible role of inflammation in the immune regulation of CAPD patients. The presence of a systemic inflammatory state in patients on PD has been explored in many studies. As reported above, we have already demonstrated that PBMC drawn from PD patients released large amounts of IL-6, which correlated to the concentrations of the serum amyloid A protein, a marker of systemic inflammation [13]. In the present study, we confirmed these previous findings, showing that PD patients had lower albumin and higher CRP serum levels when compared to healthy control subjects. Moreover, in spite of reductions in CRP serum levels in the LAC/BIC-PD and ICO-PD groups *versus* LAC-PD, there was no significant difference among PD patients,

the lack of statistical significance being probably due to the small number of subjects enrolled. However at this moment, whether different PD treatments actually affect inflammation and what impact the modulation of local and systemic inflammatory processes could have on T cell function have not been exhaustively investigated. Our study present some limitations, which are mainly due to the limited number of the subjects studied and the lack of clinical follow-up, which meant that we were unable to better characterize the clinical outcome and the risk profile of PD patients undergoing different PDF treatments. In addition, we did not investigate the effects of Th1/Th2 imbalance on the immune tolerance status, or the other T cell subsets, such as Treg and Th17 cells. However, in spite of such limitations, we think that our data add new and interesting information, suggesting that the *in vivo* exposure to PDFs results in inhibition of both systemic and peritoneal Th cells. CAPD performed with more biocompatible PDFs may help to decrease the incidence of peritonitis by exerting fewer inhibitory effects on Th1 and reducing Th2 polarization. The clinical impact and the long-term effects of these findings, as well as the potential influence of inflammation on immune cells function, remain unknown and further longitudinal studies are needed.

Disclosure. None of the authors has any conflict of interest or financial support to disclose.

REFERENCES

1. Eleftheriadis T, Antoniadi G, Liakopoulos V, Kartsios C, Stefanidis I. Disturbances of acquired immunity in hemodialysis patients. *Semin Dial* 2007; 20: 440.

2. Lim WH, Kireta S, Leedham E, Russ GR, Coates PT. Uremia impairs monocyte and monocyte-derived dendritic cell function in hemodialysis patients. *Kidney Int* 2007; 72: 1138.

3. Nitta K, Nitta K, Akiba T, *et al.* Characterization of TH1/TH2 profile in uremic patients. *Nephron* 2002; 91: 492.

4. Street NE, Mosmann TR. Functional diversity of T lymphocytes due to secretion of different cytokine patterns. *FASEB J* 1999; 5: 171.

5. Park H, Li Z, Yang XO, *et al.* A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005; 6: 1133.

6. Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 2008; 28: 454.

7. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; 299: 1057.

8. Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 2005; 6: 345.

9. Cabrera R, Tu Z, Xu Y, *et al.* An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. *Hepatology* 2004; 40: 1062.

10. Velthuis JH, MolWM, WeimarW, *et al.* CD4+CD25bright+ regulatory T cells can mediate donor nonreactivity in long-term immunosuppressed kidney allograft patients. *Am J Transplant* 2006; 6: 2955.

11. Libetta C, Rampino T, Dal Canton A. Polarization of T-helper lymphocytes toward the Th2 phenotype in uremic patients. *Am J Kidney Dis* 2001; 38: 286.

12. Zamauskaite A, Perez-Cruz I, Yaqoob MM, Madrigal JA, Cohen SB. Effect of renal dialysis therapy modality on T cell cytokine production. *Nephrol Dial Transplant* 1999; 14: 49.

13. Libetta C, De Nicola L, Rampino T, De Simone W, Memoli B. Inflammatory effects of peritoneal dialysis: evidence of systemic monocyte activation. *Kidney Int* 1996; 49: 506.

14. Chiesa S, Vigo G, Cappa F, *et al*. Peritoneal T cell responses can be polarized toward Th1 or Th2 in children on chronic peritoneal dialysis. *Artif Organs* 2004; 28: 750.

15. Dasgupta MK, Larabie M, Halloran PF. Interferon-gamma levels in peritoneal dialysis effluents: relation to peritonitis. Kidney Int 1994; 46:475.

16. ter Wee PM, Beelen RH, van den Born J. The application of animal models to study the biocompatibility of bicarbonate-buffered peritoneal dialysis solutions. *Kidney Int* 2003; 88: S75.

17. Mortier S, Faict D, Schalkwijk CG, Lameire NH, De Vriese AS. Long-term exposure to new peritoneal dialysis solutions: Effects on the peritoneal membrane. *Kidney Int* 2004; 66: 1257.

18. Williams JD, Topley N, Craig KJ, *et al*. Euro Balance Trial Group. The Euro-Balance Trial: the effect of a new biocompatible peritoneal dialysis fluid (balance) on the peritoneal membrane. *Kidney Int* 2004; 66: 408.

19. Fan SL, Pile T, Punzalan S, Raftery MJ, Yaqoob MM. Randomized controlled study of biocompatible peritoneal dialysis solutions: effect on residual renal function. *Kidney Int* 2008; 73: 200.

20. Li FK, Chan LY, Woo JC, *et al*. A 3-year, prospective, randomized, controlled study on amino acid dialysate in patients on CAPD. *Am J Kidney Dis* 2003; 42: 173.

21. Zhe XW, Tian XK, Cheng L, Wang T. Effects of peritoneal resting on peritoneal fluid transport kinetics. *Perit Dial Int* 2007; 27: 575.

22. Libetta C, Sepe V, Zucchi M, Portalupi V, Meloni F, Dal Canton A. The effect of sirolimus- or cyclosporine-based immunosuppression effects on T-cell subsets in vivo. *Kidney Int* 2007; 72: 114.

23. Wang HH, Lin CY. Interleukin-12 and -18 levels in peritoneal dialysate effluent correlate with the outcome of peritonitis in patients undergoing peritoneal dialysis: implications for the Type I/Type II T-cell immune response. *Am J Kidney Dis* 2005; 46: 328.

24. Memoli B, Libetta C, Rampino T, Dal Canton A, Conte G, Andreucci VE. Hemodialysis related induction of interleukin-6 production by blood mononuclear cells. *Kidney Int* 1992; 42: 320.

25. Williams JD, Craig KJ, Topley N, *et al*. Peritoneal Biopsy Study Group. Morphologic changes in the peritoneal membrane of patients with renal disease. *J Am Soc Nephrol* 2002; 13: 470.

26. Mortier S, Lameire NH, De Vriese AS. The effects of peritoneal dialysis solutions on peritoneal host defense. *Perit Dial Int* 2004; 24: 123.

27. Bos HJ, Struijk DG, Tuk CW, *et al*. Peritoneal dialysis induces a local sterile inflammatory state and the mesothelial cells in the effluent are related to the bacterial peritonitis incidence. *Nephron* 1991; 59: 508.

28. Tauer A, Zhang X, Schaub TP, *et al*. Formation of advanced glycation end products during CAPD. *Am J Kidney Dis* 2003; 41(Suppl. 1): S5.

29. Kim YL. Update on mechanisms of ultrafiltration failure. *Perit Dial Int* 2009; 29: S123.

30. Hoff CM. *In vitro* biocompatibility performance of Physioneal. *Kidney Int* 2003; (Suppl.): S57.

31. Park MS, Kim JK, Holmes C, Weiss MF. Effects of bicarbonate/lactate solution on peritoneal advanced glycosylation end-product accumulation. *Perit Dial Int* 2000; 20: S33.

32. Park SH, Lindholm B. Definition of metabolic syndrome in peritoneal dialysis. *Perit Dial Int* 2009; 29 (Suppl. 2): S137.

33. Frampton JE, Plosker GL. Icodextrin: a review of its use in peritoneal dialysis. *Drugs* 2003; 63: 2079.

34. Wu HY, Hung KY, Hu FC, *et al*. Risk factors for high dialysate glucose use in PD patients –A retrospective 5-year cohort study. *Perit Dial Int* 2010; 30: 448.

35. Mistry CD, Gokal R, Peers E. A randomized multicenter clinical trial comparing isosmolar icodextrin with hyperosmolar glucose solutions in CAPD. MIDAS Study Group. Multicenter Investigation of Icodextrin in Ambulatory Peritoneal Dialysis. *Kidney Int* 1994; 46: 496.

36. Davies SJ, Woodrow G, Donovan K, *et al*. Icodextrin improves the fluid status of peritoneal dialysis patients: results of a double-blind randomized controlled trial. *J Am Soc Nephrol* 2003; 14: 2338.

37. Lin A, Qian J, Li X, *et al*. Icodextrin National Multi-center Cooperation Group. Randomized controlled trial of icodextrin versus glucose containing peritoneal dialysis fluid. *Clin J Am Soc Nephrol* 2009; 4: 179.

38. Davies SJ, Brown EA, Frandsen NE, *et al*. EAPOS Group. Longitudinal membrane function in functionally anuric patients treated with APD: data from EAPOS on the effects of glucose and icodextrin prescription. *Kidney Int* 2005; 67: 1609.

39. Ha H, Cha MK, Choi HN, Lee HB. Effects of peritoneal dialysis solutions on the secretion of growth factors and extracellular matrix proteins by human peritoneal mesothelial cells. *Perit Dial Int* 2002; 22: 171.

40. Posthuma N, ter Wee PM, Niessen H, Donker AJ, Verbrugh HA, Schalkwijk CG. Amadori albumin and advanced glycation end-product formation in peritoneal dialysis using icodextrin. *Perit Dial Int* 2001; 21: 43.

41. Plum J, Lordnejad MR, Grabensee B. Effect of alternative peritoneal dialysis solutions on cell viability, apoptosis/necrosis and cytokine expression in human monocytes. *Kidney Int* 1998; 54: 224.

42. Moriishi M, Kawanishi H. Icodextrin and intraperitoneal inflammation. *Perit Dial Int* 2008; 28: S96.

43. Martikainen TA, Teppo AM, Grönhagen-Riska C, Ekstrand AV. Glucose-free dialysis solutions: inductors of inflammation or preservers of peritoneal membrane?. *Perit Dial Int* 2005; 25: 453.

44. Bacharaki D, Thodis E, Passadakis P, Kantartzi K, Kriki P, Vargemezis V. Comparative in vitro study of different peritoneal dialysis solutions on cytokine production by peripheral blood mononuclear cells. *Nephron Clin Pract* 2009;113: c321.

45. Mortier S, De Vriese AS, McLoughlin RM, *et al*. Effects of conventional and new peritoneal dialysis fluids on leukocyte recruitment in the rat peritoneal membrane. *J Am Soc Nephrol* 2003; 14: 1296.

46. Davenport A. Peritonitis remains the major clinical complication of peritoneal dialysis: the London, UK, peritonitis audit 2002-2003. *Perit Dial Int* 2009; 29: 297.