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Vitamin E–loaded dialyzer resets PBMC-operated cytokine network in dialysis patients

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Vitamin E–loaded dialyzer resets PBMC-operated cytokine network in dialysis patients.

Background. In hemodialysis patients the activity of stimulated Th1 lymphocytes is depressed, while Th2 cells are constitutively primed. Such phenomena may depend on monocyte activation and altered release of interleukin (IL)-12 and IL-18, which regulate Th cell differentiation. Reactive oxygen species (ROS) activate monocytes; therefore, a hemodialyzer with antioxidant activity would contrast ROS, prevent monocyte activation, reset IL-12 and IL-18 release, and restore Th1/Th2 balance.

Methods. Ten patients on regular dialysis treatment (RDT) with cellulosic membrane (CM) were shifted to vitamin E–coated dialyzer (VE). During treatment with CM and after 3, 6, and 12 months of treatment with VE, peripheral blood mononuclear cells (PBMC) and purified CD4+ cells were isolated, and cultured, resting, mitogen-stimulated, and interferon γ (IFNγ), IL-4, IL-10, IL-12, and IL-18 release was measured. Vitamin E and A plasma levels and the effects of a single dialysis session on peripheral blood NO levels were assayed.

Results. The constitutive release of IL-4 and IL-10 by CD4+ cells was abated significantly by treatment with VE (nadir -77.8% and −55.3%, respectively, at 12 months). INFγ release by mitogen-stimulated CD4+ recovered with VE (zenith +501% at 12 months). PBMC constitutive production of IL-12 and IL-18 was significantly reduced by VE (nadir at 12 months −64.7% and −51.3%, respectively). VE increased plasma levels of vitamins E and A. NO plasma levels fell after a single dialysis treatment with VE (−17%, $P < 0.05$) in contrast with CU (+27.1%, $P < 0.05$).

Conclusion. The network of cytokines released by monocytes and Th cells is reset toward normality by treatment with vitamin E–coated dialyzer.

Key words: Th lymphocytes, vitamin E, hemodialysis, biocompatibility, cytokines, nitric oxide.

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Patients with end-stage renal disease (ESRD) on regular dialysis treatment (RDT) have an impaired immune defense that depends mainly on defective cell-mediated response [1–2]. We have described [3] a novel aspect of the immune dysfunction associated with RDT [i.e., an imbalanced activity of the two T helper (Th) lymphocyte subsets], which results in an overwhelming prevalence of Th2 over Th1 cells. This imbalance leads to high constitutive production of interleukin (IL)-4 and IL-10, and suppression of interferon-γ (IFN-γ) (i.e., a configuration of cytokine network that may play a pivotal role in causing immunodeficiency) [4–7].

The reason(s) for the altered Th cell polarization associated with hemodialysis is obscure. Hemodialysis causes a rise in plasma levels of interleukin 2 receptor due to abnormal T-cell preactivation [8–10]; nonetheless, PBMC of patients on RDT are unable to enhance IL-2 production when stimulated [11]. Because IL-2 is a product of Th1 lymphocytes, these findings suggest that RDT causes a chronic activation of Th1 cells, and exhausts their capacity to respond to an acute challenge. The activation and proliferation of Th1 lymphocytes are regulated by macrophages/monocytes through the release of IL-12 and IL-18 [12–17], two cytokines that induce the production of IFN-γ, the most powerful Th1 cell promoter. Hemodialysis activates circulating mononuclear phagocytes so that they release increased amounts of IL-12 [3, 18]. Thus, the available evidence suggests that in patients on RDT, repeated stimulation of monocytes is a determinant of chronic priming of Th1 lymphocytes.

An effect of the contact of blood with biomaterials in hemodialysis is the production of reactive oxygen species (ROS) by monocytes and polymorphonuclear leukocytes [19–20]. Interestingly, ROS amplify the phagocytic capacity of monocytes [21], advanced oxidation products present in the plasma of patients on RDT are capable of triggering monocyte oxidative burst [19–21], oxidized lipids activate monocytes [22], and the antioxidant α-tocopherol decreases IL-1β release from

activated human monocytes [23]. These findings point to oxidative stress as a mechanism of monocyte activation in hemodialysis.

α-Tocoferol is a powerful scavenger that protects plasma molecules and cell membranes from peroxidative damage [24–25]. To contrast the effects of ROS produced during hemodialysis [26], a dialyzer has been developed that consists of a multilayer cellulose membrane coated with vitamin E on the blood surface [27]. Studies in vivo have shown that short-term treatment with this dialyzer increases vitamin E blood levels [19, 28] and affords protection against dialysis-induced oxidative stress [29–30]. In addition, the high IL-6 release from PBMC, a sign of dialysis-induced monocyte activation [31–32], is reduced by vitamin E–coated dialyzer [33].

Based on the aforesaid premises, the present study tests the hypothesis that, by contrasting the oxidative stress generated during dialysis, the α-tocoferol–bonded dialyzer attenuates monocyte priming, and reduces the release of cytokines IL-12 and IL-18. Should this hypothesis hold, the vitamin E–loaded dialyzer might prevent the chronic stimulation and exhaustion of Th1 cells driven by monocytes, and would restore the capacity of Th1 cells to respond to an acute challenge.

METHODS

Patients and controls

Ten patients (7 male, 3 female) aged 31–65, mean 52.4 ± 16.8 years, with end-stage renal disease were studied. The causes of renal failure were polycystic kidney disease ($N = 2$), chronic glomerulonephritis ($N = 3$), chronic pyelonephritis ($N = 1$), interstitial nephritis ($N = 1$), and nephroangiosclerosis ($N = 3$). The patients were on RDT with non-reused dialyzers 4 hours thrice weekly. The dialytic procedure was a standard bicarbonate dialysis, and the Kt/V index ranged from 1.22 to 1.34. The surface of the filters was tailored to the needs of the patients, and the patients were clinically stable throughout the study. In particular, no relevant changes occurred in plasma levels of urea, phosphate, calcium, and creatinine, or in hematocrit, Kt/V, and leukocyte counts. Patients with clinical or humoral signs of infection, inflammation, or malignancy, and diabetic patients were excluded from the study. No patient was under treatment with drugs acting directly on the immune system, such as steroids or other immunosuppressants, and none received drugs known as immunomodulators [e.g., calcitriol, angiotensin-converting enzyme (ACE) inhibitors]. Calcitriol in particular was stopped in all patients for at least six months before the study, and serum calcium levels were maintained by oral calcium supplementation.

The control group consisted of eight healthy volunteers (3 male, 5 female) aged 24–48, mean 34.5 ± 11.7 years, from our medical staff. All subjects gave informed consent to the study.

Study design

The patients were studied while on RDT with cellulosic dialyzer (cellulose acetate) for at least 12 consecutive months (RDT-CM), then were shifted to dialysis with vitamin E–loaded filter (Excebrane®) (Terumo, Italy) and studied again after 3, 6, and 12 months on the new treatment (RDT-VE). Blood was collected from patients just before starting the dialysis session and from controls after overnight fast. Part of the blood was used to isolate and culture peripheral blood mononuclear cells (PBMC) and purified CD4+ lymphocytes, and part was used to measure plasma levels of vitamin E and A, and of nitric oxide (NO). PBMC and CD4+ cells were cultured for 48 hours either in unstimulated condition or stimulated with mitogens (see below), and cytokines (IFNγ, IL-4, IL-10, IL-12, IL-18) were measured in culture supernatant. Cytokines prototypic of T- helper lymphocytes (INF-γ, IL-4, and IL-10) were measured in CD4+ purified cell cultures, while cytokines produced by monocytes (IL-12 and IL-18) were measured in PBMC cultures; the latter policy was preferred rather than isolating monocytes from PBMC because isolation procedures activate monocytes. The way in which blood was collected and supernatants were stored has been described in our previous studies [34].

Isolation and culture of PBMC

PBMC were isolated by gradient centrifugation as we described in a previous paper [35]. In brief, the cells were aspirated from the interface, washed three times in phosphate-buffered saline (PBS) (Life Technologies, Milan, Italy), and resuspended at a cell density of 2×10^6 cells/mL in RPMI 1640 medium (Life Technologies) containing 1% decomplemented fetal calf serum (FCS) inactivated at 56°C (Sigma-Aldrich, Milan, Italy), 2 mmol/L L-glutamine, 100 IU/mL penicillin (Life Technologies), and 100 μg/mL streptomycin (Life Technologies). After 48 hours of incubation at 37°C in humidified atmosphere containing 5% CO2, cell-free supernatants were collected by centrifugation for 10 minutes at 400*g*, passed through a millipore filter (0.2 μ; Sigma-Aldrich), and stored at −20°C.

On average, PBMC contained 74% (range, 72%–77%) lymphocytes and 26% (range, 22%–29%) monocytes. The average fraction represented by lymphocytes was similar in PBMC harvested from control patients (76%) and RDT patients at the various steps of the study: 74% during treatment with the cellulosic membrane, 76%, 77%, and 75% after 3, 6, and 12 months, respectively, of treatment with vitamin E–coated dialyzer. Cell

viability, determined by Trypan blue dye exclusion test, was >95%.

Purification and culture of CD4+ cells

T-helper lymphocytes were isolated from PBMC as we previously described [3]. In brief, PBMC [10^7 cells in 80 μL of PBS buffer supplemented with 2 mmol/L EDTA and 0.5% bovine serum albumin (BSA)] were incubated for 10 minutes at 6 to 12°C with a cocktail of hapten-conjugated antibodies recognizing CD8, CD11b, CD19, CD56, CD16, and CD36 (20 μL of cocktail solution/10^7 cells). After wash and resuspension in 80 μL of buffer, the cells were incubated for 15 minutes at 6 to 12°C with an antihapten antibody coupled with magnetic microbeads (MACS System; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). The procedure resulted in indirect magnetization of non-Th cells (i.e., B cells, monocytes, natural killer cells, CD8+ cells, granulocytes, basophils, dendritic cells, early erythroid cells, and platelets). After magnetization, PBMC were passed over a separation column (LS+) and placed in a strong magnetic field so that the cells loaded with magnetic beads were retained in the column. The purity of the isolated Th cells was analyzed by flow cytometry (Epics Profile II; Coulter Immunology, Miami, FL, USA) with monoclonal anti-CD3, anti-CD4, and anti-CD8 antibodies. CD4+ cells averaged 95.9% ± 3.7%. Purified CD4+ cells were cultured at a cell density of 5×10^5 cells/mL in the same medium as that used for PBMC. After 48 hours of incubation at 37°C in humidified atmosphere containing 5% CO2, cell-free supernatants were collected and stored at −20°C.

Stimulation studies

In stimulation studies, PBMC were cultured for 48 hours in the presence of lipopolysaccharide (LPS, 10 μg/mL; Sigma-Aldrich), while CD4+ cells were cultured for 48 hours in the presence of phytohemagglutinin (PHA) (10 μg/mL).

Cytokine assay

Cytokines (IL-4, IL-10, IL-12 p70, IL-18, and IFNγ) were assessed by enzyme-linked immunosorbent assay (ELISA) on cell-free supernatant. Antibody pairs were used according to instructions of the supplier firms. IL-18 assay was performed with reagents obtained from R&D Systems (Minneapolis, MN, USA) (primary, MoAb: clone 52713.11, 1 μg/mL; secondary, PolyAb 250 ng/mL); assay sensitivity was 4.3 pg/mL. IFNγ and IL-10 assays were conducted with reagents purchased from Immunokontact (Frankfurt am Main, Germany) as follows: IFNγ: primary, MoAb clone 43.11 at 5 μg/mL, and secondary, PolyAb at 50 ng/mL (assay sensitivity 0.72 pg/mL); IL-10: MoAb clone 9016.2 at 4 μg/mL, detecting MoAb clone JES3-1248 at 50 ng/mL (assay sensitivity 1.6 pg/mL). Bioactive p70 IL-12 assay was performed with reagents supplied by Endogen (Woburn, MA, USA) as follows: IL-12 MoAb clone 20C2 at 4 μg/mL, detecting MoAb clone C8.6 at 1 μg/mL (assay sensitivity 2.5 pg/mL). Finally, IL-4 assay was performed with a commercially available kit purchased from Endogen.

Plasma levels of vitamin E and vitamin A

Plasma levels of γ- and α-tocoferol were measured by high-performance liquid chromatography (HPLC) analysis as we previously described [36]. Plasma (200 μL) was mixed with 200 μL of tocopherol acetate in methanol (32.5 μg/mL) and extracted with 500 μL of hexane. The organic solvent recovered after centrifugation was dried with nitrogen and resuspended in 400 μL of HPLC grade methanol. The samples were then analyzed with a Jasco HPLC system (Jasco Corporation, Tokyo, Japan) equipped with an UV detector. The mobile phase was 98% methanol + 2% HPLC grade water, and the flow rate was 1.6 mL/min. Extracted samples (100 μL) were injected onto an ALLTIMA C18 5 μm, 250 mm × 4.6 mm inner dimension (i.d.) column (ALLTECH, Italia), preceded by an ALLTECH 7.5 × 4.6 mm i.d. All-guard™ Cartridge Holder. The UV detector was set at 325 nm for 7 minutes and 292 nm to end of run, and data were collected and analyzed with the software Borwin 1.5 (Jasco Corporation). Solvents for sample extractions and the main chemicals used were from Sigma (Steinheim, Germany) or Merck (Darmstadt, Germany). HPLC grade solutions were from Carlo Erba (Milano, Italy).

Total nitric oxide (NOx) assay

We investigated the effects of a single dialysis on NO levels in peripheral blood (i.e., in blood sampled from arterial dialysis line at the start and the end of dialysis session). In addition, we investigated the effect of blood passage through the filter on NO plasma levels. To this purpose we obtained simultaneous blood samples from arterial and venous dialysis lines 60 minutes after starting dialysis session. We measured NO by photometric analysis with a nitrate/nitrite colorimetric assay kit (R&D Systems). Nitric oxide production was determined as NO2 + NO3 (NOx) with the Griess reagent after reduction of nitrate to nitrite with nitrate reductase. Readings were at 540 nm, and baseline correction was carried out at 620 nm. The sensitivity limit of the assay was 1.35 μmol/L.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical analysis was carried out by repeated measures analysis of variance (ANOVA) analysis followed by Bonferroni's multiple comparison test

Table 1. Plasma levels of vitamin E and vitamin A in control subjects (CON) and in patients on RDT

	CON	RDT-CM	RDT-VE 3m	RDT-VE 6m	RDT-VE 12m
Vit E μ*mol/L*	30.7 ± 7.5	26.1 ± 10.3	32.3 ± 12.7[a]	34.7 ± 14.7[a]	35.0 ± 15.1[a]
Vit A μ*mol/L*	3.75 ± 0.87	3.39 ± 1.60	4.23 ± 1.49[a]	4.40 ± 4.46[a]	3.72 ± 0.79

Vit E, vitamin E; Vit A, vitamin A. Data are mean ± SD. Patients were treated with cellulosic membrane (RDT-CM), or Vitamin E-coated filter (RDT-VE) for 3 months (3m), 6 months (6m), and 12 months (12m).
[a] $P < 0.05$ vs. RDT-CM.

Table 2. Plasma levels of NOx (μmol/L) in control subjects (Con) and in patients on RDT treated with cellulosic membrane (RDT-CM) or vitamin E–coated filter (RDT-VE)

	Pre	Post	(A)	(V)
CON	42.8 ± 17.3	–	–	–
RDT-CM	100.7 ± 39.9[a]	128 ± 45.5[c]	126 ± 40.3	137.5 ± 44.4
RDT-VE	69.6 ± 31.2[a,b]	57.8 ± 20.3[b,c]	67.5 ± 23.9[b]	49.5 ± 18.7[b,d]

Pre and Post refer to peripheral blood (i.e., blood sampled from the arterial dialysis line) at the start and the end of the dialysis session. A and V refer to blood sampled from the arterial (A) or venous (V) dialysis line 60 minutes after starting the dialysis session. Data are mean ± SD.
[a] $P < 0.05$ vs. Con.
[b] $P < 0.05$ vs. RDT-CM.
[c] $P < 0.05$ vs. Pre.
[d] $P < 0.05$ vs. (A).

(STATVIEW software for the Macintosh; Cary, NC, USA); differences were estimated significant at P values <0.05.

RESULTS

Circulating levels of antioxidant vitamins

Table 1 shows levels of vitamins E and A in plasma of patients on RDT sampled just before starting the dialysis session. Levels of vitamin E were increased after 3 months of treatment with the vitamin E–loaded dialyzer and remained high throughout the rest of the study. Levels of vitamin A were increased significantly after 3 and 6 months of treatment.

Plasma nitric oxide

Data are shown in Table 2. NOx levels in peripheral blood sampled before starting dialysis session were significantly higher in dialysis patients, either RDT-CM or RDT-VE, than in normal volunteers. Peripheral blood NOx levels increased at the end of dialysis with cellulose filter, while at the end of dialysis with vitamin E–loaded filter they decreased, becoming similar to levels in normal volunteers. Passage of blood through vitamin E–loaded filter caused a significant decrease in NOx levels.

Release of IFNγ, IL-4, and IL-10 by CD4+ cells

Figure 1 shows the release of IFNγ by unstimulated and PHA-stimulated cells. The release of IFN-γ was similarly low in unstimulated control cells as in cells of patients on dialysis, either RDT-CM or RDT-VE. PHA increased the release of IFN-γ in all cells, but the response to PHA was about 10-fold lower in RDT-CM than in control patients (89.9 ± 18.2 pg/5 × 10^5 cells vs. 838.4 ± 142.7, respectively, $P < 0.001$). A significant recovery of IFN-γ release by CD4+ cells of dialysis patients occurred after treatment with vitamin E–loaded filter (in pg/5 × 10^5 CD4+ cells: 402.6 ± 78.6 at 3 months, 509.5 ± 76.1 at 6 months, and 540.8 ± 106.3 at 12 months, all $P < 0.05$ vs. RDT-CM).

The release of IL-4 by CD4+ cells is shown in Figure 2. Unstimulated control patients produced significantly less IL-4 than unstimulated RDT-CM (3.3 ± 0.62 pg/5 × 10^5 cells vs. 61.0 ± 13.4, respectively, $P < 0.01$). The excess constitutive production of IL-4 associated with cellulose dialysis was significantly attenuated by treatment with vitamin E–loaded dialyzer; IL-4 release decreased to 17.1 ± 4.7 pg/5 × 10^5 cells at 3 months, 16.3 ± 6.0 pg/5 × 10^5 cells at 6 months, and 13.5 ± 2.2 pg/5 × 10^5 at 12 months, all $P < 0.05$ versus RDT-CM. Stimulation with PHA strikingly increased IL-4 release in control patients (87.5 ± 21.6 pg/5 × 10^5 CD4+, $P < 0.01$) but did not raise it further in RDT-CM. In other words, RDT-CM behaved as if they were prestimulated. In fact, a significant response to stimulation was restored after treatment with vitamin E–loaded dialyzer (i.e., after the spontaneous production of IL-4 was lowered).

The release of IL-10 by CD4+ cells is shown in Figure 3. Unstimulated control patients produced significantly less IL-10 than cells of dialysis patients, either treated with cellulose, or with vitamin–E coated dialyzer. However, the excess spontaneous production of IL-10 in RDT-CM (144.7 ± 37.4 pg/5 × 10^5 cells) was significantly reduced by shifting patients from cuellulose to vitamin E–coated dialyzer (82.5 ± 27.2 pg/5 × 10^5 cells at 3 months, 67.7 ± 16.8 pg/5 × 10^5 cells at 6 months, and 64.7 ± 13.5 pg/5 × 10^5 cells at 12 months, all $P < 0.05$ vs. RDT-CM). Stimulation raised IL-10 production in all cells, but control patients attained higher levels than RDT (all groups).

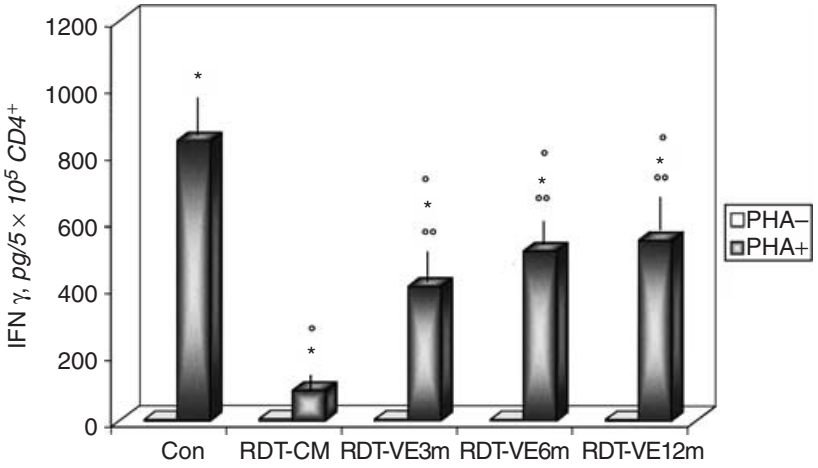

Fig. 1. Interferon γ (IFNγ) production by CD4+ of normal subjects (Con), and patients on regular hemodialysis treatment with cellulosic membranes (RDT-CM) and vitamin E–coated membranes (RDT-VE) at 3, 6, and 12 months (3m, 6m, and 12 m) of therapy. *Indicates a significant difference between stimulated phytohemagglutinin (PHA+) and unstimulated (PHA−) cells of the same group ($P < 0.001$ Con; $P < 0.01$ RDT-VE 3m, RDT-VE 6m and RDT-VE 12m; $P < 0.05$ RDT-CM). ° Indicates a significant difference vs. stimulated cells of Con ($P < 0.01$ RDT-CM; $P < 0.05$ all RDT-VE). °°$P < 0.05$ vs. stimulated RDT-CM.

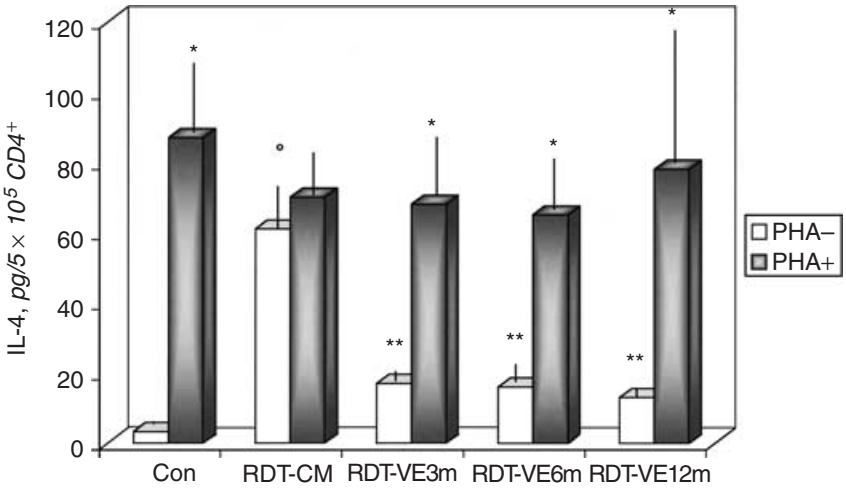

Fig. 2. Interleukin (IL)-4 production by CD4+ of normal subjects (Con), and patients on regular hemodialysis treatment with cellulosic membranes (RDT-CM) and vitamin E–coated membranes (RDT-VE) at 3, 6, and 12 months of therapy. *Indicates a significant difference between stimulated phytohemagglutinin (PHA+) and unstimulated (PHA−) cells of the same group ($P < 0.001$ Con; $P < 0.05$ all RDT-VE). ° Indicates a significant difference vs. unstimulated cells of Con ($P < 0.02$ RDT-CM; $P < 0.05$ all RDT-VE). **$P < 0.05$ vs. unstimulated RDT-CM.

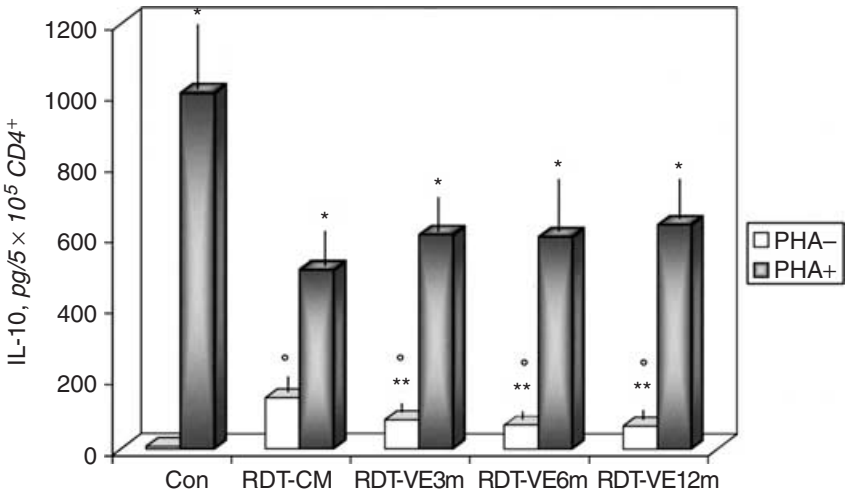

Fig. 3. Interleukin (IL)-10 production by CD4+ of normal subjects (Con), and patients on regular hemodialysis treatment with cellulosic membranes (RDT-CM) and vitamin E–coated membranes (RDT-VE) at 3, 6, and 12 months of therapy. *Indicates a significant difference between stimulated (PHA+) and unstimulated (PHA−) cells of the same group ($P < 0.001$ Con; $P < 0.05$ all RDT). °$P < 0.03$ vs. unstimulated cells of Con. **$P < 0.05$ vs. unstimulated RDT-CM.

Release of IL-12 and IL-18 by PBMC

The release of IL-12 by PBMC is shown in Figure 4. Unstimulated control patients produced significantly less IL-12 than all unstimulated RDT. However, the excess spontaneous production of IL-12 was significantly attenuated by shifting patients from cellulose to vitamin E–loaded dialyzer (RDT-CM: 54.0 ± 17.7 pg/2×10^6 PBMC; RDT-VE at 3 months: 21.7 ± 7.0 pg/2×10^6 PBMC;

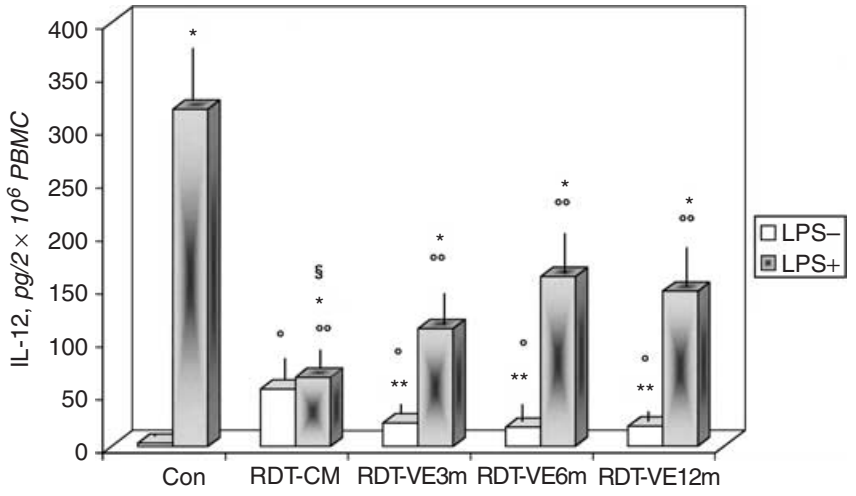

Fig. 4. Interleukin (IL)-12 production by peripheral blood mononuclear cells (PBMC) of normal subjects (Con), and patients on regular hemodialysis treatment with cellulosic membranes (RDT-CM) and vitamin E–coated membranes (RDT-VE) at 3, 6, and 12 months of therapy. *Indicates a significant difference between stimulated phytohemagglutinin (PHA+) and unstimulated (PHA−) cells of the same group ($P < 0.001$ Con; $P < 0.03$ all RDT-VE). °Indicates a significant difference vs. unstimulated cells of Con ($P < 0.01$ RDT-CM; $P < 0.05$ all RDT-VE). **$P < 0.05$ vs. unstimulated RDT-CM. °°Indicates a significant difference vs. stimulated cells of Con ($P < 0.01$ RDT-CM; $P < 0.05$ all RDT-VE). $P < 0.05$ vs. stimulated RDT-VE.

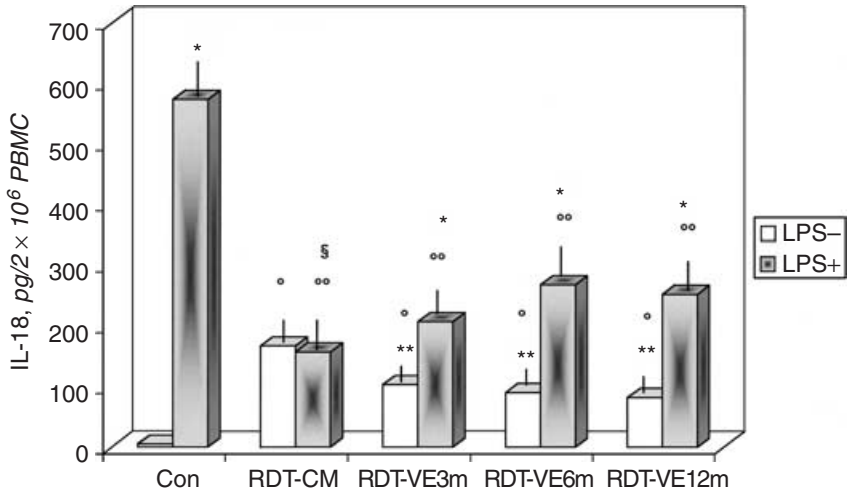

Fig. 5. Interleukin (IL)-18 production by peripheral blood mononuclear cells (PBMC) of normal subjects (Con), and patients on regular hemodialysis treatment with cellulosic membranes (RDT-CM) and vitamin E–coated membranes (RDT-VE) at 3, 6, and 12 months of therapy. *Indicates a significant difference between stimulated phytohemagglutinin (PHA+) and unstimulated (PHA−) cells of the same group ($P < 0.001$ Con; $P < 0.05$ all RDT-VE). ° Indicates a significant difference vs. unstimulated cells of Con ($P < 0.01$ RDT-CM; $P < 0.02$ all RDT-VE). **$P < 0.05$ vs. unstimulated RDT-CM. °°Indicates a significant difference vs. stimulated cells of Con ($P < 0.04$). $P < 0.05$ vs. stimulated RDT-VE6m and RDT-VE12m.

RDT-VE at 6 months: 18.1 ± 4.3 pg/2 × 10^6 PBMC; RDT-VE at 12 months: 19.1 ± 3.7 pg/2 × 10^6 PBMC). Stimulation with LPS significantly raised IL-12 production in control patients but not in RDT-CM. In contrast with the unresponsiveness of RDT-CM, stimulated RDT-VE were able to significantly increase IL-12 production, a result of two effects of treatment with vitamin E–coated dialyzer (i.e., the decreased unstimulated release combined with the increased stimulated release).

The release of IL-18 by PBMC is shown in Figure 5. The behavior of IL-18 replicates that of IL-12. Cellulose dialysis was associated with a more than 30-fold excess spontaneous release of IL-18 and unresponsivenes to stimulation. Vitamin E–loaded dialyzer significantly reduced the spontaneous production of IL-18 (from 167.5 ± 33.5 pg/2 × 10^6 PBMC in RDT-CM to 102.7 ± 16.9 pg/2 × 10^6 PBMC in RDT-VE at 3 months, 90.1 ± 16.9 pg/2 × 10^6 PBMC in RDT-VE at 6 months, and 81.7 ± 17.3 pg/2 × 10^6 PBMC in RDT-VE at 12 months), and raised the stimulated production of the cytokine, thus restoring a significant response of PBMC to LPS.

IFNγ/IL-4 ratio

The ratio between the amounts of IFNγ and IL-4 released by CD4+ cells (an index of Th1/Th2 cell balance) is shown in Figure 6. The ratio was significantly higher in control patients than in RDT-CM, both in the unstimulated state (1.08 ± 0.24 vs. 0.08 ± 0.03, respectively, $P < 0.01$), and after stimulation with PHA (9.7 ± 2.7 vs. 1.34 ± 0.45, respectively, $P < 0.01$). Treatment with vitamin E–loaded dialyzer increased significantly the IFNγ/IL-4 ratio, both in unstimulated cells (0.24 ± 0.11 at 3 months, 0.24 ± 0.12 at 6 months, and 0.25 ± 0.08 at 12 months) and in stimulated cells (6.3 ± 2.2 at 3 months, 8.5 ± 3.4 at 6 months, and 8.1 ± 1.7 at 12 months). In fact, after 6 and 12 months of treatment with vitamin E–loaded dialyzer the IFNγ/IL-4 ratio was not different in stimulated control patients and RDT-VE.

DISCUSSION

The results of this study confirm and extend our previous observation that treatment with cellulose

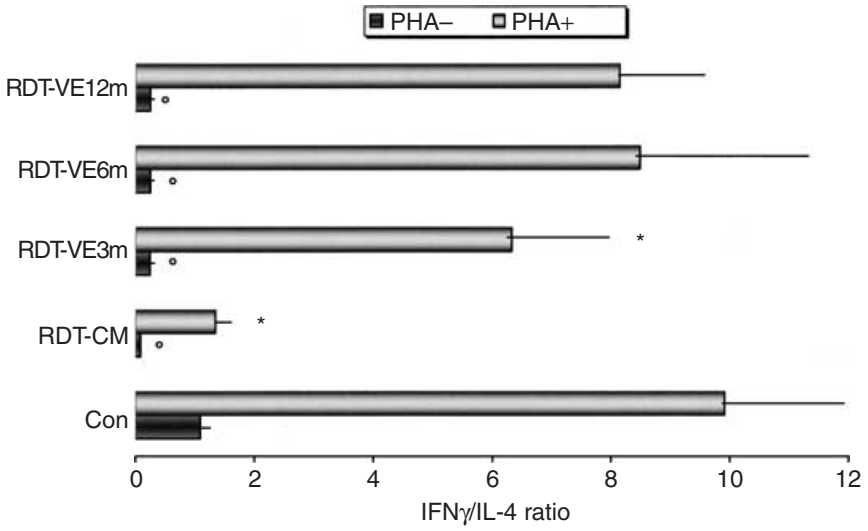

Fig. 6. Interferon (IFN)γ/interleukin (IL)-4 ratio of normal subjects (Con), and patients on regular hemodialysis treatment with cellulosic membranes (RDT-CM) and vitamin E–coated membranes (RDT-VE) at 3, 6, and 12 months of therapy. ° Indicates a significant difference vs. unstimulated cells of Con ($P < 0.01$ RDT-CM; $P < 0.05$ all RDT-VE). *Indicates a significant difference vs. stimulated cells of Con ($P < 0.01$ RDT-CM; $P < 0.05$ all RDT-VE3m).

dialyzer is associated with a dysregulation of the mononuclear cell-operated cytokine network. Such dysregulation encompasses the following phenomena: (*1*) PBMC spontaneously produce supranormal amounts of IL-12 and IL-18; (*2*) LPS-stimulated PBMC cannot further increase the release of IL-12 and IL-18, and cannot achieve production rates as high as those attained by stimulated normal cells; (*3*) Th lymphocytes spontaneously produce supranormal amounts of IL-4 and IL-10. The constitutive production of IL-4 is particularly impressive because it equals the production of PHA-stimulated normal cells, and represents an apparent ceiling that cannot be further increased by stimulation with mitogen; and (*4*) Th lymphocytes stimulated with PHA produce much less INFγ than stimulated control cells.

These observations support two conclusions. The first is that dialysis with cellulose activates circulating monocytes, as indicated by the spontaneous overproduction of IL-12 and IL-18 by PBMC. The finding that LPS-stimulated PBMC cannot further increase the release of IL-12 and IL-18 is consistent with a state of continued stimulation, resulting in loss of reactivity to an acute challenge [30]. The second is that dialysis with cellulosic membrane is associated with an overwhelming prevalence of Th2 over Th1 cells, as indicated by the striking amount of IL-4 (the prototypic Th2 cytokine) produced by unstimulated Th cells, and by the suppression of IFNγ release (the prototypic Th1 cytokine) in PHA-stimulated Th cells. The functional imbalance between the two Th subsets is also witnessed by the high constitutive release of IL-10, another Th2-related cytokine.

The prevalence of Th2 lymphocytes in a setting in which PBMC produce increased amounts of IL-12 and IL-18 (i.e., two cytokines that stimulate the production of IFNγ and drive the differentiation of Th cells to the Th1 phenotype) [13, 15] is intriguing. We suggest that the two phenomena are causally related (i.e., that the default of Th1 cells results from their continued stimulation), eventually causing functional exhaustion [31]. In summary, our findings delineate a sequence of events that starts with activation of monocytes provoked by bioincompatible membranes, and proceeds with release of IL-12 and IL-18, recruitment of Th1 cells, exhaustion of Th1 cell function, loss of the inhibitory control of Th1 on Th2 cells, and eventual prevalence of Th2 cells [3].

Based on the evidence that oxidative stress generated by dialysis is a leading cause of monocyte activation [19–21], and assuming that monocyte activation sparks the disturbance of cytokine network [37–39], we supposed that treatment with a vitamin E–coated dialyzer might correct the altered cytokine production. The results of this study seem to support this hypothesis. In fact, treatment with vitamin E–loaded dialyzer significantly lowered the spontaneous release of IL-12 and IL-18 by PBMC, and restored the ability of PBMC to increase IL-12 and IL-18 production in response to stimulation. In addition, vitamin E–coated dialyzer caused a fall in constitutive release of IL-4 and IL-10 by Th lymphocytes and significantly raised IFNγ production by stimulated Th cells. It is reasonable to hold that these effects of vitamin E–loaded dialyzer were the consequence of the antioxidant property of vitamin E [19]. In fact, vitamin E–loaded dialyzer is a dialyzer with a cellulose backbone, whose unique differential trait is its vitamin E coat. In addition, we showed that treatment with vitamin E–loaded dialyzer was associated with a consistent increase in plasma levels of vitamin E. Furthermore, serum levels of nitric oxide, a representative member of the ROS family [20], fell at the passage of blood through vitamin E–loaded dialyzer, while it increased at the passage through the cellulosic filter, and peripheral blood levels of NO were significantly reduced by a dialysis session with vitamin E–coated filter, but not with cellulosic dialyzer. Free radicals produced during hemodialysis have very short half-lives

and exhaust almost completely during passage of blood through the filter [19]. Therefore, the local availability of abundant vitamin E anchored to dialysis membrane may have been particularly effective in contrasting dialysis-generated ROS. However, the interdialytic increase in plasma levels of vitamin E and A caused by treatment with vitamin E–loaded dialyzer may have contributed to contrast oxidative stress. In fact, oral supplementation of vitamin E has been shown to reduce lipid peroxidation and to increase LDL resistance to ex vivo oxidation in uremic patients [40]. However, peroral administration of vitamin E provides less effective antioxidant defense that vitamin E–dialyzer in hemodialysis patients [41].

The partial reset of PBMC-operated cytokine network caused by vitamin E–loaded dialyzer represents a significant improvement of mononuclear cell function, not the achievment of full normality. Possibly, the incomplete normalization of PBMC activity depended on too short a period of use of vitamin E–coated filter. However, the benefit induced by vitamin E–loaded dialyzer was apparent in three months, and no further improvement occurred in the next nine months of treatment. In addition, we have shown that a disturbance of PBMC-operated cytokine network, characterized by increased spontaneous production of IL-4 and attenuated mitogen-induced IFN-γ release, is present in patients with chronic renal failure on conservative treatment [3]. Therefore, in uremic patients there exist mechanisms that alter PBMC activity, independent of the insult caused by dialysis with bioincompatible cellulose, on which vitamin E–coated filter may not be influent.

CONCLUSION

This study demonstrates that treatment with vitamin E–coated dialyzer improves the defect of PBMC function associated with cellulosic dialyzer consisting of altered spontaneous and mitogen-stimulated cytokine release. The effects of vitamin E–coated filter, in particular the recovery of reactive INFγ production by Th1 cells and the restriction of spontaneous IL-4 release by Th2 cells, may have clinical importance. In fact, the inhibition of Th1 cytokines and the prevalence of Th2 over Th1 lymphocytes may play a major role in impairing cell-mediated immunity and phagocytic activity in patients on regular hemodialysis treatment.

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