On-Line Hemodiafiltration Reduces the Proinflammatory CD14$^+$CD16$^+$ Monocyte-Derived Dendritic Cells: A Prospective, Crossover Study

Julia Carracedo,* Ana Merino,* Sonia Noguera,* Diana Carretero,* Isabel Berdud,* Rafael Ramírez,* Ciro Tetta,† Mariano Rodríguez,* Alejandro Martín-Malo,* and Pedro Aljama*

*Unidad de Investigación, Servicio de Nefrología, Hospital Universitario Reina Sofía, Córdoba, Spain; and †Fresenius Medical Care, Research Extracorporeal Therapies, Bad Homburg, Germany

It is not known whether high convective transport may have a role in modulating the chronic inflammation of hemodialysis (HD) patients. The aim of this study was to evaluate the effect of on-line hemodiafiltration (OL-HDF) on proinflammatory peripheral monocytes: Percentage of CD14$^+$CD16$^+$ cells and their telomere length and spontaneous or bacterial DNA-induced production of cytokines (TNF-$\alpha$ and IL-6). In a prospective, crossover study, 31 patients who were on high-flux HD (HF-HDF) were evaluated. Patients underwent the following sequence of treatments (4 mo each): HF-HD (basal), OL-HDF (period 1), HF-HD (period 2), OL-HDF (period 3), and HF-HD (period 4). The dialysis characteristics were similar in the two modalities; the only difference was a higher convective transport in the OL-HDF than in the HF-HD. All patients who were on OL-HDF showed a significantly lower number of CD14$^+$CD16$^+$ cells than on HF-HD (18.5 ± 2.3 basal versus 13.6 ± 2.9 period 1 and 13.9 ± 2.3 period 3; P = 0.001). By contrast, HF-HD restored the number of CD14$^+$CD16$^+$ cells to the basal values (19.2 ± 2.8 and 18.6 ± 1.4, periods 2 and 4, respectively; NS). During OL-HDF periods, the reduction of CD14$^+$CD16$^+$ was paralleled by a decreased number of short telomere cells. Spontaneous or bacterial DNA-induced production of cytokines (TNF-$\alpha$ and IL-6) was increased in HF-HD as compared with OL-HDF. In conclusion, these results demonstrate that as compared with HF-HD, OL-HDF markedly reduces the number of proinflammatory CD14$^+$CD16$^+$ cells and the production of TNF-$\alpha$ and IL-6. Future studies are needed to assess the possible therapeutic effect of convective transport on chronic inflammation that is associated with HD.


C ells of the monocyte lineage are important elements of immune defense because these cells can phagocyte foreign material; present antigens to T cells; and produce a host of cytokines, including TNF-$\alpha$, IL-1, and IL-6 (1). Cytokine expression by monocytes is triggered efficiently by stimulation with bacterial products such as LPS or bacterial DNA via the Toll-like receptors (2). Whereas the heterogeneity of tissue macrophages is well established, circulating monocyte heterogeneity has been demonstrated clearly only recently (3,4). This cell population is present in 5 to 10% of total monocytes but can increase up to 40% in several acute and chronic inflammatory states, such as in HIV infection (5), autoimmune diseases (6), and sepsis (7), and in hemodialysis (HD) (8,9). We reported that in HD patients, there is an increase in prematurely senescent cells that is characterized by the association of CD14 dim/CD16 bright expression, increased p53 expression, cytokine overproduction, and short telomeres (10,11). These cells accumulate in the peripheral blood as a result of their enhanced resistance to proapoptotic stimuli. Their proinflammatory potential is underlined by their capacity to respond to subliminal concentrations of LPS or bacterial DNA. Therefore, these features make the determination of these cells of particular relevance in the elucidation of the mechanisms underlying the chronic inflammation. Besides the effect of uremia-retained solutes, HD may induce chronic inflammation, which has been related to complement-activated fragments as a result of membrane biocompatibility and dialysate-born products (12,13).

Use of synthetic membranes and production of ultrapure dialysate have been proved to have an impact on improving treatment biocompatibility and reducing the chronic inflammatory state (13–15). It still is not clear whether high convective transport may have a role in modulating the mechanisms that are involved in inflammation in HD patients (16). Because on-line hemodiafiltration (OL-HDF), a mixed convective-diffusive modality, is performed using high-flux synthetic membranes and ultrapure dialysis fluids, our working hypothesis was that any difference observed in patients who are treated with high-flux HD (HF-HD) and OL-HDF potentially could be related to the convective transport in HDF. In this prospective,
crossover study, we evaluated the effect of convective transport on the phenotypic expression of CD14+ and CD14+CD16+ cells and their telomere length and spontaneous or bacterial DNA-induced production of cytokines (TNF-α and IL-6) in peripheral monocytes.

Materials and Methods

A total of 31 stable HD patients were included. All patients had been treated in the satellite dialysis facility of Reina Sofia University Hospital, Cordoba, Spain, for at least 6 mo before inclusion and remained in the same facility for the whole duration of the study. The first patient inclusion was in June 2003, and the last was in March 2004. We excluded patients with symptoms or signs of inflammation, infection, and neoplastic disease. Informed consent was obtained from all patients. Six healthy individuals served as control subjects. The follow-up lasted 16 mo and consisted of four periods of 4 mo each. The crossover had the following sequence: HF-HD (basal), OL-HDF (period 1), HF-HD (period 2), OL-HDF (period 3), and HF-HD (period 4). The last patient finished in July 2005.

Patients and Baseline Data

At inclusion, all patients had been on HF-HD for at least 2 mo and using polysulfone membranes (1.8 m², HF80; Fresenius Medical Care, Bad Homburg, Germany). OL-HDF was performed with ultrapure bicarbonate-buffered dialysate three times a week. Analysis of the dialysis system always revealed absence of bacteria (<100 colony-forming units/ml) or bacteriological contaminant products (endotoxin levels <0.025 endotoxin units) during the whole period of the study. The dialysis characteristics were similar in the two modalities, the only difference being a higher convective transport in the OL-HDF than in HF-HD. In OL-HDF, the mean volume infused was recorded. Dialysis efficiency was estimated using eKt/V ratio. All patients but one had a native arteriovenous fistula, and one patient had an arteriovenous graft. Medical charts were reviewed to record age, gender, weight, height, underlying renal disease, dialysis vintage, history of transplantation, diabetes, comorbid conditions, and current medication. The initial renal diagnosis was chronic glomerulonephritis in four, tubulointerstitial nephritis in three, polycystic kidney disease in nine, nephroangiosclerosis in two, and unknown cause in 13. None of the patients was on anti-inflammatory or immunosuppressive drugs during the 3 mo before inclusion and for the whole period of the study. None of them had diabetes. The therapeutic regimen, which consisted of erythropoietin (EPO), calcium-containing phosphate binders, antihypertensive drugs, and intravenous iron, was changed according to the clinical needs. All patients were on EPO from the beginning of the study. EPO dosage was adjusted to maintain the hemoglobin target of 11.5 to 12 g/dl. Weekly EPO dose was similar in both modalities of HD during the study. All routine laboratory measurements were done using certificate assay methods.

Blood samples were obtained in all patients just before the first (long interval) dialysis session of the following period. This means that the effect of each 4-mo period could be observed. Kt/V was determined with r-glutamine (2 mM), HEPES (20 mM), sodium pyruvate (1 mM), streptomycin (50 ng/ml), penicillin (100 UI/ml), and 10% human autologous serum (Bio-Whittaker, Walkersville, MD). Serum was heated (56°C for 60 min) to inactivate complement.

Evaluation of CD14 and CD16 Mononuclear Expression. Cells (10^5/ml) were incubated for 30 min at 4°C with the mAb M5E2 against the molecule CD14 or 3G8 against the molecule CD16 (Becton Dickinson, San Jose, CA). Cytofluorometric analysis was performed with a FACScan cytometer (Becton Dickinson).

Assessment of Telomere Length by Fluorescence In Situ Hybridization in Flow Cytometry. Cells (1×10^6) were washed and centrifuged (30 s at 13,000 rpm). Cell pellets were resuspended in hybridization buffer that contained 70% deionized formamide (Sigma, Chemical Co., St. Louis, MO), 10 mM Tris (pH 7.0), 10% FCS, and 0.3 μg/ml of the telomere-specific FITC-conjugated probe (FITC-O-CCCATAACTAAA-CAC-NH2). DNA from samples was heat-denatured followed by hybridization for 2 h at room temperature. Thereafter, cells were resuspended for 1 h in PBS with 10% FCS, RNAse (10 μg/ml; Boehringer Mannheim, Laval, CA), and propidium iodide. Cells then were analyzed in a FACScan flow cytometer. The telomere fluorescence signal was defined as the mean fluorescence signal in G1/G0 cells after subtraction of the background fluorescence signal (fluorescence in situ hybridization procedure without probe); the results were expressed in molecular equivalents of soluble fluorochrome units. The telomere length was determined by comparing the molecular equivalents of soluble fluorochrome units values of the test sample with those obtained in three cell lines (K562, U937, and Daudi) that are known to have different telomere lengths, which served as standard. The intra-assay coefficient of variation was 4.6%.

Measurement of Intracellular Cytokines

Intracellular cytokine production was measured in freshly isolated cells (1×10^5 cells/ml) and after 24 h in culture with cytokine-phosphate-guanosine oligodeoxynucleotide (CpG-ODN) (10 μg/ml; Invitrogen Life Technologies, Eugene, OR) at 37°C. Incubation with LPS (25 ng/ml) was used as positive control for maximal stimulation. Briefly, 10^6 cells/ml were washed, fixed, and permeabilized using the FIX & PERM cell permeabilization kit (Caltag Laboratories, Burlingame, CA). Thereafter, cells were incubated with the appropriate phycoerythrin (PE)-conjugated antibody: Anti-TNF-α, and anti–IL-6 (Becton Dickinson), or the corresponding isotype antibody, which served as negative control. The values shown are the percentage of cells that expressed the cytokine relative to the value obtained with LPS. Each result (one single value) was the average of three independent determinations in the same sample. The intra-assay coefficient of variation was 3.9%.

Cytometric Bead Array Immunoassay

The cytometric bead array technique was based on the binding of microparticles that were labeled with different fluorescence intensities to antibodies. The maximum wavelength emission was approximately 650 nm (fluorescence-3 channel). The particles were bound by a covalent bond to an antibody against one of the cytokines, IFN-γ or IL-10 (Pharmingen, San Diego, CA). This method allowed the simultaneous determination of different cytokines in the same sample. The cytokines were captured directly in the immunoassay using different antibodies bound to PE, which emitted at 585 nm (fluorescence-2 channel). The PE-conjugated detector antibody completed the sandwich, and the intensity that was measured in fluorescence-2 channel was proportional to the concentration of cytokines in the sample, which was quantified from a calibration curve. An important characteristic of the assay system was that the calibrators, the antibody–bead reagent, and
the second antibody reagent, antibody–PE, were made from mixtures of cytokines. Standard curves (0 to 5000 pg/ml) were derived from a set of calibrators, and the same set was used for all assays. Then, 50 μl of the sample or cytokine standard was added to the mixture of 50 μl each of antibody–PE detector and antibody–bead reagent. The mixture (150 μl) was incubated for 160 min in the dark at room temperature and washed, and the test samples were acquired using the flow cytometer.

**Statistical Analyses**

Comparison between means from different periods were performed by repeated measures ANOVA followed by Bonferroni test. Comparison of two means was done by t test for paired or unpaired data. Nonparametric data were analyzed by Wilcoxon or Mann-Whitney test for paired and unpaired comparison, respectively. Differences were considered significant at \( P < 0.05 \).

**Results**

Table 1 shows demographics and biochemical parameters of patients throughout the study. Twelve of the 31 patients finished the study. Seven patients dropped out during period 1 (three for kidney transplantation, one had colon carcinoma, one had abdominal surgery, one for consent withdrawal, and one for acute infection), two patients dropped out during period 2 (two for kidney transplantation), six patients dropped out during period 3 (one for kidney transplantation, three for pulmonary infection, one for the thrombosis of the vascular access, and one for consent withdrawal), and four patients dropped out during period 3 (two for vascular access thrombosis, one for acute infection, and one for consent withdrawal). There was no statistical difference for any of the indicated parameters evaluated in each of the four study periods.

**Evaluation of CD14 and CD16 Mononuclear Expression**

In patients who were on HF-HD at baseline, a high percentage of PBMC expressed CD14 and CD16 as compared with healthy control subjects (18.5 ± 2.3 versus 5.45 ± 2.5; \( P = 0.001; \) Figure 1). After 4 mo on OL-HDF, these same patients showed a marked decrease in the percentage of CD14\(^+\)CD16\(^+\) cells (13.6 ± 2.9 versus 18.5 ± 2.3; \( P = 0.001 \)). Actually, in all patients, the percentage of CD14\(^+\)CD16\(^+\) decreased after period 1 (OL-HDF). During period 2 (back to HF-HD), the percentage of CD14\(^+\)CD16\(^+\) increased to 19.2 ± 2.8, a value significantly higher than that in period 1 (\( P = 0.001 \)). In general, all patients showed an increase in CD14\(^+\)CD16\(^+\) cells during period 2. After period 3 (OL-HDF), the percentage of CD14\(^+\)CD16\(^+\) was decreased as compared with that in period 2 (13.9 ± 2.3 versus 19.2 ± 2.8; \( P = 0.001 \)). During the last period, the percentage of CD14\(^+\)CD16\(^+\) increased back to 18.6 ± 1.4, which was significantly greater than values that were obtained in period 3 (\( P = 0.004 \)). It also was evident that in patients who were on OL-HDF in both periods 1 and 3, CD14\(^+\)CD16\(^+\) cells were similarly reduced (\( P < 0.05 \)). Conversely, in patients who were on HF-HD in both periods 2 and 4, CD14\(^+\)CD16\(^+\) cells were sim-

**Table 1. Characteristics and laboratory values of the included patients at baseline and during the four periods of the crossover study**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Basal HF-HD</th>
<th>OL-HDF</th>
<th>HF-HD</th>
<th>OL-HDF</th>
<th>HF-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>31</td>
<td>24</td>
<td>22</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Dropouts</td>
<td>—</td>
<td>7</td>
<td>2</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>18/13</td>
<td>14/10</td>
<td>13/9</td>
<td>11/5</td>
<td>8/4</td>
</tr>
<tr>
<td>Age (yr; mean [minimum–maximum])</td>
<td>54.8 (22 to 77)</td>
<td>54.9 (22 to 77)</td>
<td>55.9 (22 to 77)</td>
<td>53.6 (22 to 77)</td>
<td>57.2 (22 to 77)</td>
</tr>
<tr>
<td>Dialysis vintage (mo; mean [minimum–maximum])</td>
<td>55.2 (1 to 162)</td>
<td>55.7 (14 to 158)</td>
<td>58.9 (14 to 158)</td>
<td>50.6 (14 to 122)</td>
<td>50.6 (14 to 122)</td>
</tr>
<tr>
<td>Duration of dialysis treatment (min; mean [minimum–maximum])</td>
<td>221 (180 to 270)</td>
<td>222.5 (180 to 270)</td>
<td>220.9 (180 to 270)</td>
<td>230 (180 to 270)</td>
<td>227.5 (180 to 270)</td>
</tr>
<tr>
<td>Volume infused (L; mean [minimum–maximum])</td>
<td>—</td>
<td>19.43 (16 to 23.5)</td>
<td>—</td>
<td>19.52 (16 to 23.5)</td>
<td>—</td>
</tr>
<tr>
<td>eKt/V (mean [minimum–maximum])</td>
<td>1.46 (1.08 to 1.98)</td>
<td>1.46 (1.05 to 1.99)</td>
<td>1.45 (0.9 to 1.89)</td>
<td>1.51 (1.02 to 1.99)</td>
<td>1.5 (1.1 to 1.99)</td>
</tr>
<tr>
<td>Time average concentration urea (mean [minimum–maximum])</td>
<td>85.85 (50.5 to 120)</td>
<td>89.83 (64.5 to 136)</td>
<td>84.3 (20 to 112.5)</td>
<td>90.8 (63.5 to 127)</td>
<td>88.5 (59.5 to 124.5)</td>
</tr>
<tr>
<td>Leucocytes (mean [minimum–maximum])</td>
<td>8142.7 (4520 to 10700)</td>
<td>7500.5 (5170 to 10890)</td>
<td>7592.0 (4160 to 11300)</td>
<td>6477.8 (3404 to 8310)</td>
<td>7466.67 (4910 to 11160)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl; mean [minimum–maximum])</td>
<td>12.34 (9.2 to 14.3)</td>
<td>12.15 (9.1 to 13.8)</td>
<td>11.8 (9.4 to 13.6)</td>
<td>12.5 (11.3 to 15.1)</td>
<td>12.5 (10.5 to 14.9)</td>
</tr>
<tr>
<td>Ferritin (μg/ml; mean [minimum–maximum])</td>
<td>504.3 (139 to 1064)</td>
<td>402.6 (60 to 788)</td>
<td>472.76 (126 to 1074)</td>
<td>397.8 (340 to 644)</td>
<td>374 (229 to 644)</td>
</tr>
<tr>
<td>Albumin (g/dl; mean [minimum–maximum])</td>
<td>4.03 (3.4 to 4.7)</td>
<td>4.05 (3.7 to 4.5)</td>
<td>4.1 (3.6 to 4.7)</td>
<td>4.08 (3.5 to 4.4)</td>
<td>4.05 (3.6 to 4.7)</td>
</tr>
<tr>
<td>CRP (mg/L; median [minimum–maximum])</td>
<td>4.0 (0.7 to 22.9)</td>
<td>2.0 (0.9 to 17.6)</td>
<td>2.4 (0.8 to 9.2)</td>
<td>2.0 (0.6 to 38.8)</td>
<td>5.6 (0.8 to 41.4)</td>
</tr>
<tr>
<td>β2-microglobulin (mean [minimum–maximum])</td>
<td>25.24 (13.6 to 39.5)</td>
<td>26.1 (20.8 to 31.9)</td>
<td>25.7 (16.5 to 34.3)</td>
<td>24.5 (20 to 32.1)</td>
<td>24.7 (23 to 27.4)</td>
</tr>
<tr>
<td>PO4 (mg/dl; mean [minimum–maximum])</td>
<td>5.09 (3.1 to 1.5)</td>
<td>5.56 (3.6 to 9.4)</td>
<td>5.3 (1.6 to 8.7)</td>
<td>5.4 (3.2 to 8)</td>
<td>5.0 (2.2 to 7.7)</td>
</tr>
<tr>
<td>Ca (mg/dl; mean [minimum–maximum])</td>
<td>9.6 (8.1 to 11.2)</td>
<td>9.6 (7.5 to 11)</td>
<td>9.4 (8.3 to 11.1)</td>
<td>9.8 (9.1 to 10.9)</td>
<td>9.7 (8.4 to 10.9)</td>
</tr>
<tr>
<td>Ca X PO4 (mg/dl^2; mean [minimum–maximum])</td>
<td>47.35 (38.9 to 66.6)</td>
<td>53.82 (34.2 to 88.3)</td>
<td>50.7 (13.2 to 80.9)</td>
<td>53.3 (30.08 to 77.6)</td>
<td>48.6 (22.4 to 70.8)</td>
</tr>
<tr>
<td>Plasma TNF (pg/ml; mean [minimum–maximum])</td>
<td>41.9 (33.2 to 52.9)</td>
<td>30.6 (29.4 to 45.6)</td>
<td>39.7 (30.3 to 47.6)</td>
<td>33.6 (27.9 to 46.8)</td>
<td>43.5 (31.3 to 49.2)</td>
</tr>
<tr>
<td>Plasma IL-6 (pg/ml; mean [minimum–maximum])</td>
<td>18.9 (10.6 to 17.8)</td>
<td>13.2 (5 to 19)</td>
<td>23.3 (14 to 26)</td>
<td>16.4 (11 to 25)</td>
<td>21.6 (15.2 to 31.9)</td>
</tr>
</tbody>
</table>

*CRP, C-reactive protein; HF-HD, high-flux hemodialysis; OL-HDF, on-line hemodiafiltration.*
ilarly increased ($P < 0.05$). It was noteworthy that the changes described in the crossover study were observed in all the studied patients (Figure 1).

**Evaluation of Telomere Length**

Figure 2 shows a representative example of simultaneous analysis by flow cytometry of telomere fluorescence of the CD14$^+$ and of the CD14$^+$CD16$^+$ cells from healthy control subjects (Figure 2A), patients who were on HF-HD (Figure 2B), and patients who were on OL-HDF (Figure 2C). Figure 2A shows a single peak by fluorescence in situ hybridization by flow cytometry, which represents normal telomere length. By contrast, Figure 2B displays a two-peak bimodal distribution that indicates a clear shortening of the telomeres in the CD14$^+$CD16$^+$ cell population. Figure 2C shows that the number of CD14$^+$CD16$^+$ cells is remarkable reduced, and this was paralleled by a reduced amount of cells with shortened telomeres.

**Measurement of Intracellular Cytokines**

Table 2 illustrates the percentage of unstimulated and CpG-ODN-stimulated CD14$^{++}$ and CD14$^+$CD16$^+$ cells that had intracellular cytokine levels detectable by anti–TNF-α and anti–IL-6 antibodies. The CD14$^+$CD16$^+$ population showed significantly higher percentages of both cytokines as compared with CD14$^{++}$ in experiments with both unstimulated and CpG-ODN-stimulated cells in the two dialysis modalities. Among OL-HDF patients, there was a statistically significant increase in the percentage of CD14$^+$CD16$^+$ expression cytokines (IL-6 and TNF-α) when these cells were stimulated with CpG-ODN ($P = 0.01$). By the mean fluorescence channel, both TNF-α and IL-6 concentrations were highly significantly increased in the CD14$^+$CD16$^+$ population without any difference related to stimulation, in both HF-HD and OL-HDF.

**Cytometric Bead Array Immunoassay**

Using a cytometric bead array immunoassay, the concentrations of the two cytokines in the supernatant from unstimulated or CpG-ODN–stimulated PBMC were evaluated (Table 3). There seemed to be a striking ($P = 0.005$) increase in TNF-α and IL-6 in CpG-ODN–stimulated PBMC from HF-HD patients. For both cytokines, unstimulated levels in HF-HD and OL-HDF, as well as the levels after CpG-ODN stimulation in OL-HDF, were in negligible amounts (Table 3).

**Discussion**

In this study, we show for the first time that the convective component of OL-HDF reduces CD14$^+$CD16$^+$ monocyte-derived dendritic cells and their proinflammatory potential. Both dialysis modalities HF-HD and OL-HDF were in fact equal for the membrane used and water quality. The study was performed in patients who were treated in one single center to ensure uniform procedures and standards of ultrapure dialysis fluids during the whole duration of the study.

We adopted a crossover design that allowed us to evaluate the response of each patient’s cells to HF-HD and OL-HDF during four periods, each consisting of 4 mo. The crossover design allowed us to complete the study with a reasonable...
number of patients despite the high rate of dropouts, which was related mainly to the high incidence of kidney transplantation.

Our results clearly show that HF-HD was associated with a higher number CD14\(^{++}\)/CD16\(^{+}\) monocyte-derived dendritic cells in respect to seemingly healthy control subjects. It is known that whereas in young normal individuals most mononuclear cells present a high expression of CD14 together with a high baseline activity, and other phenotypes described in replicative senescent cells. In addition, these cells show similarities of dendritic cells because they have high activity as allogenic antigen-presenting cells. Furthermore, most CD14\(^{++}\)/CD16\(^{+}\) cells express the CD209 molecule, a marker for dendritic cells (data not shown).

Using the crossover design, it was observed that using each patient as his or her own control, all patients while they were on OL-HDF showed a significantly lower number of the CD14\(^{++}\)/CD16\(^{+}\) cells than when they were on HF-HD. By contrast, HF-HD even if performed with the same highly biocompatible, high-flux polysulfone membrane, increased the number of CD14\(^{++}\)/CD16\(^{+}\) monocyte-derived dendritic cells (periods 2 and 4 of the study design), similar to the basal values (\(P > 0.05\)).

The CD14\(^{++}\)/CD16\(^{+}\) cells showed an enhanced capacity to produce intracellular TNF-\(\alpha\) and IL-6 with respect to the conventional CD14\(^{++}\) cells. This occurred already in unstimulated cells from patients who were on HF-HD and OL-HDF and even more when cells were stimulated with CpG-ODN. By the mean fluorescence channels, both TNF-\(\alpha\) and IL-6 concentrations were highly significantly increased in the CD14\(^{++}\)/CD16\(^{+}\) population without any difference related to stimulation, HF-HD, or OL-HDF.

Using a system that involved stimulation of whole peripheral blood followed by staining and analysis of cytokine expression, Belge et al. (18) demonstrated a higher level of TNF-\(\alpha\) protein expression in CD14\(^{++}\)/CD16\(^{+}\) cells as compared with the CD14\(^{++}\) cells. These authors also showed that depletion of these cells from PBMC may reduce the amount of stimulus-induced TNF-\(\alpha\) in the supernatant by 60%, although the CD14\(^{++}\)/CD16\(^{+}\) monocytes accounted for only 10% of the monocytes. This suggested that in vitro and even more so in vivo, during bacterial infection, the minor population of CD14\(^{++}\)/CD16\(^{+}\) cells may be a major source of inflammatory cytokines such as TNF-\(\alpha\). In agreement with Belge et al. (18), we confirm that the CD14\(^{++}\)/CD16\(^{+}\) cells are the major source of cytokines that are released in the supernatant. Furthermore, this occurred for cultured CD14\(^{++}\)/CD16\(^{+}\) cells from HF-HD but not from OL-HDF patients. It also is noteworthy that despite a high intracellular concentration of both cytokines (Table 2), soluble IL-6 and TNF-\(\alpha\) could be detected only in cultured CpG-ODN-stimulated CD14\(^{++}\)/CD16\(^{+}\) cells. This suggests that a co-stimulatory effect is needed for the overt biologic expression by CD14\(^{++}\)/CD16\(^{+}\) cells.

Mortality of dialysis patients remains elevated despite advances in dialysis technology, significant improvement in dialysis quality, and better global care of patients (19,20). On one side, it is interesting to note that a preliminary report from the international Dialysis Outcomes and Practice Patterns Study

### Table 2. Effect of CpG-ODN on intracellular cytokines in CD14\(^{++}\) and CD14\(^{+}\)/CD16\(^{+}\) cells from patients on HF-HD and OL-HDF

<table>
<thead>
<tr>
<th></th>
<th>CD14(^{++})</th>
<th>CD14(^{+})/CD16(^{+})</th>
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<tbody>
<tr>
<td></td>
<td>IL-6</td>
<td>TNF-(\alpha)</td>
</tr>
<tr>
<td>HF-HD Unstimulated</td>
<td>1.6 ± 0.5</td>
<td>4.2 ± 1.6</td>
</tr>
<tr>
<td>CpG-ODN Unstimulated</td>
<td>2.7 ± 1.5</td>
<td>4.8 ± 3.1</td>
</tr>
<tr>
<td>OL-HDF Unstimulated</td>
<td>5.9 ± 0.2</td>
<td>4.2 ± 1.6</td>
</tr>
<tr>
<td>CpG-ODN Unstimulated</td>
<td>6.3 ± 2.9</td>
<td>4.8 ± 3.1</td>
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</tbody>
</table>
| aData are means ± SD from 24 patients studied at basal and at period 1. MFC, mean fluorescence channel; bP = 0.005 versus CD14\(^{++}\) cells. cP = 0.01 versus unstimulated cells.

### Table 3. Effect of CpG-ODN on cytokine (ng/ml) release in the supernatant from 10\(^6\) cells/ml from HF-HD and OL-HDF patients

<table>
<thead>
<tr>
<th>HF-HD</th>
<th>OL-HDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated</td>
<td>IL-6</td>
</tr>
<tr>
<td></td>
<td>TNF-(\alpha)</td>
</tr>
<tr>
<td>CpG-ODN</td>
<td>68 ± 9b</td>
</tr>
</tbody>
</table>

aData are means ± SD from 24 patients studied at basal and at period 1. bP = 0.005 versus unstimulated cells.
(DOPPS) showed that patients who received HDF treatment had a reduced risk for death compared with those who were treated by conventional HD (21). This is a unique report that deserves further analysis and shows for the first time that high-efficiency convective therapies are associated with a reduced death risk, accounting for comorbid condition of patients and dialysis dose increase. The spectrum of eliminated uremic toxins (22–25) together with the adoption of ultrapure dialysate may contribute to explain the reduction of the chronic inflammation (26). The latter has been associated with an elevated number of circulating monocytes; an increased percentage of mature proinflammatory monocytes; and an overproduction of IL-1, TNF-α, and IL-6, without the ability to synthesize the anti-inflammatory cytokine IL-10 (26–28). Overt signs of chronic inflammation as evidenced by high plasma levels of the acute-phase response proteins such as C-reactive protein and substance. Amyloid A has been recognized as an independent risk factor for gross and cardiovascular mortality in ESRD patients (29,30). The patients included in this study showed low plasma levels of C-reactive protein, had normal plasma albumin, and showed no evidence of otherwise clinically evident inflammatory disease. Therefore, the observed changes in the CD14+/CD16+ cell population were observed in well-nourished patients with very mild inflammation. Further studies will be needed to assess whether these observations may be confirmed in HD patients with overt inflammation as well.

Our study has some drawbacks. Although the reduction of the CD14+/CD16+ cell population in the two study periods of OL-HDF was highly reproducible, we do not know for how long this improvement may last in patients who continue to be treated with OL-HDF. It is suggested that the convective component of OL-HDF is the major factor in the reduction of CD14+/CD16+ cell population. However, we did not evaluate the biologic response with respect to different volume exchange, which might give more precise information on the relative role of convective transport. Uremia should be playing a role because, as previously reported, the CD14+/CD16+ cell population was significantly reduced in nondialyzed uremic patients (31).

Conclusion
The CD14+CD16+ cells are increased in HF-HD patients and show features of senescent cells. These cells have an enhanced capacity to exert proinflammatory activities in both unstimulated and stimulated conditions. OL-HDF markedly reduces the number of these proinflammatory CD14+CD16+ cells. Future studies are needed to assess the relevance of these immunologic changes in larger patient populations and the possible therapeutic effect of OL-HDF on overt chronic inflammatory states.

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References


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