Cytokine production in haemodiafiltration: a multicentre study

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Abstract

Background. Bacterial contamination of dialysate may enhance cytokine production in haemodialysis. We tested the hypothesis that intracellular cytokines could be enhanced in a large group of patients exposed to backfiltration of dialysate over a long period of observation.

Methods. The intracellular cytokine (interleukin-1 receptor antagonist and interleukin-1β) concentrations in chronic uraemic patients undergoing haemodiafiltration, which is known to be associated with backfiltration (Group II, 12 patients), were compared to those found in patients treated with a modified haemodiafiltration modality without backfiltration (Group I, 16 patients), in patients shifted from one modality to the other (Group III, 27 patients) and in 10 patients on haemodialysis (Group IV) in a 1-year multicentre study. Group V comprised 10 healthy volunteers. All dialysis monitors were equipped with dialysate ultrafiltration systems. Dialysate contamination was studied by the LAL and the peripheral mononuclear cell/interleukin-1β assays in the presence or absence of polymyxin B.

Results. Intracellular interleukin-1 receptor antagonist and interleukin-1β both increased significantly (P < 0.002) but slowly (after 8 months) in Groups II vs I, and during the 4-month period in haemodiafiltration with backfiltration in Group III. The incidence of post/predialysis concentration ratio (over 1.5) increased two- to threefold in patients treated with haemodiafiltration with backfiltration with respect to haemodiafiltration without backfiltration. Results on the assays for LAL (<0.5 E/ml) and interleukin-1β (range 80.1–90.2 pg/5×10^6 cells; 70.2–81.3 pg/5×10^6 cells with polymyxin B) showed a moderate-to-low degree of dialysate contamination.

Conclusions. Backfiltration of dialysate with moderate-to-low degree of contamination may enhance cytokine synthesis in the long term. Thus, the relevance for dialytic strategies aiming at improving dialysate quality and/or at reducing backfiltration is highlighted.

Key words: backfiltration; biocompatibility; dialysate sterility; haemodialysis membranes; haemodialysis (chronic complications)

Introduction

Despite multiple technological advances including the introduction of reverse osmosis, dialysate ultrafiltration, single-pass hydraulic circuit in dialysis machines, the issue of bacterial contamination of dialysate still represents a challenge for the attending nephrologists [1–5]. The real incidence of pyrogen-related clinical effects may be underestimated or even overlooked. Furthermore, dialysate contamination by bacterial products is recognized as a major factor predisposing to the enhanced cytokine production in haemodiafiltration [6–8].

Pyrogenic substances released from bacterial debris or actively secreted from growing Gram-negative bacteria include cell-wall components such LAL-reactive, lipid A-containing components (lipopolysaccharides, LPS) but also LAL-unreactive, biologically-active compounds (low molecular weight LPS fragments, peptidoglycans, muramylpeptides, exotoxin A from Pseudomonas aeruginosa or hemolysin from Escherichia coli [9,10]. The permeability of dialyser membranes to bacteria-derived pyrogens has been the
The potential transfer of cytokine-inducing substances (CIS) in in vivo haemodiafiltration and the relevance of backfiltration still represent a matter of concern. Most of the studies involving the passage of CIS have been performed in vitro and conclusions have been based on the production of cytokines either by recirculated human donor blood or using the peripheral blood mononuclear cell stimulation (PBMC/cytokine) assay (as reviewed in [13]). In vivo studies dealing with cytokine generation by circulating monocytes have been performed for rather short periods of time (1 month) and have yielded conflicting results [21, 22]. Our hypothesis was to verify cytokine generation in a large group of patients exposed or not to backfiltration of dialysate over a long period of observation (1 year).

To this aim, we studied intracellular cytokine (interleukin-1β (IL-1β) and interleukin-1 receptor antagonist (IL-1ra)) concentrations in chronic uraemic patients undergoing conventional haemodiafiltration, which is known to be associated with backfiltration [23]. The in vivo intracellular concentrations of IL-1ra and IL-1β were compared to those found in patients treated with a modified haemodiafiltration modality that excludes backfiltration [23] or in patients shifted from one modality to the other.

Subjects and methods

Patients

The study lasted 1 year (from April 1995 to May 1996) and was conducted in 13 haemodialysis centres. Different centres chosen for their use of haemodiafiltration techniques were recruited in order to achieve a sufficient number of patients given the expected variability of cytokine measurements. Fifty-five chronically uraemic patients on haemodiafiltration with backfiltration or haemodiafiltration without backfiltration were enrolled. Patients with conditions that could influence cytokine production (such as acute infection or blood transfusion in the past month, chronic infections, active immunological disease, immunosuppressive therapy, previous transplantation or history of malignancy) were excluded from this study.

Patients were randomized into three groups according to the type of treatment (Figure 1). Group I: 18 patients were treated for 1 year with haemodiafiltration with backfiltration (2 drop-outs, 1 left for renal transplantation and 1 because of acute infection; mean age 55.1 ± 12.5 years; mean dialytic age 73.5 ± 63.2 months). Haemodiafiltration without backfiltration was performed using a haemofilter and a haemodialyser in series (see below). Group II: 14 patients were treated with haemodiafiltration with backfiltration (2 drop-outs: 1 died from cardiac infarction, 1 left for renal transplantation; mean age 48.9 ± 18.8 years; mean dialytic age 74.5 ± 67.5 months). Group III: 29 patients (2 drop-outs: 1 died from vascular accident and 1 had vascular access infection; mean age 59.6 ± 18.9 years; mean dialytic age 77.8 ± 59.1 months) started with haemodiafiltration without backfiltration. After 4 months, patients were switched to haemodiafiltration with backfiltration. Finally, after 4 months they were returned to haemodiafiltration without backfiltration. The following controls were made: group IV: 10 patients were treated with conventional haemodialysis (mean age 58.1 ± 17.5 years; mean dialytic age 84.5 ± 71.2 months); group V: 10 healthy normal volunteers (mean age 37.1 ± 7.5 years) served as control for baseline total intracellular cytokine concentration. Informed consent was requested and given in written form whenever patients would be switched to another dialytic modality. Patients were centrally randomized to the dialytic modality. Each patient spent 1 month as run-in period. Blood samples were obtained at the beginning and at the end of each 4-month period before (predialysis) and after (postdialysis) of each session. All centres adhered to a protocol for PBMC purification. In order to standardize the materials and methods used, all reagents (RPMI 1640, buffers, Lymphoprep) were from the same stock. A laboratory technician, skilled in cell purification techniques, performed all purification steps in each of the 13 centres. After drawing, blood samples were immediately processed (average time interval between blood sampling and PBMC purification: approximately 90 min) and the cell suspensions (see below) were stored at −80°C until assayed for intracellular cytokine determination. Over the 12

Fig. 1. Patients’ groups and protocol of studies. For groups IV and V see text. Arrows indicate the timing of pre- and post-dialysis draws for intracellular measurements of IL-1ra and IL-1β as well as for mid-dialysis sampling of dialysate for the LAL and PBMC/cytokine assays. HDF–BF, haemodiafiltration without backfiltration; HDF + BF, haemodiafiltration with backfiltration.
months of the trial, no specimen was inadvertently thawed during storage. One dialysate sample was obtained in a sterile manner mid-dialysis.

**Dialytic modalities**

Haemodiafiltration without backfiltration: haemofiltration and haemodialysis were associated but physically separated by using a double filter connected in series and encompassing a high-permeability membrane (HFT 05, Bellco, Mirandola, Italy, high-permeability polysulphone membrane, 0.5 m²) and (NT1475H, Bellco, Haemophan, 1.37 m²).

Haemodiafiltration with backfiltration: conventional haemodiafiltration was performed with high-permeability haemodialyser (BLS 632, polysulphone, 1.8 m², Bellco) (group II). The two modalities were identical as far as UF/h, reintroduction fluid, Qb and Qd (Table 2). In these operating conditions, backfiltration occurs with the use of highly permeable membranes [23]. Conventional haemodialysis was performed for 4 h, using a synthetically modified, benzyl-substituted cellulose (NC2085, Bellco; 1.95 m²) (group IV). All dialysis monitors were equipped with dialysate ultrafiltration systems.

**Preparation of PBMC**

PBMC were separated from 20 ml heparin-anticoagulated uraemic and normal whole blood by centrifugation (700 g, 20 min) using sterile lymphocyte separator tubes (FAR Italy, Verona, Italy) and undiluted Lymphoprep (Nycomed, Oslo, Norway) as described in [24]. The PBMC layer was harvested into saline and centrifuged at 400 g, for 10 min. The cells were washed in saline twice more. The cells were then resuspended in ultrafiltered tissue culture medium (RPMI 1640, pH 7.4, Sigma Chemical Co., St. Louis, Mo). PBMC were counted using standard haemocytometers. The light density fractions containing mainly 85–90% lymphocytes, 10–15% monocytes, and less than 2% neutrophils was resuspended at a concentration of 2.5 × 10⁶/ml and immediately frozen at −70 °C. In selected experiments, freshly purified PBMC from uraemic patients were centrifuged (1200 g, 20 min) and the supernatants were separated to evaluate the concentrations of extracellular cytokines. In experiments designed to investigate on the cytokine (IL-1β)-inducing property of the dialysate samples, the light density fraction cells were allowed to adhere (1 h, 37°C, 5% CO₂) in 96-well plates at a concentration of 5 × 10⁵ monocytes/0.150 ml RPMI 1640 supplemented with 2% heat-inactivated autologous AB serum (Gibco, Haisley, UK), 100 IU/ml penicillin and 100 μg/ml streptomycin. In selected experiments, dialysate samples were incubated at 37°C for 30 min with polymyxin B (PMX B, 10 μg/ml, Sigma Chemical Co, St Louis, Mo), an inhibitor of lipid A [25], and added to the cells. In control experiments, PMX-B per se at the same final concentrations did not significantly alter the production of IL-1β induced by 5 ng/ml of LPS from *Escherichia coli* (strain serotype 055:B5, Sigma, L-4005). RPMI contained no detectable endotoxin as assessed by the LAL test. Non-adherent cells were eliminated by three washes. Standard dose response curve was performed with filtrates from *Pseudomonas aeruginosa* (kind gift of Dr G. Lonnemann). All samples were stored for not more than 1 month.

**Laboratory methods**

Cells were lysed by three freeze–thaw cycles. Intracellular cytokine concentrations were determined by ELISA (for IL-1ra, Medgenix Diagnostics, Brussels, Belgium; for IL-1β, Immunotech, Marseille, France). The percentage of intra- and interassay variabilities were 11.9 and 12.5; for IL-1ra, intra-assay and interassay % variability were 9.3 and 11.3, for IL-1β, intra-assay and interassay % variability were 10.9 and 10.4. The sensitivity thresholds (pg/ml) were 5.5 for IL-1β, and 6.5 (for plasma) or 22 (for aqueous samples) for IL-1ra. Samples were assayed in duplicate. Results were normalized to the cell concentration of 2.5 × 10⁶ and expressed in pg/2.5 × 10⁶ cells.

**Presentation of results**

The average ± standard deviation of the mean was calculated per each cytokine within the groups. Paired or unpaired t test was performed to assess statistical significance (P<0.05). Furthermore, we calculated the post/pre ratio (for IL-1β and IL-1ra). Ratios over 1.5 were considered as indicative of stimulation. The ratios (means ± standard deviation) of each group were calculated and compared by the Newmann–Keuls method. Furthermore, the incidence of ratios over 1.5 for each cytokine on the total number of samples tested was calculated in all the groups.

**Results**

Table 1 summarizes the different dialytic modalities used in groups I, II and IV. In group I and II, haemodiafiltration (without and with backfiltration respectively) was performed using similar parameters (i.e. duration, blood flow rate, dialysate flow rate, substituting cellulose) and showed a moderate-to low property of the dialysate samples, the light density fraction inducing property of dialysate using the PBMC haemodiafiltration (without and with backfiltration respectively) was performed for 4 h, using a synthetically modified, benzyl-substituted cellulose (NC2085, Bellco; 1.95 m²) (group IV). All dialysis monitors were equipped with dialysate ultrafiltration systems.

Table 2. The average ± standard deviation of the mean was calculated per each cytokine within the groups. Paired or unpaired t test was performed to assess statistical significance (P<0.05).

Furthermore, we calculated the post/pre ratio (for IL-1β and IL-1ra). Ratios over 1.5 were considered as indicative of stimulation. The ratios (means ± standard deviation) of each group were calculated and compared by the Newmann–Keuls method. Furthermore, the incidence of ratios over 1.5 for each cytokine on the total number of samples tested was calculated in all the groups.

**Results**

Table 1 summarizes the different dialytic modalities used in groups I, II and IV. In group I and II, haemodiafiltration (without and with backfiltration respectively) was performed using similar parameters (i.e. duration, blood flow rate, dialysate flow rate, dialysate ultrafiltration, reinfusion flow rate, reinfusion volume). The effectiveness of the treatment as indicated by the treatment time, blood flow, differences in serum urea and the weight loss were the same in all groups (Table 1). As shown in Table 2, endotoxin contamination of the dialysate (LAL assay), and the IL-1β-inducing property of dialysate using the PBMC/cytokine assay collected in groups I, II, III and IV were not different during the study period between the two dialytic modalities and showed a moderate-to low degree of dialysate contamination (for comparison see [3,7]). Furthermore, incubation with PMX B reduced by 12.5, 9.6 and 9.15%, the IL-1β-inducing properties by dialysates from group I, II, and IV respectively.

Figure 2 summarizes the post/predialysis ratios of intracellular IL-1ra and IL-1β in patients of group I, II, III (and group IV, see legend to Figure 2) over the 12-year study divided into three 4-month periods. The ratios were calculated on the absolute values (in pg/2.5 × 10⁶ see legend to Figure 2). While ratios (post/pre IL-1ra and IL-1β) of group I never significantly differed from basal values, ratios of group II significantly (P<0.002) increased at 8 months and remained so after 12 months. Furthermore, a highly significant (P<0.002) difference was found for IL-1ra and IL-1β between group I and II after 8 months and for IL-1β after 12 months.
In group III, by analysing the post/predialysis ratios at each 4-month period, intracellular IL-1ra and IL-1β significantly rose after 8 months ($P<0.001$ with respect to basal and 4th-month data) and returned to basal values ($P>0.05$) in the following 4-month period (12th month).

As shown in Figure 3, the incidence of ratios over 1.5 for IL-1ra and IL-1β increased from 25 and 33% at 4 months, to 50 and 42% at 8 months, and to 48 and 49% at 12 months. No significant increase of post/pre ratios of intracellular concentrations of IL-1ra and IL-1β could be detected in patients on haemodiafiltration without backfiltration (group I) in none of the study periods. Likewise, the incidence of ratios over 1.5 for IL-1ra and IL-1β in group I was not increased over the three 4-month periods (18 and 29% at 4 months, 10 and 25% at 8 months, 39 and 30% at 12 months).

**Discussion**

Backfiltration and backdiffusion of contaminated dialysate have been recognized as driving forces for the transmembrane passage of endotoxin and other CIS during dialysis [11,26]. Haemodiafiltration has recently received new interest due to its potential of greater efficiency and the possibility of reducing dialysis treatment time. However, haemodiafiltration, but not haemodialysis performed with low-permeability membranes, is associated with the risk of backfiltration [23]. During haemodiafiltration or high-flux haemodiafiltration with high-permeability membranes, the extreme variations of transmembrane pressure during each influx/reflux cycle may induce a small increase in LAL-reactive endotoxin in the blood of patients without clinically relevant reactions [27–29]. Although polysulphone membranes have been shown in vitro to be impermeable to large amounts of LAL-reactive endotoxin and other bacterial and IL-1β-derived fragments in conditions where backfiltration occurred, the incidence of pyrogen reactions was ascribed to the practice of reuse.

**Table 1.** Dialysis parameters of the three modalities used in this study. Haemodiafiltration without BF (group I), haemodiafiltration with backfiltration (BF, group II), and haemodialysis (HD, group IV)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Haemodiafiltration ± BF</th>
<th>Haemodiafiltration–BF</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (min)</td>
<td>210 ± 26</td>
<td>195 ± 20</td>
<td>220 ± 15</td>
</tr>
<tr>
<td>Blood flow rate (ml/min)</td>
<td>351 ± 41</td>
<td>320 ± 23</td>
<td>350 ± 41</td>
</tr>
<tr>
<td>Dialysate flow rate (ml/min)</td>
<td>500 ± 98</td>
<td>510 ± 97</td>
<td>550 ± 100</td>
</tr>
<tr>
<td>Dialysate ultrafiltration</td>
<td>Psu Amicon 1.8 m²</td>
<td>Psu Amicon 1.8 m²</td>
<td>Psu Amicon 1.8 m²</td>
</tr>
<tr>
<td>Reinfusion fluid</td>
<td>Lactate 42 mmol</td>
<td>Lactate 42 mmol</td>
<td></td>
</tr>
<tr>
<td>Reinfusion volume (l)</td>
<td>9–10</td>
<td>9–10</td>
<td></td>
</tr>
<tr>
<td>Dialysate surface (m²)</td>
<td>1.8</td>
<td>0.5 ± 1.6</td>
<td>1.95</td>
</tr>
<tr>
<td>Dialyser membrane</td>
<td>Psu</td>
<td>Psu + He</td>
<td>SMC</td>
</tr>
<tr>
<td>Δ body weight (%)</td>
<td>4</td>
<td>3.7</td>
<td>3.9</td>
</tr>
<tr>
<td>Urea predialysis (mmol/l)</td>
<td>25.2 ± 1.2</td>
<td>25.7 ± 0.8</td>
<td>27.0 ± 0.9</td>
</tr>
<tr>
<td>Urea postdialysis (mmol/l)</td>
<td>8.9 ± 0.5</td>
<td>8.7 ± 0.8</td>
<td>9.2 ± 0.8</td>
</tr>
</tbody>
</table>

**Table 2.** Bacterial contaminants in the dialysates of the three modalities used in this study. Haemodiafiltration without BF (group I), haemodiafiltration with backfiltration (BF, group II), and haemodialysis (HD, group IV)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Haemodiafiltration + BF</th>
<th>Haemodiafiltration–BF</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAL (Eu/ml)</td>
<td>0.13 ± 0.06</td>
<td>0.16 ± 0.05</td>
<td>0.18 ± 0.06</td>
</tr>
<tr>
<td>IL-1β (pg/5 × 10⁶ cells)</td>
<td>80.12 ± 11.5</td>
<td>90.23 ± 14.11</td>
<td>84.32 ± 13.94</td>
</tr>
<tr>
<td>IL-1β (pg/5 × 10⁶ cells) + PMX-B</td>
<td>70.23 ± 9.41</td>
<td>81.32 ± 21.12</td>
<td>76.31 ± 23.32</td>
</tr>
</tbody>
</table>

In selected experiments, dialysates were preincubated with PMX-B (10 μg/ml) for 20 min at 37°C and added to 2 × 10⁶ cells (see Subjects and methods). Group III values for LAL and PBMC/cytokine assays were 0.24 ± 0.08 and 97 ± 43 pg/5 × 10⁶ cells. Data are given as means ±1 standard deviation. Number of tested samples for LAL and PBMC assays were 60 for group I, 39 for group II, 90 for group III, and 35 for group IV.
Cytokine production in haemodiafiltration

Fig. 2. Post/pre-dialysis (for IL-1ra and IL-1β) ratios at the start and at each 4-month period in group I (○), group II (■) and group III. Dotted line indicates the ratio of 1.5 arbitrarily taken as net production index (see Subjects and methods). Bars are the standard deviation. *P<0.05 with respect to the basal value. No indication indicates the absence of statistical significance. Ratios were calculated on post/pre-dialysis intracellular cytokine concentrations from purified PBMC. The total leukocyte count was 2.01 × 10^6/ml (±0.79; % monocytes 11.68 ± 9.31, average resuspension volume 2.78 ± 0.31 ml). Thus the concentrations of cytokines were well above the threshold limits (see Subjects and methods) and were normalized to 2.5 × 10^6 PBMC The concentrations of post/pre-dialysis IL-1ra and IL-1β are listed herein (the cumulative means ± 1 standard of 152, 144, 324 and 40 samples from group I, group II, group III, group IV patients for IL-1ra and IL-1β respectively, drawn over the 12-month study): 1148 ± 1298/1167 ± 1228 and 7.5 ± 9.7/7.4 ± 11.8, for group I; 1212 ± 1109/808 ± 737 and 11.6 ± 12.5/6.5 ± 7.1 for group II; 2819 ± 2396/2561 ± 1494, and 11.3 ± 7.8/9.2 ± 4.9 for group III; 1256 ± 1367/1231 ± 1354 and 6.8 ± 8.7/6.9 ± 10.3 for group IV patients. No statistically significant differences could be observed between pre-and post (IL-1ra)-(IL-1β)-dialysis absolute values in the different groups. In group IV, no significant increase was observed for all cytokines and the relative ratios were superimposable to those of group I (P>0.05). Intracellular cytokine concentrations in PBMC from group V healthy subjects were below the sensitivity thresholds for the cytokine assays (see Subjects and methods).

dialyser geometry and a Kf of 28.11 ml/h/mm Hg/m^2 for polysulphone (Qb 300 ml/min, Qd 500 ml/min), a 5760 ml/h is the ultrafiltration rate that was shown to be required to abrogate backfiltration. Only by increasing positive pressure on the dialysate compartment, one can reduce ultrafiltration rate to below 3600 ml/h, thereby increasing the net backfiltration. Physical separation of convection and diffusion (haemofilter + low-flux membrane dialyser) [32] abrogates backfiltration provided that net ultrafiltration is imposed not only in the haemofilter (3000 ml/h) but also in the dialysers (360–600 ml/h) [23]. Based on a Kf of 5 ml/h/mm Hg/m^2 for cellulose membranes (Cuprophan®) or on a Kf of 7.5 ml/h/mm Hg/m^2 for diethylaminoethyl-substituted cellulose (Haemophan®) membranes (at Qb 300 ml/min and Qd 500 ml/min), no backfiltration could be expected at ultrafiltration rates of 600 ml/min. The patients reported in this study underwent haemodiafiltration for 1 year (group II) with a mean of 3384 ml/h net ultrafiltration using a 1.6 m^2 polysulphone haemodialysers, a condition that would certainly allow backfiltration to occur.

The present multicentre study aimed at testing the hypothesis that cytokine generation could be enhanced in patients exposed to backfiltration over a long period (1 year). This hypothesis was confirmed by our findings. In fact, haemodiafiltration with backfiltration was associ-
Fig. 3. Percentage incidence of post/pre-dialysis (for IL-1ra and IL-1β) (over 1.5) in group I (○), group II (■) and group III. This was calculated on the number of ratios over 1.5 on a total of 192 samples for each cytokine (basal+pre- and post- at each 4-month period, group I), of 144 samples (basal+pre- and post- at each 4-month period, group II), and of 324 samples (basal+pre- and post- at each 4-month period, group III). As control, group IV (data not shown) showed an incidence of ratios over 1.5 less than 23% for all the cytokines tested. *P < 0.01 vs basal values.

ated with a significant increase of intracellular concentration of IL-1ra and IL-1β (as inferred from the post/pre ratios) (group II) and group III (at 8 months). Such an increase appeared to be significant only after 8 months. The possibility that backfiltration could be implicated in the enhanced intracellular concentrations of IL-1ra and IL-1β was further strengthened by post/pre ratios and in the incidence of ratios over 1.5 in patients of group III at 8 and 12 months. Post/pre ratios of intracellular concentrations of IL-1ra and IL-1β significantly (P < 0.002 vs basal values) increased only during haemodiafiltration with backfiltration (8 months). Furthermore, when patients were shifted from haemodiafiltration with backfiltration to haemodiafiltration without backfiltration, post/pre ratios of intracellular concentrations of IL-1ra and IL-1β significantly decreased to basal values (P < 0.05).

There was an unexpected delay in the increase (group II) as well as a rapid increase/decrease (group III) of the intracellular concentration of IL-1ra and IL-1β. We suggest the following explanations: (1) dialysates had a moderate-to-low degree of contamination of the (for comparison, see [3,7]); although group IV had a significant higher LAL concentration, no significant increase could be observed in the post-/pre-ratios of IL-1ra and IL-1β in agreement with the lack of passage of LAL-reactive material through haemodialysis membranes [13]; (2) the data analysis enabled us to reduce the impact of high variability of absolute values, thus making the sample size appropriate; (3) the criteria used to consider the occurrence of stimulation was intentionally conservative as only ratio values of 1.5 or over (and not within 1 and 1.5) were considered in the statistical analysis. However, we cannot exclude that the time frame for such increases or decreases in intracellular concentrations of cytokines may have been affected by the sampling interval.

Studies concerning cytokine concluded for relatively low or no significant differences to the large interindividual variability [34,35]. A significant portion
of IL-1 synthesized by activated PBMC remains intracellular, and is not released into the circulation [36,37]. Isolation of PBMC removes circulating factors such as proteases, soluble receptors or non-specific binding proteins [38–40] that could have potentially interfered with our cytokine measurements. Our PBMC preparations contained less than 2% neutrophils. These cells may accelerate cytokine breakdown by releasing proteases, or they may promote clearance by binding and internalizing cytokines [41]. Finally, the absence of any incubation allowed us to rule out any possible contamination.

Previous studies have indicated that secreted IL-1ra is a sensitive marker of enhanced cytokine synthesis induced the bacterial contamination of the dialysate [42,43]. In this study, the assessment of the intracellular IL-1ra amplified the cytokine production as later evidenced by increases in intracellular IL-1β (see group III at 8 months). There are two forms of IL-1ra: a form possessing a 25 amino acid peptide that is secreted to more than 90% [43]. The intracellular form of IL-1ra does not have a typical signal sequence and therefore remains cell-associated [44].

The key question remains on what is the relevance of these observations in the clinical outcome. Although no acute symptoms or clinical evidence of backfiltration occurred, the period of observation and the type of study design were not made to evaluate morbidity or mortality. In search for a clinical link with bioincompatibility-related enhanced cytokine production, we have recently analysed indexes of systemic inflammation in patients exposed to backfiltration. Preliminary data have suggested that patients of group II have the highest levels of plasma C-reactive protein that correlated with plasma IL-6 (Tetta, Panichi, in preparation). Whether acute phase proteins such as C-reactive protein may be predictive for a higher risk of developing cardiovascular disease in the uraemic dialysed population as it has been shown in normal healthy subjects [45], still awaits confirmation.

Future prospective, randomized, case control multicentre studies will have to define the clinical outcome in long-term dialysis patients exposed to backfiltration. These studies will need to evaluate the clinical relevance of patients’ exposure to bacteria contaminated dialysates. The present and most recent observations suggest that backfiltration induces a chronic, slowly-developing inflammatory state that may be abrogated by avoiding backfiltration of contaminated dialysate. Future studies will have also to assess whether the adoption of ultrapure dialysis solutions is able to abrogate and/or to minimize the effects related to backfiltration.

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