Effects of dialyzer membrane on interleukin-1β (IL-1β) and IL-1β-converting enzyme in mononuclear cells

SILVIA LINNENWEBER and GERHARD LONNEMANN
Division of Nephrology, Medizinische Hochschule Hannover, Hannover, Germany

Effects of dialyzer membrane on interleukin-1β (IL-1β) and IL-1β-converting enzyme in mononuclear cells.

Background. In vitro stimulation of mononuclear cells (peripheral blood mononuclear cells; PBMCs) with an endotoxin (lipopolysaccharide; LPS) revealed elevated cell-associated levels of interleukin-1β (IL-1β) in end-stage renal disease (ESRD) patients on Cuprophan hemodialysis (HD), suggesting a defect in the process of IL-1β’s release from activated cells. IL-1β is initially synthesized as an inactive precursor called proIL-1β. ProIL-1β is processed into the biologically active mature form of IL-1β (mIL-1β) requiring the specific IL-1β-converting enzyme (ICE).

Methods. Using specific immunoassays (enzyme-linked immunosorbent assays), we measured cell-associated and extracellular proIL-1β as well as mIL-1β in LPS-stimulated PBMCs to test whether ICE-dependent processing of proIL-1β and/or secretion of mIL-1β was impaired in ESRD patients compared with healthy controls. PBMCs of healthy controls (N = 9), of ESRD patients on peritoneal dialysis (PD, N = 10), and of those patients on intermittent HD (N = 8) were studied. The same HD patients were studied three times with low-flux Cuprophan (GFS 12), low-flux polysulfon (F6 HPS), and high-flux polysulfon (F60 S) in random order. PBMCs were separated from whole blood by Ficoll-Hypaque centrifugation and incubated in vitro for 18 hours in the presence LPS (10 ng/mL). Extracellular (PBMC culture supernatants) and cell-associated (cell lysates) levels of proIL-1β and mIL-1β were measured.

Results. The total production (cell-associated plus extracellular) of LPS-induced IL-1β (proIL-1β plus mIL-1β) was similar in healthy controls (25.96 ± 0.84 ng/2.5 × 10⁶ PBMC), PD patients (29.53 ± 1.31 ng/2.5 × 10⁶ PBMC), and in Cuprophan-treated HD patients (23.28 ± 1.24 ng/2.5 × 10⁶ PBMC). In normal controls, 43.6% of the total IL-1β was processed into mIL-1β, which was significantly more than that in PD patients (27.3%, P < 0.02) but was similar to that in Cuprophan-treated HD patients (37.1%). Comparing cell-associated and extracellular concentrations of mIL-1β, PBMCs of normal controls secreted 82.2% of mIL-1β; this was significantly more than that in PD patients (59.4%, P < 0.01) and that in Cuprophan HD patients (54.2%, P < 0.01). When HD patients were switched from Cuprophan to F6 HPS or F60 S, neither total IL-1β production nor processing of IL-1β changed. However, secretion of mIL-1β increased significantly with F6 HPS (80.6%, P < 0.01) as well as with F60 S (76.6%, P < 0.02) compared with Cuprophan.

Conclusion. We conclude that the ability of PBMCs to produce IL-1β in response to LPS is normal in PD patients as well as in HD patients. ICE-dependent processing of inactive proIL-1β into biologically active mIL-1β is reduced in PD patients, but not in HD patients. Secretion of mIL-1β is impaired in PD and HD patients treated with Cuprophan. This impaired ability to secrete active mIL-1β seems to be independent of ICE activity and is normalized when HD-patients are switched from Cuprophan to low- or high-flux polysulfon. Increased cell-associated levels of biologically active mIL-1β in circulating PBMCs represent a state of inflammation that may contribute to chronic inflammatory diseases such as β2-microglobulin amyloidosis. Replacement of Cuprophan by synthetic membranes normalizes PBMC function and reduces the state of inflammation in ESRD patients.

Proinflammatory cytokines such as interleukin-1β (IL-1β) are mediators of acute and chronic inflammation. Cytokines are predominantly produced by circulating mononuclear cells (PBMCs). Several experimental and clinical studies provided evidence that hemodialysis (HD) with complement-activating dialyzer membranes and/or contaminated dialysate cause activation of PBMCs, as indicated by increased production of cytokines [1, 2]. IL-1β is not constitutively produced but is always de novo synthesized in response to stimulation. Activation of cytokine production is best described following the sequence of events when PBMCs are stimulated with endotoxin (LPS). LPS binds to a specific plasma protein called LPS-binding protein (LBP) and the LPS-LBP complex triggers the CD14 receptor on PBMCs. Using several signal transduction pathways, activation of the CD14 receptor results in transcription of IL-1β mRNA [3]. IL-1β mRNA is subsequently translated into a biologically inactive pro-peptide (proIL-1β), which needs to be processed to gain biological activity. Processing of proIL-1β requires a specific enzyme called IL-1β-converting enzyme (ICE), which cleaves off a pro-piece to produce the biologically active mature mIL-1β molecule of 17 kD molecular weight, which

Key words: end-stage renal disease, hemodialysis, Cuprophan membranes, polysulfone membranes, endotoxin.

© 2001 by the International Society of Nephrology
is secreted from PBMCs. The mechanism of mIL-1β secretion is not fully understood because IL-1β is missing a signal peptide typical for proteins meant to be secreted from cells. Some authors speculate that ICE is not only required for processing of IL-1β but is also involved in the process of secretion of mIL-1β [3]. Alternatively, mIL-1β could be released from activated PBMCs by a mechanism independent of ICE activity. Once released into the circulation, mIL-1β acts predominantly in the cell–cell interaction with circulating cells and resident tissue cells via specific receptors and has a very short half-life because of rapid endogenous clearance [3].

We have previously demonstrated that PBMCs of end-stage renal disease (ESRD) patients on long-term Cuprophan HD have increased cell-associated levels of biologically active IL-1β compared with PBMCs of healthy controls [4]. When the same patients were switched to HD with a synthetic high-flux membrane (AN69, Hospal), LPS-induced total production of IL-1β (total = cell-associated plus extracellular) remained unchanged, but significantly more IL-1β was released into the cell supernatants, indicating improved PBMC function when ESRD patients were treated with a more biocompatible dialyzer membrane [4]. In this study, we used a radio-immunoassay that detects both proIL-1β and mIL-1β. For this reason, we could not distinguish whether increased cell-associated levels of IL-1β in Cuprophan-HD were due to impaired processing of proIL-1β into mIL-1β (suggesting reduced ICE activity) or due to a defect in secretion of biologically active mIL-1β from activated PBMCs. In the present study, we therefore used two specific immunoassays to measure proIL-1β and mIL-1β quantitatively in LPS-stimulated PBMCs of ESRD patients. In order to assess the role of treatment modality, ESRD patients on HD were compared with age-matched patients on peritoneal dialysis (PD) and to age-matched healthy controls. To test the effect of the dialyzer membrane, patients on HD were treated with low-flux Cuprophan, low-flux polysulfon, and high-flux polysulfon dialyzers in randomized order in three consecutive study periods.

METHODS

After giving informed consent, 18 ESRD patients and 9 age-matched healthy controls [4 women and 5 men, mean age (range) 38.9 (25 to 56) years] were included in the study. Ten ESRD patients [5 women and 5 men, mean age 39.7 (24 to 55) years] were on cyclilor-assisted PD. Eight ESRD patients [4 women and 4 men, mean age (range) 41.1 (22 to 71) years] were on chronic intermittent HD. The same eight patients were randomized to three consecutive study periods of five weeks, each with three HD sessions of four to five hours per week, in which the following dialyzers were tested: low-flux Cuprophan (GFS 12, surface area 1.3 m²; Gambro, Hechingen, Germany), low-flux polysulfon (F6 HPS, surface area 1.3 m²; Fresenius, Bad Homburg, Germany), and high-flux polysulfon (F60S, surface area 1.3 m²; Fresenius).

Blood sampling was performed twice within three weeks in all subjects and study periods. In HD patients, blood was drawn from the arteriovenous fistula before the start of the HD session after the long interdialytic interval. In PD patients, blood was drawn from a cubital vein in the morning after the overnight drain of peritoneal dialysate. PBMCs were separated from heparinized whole blood samples by Ficoll-Hypaque centrifugation. After washing in saline, PBMCs were resuspended in pyrogen-free polypropylene tubes containing tissue culture medium (RPMI 1640, supplemented with antibiotics) at a final concentration of 2.5 × 10⁶ PBMCs per mL. PBMCs were incubated in the presence of LPS (10 ng/mL) for 18 hours at 37°C in a humidified atmosphere containing 5% CO₂. Following incubation, culture supernatants were separated from cells to obtain two fractions in which extracellular (supernatants) and cell-associated (cell-lysates) IL-1β was measured. PBMCs were lyzed by three freeze-thaw cycles.

Enzyme-linked immunosorbent assays for the detection of proIL-1β and mIL-1β

The two enzyme-linked immunosorbent assays (ELISAs) were purchased from Cistron (Pine Brook, NJ, USA). Both assays use monoclonal capture antibodies and polyclonal detection antibodies. The proIL-1β ELISA detects 100% of proIL-1β and 0% of mIL-1β and is therefore highly specific. In contrast, the polyclonal detection antibody of the mIL-1β assay detects 100% of mIL-1β but also a small amount of proIL-1β (10%).

ProIL-1β and mIL-1β concentrations were expressed in ng/2.5 × 10⁶ PBMCs. Results of the two determinations per subject and study period were averaged. In order to compare study groups (and study periods in HD-patients), the results of groups per periods were expressed as means ± SEM. Statistics were performed using the Student’s t-test for unpaired (comparison of controls with PD and HD patients) and paired (effect of different dialyzers in the same HD patients) observations.

RESULTS

The total production (cell-associated plus extracellular) of LPS-induced IL-1β (proIL-1β plus mIL-1β) was 25.96 ± 0.84 ng/2.5 × 10⁶ PBMCs in healthy controls, which was not significantly different from that in PD patients (29.53 ± 1.31 ng/2.5 × 10⁶ PBMC) and that in Cuprophan-treated HD patients (23.28 ± 1.24 ng/2.5 × 10⁶ PBMC). ProIL-1β was not detectable in PBMC culture supernatants, indicating that the inactive pro-peptide of
IL-1β remains inside intact mononuclear cells. Cell-associated concentrations of proIL-1β were significantly ($P < 0.01$) higher in PD patients compared with controls and with HD patients on Cuprophan (Fig. 1). These elevated levels of proIL-1β were accompanied by lower levels of mIL-1β (cell-associated plus extracellular) in PD patients, indicating less processing of proIL-1β. In PBMCs of PD patients, 27.3% of total IL-1β was processed into active mIL-1β. This was significantly less than that in healthy controls (43.6%, $P < 0.02$) and that in Cuprophan-treated HD patients (37.1%, $P < 0.02$). These data suggest impaired IC activity in PD patients but not in HD patients. Comparing levels of biologically active mIL-1β in the cell-associated and extracellular compartments, PBMCs of normal controls secreted 82.2% of mIL-1β into the culture supernatant. This was significantly more than that in PD patients (59.4%, $P < 0.01$) and that in Cuprophan HD patients (54.2%, $P < 0.01$). According to these data, the process of secretion of mature IL-1β is impaired in all ESRD patients independently of the modality of renal replacement therapy (HD vs. PD).

When the same HD patients were randomly assigned to three consecutive study periods of five weeks each using Cuprophan, F6 HPS, or F60S, dialyzer membrane-dependent differences were observed (Fig. 2). Compared with Cuprophan HD, neither total LPS-induced IL-1β production (proIL-1β + mIL-1β) nor the percentage of processed IL-1β changed significantly when low-flux or high-flux polysulfon dialyzers were used. However, the percentage of secreted mIL-1β increased significantly with F6 HPS (80.6%, $P < 0.01$) as well as with F60S (76.6%, $P < 0.02$) compared with Cuprophan. These data suggest that replacement of the complement activating Cuprophan membrane by the biocompatible low-flux polysulfon membrane is able to normalize impaired secretion of biologically active mIL-1β in the presence of unchanged and normal ICE-dependent processing of proIL-1β. The use of high-flux polysulfon instead of low-flux polysulfon is not necessary to reach this effect.

**DISCUSSION**

It is a well-known phenomenon that ESRD patients on long-term HD with complement-activating Cuprophan membranes show signs of mononuclear cell (PBMC) activation, as indicated by increased cell-associated levels of the proinflammatory cytokine IL-1β [5, 6]. In additional studies, we demonstrated that ex vivo LPS stimulation of PBMC induced similar total production of IL-1β in cells of Cuprophan-treated ESRD patients compared with PBMCs of healthy controls [4]. However, the amount of IL-1β secreted from LPS-stimulated PBMCs was significantly reduced in Cuprophan HD patients compared with normal controls, indicating a defect in the mechanism of IL-1β secretion [4]. When the same patients were switched to synthetic high-flux AN69 dialyzers, IL-1β secretion improved significantly. In parallel, prostaglandin E2 (PGE2) production decreased significantly during AN69 HD. Because the addition of indomethacin to LPS-stimulated PBMCs of Cuprophan HD patients also improved secretion of IL-1β in the presence of unchanged total IL-1β production, we concluded that the
endogenous PGE₂ production inhibits the unknown mechanism of IL-1β secretion, and we speculated that PGE₂ could inactivate ICE, which is essential for processing and which may be important for the secretion of mature IL-1β as well [4].

To investigate impaired IL-1β secretion in ESRD patients further, we conducted the present study in which proIL-1β, the substrate of ICE, and mIL-1β, the product of ICE, were specifically and quantitatively measured in the cell-associated as well as in the extracellular compartment of LPS-stimulated PBMCs. First, we compared PBMCs of ESRD patients on Cuprophan HD with those of age-matched patients on PD and those of age-matched healthy controls. ProIL-1β was not detectable in any PBMC culture supernatant, confirming published data showing that the inactive precursor is not released from intact PBMCs [3]. The comparison of cell-associated levels of proIL-1β with total production of mIL-1β offers the possibility to describe ICE-dependent processing of IL-1β in the three study groups. Turnover of proIL-1β into mIL-1β was significantly reduced in PD patients but not in HD patients on Cuprophan HD compared with healthy controls. These data suggest that ICE activity is reduced in PD patients, but not in HD patients. When cell-associated and extracellular levels of the biologically active form of IL-1β were compared, our data demonstrate that PBMCs of healthy controls contained significantly less mIL-1β inside the cells, which was accompanied by significantly higher levels of mIL-1β in the cell supernatants compared with that of PD and Cuprophan HD patients. These data suggest that secretion of mIL-1β is impaired in PD patients as well as in HD patients on Cuprophan HD. With respect to Cuprophan HD, these data confirm our previous study discussed previously in this article [4]. Since ICE-dependent processing of proIL-1β seems to be normal in HD patients on Cuprophan, we speculate that the defect in mIL-1β secretion is not due to inadequate activity of ICE. There seems to be an ICE-independent mechanism of mIL-1β release from activated PBMCs that is inhibited, possibly due to increased PGE₂ production [4].

The second part of our study demonstrates a clear effect of the dialyzer membrane on mIL-1β secretion from LPS-stimulated PBMCs. When Cuprophan dialyzers were replaced by low-flux polysulfone for five weeks, secretion of mIL-1β was no longer different from that in healthy controls. The ratio of processed mIL-1β over unprocessed proIL-1β remained unchanged, indicating that ICE activity did not differ under the two HD conditions. These data support the concept that there is an ICE-independent mechanism of mIL-1β secretion, which is improved when biocompatible dialyzer membranes are used. The factor influencing mIL-1β secretion seems to be induced during Cuprophan HD—for example enhanced PGE₂ due to complement activation—because normalization of mIL-1β secretion was reached with a low-flux biocompatible membrane. The use of high-flux polysulfone did not further improve PBMC function, suggesting that the observed beneficial effect was not due to removal of any unknown factor of middle molecular size influencing mIL-1β secretion. Biocompatibility seems to be more important than high-flux dialysis if improved PBMC function is the goal in ESRD patients on HD.

The reason for impaired mIL-1β secretion from PBMCs in PD patients remains to be elucidated. We cannot exclude that in this population impaired ICE activity may also influence secretion of mIL-1β.

In conclusion, secretion of mature IL-1β from LPS-stimulated PBMCs is impaired in ESRD patients on HD with bioincompatible Cuprophan dialyzer membranes. This change in PBMC function is reversible when biocompatible membranes are used. The impaired ability of PBMC to secrete mIL-1β contributes to high cell-associated levels of biologically active IL-1β detectable in circulating PBMCs of Cuprophan-treated patients as late as three days after the end of the previous HD session [5, 6]. Circulating PBMCs carrying bioactive IL-1β have to be considered as primed or preactivated cells, which are ready to release this proinflammatory cytokine in response to a second stimulus such as AGE peptides [7] or others. In order to improve the state of inflammation associated with long-term intermittent HD, biocompatible HD membranes are recommended.

Reprint requests to Gerhard Lonnenmann, M.D., Gemeinschaftspraxis für Nephrologie/Dialyse, Eichendorf 15, D-30851 Langenhagen, Germany.
E-mail: G.Lonnenmann@t-online.de

REFERENCES