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Impaired endotoxin-induced interleukin-1β secretion, not total production, of mononuclear cells from ESRD patients

GERHARD LONNEMANN, IRIS BARNDT, VOLKER KAEVER, MARION HAUBITZ, RALF SCHINDLER, STANLEY SHALDON, and KARL M. KOCH

Department of Nephrology and Department of Molecular Pharmacology, Medizinische Hochschule Hannover, Hannover, Germany, and Department of Nephrology, University Hospital Nimes, Nimes, France

Impaired endotoxin-induced interleukin-1β secretion, not total production, of mononuclear cells from ESRD patients. Lipopolysaccharide (LPS)-induced interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNFα) production and secretion from peripheral blood mononuclear cells (PBMC) were determined in a longitudinal study with repeated measurements in PBMC from patients with chronic uremia not on hemodialysis ($N = 8$), end-stage renal disease (ESRD) patients ($N = 8$), and healthy controls ($N = 7$). ESRD patients were studied while using low-flux Cuprophan dialyzers and again using high-flux AN 69 dialyzers. Total (cell-associated plus secreted) LPS-induced IL-1β production was enhanced in uremic patients, but similar to controls in ESRD patients on Cuprophan. In contrast, LPS-induced IL-1β secretion (secreted amounts in % of total production) was similar to controls in uremic patients, but significantly reduced in ESRD patients on Cuprophan ($P < 0.01$). During AN 69 hemodialysis, LPS-induced total IL-1β production remained unchanged but IL-1β secretion increased significantly ($P < 0.05$) compared to Cuprophan dialysis. Increased IL-1β secretion coincided with a suppression in PGE_2 synthesis ($P < 0.02$). Similarly, blockade of endogenous PGE_2 by indomethacin increased LPS-induced IL-1β secretion ($P < 0.01$) but did not enhance total IL-1β production in PBMC from controls and patients on Cuprophan hemodialysis. Neither total production nor secretion of TNFα was different comparing the three study groups. We conclude that LPS-induced IL-1β secretion, but not total production, is impaired in PBMC from ESRD patients on long-term Cuprophan hemodialysis. This functional change in the PBMC response is specific for IL-1β, not due to uremia *per se* but hemodialysis-dependent and reversible. Hemodialysis with AN 69 suppresses endogenous PGE_2 synthesis in PBMC which is associated with increased LPS-induced IL-1β secretion in the presence of unchanged total IL-1β production. We speculate that PGE_2 could inactivate the IL-1β converting enzyme which is essential for processing and secretion of mature IL-1β.

The *in vitro* production of proinflammatory cytokines such as IL-1 and TNF from PBMC is one way to study differences in the response of these cells between chronic disease and health [1, 2]. As cytokines play an important role in cell-cell communication, the total cellular production of IL-1 and TNF as well as the release of these mediators from activated PBMC are important in the orchestration of the host response system [3, 4]. Although TNFα has a signal peptide similar to that of secretory proteins, TNFα can remain inside the cell [5, 6]. However, in sharp contrast, the two interleukin-1 molecules (IL-1α and IL-1β) clearly lack signal peptides [4]. Despite the missing signal peptide, as much as 60% of IL-1β has been found in the supernatant of lipopolysaccharide- (LPS) stimulated PBMC of healthy volunteers *in vitro* [7, 8].

Cytokine production of PBMC has been used to describe the effect of orally administered drugs (such as cyclooxygenase inhibitors) [9] or dietary supplementation [1, 10]. However, there is a remarkable intra-individual variability in LPS-induced IL-1β production from PBMC studied on three different days within one week [1, 9]. Therefore, in order to judge whether a change in PBMC function due to disease or therapy is meaningful, it is a prerequisite to describe the intra-individual variability of *in vitro* PBMC cytokine production under stable conditions over a defined period of time.

Patients on chronic hemodialysis therapy have elevated cell-associated levels of cytokines such as IL-1β and TNFα in PBMC determined by immediate lysis after separation or after incubation [2, 11–13]. Using single point measurements, we have demonstrated that PBMC from patients on hemodialysis with low-flux Cuprophan dialyzers produce similar amounts of total (secreted plus cell-associated) IL-1β in response to LPS *in vitro* compared to PBMC from an age-matched control group [14]. In that study, LPS-stimulated PBMC secreted significantly less IL-1β than PBMC from healthy controls (controls, 56.2 ± 8.3%; Cuprophan hemodialysis, 31.2 ± 6.3%, $P < 0.02$). The reduction in IL-1β secretion seems to be stimulus dependent since *Staphylococcus epidermidis* induced the release of approximately 90% of total IL-1β in both groups (controls, 88.1 ± 4.1%; Cuprophan hemodialysis, 89.0 ± 2.7%).

The mechanisms involved in the reduced ability to release IL-1β in response to LPS remained unclear. Furthermore, it has not been determined whether the change in PBMC function is due to uremia *per se* or to repeated stimulation by the hemodialysis procedure. Also, the question whether the inflammatory nature of the dialyzer membrane-induced dysfunction [14, 15] was reversible remained unanswered.

In order to address these issues, we studied the variability of TNFα and IL-1β production and release from LPS stimulated PBMC from healthy controls, uremic patients with chronic renal failure as well as from patients with end-stage renal disease (ESRD) on chronic hemodialysis. We used repeated measurements in PBMC per individual during a period of eight weeks. The

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same ESRD patients were studied in two consecutive eight-week periods in which they were dialyzed using low-flux Cuprophan versus high-flux AN 69 dialyzers. We measured PGE_2 concentrations in PBMC supernatants and studied the effect of indomethacin on LPS-induced IL-1β and TNFα production and release from PBMC.

Methods

Patients and study groups

Two control groups were included in the study. For the longitudinal study over a time period of 8 weeks, the first group included seven healthy male volunteers with a mean age of 32.4 years (27 to 42). Blood was drawn from an anticubital vein once a week in the morning of the same weekday. In a second control group, 10 healthy volunteers, seven males, three females, donated blood once for PBMC separation in order to study the effect of indomethacin on LPS-induced IL-1β and TNFα production and secretion. None of the volunteers took any medication (cortison, aspirin or other prostaglandin inhibitors) throughout the study period.

The first study group included eight uremic patients with chronic renal failure, two males, six females with a mean age of 53.9 years (31 to 78) who gave informed consent. The diagnosis of the kidney diseases were polycystic kidney disease in one, diabetic nephropathy in two, chronic glomerulonephritis in three and chronic interstitial nephropathy in two. These patients were studied on each occasion for which they were scheduled for a routine blood test in the out patient unit. This were three occasions during the eight-week study period with at least a two week interval between two samples. In this group, serum concentrations were 28.2 ± 4.0 mmol/liter for urea and 699 ± 168 μmol/liter for creatinine. The creatinine clearance was 12.7 ± 4.9 ml/min.

The second group included eight male ESRD patients, mean age 52.9 years (34 to 64), who gave informed consent. These patients were on hemodialysis therapy with Cuprophan dialyzers for an average time of 61 months (16 to 132) when entering the study. The diagnosis leading to end-stage renal failure was polycystic kidney disease in four, chronic glomerulonephritis in three and chronic interstitial nephritis in one. Patients with hepatitis B or C, active hyperparathyroidism or requiring anti-inflammatory medication were not included.

During the first study period of eight weeks, hemodialysis was continued using Cuprophan® dialyzers (GF 120 H®, Gambro, Lund, Sweden) for three hemodialysis sessions per week, with four to five hours each. Blood was drawn once per week from the arterio-venous fistula before the start of the dialysis session following the long inter-dialytic interval. Then all patients switched to AN 69® dialyzers (Filtral 12®, Hospal, Nürnberg, Germany). Following a washout period of eight weeks in which no blood was drawn, the patients entered the second study period of eight weeks where blood was taken again once a week before hemodialysis after the long inter-dialytic interval. The effectiveness of the hemodialysis as indicated by the treatment time, blood flow in the dialysis machine, post-pre-treatment differences in serum urea and the weight loss over treatment, was the same in both study periods (Table 1). In addition, the endotoxin contamination of the dialysate (Limulus test: Coatest® Endotoxin, KabiVitrum GmbH, München, Germany), which is recognized to be a potent inducer of PBMC cytokine production [16, 17], was less than 21 pg/ml and did not differ significantly during the two study periods (Table 1).

Table 1.

Parameter	Cuprophan hemodialysis	AN 69 hemodialysis
Δ Body weight *kg*	3.00 ± 0.18	3.05 ± 0.24
RR pre-HD *mm Hg*	149 ± 2.1/88.9 ± 2.7	154.5 ± 3.9/90.6 ± 2.3
RR post-HD *mm Hg*	140.6 ± 4.8/86.3 ± 3.5	140.6 ± 3.9/85.8 ± 4.4
Urea pre-HD *mmol/liter*	25.36 ± 0.8	26.34 ± 1.1
Urea post-HD *mmol/liter*	8.94 ± 0.43	8.57 ± 0.54
Δ Urea *mmol/liter*	16.43 ± 0.71	17.77 ± 0.84
Dialysate endotoxin[a] *pg/ml*	19.5 ± 1.5	20.3 ± 0.6

Numbers represent the means ± SEM of $N = 8$ averaged values per patient.

[a] Dialysate samples were taken from the dialyzer outlet after 3 hours of hemodialysis

To test the effect of indomethacin on LPS-induced IL-1β production and secretion, PBMC were separated from 10 ESRD patients on Cuprophan hemodialysis (6 males, 4 females). These patients are different from those in the study group mentioned above.

Preparation of human blood mononuclear cells (PBMC)

For the longitudinal study blood was drawn under aseptic conditions into pyrogen-free tubes containing EDTA (1.5 mg/ml of blood) (Sarstedt, Nümbrecht, Germany) to which the proteinase inhibitor aprotinin was added (0.67 TIU/ml of blood) (Sigma, St. Louis, MO, USA). The blood was kept on ice and subsequently processed within 60 minutes. To study the effect of indomethacin, blood was drawn into pyrogen-free syringes containing 10 IU/ml heparin.

From each blood sample PBMC were separated by Ficoll-Hypaque (Seromed, Biochrom KG, Berlin, Germany) centrifugation. The PBMC were re-suspended to a concentration of 5×10^6 cells/ml in ultrafiltered [18] tissue culture medium (MEM, Whittaker M.A. Bioproducts Inc., Walkersville, MD, USA) supplemented with 2 mM L-glutamine, 10 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin (all from Whittaker M.A.) and 2% heat-inactivated human AB serum. One milliliter aliquots (final concentration 2.5×10^6 PBMC/ml) were incubated in 24-well flat bottom polystyrene plates (NunclonR, A/S Nunc, Roskilde, Denmark). In the longitudinal study, PBMC were stimulated with 100 ng/ml LPS from *E. coli* (phenol-extracted LPS: O55:B5, Sigma), a dose which induces maximal cytokine production in normal PBMC [8]. In additional experiments, PBMC were stimulated with 1 to 100 ng/ml LPS in the presence and absence of 0.5 μg/ml indomethacin (Sigma).

During 18 hours of PBMC incubation, temperature was maintained at 37°C in an atmosphere containing 5% CO_2. After this period, cell culture supernatants were removed, transferred into 1.5 ml Eppendorf tubes and centrifuged for five minutes at 4°C to remove remaining cells. Following this centrifugation step, supernatants containing the extracellular amounts of cytokines were transferred into new 1.5 ml eppendorf tubes and frozen at −70°C. The cell pellets (in approximately 20 μl of remaining medium) in the eppendorf tubes were re-suspended in 1 ml of fresh MEM, added to the adhering PBMC in the corresponding wells of the 24 well plates and kept frozen at −70°C. On the day of the cytokine

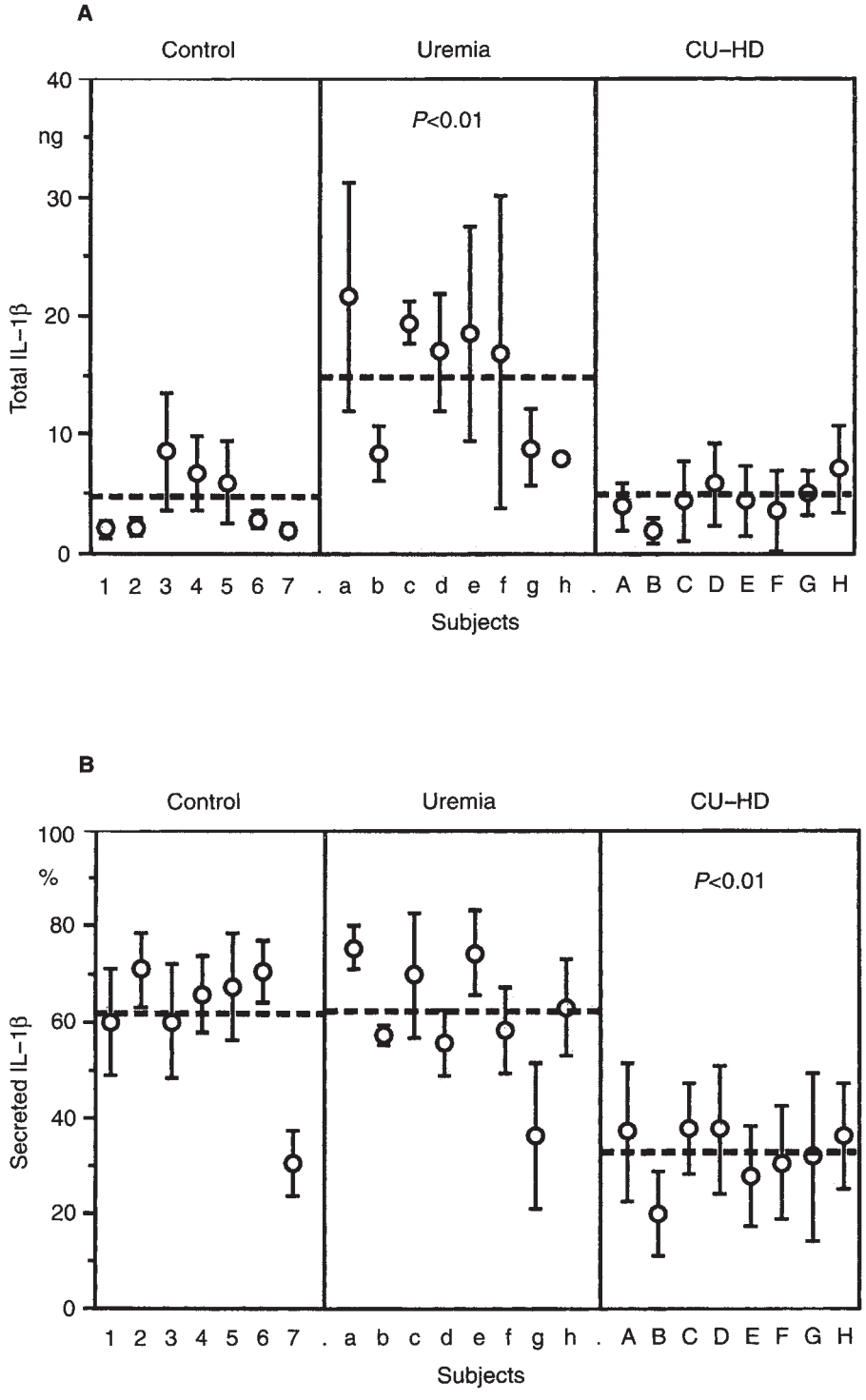

Fig. 1. *Total LPS (100 ng/ml)-induced production (A) and release of IL-1β (B) from PBMC.* Each number or letter under the horizontal axis represents a different individual (subject). Symbols represent the mean ± SD of 6 determinations per subject in controls, 3 determinations per subject in uremic patients (uremia), and 6 to 8 determinations per subject in ESRD patients on Cuprophan hemodialysis (CU-HD). Broken horizontal lines indicate the means per study group: Controls $N = 7$, uremia $N = 8$, and CU-HD $N = 8$. Total IL-1β production is expressed as ng/2.5 × 10^6 PBMC/18 hours of incubation; secreted amounts are depicted as percent of total production. Total IL-1β production was higher in uremia compared to controls and CU-HD ($P < 0.01$); IL-1β secretion was significantly reduced in CU-HD ($P < 0.01$) compared to controls and uremia.

measurements by radioimmunoassays the 24 well plates were subjected to three freeze- (−70°C) thaw (37°C) cycles in order to lyse the PBMC for measurement of cell-associated cytokines.

Cytokine measurements

IL-1β and TNFα were measured by radioimmunoassay (RIA) in cell culture supernatants and cell lysates as described previously [7, 19]. The polyclonal antibodies used in each RIA (rabbit anti-human TNFα and rabbit anti-human IL-1β) were gifts from Prof. C.A. Dinarello (New England Medical Center, Boston, MA, USA). The anti-human IL-1β antiserum detects the mature IL-1β peptides (17 kD and 21 kD) as well as 50% of the IL-1β precursor which is not recognized by monoclonal antibodies produced against the mature form of IL-1β [20, 21]. The sensitivity of both RIAs is approximately 80 pg/ml in PBMC culture supernatants and cell lysates.

To validate the cytokine assays for repeated measurements, we determined the intra- and inter-assay variability expressed by the coefficient of variation (V = SD/mean × 100). Aliquotes of the same PBMC supernatant and corresponding cell-lysate were assayed 10 times in one assay, yielding the intra-assay variability of V_{intra} = 11.4%; testing the same sample in ten different assays, the inter-assay variability was V_{inter} = 11.7%. Hence, to obtain the total cytokine production level, we added the cell-associated levels to the extracellular levels. This number was not significantly different from the actual measured total production in PBMC when supernatants and cells were lysed together (calculated total IL-1β = 9.9 ± 1.0; measured total IL-1β = 10.9 ± 1.3 ng/2.5 × 10^6 PBMC/18 hr; N = 10).

Prostaglandin E_2 (PGE_2) in the supernatants of LPS-stimulated PBMC was measured by specific ELISA as previously described [22]. The detection limit of this assay is 0.25 ng/ml.

Statistics

Results are given as means ± standard deviation (SD). Statistical significance was assessed using one Factor ANOVA analysis. Post-hoc method of data evaluation was Fisher's PLSD test. Differences were considered to be significant when $P < 0.05$.

Results

Decreased release of LPS-induced IL-1β from PBMC associated with hemodialysis

Figure 1 depicts LPS-induced IL-1β production as well as release from PBMC in the three study groups. The total LPS-induced IL-1β production (Fig. 1A) was similar to that in controls (4.3 ± 2.7 ng/2.5 × 10^6 PBMC/18 hrs) and ESRD patients (4.5 ± 1.6) but higher in uremic patients (14.8 ± 5.5, $P < 0.01$). Secretion of LPS-induced IL-1β (Fig. 1B) was significantly reduced in PBMC from ESRD patients on chronic hemodialysis using low-flux Cuprophan dialyzer membranes (32.5 ± 6.3%; $P < 0.01$) compared to that of controls (60.8 ± 14.1%) and uremic patients (61.4 ± 12.6%).

There was intra-individual variability in total IL-1β production in each of the three study groups (Fig. 1A). The coefficient of variation was significantly higher ($P < 0.02$) in ESRD patients using Cuprophan dialyzers (62.1 ± 16.7%) compared to uremic patients (43.4 ± 23.9%) and healthy controls (40.1 ± 13.5%). The intra-individual variability was significantly reduced in each study group when the levels of extracellular IL-1β were expressed as percent of total LPS-induced IL-1β production. Similar to the differences in total IL-1β production, the intra-individual variability for secreted amounts of IL-1β was higher in ESRD patients (36.3 ± 6.34%; $P < 0.01$) than in normal controls (15.5 ± 5%) and in uremic patients (15.7 ± 11.8%). In six out of seven control individuals and seven out of eight uremic patients IL-1β release from PBMC was greater than 50%. However, despite the higher intra-individual variability, PBMC from patients on hemodialysis with Cuprophan dialyzers consistently secreted less than 50% of LPS-induced IL-1β.

LPS-induced TNFα production and release from PBMC

In contrast to IL-1β, neither the total production nor the release of LPS-induced TNFα from PBMC differed significantly between the three study groups (Fig. 2). Total TNFα production was 6.8 ± 3.9 ng/2.5 × 10^6 PBMC/18 hours in controls, 5.2 ± 3.0 ng in uremic patients and 8.8 ± 3.7 ng in ESRD patients. TNFα release from PBMC was 91.1 ± 4.6% in controls, 88.9 ± 2.5% in uremic patients and 88.6 ± 4.7% in ESRD patients on Cuprophan dialyzers.

The intra-individual variability of LPS-induced TNFα production (Fig. 2A) was not significantly different from the one described for IL-1β. The coefficient of variation was 53.3 ± 22.1% in healthy controls, 32.3 ± 18.8% in uremic patients and 58.5 ± 14.4% in ESRD hemodialysis. When the variability was calculated for secreted TNFα (Fig. 2B), the levels were in the range of 10%, which falls into the variability of the method used.

LPS-induced PGE_2 production from PBMC

As shown in Figure 3, LPS-induced PGE_2 production was decreased in PBMC from uremic (20.5 ± 8.3 ng; $P < 0.05$) and ESRD patients on hemodialysis with Cuprophan dialyzers (15.2 ± 9.7 ng; $P < 0.01$) or AN 69 dialyzers (3.9 ± 5.2 ng; $P < 0.001$) compared to healthy controls (30.7 ± 9.7). The intra-individual variability was higher for PGE_2 than for IL-1β and TNFα with coefficients of variation of 74.4 ± 27.0% in controls, 55.8 ± 43.3% in uremic patients, 78.8 ± 25.7% in Cuprophan hemodialysis, and 61.4 ± 26.1% in AN 69 hemodialysis.

The suppression of LPS-induced IL-1β release is reversible

The ESRD patients on Cuprophan dialyzers were switched to AN 69 dialyzers and were studied again after a washout period of eight weeks. Figure 4 depicts the average of eight values for each patient for total LPS-induced IL-1β and TNFα production and secretion as well as PGE_2 synthesis. The intra-individual variability was not significantly different between the two hemodialysis periods. The total IL-1β and TNFα production decreased in six out of eight patients but increased in the same two patients, resulting in a non-significant change when comparing the Cuprophan with the AN 69 period. Furthermore, TNFα release did not change under the two treatment conditions. After 8 to 16 weeks of hemodialysis using AN 69 dialyzers, IL-1β release increased consistently in each of the eight patients ($P < 0.05$). This reversal in IL-1β release coincided with a decrease in PGE_2 production in seven out of eight patients ($P < 0.02$) when Cuprophan dialyzers were replaced by AN 69 (Fig. 4).

As there was no time related trend to increased IL-1β release and decreased PGE_2 production during the AN 69 period, the washout period of eight weeks was sufficient to reach stable conditions on AN 69 hemodialysis. Therefore, it was legitimate to average the eight values per study period.

Inhibition of endogenous PGE_2 production by indomethacin enhances IL-1β release from PBMC

Although the absolute concentrations of PGE_2 are reduced in PBMC supernatants from ESRD patients compared to normal controls, the data shown in Figure 4 suggest that relative changes in PGE_2 synthesis influence IL-1β secretion. In order to test this hypothesis, PBMC from healthy volunteers were incubated in the absence and presence of indomethacin (Table 2). Inhibition of PGE_2 did not enhance total IL-1β production in response to LPS (Fig. 5). However, in these same cultures, IL-1β secretion increased in the presence of indomethacin in response to 10 ng/ml

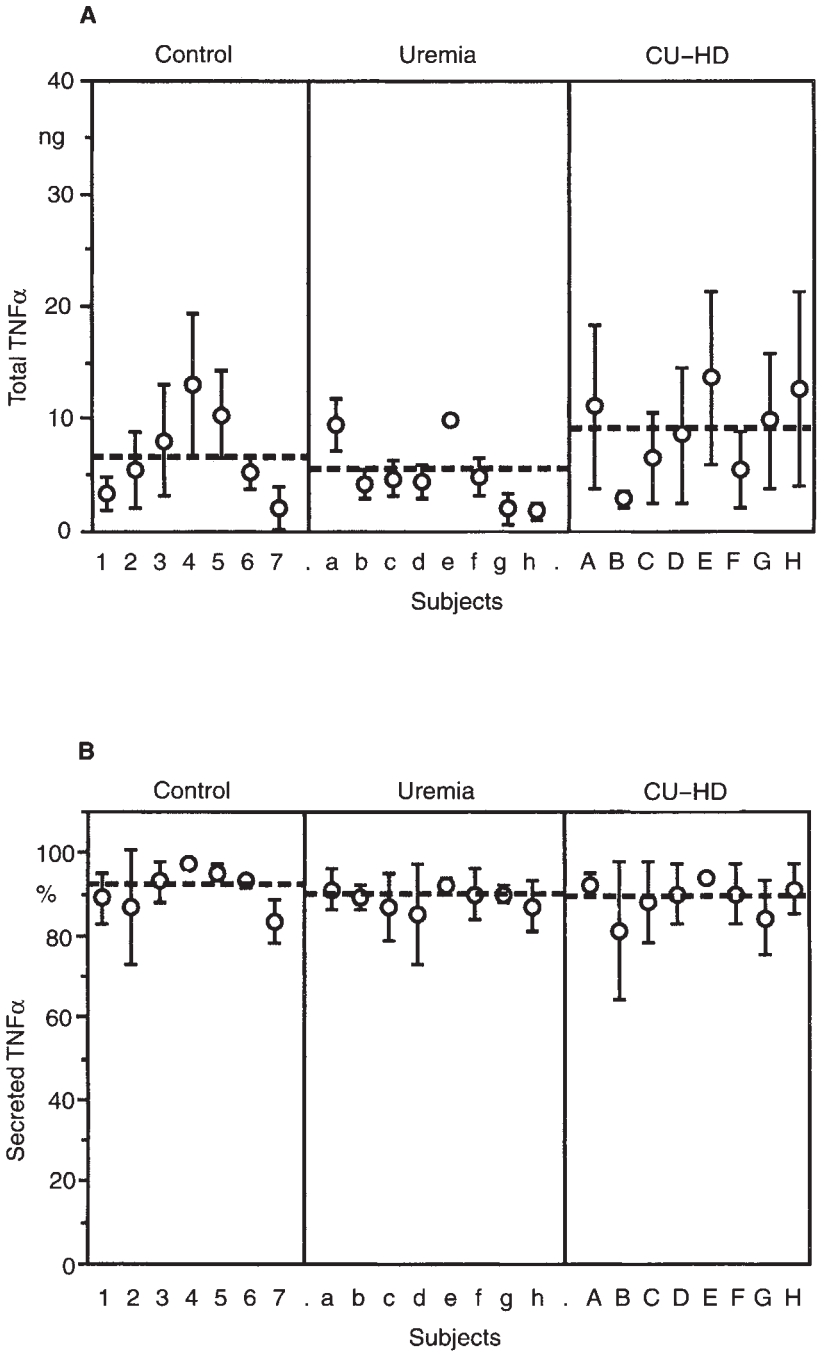

Fig. 2. *Total TNFα production (A) and TNFα release (B) from LPS (100 ng/ml)-stimulated PBMC of healthy controls (N = 7), uremic patients (N = 8) and ESRD patients on Cuprophan hemodialysis (CU-HD, N = 8).* Symbols represent the mean ± SD of 6 determinations per control subject, 3 determinations per uremic patients (uremia), and 6 to 8 determinations per ESRD patient on Cuprophan hemodialysis (CU-HD). Broken horizontal lines indicate the means per study group. Differences in total TNFα production or TNFα secretion between the three groups were not statistically significant.

LPS from 53.1 ± 17.6% to 63.1 ± 15.7% ($P < 0.02$). In response to 100 ng/ml LPS, IL-1β secretion increased from 57.7 ± 19.1% to 71.1 ± 16.2% ($P < 0.01$; Fig. 5). In response to 100 ng/ml LPS, the total TNFα production increased from 10.7 ± 3.47 ng to 14.6 ± 5.03 ng in the presence of indomethacin ($P < 0.01$), but secretion of TNFα did not change significantly.

When PBMC from ESRD patients on Cuprophan hemodialysis were studied, total IL-1β production in response to 100 ng/ml LPS was not enhanced by indomethacin, but IL-1β secretion increased from 44.2 ± 17% to 57.9 ± 18% ($P < 0.01$) in the presence of indomethacin (Fig. 5).

Discussion

Suppressed IL-1β secretion associated with Cuprophan hemodialysis

LPS-stimulated PBMC from ESRD patients on chronic hemodialysis with low-flux Cuprophan dialyzers are suppressed in their ability to secrete IL-1β, but not TNFα. These data confirm previous observations [14] and are in agreement with other studies describing reduced extracellular levels of IL-1β in the supernatants of LPS-stimulated PBMC from ESRD patients [15, 23, 24].

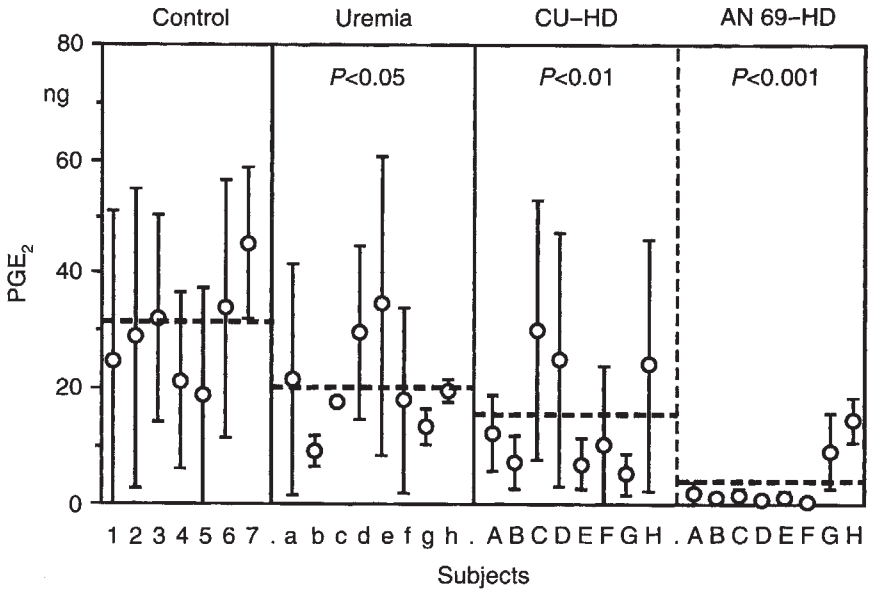

Fig. 3. *LPS-induced PGE_2 synthesis by PBMC from healthy controls, uremic patients and ESRD patients on hemodialysis with Cuprophan and AN 69 dialyzers.* Symbols represent the mean ± SD of 6 determinations per control subject, 3 determinations per uremic patient, and 6 to 8 determinations per ESRD patient on Cuprophan hemodialysis (CU-HD). Broken horizontal lines indicate the means per study group. Compared to controls, PGE_2 synthesis was reduced in uremia ($P < 0.05$), Cuprophan hemodialysis ($P < 0.01$) and AN 69 hemodialysis ($P < 0.001$). Switching the same ESRD patients from Cuprophan to AN 69 decreased *in vitro* LPS-PGE_2 synthesis ($P < 0.02$).

On the other hand, PBMC from uremic patients not on hemodialysis are not different from those of healthy controls. Thus, the impaired IL-1β release is not caused by uremia *per se* but is hemodialysis-dependent. In the present study, suppressed IL-1β secretion was reversed when low-flux Cuprophan dialyzers were replaced by highly permeable AN 69 dialyzers for at least eight weeks. When four out of eight ESRD patients were studied again after one year of AN 69 hemodialysis, their PBMC secreted 60% of LPS-induced IL-1β which is similar to that in healthy or uremic PBMC (unpublished observation). More IL-1β secretion occurred without a change in total LPS-induced IL-1β production and this reversal was associated with a decrease in PGE_2 synthesis. Similarly, inhibition of endogenous PGE_2 by indomethacin increased IL-1β secretion from LPS-stimulated PBMC of healthy controls and ESRD patients on Cuprophan. These data suggest that in ESRD patients as well as in healthy volunteers, down-regulation of endogenous PGE_2 increases IL-1β secretion rather than total IL-1β production by PBMC. However, as PGE_2 synthesis is reduced in PBMC from ESRD patients compared to normal controls, the relative changes in PGE_2 production as opposed to the absolute amounts seem to influence IL-1β secretion.

We studied PBMC *in vitro* approximately 2.5 days after termination of the preceding hemodialysis session. Therefore, our data indicate that blood-dialyzer interactions during hemodialysis may cause functional changes in PBMC which persist as long as two days after the end of the hemodialysis procedure. Switching the dialyzer membrane may persistently modify the PBMC response to LPS-stimulation. It remains to be established, however, whether the increased IL-1β secretion observed under AN 69 hemodialysis reflects an improved or even a normalized PBMC function in ESRD patients.

Mechanisms involved in the suppression of IL-1β secretion from PBMC of ESRD patients on long-term Cuprophan hemodialysis are unknown. However, as the dialyzer clearance may play a role, it is important to state that serum urea levels were not different comparing the two hemodialysis conditions. Therefore, it is unlikely that the modified PBMC function during the AN 69 phase was due to better dialyzer clearance of these small molecular substances. We cannot exclude, however, a beneficial effect of an increased clearance for undefined uremic toxins with a molecular weight of up to 15,000 Daltons which may have an inhibitory effect on PBMC and which are cleared by the high-flux AN 69 but not by the low-flux Cuprophan membrane.

The role of PGE_2

The question whether PGE_2 synthesis from PBMC in uremic patients and ESRD patients is altered is controversial. There are reports of increased [25], unchanged [24] and decreased [26] PGE_2 production in PBMC from ESRD patients compared to cells of healthy controls. In the present study, PGE_2 production was reduced in cells from patients during Cuprophan hemodialysis and decreased further during AN 69 usage in the same patients. This further reduction was associated with an increase in IL-1β secretion. However, when individual data were analyzed, we could not demonstrate a significant inverse correlation between decreasing PGE_2 levels and increasing secretion of IL-1β under the two dialysis conditions. In a second set of experiments, blockade of endogenous PGE_2 production by indomethacin resulted in increased IL-1β secretion, not total IL-1β production, from cells from normal subjects as well as from ESRD patients on Cuprophan hemodialysis. These data suggest that endogenous PGE_2 inhibits LPS-induced IL-1β secretion but not total synthesis.

Previous reports described that PGE_2 down-regulates LPS-induced IL-1β production [27]. In that study, the secretion of IL-1 into the cell supernatant was measured and, therefore, is consistent with our finding. As in response to 100 ng/ml LPS total TNFα production (but not IL-1β) was significantly enhanced in the presence of indomethacin, the results presented here are in agreement with Endres et al who described that PGE_2 influences IL-1β and TNFα production in normal PBMC differently [28].

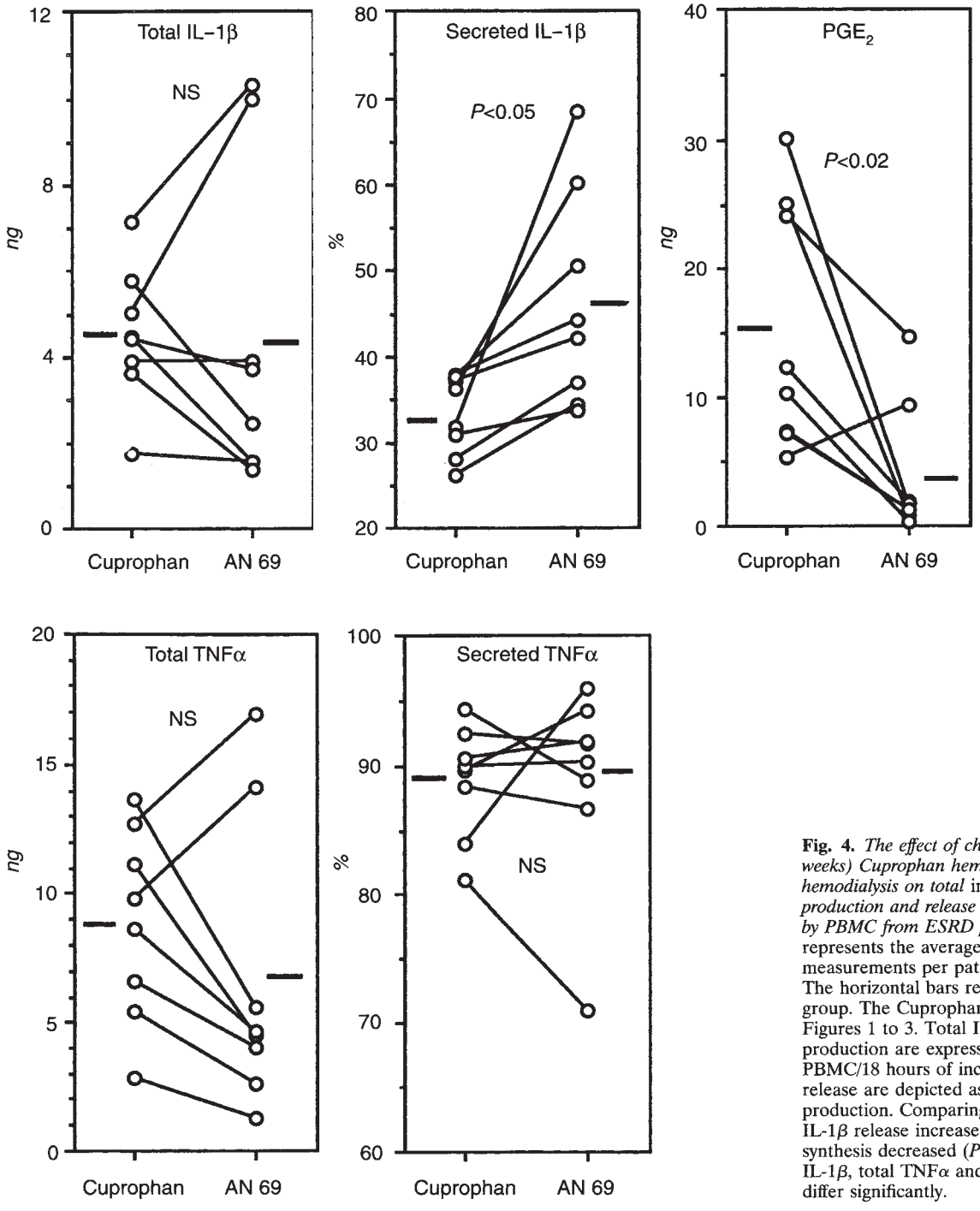

Fig. 4. *The effect of chronic (a minimum of 8 weeks) Cuprophan hemodialysis versus AN 69 hemodialysis on total* in vitro *IL-1β and TNFα production and release as well as PGE_2 synthesis by PBMC from ESRD patients.* Each symbol represents the averaged value of 6 to 8 measurements per patient and study period. The horizontal bars represent the means per group. The Cuprophan data are the same as in Figures 1 to 3. Total IL-1β, TNFα, and PGE_2 production are expressed as ng/2.5 × 10^6 PBMC/18 hours of incubation. IL-1β and TNFα release are depicted as percent of total production. Comparing the two study periods, IL-1β release increased ($P < 0.05$) and PGE_2 synthesis decreased ($P < 0.02$) whereas total IL-1β, total TNFα and TNFα release did not differ significantly.

Mechanisms of IL-1β secretion

Several mechanisms of IL-1β release from LPS-stimulated PBMC have been introduced. IL-1β is bound in the cytosol, processed by a specific enzyme and translocated to the plasma membrane. Release from the cell may involve a process which probably involves tissue plasminogen activator and serum enzymes such as plasmin [29, 30]. A role of protein kinase C (PKC) has been proposed [31] because PBMC activated by LPS in the presence of PKC inhibitors do not secrete IL-1β, whereas total IL-1β production is unaffected. Furthermore, IL-1β secretion seems to be regulated by temperature-sensitive short lived proteins, because preincubation of PBMC for 60 minutes at 42°C inhibits secretion but only reduces total production of IL-1β [32]. In contrast to an active process of secretion, other studies favor the concept that IL-1 is released from LPS-stimulated PBMC by a non-specific pathway as a consequence of cellular injury [33]. It remains to be elucidated whether any of these mechanisms are different in PBMC from ESRD patients.

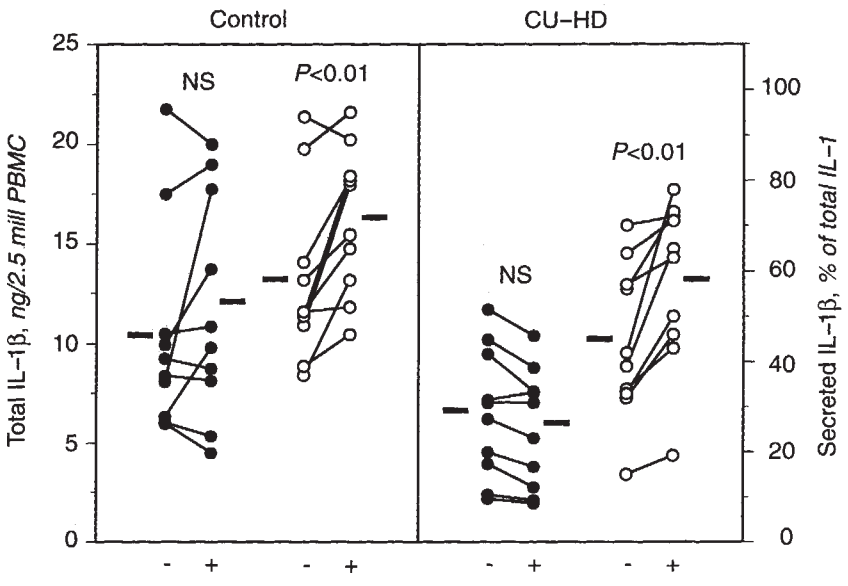

Fig. 5. *The effect of indomethacin (0.5 μg/ml) on total IL-1β production and IL-1β secretion by PBMC from healthy controls (Control) and from ESRD patients on Cuprophan hemodialysis (CU-HD).* PBMC were stimulated with 100 ng/ml LPS. Total IL-1β (●) is depicted on the left vertical axis as ng/2.5 × 10^6 PBMC/18 hours of incubation; secreted IL-1β (○) is depicted on the right vertical axis as percent of total IL-1β production. The symbols represent individual data of 10 controls and 10 CU-HD patients. The control data are the same as those depicted in Table 2. The horizontal bars represent the means per group. $P < 0.01$ compared to PBMC incubations without indomethacin; NS = not significant. − is no indomethacin; + is 0.5 μg/ml indomethacin.

Table 2. Effect of indomethacin on LPS-induced IL-1β and TNFα production and secretion

LPS *ng/ml*	Indomethacin *0.5 μg/ml*	Total IL-1β *ng/2.5 × 10^6 PBMC*	Secreted IL-1β *% of total*	Total TNFα *ng/2.5 × 10^6 PBMC*	Secreted TNFα *% of total*
0	−	<0.08	ND	0.26 ± 0.1	ND
	+	<0.08	ND	0.32 ± 0.1	ND
1	−	3.9 ± 2.3	51.2 ± 18.1	8.2 ± 2.3	94.9 ± 2.4
	+	3.7 ± 2.4	46.6 ± 21.6	8.8 ± 2.8	96.1 ± 0.4
10	−	11.3 ± 8.7	53.1 ± 17.6	10.1 ± 2.9	94.1 ± 6.2
	+	10.3 ± 6.2	63.1 ± 15.7[a]	10.8 ± 3.0	94.4 ± 2.7
100	−	10.4 ± 5.2	57.7 ± 19.1	10.7 ± 3.5	94.2 ± 3.2
	+	11.8 ± 5.6	71.1 ± 16.2[b]	14.6 ± 5.0[a]	96.0 ± 1.2

Data are means ± SD.
[a] $P < 0.02$, [b] $P < 0.01$ compared to PBMC incubated without indomethacin

Processing of IL-1

Our data are also consistent with an impaired processing of the IL-1β precursor molecule into the mature 17 kD IL-1β, which is the major extracellular form of IL-1β. Impaired processing of IL-1β results in the accumulation of the precursor in the cytosol. As the precursor is partially recognized by the polyclonal anti-human IL-1β antiserum used in this study, accumulation of the IL-1β precursor molecule would result in increased cell-associated levels of IL-1β. On the other hand, impaired processing will reduce the appearance of the mature IL-1β molecule in the extracellular compartment. The enzyme involved in the processing of pro-IL-1β is the interleukin-1β converting enzyme (ICE) [34]. This IL-1 convertase is pH sensitive, is optimally active at low (5.7) external pH and is inactivated at pH ≥8.0 [32]. One could propose from the data in this study that ICE is less active in PBMC from ESRD patients compared to healthy controls. It is possible that the increase in PGE_2 synthesis suppresses either the synthesis or activation of ICE.

Human ICE is homologous to the Nematode *c-elegans 3 (ced-3)* gene which is required for developmental programmed cell death or apoptosis [35]. Cells undergoing apoptosis express the *ced-3* gene. By down-regulating of ICE activity during Cuprophan hemodialysis, impaired programmed cell death of monocytes/macrophages could result in longer survival of monocytes in the circulation. These monocytes are likely to express a cytokine profile which is different from that of young monocytes. Further studies are necessary to investigate whether these altered monocytes are present in the circulation of ESRD patients and whether these cells could account for the immune defect described in these patients.

Variability in IL-1β and TNFα production

This study describes an intra-individual variation as high as 40 to 60% in LPS-induced production of IL-1β and TNFα in all study groups. This variability indicates that determination of cytokine production using single point measurements is not reliable. When the release of IL-1β from LPS stimulated PBMC was expressed as percent of total production, the coefficients of variation were reduced to the level of the assay variability. These data suggest that IL-1β release as opposed to total IL-1β production is a stable and reliable parameter to describe PBMC function in longitudinal studies.

Our observation that the inter-subject variation was highest in the control group is in agreement with the previously made distinction between high and low responders for LPS-induced cytokine production [9]. Studying IL-1β release, the inter-individual variability was high in controls and uremic patients due to one individual in each group who had consistently reduced amounts of secreted IL-1β. These data suggest that the mechanism of IL-1β release from PBMC may be, in part, genetically determined. However, as IL-1β secretion was less than 50% in PBMC from all ESRD patients on Cuprophan treatment, our data suggest an influence of the hemodialysis procedure on PBMC function.

Neither total TNFα production nor TNFα release was significantly different in uremic patients and hemodialysis patients compared to controls. These data indicate that neither uremia *per se* nor the hemodialysis procedure changes the ability of PBMC to synthesize and release TNFα in response to LPS. In contrast, total

LPS-induced IL-1β production was higher in PBMC from uremic patients compared to those from healthy controls and ESRD patients. Explanations for this observation remain speculative; it is possible that uremia and/or an active kidney disease causes a preactivation of PBMC leading to a higher IL-1β production. However, as different individuals were studied in the uremic group versus the ESRD group and because of the large intra-individual variability, the importance of differences in total IL-1β production, although statistically significant, should be viewed with caution.

To our knowledge, our study provides the first evidence that the impaired ability to secrete IL-1β is predominantly associated with the hemodialysis procedure itself and not by uremia *per se*. If one assumes that the reduced ability of PBMC to release IL-1β plays a role in the impaired immune response described in hemodialysis patients [36–38], the results of the present study suggest that long-term hemodialysis therapy with synthetic high-flux membranes such as AN 69 could improve the ability of PBMC to secrete IL-1β in ESRD patients. Whether this modified PBMC response observed *in vitro* is accompanied by an improved immune response remains to be established. However, as the functional changes of PBMC seem to be induced by the dialysis procedure, the reduced IL-1β secretion under the conditions of long-term Cuprophan hemodialysis may just reflect an adaptation of the PBMC to protect the patient against the inflammatory effects of repeated cytokine induction during the extracorporeal procedure.

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Reprint requests to Gerhard Lonnemann, M.D., Department of Nephrology, Medizinische Hochschule Hannover, Konstanty-Gutschow-Str. 8, D-30625 Hannover, Germany.

References

1. Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JWM, Cannon JG, Rogers TS, Klempner MS, Weber PC, Wolff SM, Dinarello CA: The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 320:265–271, 1989
2. Herbelin A, Urena P, Nguyen AT, Zingraff J, Deschamps-Latscha B: Influence of first and long-term dialysis on ureamia-associated increased basal production of interleukin-1 and tumor necrosis factor alpha by circulating monocytes. *Nephrol Dial Transplant* 6:349–357, 1991
3. Dinarello CA: Interleukin-1 and its biologically related cytokines. *Adv Immunol* 44:153–205, 1989
4. Dinarello CA: Interleukin-1 and interleukin-1 antagonism. *Blood* 77:1627–1652, 1991
5. Kriegler M, Perez C, DeFay K, Albert I, Lu SD: A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: Ramifications for the complex physiology of TNF. *Cell* 53:45–53, 1988
6. Nii A, Sone S, Orino E, Ogura T: Induction of a 26-kDa membrane-form tumor necrosis factor (TNF)-alpha in human alveolar macrophages. *J Leuk Biol* 53:29–36, 1993
7. Endres S, Cannon JG, Ghorbani R, Dempsey RA, Sisson SD, Lonnemann G, van der Meer JWM, Wolff SM, Dinarello CA: In vitro production of IL-1 beta, IL-1 alpha, TNF and IL-2 in healthy subjects: distribution, effect of cyclooxigenase inhibition and evidence of independent gene regulation. *Eur J Immunol* 19:2327–2333, 1989
8. Lonnemann G, Endres S, van der Meer JWM, Koch KM, Dinarello CA: Differences in the synthesis and kinetics of release of interleukin-1 alpha, interleukin-1 beta and tumor necrosis factor from human mononuclear cells. *Eur J Immunol* 19:1531–1536, 1989
9. Endres S, Ghorbani R, Lonnemann G, van der Meer JWM, Dinarello CA: Measurement of immunoreactive interleukin-1 beta from human mononuclear cells: Optimization of recovery, intrasubject consistency, and comparison with interleukin-1 alpha and tumor necrosis factor. *Clin Immunol Immunopathol* 49:424–438, 1988
10. Cannon JG, Meydani SN, Fielding RA, Fiatarone MA, Meydani M, Farhangmehr M, Orencole SF, Blumberg JB, Evans WJ: Acute phase response in exercise. II. Associations between vitamin E, cytokines, and muscle proteolysis. *Am J Physiol* 260:1235–1240, 1991
11. Haeffner-Cavaillon N, Cavaillon JM, Ciancioni C, Bacle F, Delons S, Kazatchkine MD: In vivo induction of interleukin-1 during hemodialysis. *Kidney Int* 35:1212–1218, 1989
12. Lonnemann G, Haubitz M, Schindler R: Hemodialysis-associated induction of cytokines. *Blood Purif* 8:214–222, 1990
13. Blumenstein M, Ziegler HH, Schiller B, Schmidt B, Ward RA, Gurland HJ: Differential activation of monocytes in haemodialysis patients exposed to different types of membranes. *Scand J Immunol* 31:183–190, 1990
14. Haubitz M, Kloeppel M, Lonnemann G, Nonnast-Daniel B: In vitro production of interleukin-1 from blood mononuclear cells of patients on chronic hemodialysis therapy. *Clin Nephrol* 38:30–35, 1992
15. Blumenstein M, Schmidt B, Ward RA, Ziegler-Heitbrock HWL, Gurland HJ: Altered interleukin-1 production in patients undergoing hemodialysis. *Nephron* 50:277–281, 1988
16. Lonnemann G, Bingel M, Floege J, Koch KM, Shaldon S, Dinarello CA: Detection of endotoxin-like interleukin-1-inducing activity during in vitro dialysis. *Kidney Int* 33:29–35, 1988
17. Laude-Sharp M, Caroff M, Simard L, Pusineri C, Kazatchkine MD, Haeffner-Cavaillon N: Induction of IL-1 during hemodialysis: Transmembrane passage of intact endotoxins (LPS). *Kidney Int* 38: 1089–1094, 1990
18. Dinarello CA, Lonnemann G, Maxwell R, Shaldon S: Ultrafiltration to reject human interleukin-1-inducing substances derived from bacterial cultures. *J Clin Microbiol* 25:1233–1238, 1987
19. van der Meer JWM, Endres S, Lonnemann G, Cannon JG, Ikejima T, Okusawa S, Gelfand JA, Dinarello CA: Concentrations of immunoreactive human tumor necrosis factor alpha produced by human mononuclear cells in vitro. *J Leuk Biol* 43:215–223, 1987
20. Schindler R, Clark BD, Dinarello CA: Dissociation between interleukin-1 beta mRNA and protein synthesis in human peripheral blood mononuclear cells. *J Biol Chem* 265:10323–10327, 1990
21. Dinarello CA: ELISA kits based on monoclonal antibodies do not measure total IL-1beta synthesis. *J Immunol Meth* 148:255–259, 1992
22. Kaever V, Wessel K, Radeke HH, Resch K: Influence of the intracellular free calcium level and calmodulin antagonists on prostaglandin and leukotriene synthesis in murine macrophages. *Agents Actions* 32:82–84, 1991
23. Kang X, Hirano T, Oka K, Tamaki T, Sakurai E, Kaji N, Yoshida M, Kozaki M: Recovery of decreased ability of peripheral blood mononuclear cells from chronic renal failure to produce interleukin-1 alpha and beta after renal transplantation. *Nephron* 58:450–455, 1991
24. Friedlander MA: Interleukin-1 beta, tumor necrosis factor, and prostaglandin E_2 response after incubation of peritoneal cells and peripheral blood mononuclear cells with lipopolysaccharide. *Adv Perit Dial* 7:142–149, 1991
25. Bloehr H, Bregengaard C, Povlsen JV: Triiodothyronine stimulates growth of peripheral blood mononuclear cells in serum-free cultures in uremic patients. *Am J Nephrol* 12:148–154, 1992
26. Langhoff E, Blaehr H, Ladefoged J: Production of prostaglandin E_2 by macrophages in uraemia. *Nephrol Dial Transplant* 6:966–970, 1991
27. Knudsen PJ, Dinarello CA, Strom TB: Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin-1 activity by increasing intracellular adenosine monophosphate. *J Immunol* 137: 3189–3194, 1986
28. Endres S, Fulle HJ, Sinha B, Stoll D, Dinarello CA, Gerzer R, Weber PC: Cyclic nucleotides differentially regulate the synthesis of

tumor necrosis factor-alpha and interleukin-1 beta by human mononuclear cells. *Immunology* 72:56–60, 1991

29. MATSUSHIMA K, TAGUCHI M, KOVACS EJ, YOUNG HA, OPPENHEIM JJ: Intracellular localization of human monocyte associated interleukin-1 (IL-1) activity and release of biologically active IL-1 from monocytes by trypsin and plasmin. *J Immunol* 136:2883–2981, 1986
30. RUBARTELLI A, COZZOLINO F, TALIO M, SITIA R: A novel secretory pathway for interleukin-1 beta, a protein lacking a signal sequence. *EMBO J* 9:1503–1510, 1990
31. BAKOUCHE O, MOREAU JL, LACHMAN LB: Secretion of IL-1: Role of protein kinase C. *J Immunol* 148:84–91, 1992
32. RUBARTELLI A, BAJETTO A, ALLAVENA G, COZZOLINO F, SITIA R: Post-translational regulation of interleukin-1 beta secretion. *Cytokine* 5:117–124, 1993
33. HOGQUIST KA, NETT MA, UNANUE ER, CHAPLIN DD: Interleukin-1 is processed and released during apoptosis. *Proc Natl Acad Sci USA* 88:8485–8489, 1991
34. THORNBERRY NA, BULL HG, CALAYCAY JR, CHAPMAN KT, HOWARD AD, KOSTURA MJ, MILLER DG, MOLINEAUX SM, WEIDNER JR, AUNINS J, ELLISTON KO, AYALA JM, CASANO FJ, CHIN J, DING GJ-F, EGGER LA, GAFFNEY EP, LIMJUCO G, PALYHA OC, RAJU SM, ROLANDO AM, SALLEY JP, YAMIN T-T, LEE TD, SHIVELY JE, MACCROSS M, MUMFORD RA, SCHMIDT JA, TOCCI MJ: A novel heterdimeric cysteine protease is required for interleukin-1 beta processing in monocytes. *Nature* 356:768–774, 1992
35. YUAN J, SHAHAM S, LEDOUX S, ELLIS HM, HORVITZ HR: The C. elegans cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1β converting enzyme. *Cell* 75:641–652, 1993
36. CAPPEL R, VAN BEERS D, LIESNARD C, DRATWA M: Impaired humoral and cell-mediated immune response in dialyzed patients after influenza vaccination. *Nephron* 33:21–25, 1983
37. LEWIS SL, VAN BEERS D: Neutrophil and monocyte alterations in chronic dialysis patients. *Am J Kidney Dis* 9:2381–2386, 1987
38. CHATENOUD L, DUGAS B, BEAURAIN G, TOUAM M, DRUEKE T, VASQUEZ A, GALANAUD P, BACH JF, DELFRAISSY JF: Presence of preactivated T-cells in hemodialysis patients: Their possible role in altered immunity. *Proc Natl Acad Sci USA* 83:7457–7461, 1986