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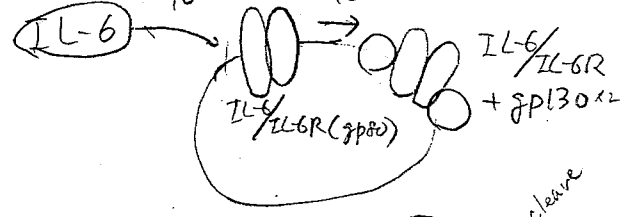

Role of different dialysis membranes in the release of interleukin-6-soluble receptor in uremic patients

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Role of different dialysis membranes in the release of interleukin-6-soluble receptor in uremic patients.

Background. Interleukin-6 (IL-6) exerts its actions through a cell-surface receptor system that consists of two transmembrane subunits: the IL-6 binding glycoprotein gp 80 (IL-6R) and the signal-transducing component (gp 130). Soluble forms of the IL-6R (sIL-6R) are generated by shedding of the membrane-associated proteins. The sIL-6R binds the ligand IL-6 with comparable affinity as the membrane-associated IL-6R and enhances the actions of IL-6.

Methods. Our aim was to evaluate the role of both uremia and different dialysis membranes on peripheral blood mononuclear cell (PBMC) release (either in absence or in presence of mitogen stimulation) and plasma levels of sIL-6R. Ten patients chronically dialyzed with cuprophan membranes (CU), eight patients on regular dialysis treatment with polymethylmethacrylate (PMMA) membranes, 11 uremic nondialyzed patients (UR), and 12 healthy subjects (CON) were included in the study.

Results. PBMCs harvested from CU spontaneously released significantly ($P < 0.01$) greater amounts of sIL-6R (881.8 ± 80.1 pg/mL), as compared with CON (267.5 ± 26.5 pg/mL), UR (258.4 ± 38.1 pg/mL), and PMMA (288.4 ± 24.6 pg/mL). Under mitogenic stimulation, the sIL-6R release was significantly ($P < 0.01$) increased in all groups. The greater PBMC production of sIL-6R in CU was followed by significantly ($P < 0.01$) higher levels of circulating soluble receptors (48.7 ± 2.5 ng/mL, 60%), as compared with CON (30.5 ± 1.9 ng/mL). UR also showed high circulating levels of sIL-6R (53.3 ± 5.9 ng/mL), probably secondary to an impaired urinary excretion. Circulating levels of sIL-6R in PMMA were comparable to CON (30.3 ± 3.3 ng/mL). Either the absence of monocyte activation or the adsorption of sIL-6R on the hydrophobic PMMA surface could explain this finding.

Conclusions. These results suggest an important role for poor dialysis biocompatibility of CU on the release of sIL-6R, which increases sIL-6R plasma levels, thereby enhancing the inflammatory effects of IL-6.

Key words: biocompatibility, hemodialysis, cytokine, cuprophan membranes, polymethylmethacrylate membranes, peripheral blood mononuclear cells, inflammation.

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Life expectancy is greatly reduced in patients undergoing dialysis therapy [1]. The causes of the high rate of mortality and morbidity (mainly cardiovascular and infection) remain unclear and the object of study [2, 3]. Some dialysis-related alterations in the immune and host-defense systems may play a relevant role in this crucial issue, and seem to correlate to the high peripheral blood mononuclear cell (PBMC) production of proinflammatory cytokines, such as interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-6 [4–6]. Other conditions resulting from an up-regulated proinflammatory cytokine production and release may include dialysis amyloidosis [7], malnutrition [8, 9], and atherosclerosis [10].

An enhanced spontaneous production of IL-6 by PBMCs harvested from dialyzed patients treated with cuprophan membranes, as compared with healthy controls and uremic nondialyzed patients, was demonstrated by our group [11, 12].

Interleukin-6 exerts its actions through a cell surface receptor system that consists of two transmembrane subunits: the IL-6 binding gp 80 (named IL-6R) and the signal-transducing component gp 130, which is not ligand binding [13, 14]. IL-6 first binds to the IL-6R, and this complex subsequently associates with two gp 130 molecules. The IL-6 binds IL-6R with low affinity ($K_d = 10^{-9}$ mol/L), but the IL-6/IL-6R complex may trigger its association with gp 130 (two molecules), forming a high-affinity, IL-6–binding site ($K_d = 10^{-11}$ mol/L). Therefore, the signal transducing complex of the IL-6 receptor system on cell membrane consists of a disulfide-linked dimer of gp 130 associated with an IL-6/IL-6R complex [15, 16].

Soluble forms of IL-6R and gp 130, detected in various body fluids of healthy donors (serum, urine) [17], are

generated mainly by the shedding of membrane-associated proteins [15]. In particular, the 80 kD membrane-bound IL-6R is proteolytically cleaved with release of a 55 kD soluble protein (named sIL-6R). The sIL-6R (55 kD) binds the ligand IL-6 with comparable affinity as the membrane-associated receptor (IL-6R 80 kD). Unlike all known soluble cytokine receptors, sIL-6R has no antagonistic role but, in contrast, can act as an agonist of IL-6 activity and may promote or enhance the response to IL-6 in some biological assay [15, 16].

While plasma levels and PBMC release of circulating receptors of other cytokines have been extensively studied [18–20], no data are available about sIL-6R PBMC release and its plasma concentrations in uremic patients. The aim of this study was therefore to investigate the sIL-6R kinetics in both dialyzed and nondialyzed uremic patients. In particular, we evaluated and compared with normal healthy subjects (control group) the sIL-6R circulating levels in uremic nondialyzed patients or in patients regularly dialyzed with two membranes of different biocompatibility toward the leukocyte and the complement system. To better understand the mechanisms determining plasma levels of sIL-6R, we also evaluated the sIL-6R production (basal and stimulated) by PBMCs harvested from the same groups of subjects and sIL-6R urinary excretion in both controls and uremic nondialyzed patients. Finally, to ascertain the role of IL-6 on influencing sIL-6R plasma levels and PBMC release, the concentration of this cytokine on the same plasma and PBMC supernatant samples was also measured.

METHODS

Patient selection

Four groups of subjects were enrolled in this study: (*1*) 10 patients (6 males and 4 females, mean age, 52 ± 12.4 SD years) on regular dialysis treatment (RDT) consisting of dialysis three times a week for at least one year prior to the study with new cuprophan membranes (CU; mean membrane surface, 1.3 m^2, thickness, 8 μ; sterilization, ethylene oxide; filters manufactured by Bellco, Mirandola, Italy); (*2*) 8 patients (5 males and 3 females; mean age, 48.6 ± 8.4 SD years) on RDT for at least one year prior to the study, with a more biocompatible synthetic membrane [polymethylmethacrylate (PMMA); model B3.1.3A, mean membrane surface 1.3 m^2, membrane thickness 20 μ, sterilization: γ-ray; filters manufactured by Toray, Tokyo, Japan)]; (*3*) 11 patients (7 males and 4 females, mean age, 49.6 ± 10.6 SD years) with advanced chronic renal failure (UR; mean glomerular filtration rate value, measured as the mean of creatinine and urea clearance, 10.4 ± 2.8 mL/min); and (*4*) 12 healthy laboratory staff volunteers (CON; 7 males and 5 females, mean age, 45.7 ± 12.2 SD years) who served as the control group.

Neither group of patients on RDT changed their membrane (cuprophan or PMMA, respectively) for one year before the study. No difference was evidenced in their residual renal function (2.2 ± 0.6 SD and 1.9 ± 0.4 mL/min in CU and PMMA, respectively).

No patient had diabetes mellitus or any clinical or laboratory evidence for infectious, neoplastic, or inflammatory disease. No patient was consuming any drug that interfered with the immune response. No difference was evidenced in the percent of smokers or in alcohol intake. All subjects gave their informed consent prior to the study.

In dialyzed patients (either on cuprophan or PMMA membrane), dialysate was filtered through hydrophobic membranes and prepared with bicarbonate dry powder cartridges; Kt/V urea, estimated according to the Daugirdas equation [21], was kept between 1.2 and 1.3, and the protein catabolic rate was kept between 1.0 and 1.3 g/kg/day.

Blood samples and cell cultures

In all groups, heparinized blood samples of 15 mL were collected in the morning for obtaining plasma samples and PBMCs. In dialyzed patients, blood samples were drawn just before the onset of the second dialysis session of the week. Although a day-to-day variability in cytokine release by PBMCs has been reported by Pereira et al [18], preliminary observations by our group, with repeated blood samples randomly drawn before the three different dialysis treatments of the week and during interdialytic periods, did not show significant differences in cytokine production by lymphomononuclear cells. These results are in agreement with those recently obtained by Balakrishnan et al [20].

Peripheral blood mononuclear cells were harvested and established in cultures as previously described [11, 12]. In brief, PBMCs were obtained by a Ficoll-Hypaque (Flow Laboratories, Irvine, UK) gradient density centrifugation (400 × *g* for 30 min). The mononuclear layer was then collected and washed twice with RPMI 1640 culture medium (Flow Laboratories), ultrafiltered to avoid microbial product contamination (Gambro, Lund, Sweden). PBMCs were then resuspended in 15 mL polypropylene round-bottom tubes (Falcon; Becton Dickinson, Lincoln Park, NJ, USA) at a concentration of 2 × 10^6/mL in an Iscove's culture medium (Flow Laboratories) supplemented with 1% heat-inactivated fetal bovine serum (Sigma Chimica, Milan, Italy) and antibiotics (penicillin and streptomycin).

Peripheral blood mononuclear cell cultures were prepared with and without a mitogenic stimulation with 10 μg/mL of bacterial lipopolysaccharide (LPS; Sigma). On the basis of previous studies from our lab and other groups [12, 22, 23], this LPS dosage and the incubation

time have been shown to induce the maximal stimulation for the PBMC release of proinflammatory cytokines.

Peripheral blood mononuclear cells were cultured for 24 hours at 37°C in a humidified atmosphere containing 5% CO_2; cell-free supernatants were collected by centrifugation and were stored at −20°C.

The cell cultures contained about 95% PBMCs with a mean value of 76% lymphocytes (range 68 to 88%) and 12% monocytes (range 8 to 16%); no difference was observed in cell distribution (lymphocytes and monocytes) in the four groups of subjects included in the study. More than 98% PBMCs were viable, as determined by trypan blue dye exclusion at the beginning of the culture, and more than 90% were viable before supernatants were collected.

Urine samples

In healthy subjects and uremic nondialyzed patients, urine was also collected for 12 hours after blood drawing. Urine collections were accurately measured, and a urine sample was stored at −20°C.

Analytical determinations

Creatinine and urea concentrations were determined on plasma and urine samples of control subjects and uremic nondialyzed patients with a Beckman autoanalyzer (Beckman Instruments, Fullerton, CA, USA).

Cytokine assays

Soluble IL-6 receptor and IL-6 concentrations in PBMC cultures supernatant, plasma, and urine samples were evaluated by enzyme-linked immunosorbent assays (ELISA) using two different commercially available kits (Quantikine; R&D Systems, Minneapolis, MN, USA, for both IL-6 and sIL-6R assays). All samples for a given assay (including standards and supernatant, plasma, and urine samples) were analyzed in duplicate at the same time. In particular, the assay for human sIL-6R measures the total amount of sIL-6R present in the samples (that is, the total amount of free receptor plus the total amount of receptor bound to IL-6) by employing a quantitative sandwich enzyme immunoassay technique. In brief, a monoclonal antibody specific for sIL-6R was precoated onto a microtiter plate. Standards and samples were pipetted into the wells; thus, any sIL-6R present was bound by the immobilized antibody. After washing away any unbound substance, an enzyme-linked polyclonal antibody specific for sIL-6R was added to the wells. After washing, a substrate solution was added to the wells, and color developed in proportion to the amount of sIL-6R bound in the initial step. The color development was stopped after 30 minutes, and the intensity of the color was measured at 450 nm. The lower detection limit of this assay was <3.5 pg/mL, and the variation coefficient of both interassay and intra-assay was <10%. This procedure was similar for the human IL-6 assay, and the lower detection limit for this assay was <0.70 pg/mL. The variation coefficient of both interassay and intra-assay was <5%.

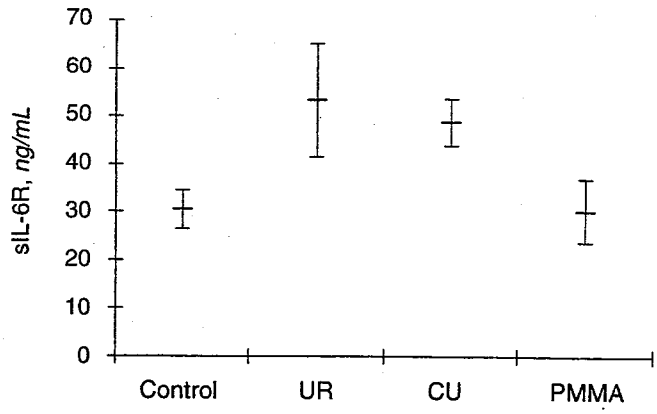

Fig. 1. Plasma circulating levels of soluble interleukin-6 receptor (sIL-6R) in 8 healthy subjects (control), 11 uremic nondialyzed patients (UR), 10 patients dialyzed with cuprophan membrane (CU), and 8 patients dialyzed with polymethylmethacrylate membranes (PMMA). The results are expressed as mean ± 95% confidence intervals (obtained as 1.96 × SEM). CU and UR show circulating levels of sIL-6R significantly ($P < 0.01$) higher than in the other two groups.

Statistical analysis

Statistical analysis was performed using the analysis of variance (ANOVA) followed by the Bonferroni test, as post hoc test, and linear regression analysis. Unless otherwise reported, results are expressed as means ± SEM. The statistical significance was defined as $P < 0.05$.

RESULTS

Plasma levels of sIL-6R

As shown in Figure 1, uremic patients dialyzed with cuprophan membranes (CU) and uremic nondialyzed patients (UR) showed significantly ($P < 0.01$) higher plasma levels (expressed as means ± 95% confidence intervals) of sIL-6R (48.7 ± 4.9 and 53.3 ± 11.6 ng/mL, respectively) as compared with circulating values found in normal healthy controls (controls, 30.5 ± 3.8 ng/mL) and uremic patients dialyzed with PMMA membranes (PMMA, 30.3 ± 6.4 ng/mL). No difference was observed in sIL-6R plasma levels between CU and UR and between controls and PMMA. In Figure 1, it is also evident that no overlap was present for data obtained from CU and UR versus controls and PMMA.

sIL-6R production by unstimulated and stimulated PBMCs

As reported in Figure 2, uremic patients dialyzed with cuprophan membranes (CU) showed a significantly ($P <$ 0.01) higher value of sIL-6R release by unstimulated PBMC (881.8 ± 80.1 pg/mL) as compared with sIL-6R

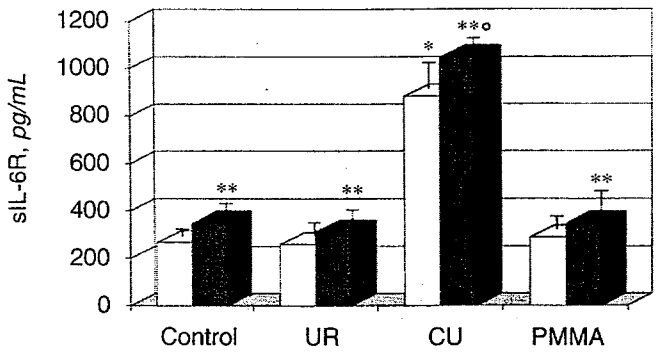

Fig. 2. Soluble IL-6 receptor (sIL-6R) release after incubating both unstimulated (□) and stimulated (■) peripheral blood mononuclear cells (PBMCs) for 24 hours. The results are obtained from the same four groups of Figure 1 and are expressed as mean ± SEM. $^{*}P < 0.01$ vs. unstimulated control, UR, and PMMA; $^{**}P < 0.01$ vs. corresponding unstimulated value; $^{\circ}P < 0.01$ vs. stimulated control, UR, and PMMA.

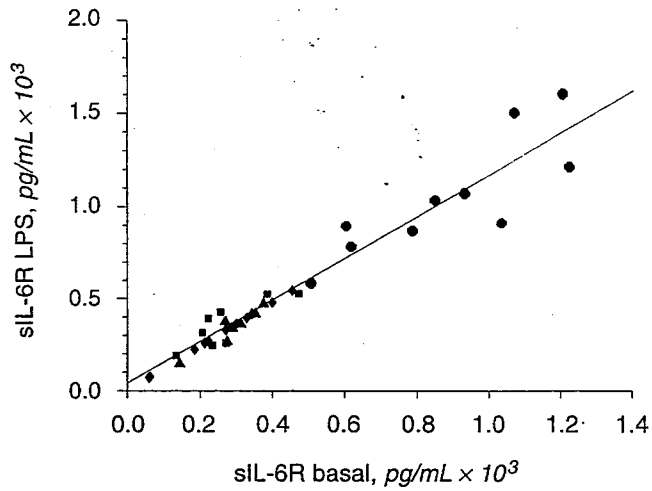

Fig. 3. Linear regression analysis between unstimulated (*x* axis, sIL-6R basal) and lipopolysaccharide (LPS)-stimulated (*y* axis, sIL-6R LPS) sIL-6R release by PBMC. The analysis includes all subjects of the same four groups of two previous figures: control (■), UR (◆), CU (●), and PMMA (▲). sIL-6R release values under LPS result highly correlated with the values obtained in basal condition ($r = 0.967$, $P < 0.001$).

production in controls (267.5 ± 26.5 pg/mL), UR (258.4 ± 38.1 pg/mL) and PMMA (288.4 ± 24.6 pg/mL). When stimulated by LPS, PBMC released a significantly ($P < 0.01$) greater amount of sIL-6R in all groups: controls, 349.0 ± 30.2 (33.6% as compared with unstimulated PBMC release); UR, 312.8 ± 45.9 (21%); CU, 1049.0 ± 100.6 (19%); and PMMA, 348.3 ± 35.0 (21%) pg/mL (Fig. 2). sIL-6R release by stimulated PBMCs resulted significantly ($P < 0.01$) higher in patients dialyzed with cuprophan membranes as compared with the other stimulated three groups (Fig. 2).

No statistically significant difference was obtained among the four groups in the absolute value of increase in sIL-6R release observed by stimulated PBMCs as compared with basal values (81.5 ± 17.5, 54.4 ± 7.8, 167.2 ± 55.0, and 59.9 ± 14.1 pg/mL in controls, UR, CU, and PMMA, respectively).

Soluble IL-6 receptor release values found under mitogenic stimulation were highly and positively correlated with the values obtained without stimulation ($r = 0.967$, $P < 0.001$; Fig. 3). Figure 3 also shows that uremic patients dialyzed with CU exhibit the highest values in both unstimulated and stimulated condition; no difference is seen among the other three groups (controls, UR, and PMMA).

sIL-6R urinary excretion

Urinary excretion of sIL-6R in uremic nondialyzed patients was significantly ($P < 0.001$) lower (0.44 ± 0.1 ng/min) as compared with values obtained in control healthy subjects (2.3 ± 0.3 ng/min).

Plasma levels of IL-6

Uremic patients dialyzed with CU had plasma levels of IL-6 that were significantly ($P < 0.05$) higher (5.4 ± 1.3 pg/mL) than those observed in all other groups (1.8 ± 0.9, 1.2 ± 0.6, and 1.7 ± 0.7 pg/mL in controls, UR, and PMMA, respectively).

Table 1. Interleukin-6 (IL-6) production (pg/mL) after incubating both unstimulated (Basal) and stimulated PBMC for 24 hours

	Control	UR	CU	PMMA
Basal	56.7 ± 7.2	120.1 ± 35.5	375.5 ± 26.1[a]	64.2 ± 9.4
Stimulated	289.2 ± 16.5[c]	234.9 ± 19.1[c]	363.0 ± 20.5[b]	257.4 ± 4.6[c]

Results were obtained from the four groups: control, uremic nondialyzed (UR), uremic dialyzed with cuprophan membranes (CU) and uremic dialyzed with polymethylmethacrylate membranes (PMMA). Data are expressed as mean ± SEM.

[a] $P < 0.01$ vs. Basal control, UR and PMMA

[b] $P < 0.01$ vs. Stimulated control, UR, PMMA

[c] $P < 0.01$ vs. Basal

Basal and stimulated PBMC release of IL-6

As reported in Table 1, uremic patients dialyzed with cuprophan membranes showed a significantly ($P < 0.01$) higher value of IL-6 production by unstimulated PBMCs as compared with values obtained in controls, UR, and PMMA. No difference was observed in basal IL-6 release among controls, UR, and PMMA groups. When the PBMC production of IL-6 was stimulated by LPS, PBMC harvested from uremic patients dialyzed with the cuprophan membranes released a significantly ($P < 0.01$) higher amount of IL-6 than PBMCs isolated from controls, UR, and PMMA. No difference, however, was observed between unstimulated and stimulated IL-6 production in CU patients. In contrast, the other groups (controls, UR, and PMMA) showed a statistically significant ($P < 0.01$) difference (increase under stimula-

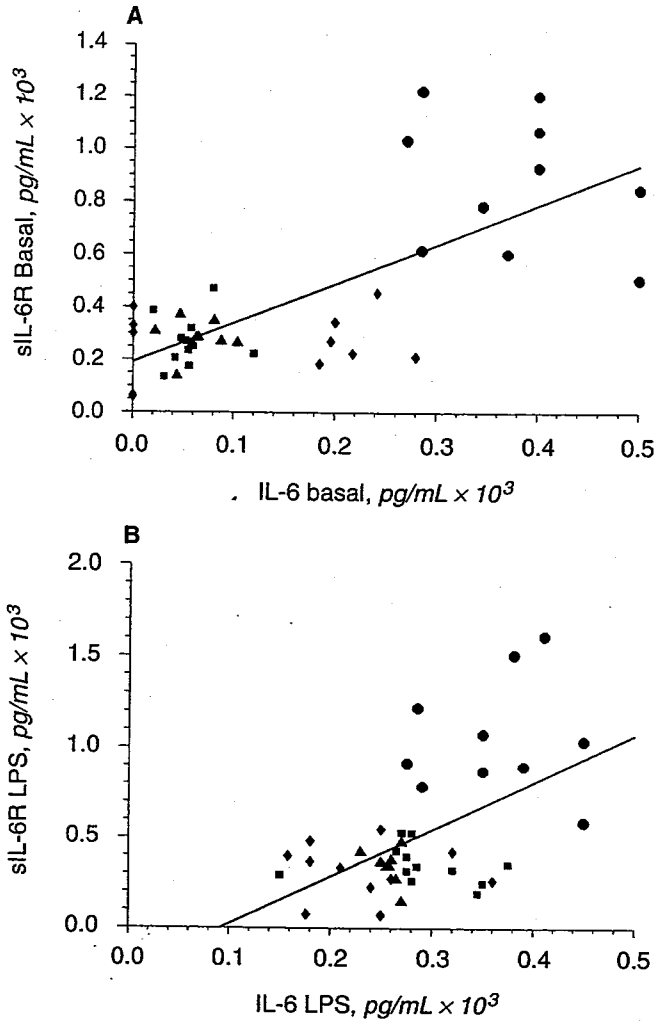

Fig. 4. Linear regression analysis between IL-6 (*x* axis) and sIL-6R (*y* axis) release by PBMCs in both unstimulated (basal, *A*) and stimulated (LPS, *B*) conditions. Symbols are: control (■), UR (◆), CU (●), and PMMA (▲). sIL-6R release values are highly correlated with IL-6-values in both the unstimulated ($r = 0.735$, $P < 0.001$) and stimulated ($r = 0.521$, $P < 0.001$) conditions.

tion) between the unstimulated and the stimulated condition.

Interleukin-6 release by PBMCs was statistically correlated to the sIL-6R PBMC release in both basal ($r = 0.735$, $P < 0.001$) and stimulated ($r = 0.521$, $P < 0.001$) conditions (Fig. 4). IL-6 production in basal condition was also statistically correlated with IL-6 release under stimulation ($r = 0.710$, $P < 0.001$).

DISCUSSION

The results of this study clearly demonstrate that uremic patients dialyzed with cuprophan membranes showed circulating levels of sIL-6R that were significantly higher (almost 60%) than healthy control subjects but similar to those observed in uremic nondialyzed patients (Fig. 1). The results also demonstrated a spontaneous sIL-6R release by PBMCs harvested from uremic patients dialyzed with cuprophan membranes that was significantly greater than that of uremic nondialyzed patients and patients regularly dialyzed with a more biocompatible, less activating complement, dialysis membrane (PMMA). The latter two groups exhibited release values similar to those of normal healthy subjects (Fig. 2). These results suggest PBMC activation, presumably secondary to the poor biocompatibility of the dialysis cuprophan membranes. Such a state of activation could be responsible for the shedding, or de novo synthesis, of the sIL-6R. LPS stimulation, in fact, induced a PBMC activation, with a statistically significant increase in sIL-6R release in all groups (Fig. 2). sIL-6R production under mitogen stimulation was highly and positively correlated with basal values, suggesting an almost similar quantitative response to the mitogenic stimulus in all groups, independently from their basal activation status (Fig. 3).

As mentioned, the circulating levels of sIL-6R were significantly higher in nondialyzed uremic patients than those of control subjects and were similar to uremic patients dialyzed with cuprophan membranes. Since sIL-6R release by PBMCs was not different in nondialyzed uremic patients and control subjects, the increased plasma levels of sIL-6R in the former group do not necessarily reflect an increased receptor release, secondary to inflammatory states and monocyte activation, but presumably are due to the reduced renal excretion of these soluble receptors. Our data on urinary excretion of sIL-6R confirm this hypothesis, demonstrating an almost fivefold reduction in urinary excretion of these receptors in nondialyzed uremic patients as compared with healthy subjects.

The uremic patients dialyzed with PMMA membranes showed low circulating plasma levels of sIL-6R despite the fact that their renal function was almost absent. This finding could be explained by either the absence of monocyte activation or the adsorption of this soluble receptor by the hydrophobic PMMA membrane. Fujimori, Naito, and Miyazaki have recently demonstrated a significant adsorption of complement fragments and cytokines (IL-1, IL-6, and TNF) by different hydrophobic dialysis membranes, particularly by polyacrylonitrile and PMMA [24]. A similar phenomenon could also be hypothesized for sIL-6R.

Regarding IL-6 plasma levels and PBMC release, our results are in agreement with previous studies [11, 12, 25] and demonstrate, in patients dialyzed with cuprophan membrane, a production of IL-6 by stimulated PBMCs not different from that obtained in an unstimulated condition (Table 1). These data confirm a reduction in the ability of PBMCs, when challenged with mitogen, to release adequate amounts of this proinflammatory cytokine in patients dialyzed with cuprophan membranes

[12]. A down-regulation of recurrently activated lymphomononuclear cells to synthesize and release cytokines, as proposed by Zaoui and Hakim [26], probably explains this phenomenon. Chronic use of a more biocompatible, less complement-activating membrane (such as PMMA) may reverse this alteration. IL-6 release resulted statistically correlated to sIL-6R release, in both basal and stimulated conditions (Fig. 4), suggesting a role of the same cytokine on sIL-6R release.

Naturally occurring sIL-6R was first detected as an IL-6–binding protein present in the urine of normal subjects [17]. The purification of this IL-6–binding molecule has further confirmed its identity with the extracellular portion of the membrane receptor gp 80 (IL-6R). IL-6, in the circulation, is bound to the sIL-6R, and there is a large depot of sIL-6R molecules that might further bind IL-6 synthesized, after stimulation, by monocytes, fibroblasts, or endothelial cells [15]. Soluble IL-6R is generated independently by either one of these two mechanisms: a shedding from the membrane-bound receptor protein (particularly under mitogenic stimulation) or, in the basal condition, an alternative splicing of mRNA leading to the loss of the transmembrane domain [27].

In contrast to soluble receptors of other cytokines, such as IL-1, IL-2, and TNF [28–30], which are known to competitively inhibit the functions of their ligands, sIL-6R is able to bind IL-6 with affinity comparable to that of the membrane-associated receptor (IL-6R or gp 80), thereby enhancing the actions of IL-6 [15]. The complex IL-6/sIL-6R may associate on cell surface with two molecules of the transmembrane signal-transducing receptor component gp 130 to mediate the IL-6 signal.

sIL-6R potentiates the IL-6 actions on cells already responsive to IL-6, that is, on cells expressing either gp80 or gp 130 on their surface. More important, sIL-6R bound to IL-6 elicits an IL-6–specific signal on cells that expresses gp 130 but not the gp 80 (IL-6R) protein on their surface and, therefore, are not able to bind IL-6. This implies that cells releasing sIL-6R protein render other cells responsive to IL-6 (trans-signaling) [15]. The soluble form of gp 130 (molecular weight, 90 to 110 kD) has no detectable binding affinity for IL-6. The association between the complex IL-6/sIL-6R and the soluble gp 130 has been detected in the serum of patients and could be demonstrated to have an antagonistic activity, negatively regulating the IL-6 signal [31].

A major role of the sIL-6R in potentiating the IL-6 effects has been shown by Gaillard et al [32]. These authors, using a new culture system in which the supernatant recirculated permanently through an anti-sIL-6R affinity column removing all sIL-6R from the culture medium, demonstrated that sIL-6R sustained the proliferation of multiple myeloma cells that need IL-6 to proliferate [32]. It is still matter of debate whether sIL-6R could play a crucial role in increasing the production of acute-phase protein by human hepatocytes [33].

The possible clinical implications of our findings are that elevated levels of sIL-6R may promote or increase the response to IL-6 and therefore enhance its inflammatory effects.

Interleukin-6 is the major regulator of the hepatic acute phase protein response during inflammation with changes in circulating protein levels and increase in synthesis (up to 1000-fold or more) of the two major acute-phase proteins, C-reactive protein (CRP) and serum amyloid A (SAA) [34–37]. It is now well known that IL-6 is the major cytokine inducing transcription of human CRP during the acute phase-response [38, 39]. Surprisingly, it should be noted that CRP in turn causes a threefold increase of sIL-6R production [40].

In a previous study, we demonstrated that the increased PBMC production of IL-6 was paralleled, in uremic patients dialyzed with cuprophan membrane, by a striking increment of the plasma levels of SAA [12]. Accordingly, a strong linear correlation was detected between IL-6 PBMC production and SAA plasma levels, indicating that IL-6 may be involved in the increase of SAA levels in patients dialyzed with cuprophan membranes [12]. Similar considerations have been proposed for CRP by Panichi et al [41].

C-reactive protein and SAA levels have shown prognostic value in predicting cardiovascular events [42]. Bergström et al first suggested that CRP may predict mortality in hemodialysis patients (abstract; *J Am Soc Nephrol* 6:573, 1995). Kaysen, Stevenson, and Depner recently found that serum albumin concentration [43], a powerful predictor of death in end-stage renal disease patients [44], was also significantly and negatively correlated with both CRP and SAA. In addition to being regulated by dietary protein intake, in fact, albumin is a negative acute-phase reactive protein, as its synthesis is actively suppressed as part of the response to inflammation [45].

Zimmermann et al have recently evaluated whether an activated acute-phase response may influence or predict cardiovascular risk in uremic dialyzed patients [46]. The authors found that a considerable number of hemodialysis patients exhibit an activated acute-phase response, which is closely related to high levels of atherogenic vascular risk factors and cardiovascular death [46]. Further studies are necessary to better clarify the role of IL-6 and of its soluble circulating receptor in eliciting the inflammatory response and the CRP synthesis in uremic dialyzed patients.

In conclusion, our results demonstrate more elevated levels of circulating sIL-6R in patients dialyzed with cuprophan membranes. These elevated levels, caused by the agonistic role of the soluble receptor, could amplify the inflammatory deleterious effects of IL-6 and signifi-

cantly contribute to the alterations induced by this proinflammatory cytokine.

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