The Effects of the Dialysis Membrane on Cytokine Release[1]

Philippe Zaoui and Raymond M. Hakim[2]

P. Zaoui, Service de Nephrologie, Université de Grenoble, Grenoble, France

R.M. Hakim, Division of Nephrology, Vanderbilt University Medical Center, Nashville, TN

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ABSTRACT

The immune response requires the coordinated release of a network of cytokines including interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-2. The potential role of the dialysis membrane on the elaboration of these cytokines by peripheral blood mononuclear cells (PBMNC) harvested from hemodialysis patients was investigated in a prospective crossover study. Eight hemodialysis patients, chronically dialyzed with a biocompatible membrane, were sequentially dialyzed for 2 wk with new cuprophane membranes (Phase I), 2 wk with a low-flux, low-complement-activating membrane (Phase II), and then switched back for a further 2 wk of dialysis with a cuprophane membrane (Phase III). At the end of 2 wk of exposure to the cuprophane membrane, during both Phase I and Phase III, the ability of PBMNC to elaborate IL-1β, tumor necrosis factor-α, and IL-2, as well as soluble IL-2 receptors, in response to phytohemagglutinin was significantly reduced compared with their respective levels at the beginning of the phase; dialysis with a biocompatible membrane increased these levels, and at the end of 2 wk, the response of the PBMNC to phytohemagglutinin was close to that in normal controls. These findings may explain some of the conflicting results in the measurement of cytokine levels in hemodialysis patients and may have clinical implications.

Key Words: *Interleukin-1β, tumor necrosis factor alpha, interleukin-2, cytokines, complement activation*

Immune response to antigens depends on the coordinated response of tissue macrophages and circulating monocytes as antigen presenting cells and lymphocytes as effector cells (1). A crucial element in this immune response is the elaboration of a network of cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), primarily by monocytes and macrophages, and IL-2, by activated T lymphocytes. After their autocrine activation, T lymphocytes express high-affinity IL-2 receptors made up of the p55 and p75 chains. Subsequently, the p55 chain is released from these activated lymphocytes as soluble IL-2 receptors (sIL-2R) (2).

Several studies have examined factors in the dialysis procedure that may play a role in the well-known immunodeficiency of uremic patients (3). Those studies were also spurred by the "interleukin hypothesis," which proposed that cytokines may participate in the acute, as well as the long-term, adverse responses associated with dialysis therapy (4–6). Many of those studies have focused on the intracellular content of cytokines such as IL-1 or on the transcriptional process (mRNA) of these cytokines with acute changes in dialysis parameters or membranes (7–13). However, the clinical relevance of these intracellular changes have not been well documented.

On the other hand, several studies have reported measurements of plasma levels of cytokines in hemodialysis patients. The levels reported in those studies have been quite variable. These variations were assumed to reflect the combined direct effects of the membrane, the extent of complement activation induced by the membrane, the degree of eosinophilia, the potential stimulation from endotoxin fragments that can cross from the dialysate to the blood during clinical dialysis, or possibly, the dialysance of these cytokines during clinical dialysis (14–22). In addition, the significance of these plasma levels has become more difficult to interpret because of the recent characterization of specific endogenous inhibitory peptides for several of these cytokines, including IL-1R antagonist and TNF-binding protein (23).

We undertook a study of the cytokine response to mitogens of peripheral blood mononuclear cells (PBMNC) harvested from eight dialysis patients in a prospective cross over design study using two different dialysis membranes: the cuprophane membrane, which is known to activate complement, neutrophils, and monocytes, and a low-flux, biocompatible mem-

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[2] Correspondence to Dr. R. Hakim, Division of Nephrology, S-3307 MCN, 1161 21st Avenue South and Garland, Nashville, TN 37232-2372.

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brane, the polymethylmethacrylate membrane (PMMA), (Toray, Tokyo, Japan), which is associated with modest complement activation and no evident activation of neutrophils or monocytes (24,25). Specifically, we studied the release of IL-1β, TNF-α, IL-2, and sIL-2R in the supernatants of long-term cultures of PBMNC harvested from the same patients, before and after the first and sixth exposures to the two different dialysis membranes, in a crossover design study.

MATERIALS AND METHODS

Patients

Eight hemodialysis patients on chronic hemodialysis for 44 ± 24 mo were selected for participation in this study. All patients were previously dialyzed with a biocompatible membrane, either the PMMA B2-2.0H or the Fresenius F-60. The mean age of the patients was 69 yr (range, 51 to 76). None of the patients had any clinical evidence for an active inflammatory disease process or malignancy, and none were diabetic or had immunoglobulin (Ig) A nephropathy as the cause of their chronic renal failure. None of the patients were on steroid or immunosuppressive therapy, and none were on aluminum-chelation therapy with desferrioxamine (which is known to inhibit transferrin receptor and IL-2R expression) (26). All patients were on oral 1,25-dihydroxy vitamin D_3 supplementation (27). Kinetic modeling is performed monthly on all patients undergoing chronic dialysis at the VA hospital. The mean ± SD Kt/V (K, dialyzer clearance; t, dialysis time; V, volume of distribution of urea) of the eight patients in the study was 1.21 ± 0.2, and the mean BUN and creatinine levels were 72 ± 11 and 12.8 ± 3.3 mg/dL, respectively. Informed consent, approved by the Vanderbilt University Medical Center and the Nashville VA Hospital IRB, was obtained from all participants.

Methods

Study Design. The design of the study was a prospective crossover design. During the first phase of the study, all patients were dialyzed for six consecutive times (three times a week) with a new cuprophane hollow-fiber membrane (GF-120, Gambro, Lund, Sweden) (Phase I). After 2 wk, the patients were switched to 2 wk of PMMA (Toray, B2-2.0H) membrane (Phase II), a low-flux, noncomplementing-activating membrane; they were then returned for an additional 2 wk of dialysis with new cuprophane membranes (Phase III). All other components of the dialysis prescription were maintained constant. The dialyzers were not reused during the study period. Because these dialyzers had similar solute clearance characteristics, the mean Kt/V, BUN, and creatinine values were not different during different phases of the study. All changes in dialysis membrane occurred after the long weekend stretch.

PBMNC were harvested from blood drawn predialysis and postdialysis at the first and sixth (i.e., beginning and end of 2 wk) dialysis with each type of membrane. Predialysis blood was drawn before the institution of dialysis, and postdialysis blood was drawn after the patient was disconnected from the dialyzer. Control samples, obtained from 18 uremic, nondiabetic, and nondialyzed patients (average serum creatinine, 7.2 ± 1.0 mg/dL) and from 12 normal volunteers without known medical problems, were assessed in parallel.

Isolation of PBMNC. Thirty milliliters of blood was mixed with equal amounts of Hank's balanced salt solution (Gibco, Grand Island, NY) and carefully layered over Histopaque (Sigma Chemical Co., St. Louis, MO) media, allowing gradient density centrifugation. The mononuclear cell layer was collected and washed twice with Hank's balanced salt solution and then resuspended in RPMI 1640 supplemented with 2 mM L-glutamine (Gibco), 100 U/ml penicillin G, 10 μg/ml streptomycin (Sigma), and 10% decomplemented, sterile-filtered, certified fetal calf serum (Gibco).

PBMNC (5×10^5 cells/mL) were placed in 24-well plates (NUNC, New York, NY), incubated for 4 days at 37°C in a 5% CO_2-saturated humidity incubator (Forma Scientific, Marietta, OH), and activated with phytohemagglutinin (PHA; Gibco) at a concentration of 1% (vol/vol). Maximal expression and release of IL-2 and IL-2R (p55) have been shown under these conditions (28–31). Viability by Trypan blue dye exclusion was always more than 99% at the beginning of the culture and more than 90% on the fourth day of culture.

Cytokine Release by Stimulated PBMNC. Supernatants of PHA-activated cultures during the three phases of dialysis, as well as supernatants of similarly activated cultures of controls, were assayed by enzyme-linked immunoassay for the presence of IL-1β, TNF-α, and IL-2 according to the manufacturer's instruction (Genzyme, Boston, MA). The tests are based on a solid-phase enzyme immunoassay using the multiple antibody sandwich principle. In brief, using TNF-α as an example, a mouse monoclonal antibody specific for human TNF-α is used to capture TNF-α present in the sample; this is followed by the addition of TNF-α polyclonal antibody, which binds multiple epitopes on TNF-α, followed in sequence by a biotin-conjugated goat anti-rabbit IgG and streptavidin-conjugated peroxidase, which acts on a peroxide-chromogen substrate to produce increased absorbance at 492 nm (26). All determinations were done in triplicate and averaged for each datum point.

The detection limit of the assay was 20 pg/mL, and the interassay and intra-assay reproducibility was 3.1%. There was no detectable cross-reactivity be-

tween IL-1, TNF-α, and IL-2. Cultures unstimulated with PHA had cytokine levels below the detection limit (<20 pg/mL).

Soluble IL-2Rα Release. Supernatants of PHA-stimulated cultures were also assayed for the presence of soluble IL-2Rα molecules by a sandwich enzyme-linked immunoassay kit (Cellfree; T-cell Sciences, Cambridge, MA). The absorbance values of standard IL-2R dilution were plotted in a second-order regression curve with SIGMAPLOT software, and the resulting fit was used to determine the concentration of the unknown samples. Interassay variation was less than 8%, and the detection limit was 20 IU/mL. Unstimulated PBMNC cultures had no detectable IL-2Rα release.

Endotoxin Contamination. Supernatants of cultures from each of the crossover studies were tested by a quantitative LAL assay (LAL; QCL 1000; Whittaker Bioproducts Inc., Walkersville, MD) and were always below 2.5 EU/mL.

Statistical Analysis

Comparisons between the different phases of the crossover study were by the use of multivariate analysis for balanced design studies with the BMDP software package; *Aposteriori* testing for differences between specific phases was done by use of the Bonferroni method for multiple comparison adjustments on all main effect means. Comparison with control groups was done with the nonpaired *t* test after testing for normality.

RESULTS

TNF-α

The TNF-α level in supernatants of PBMNC was markedly dependent on the phase of the study in which it was measured (Table 1). Thus, TNF-α levels declined from 913 ± 160 pg/mL predialysis at the start of Phase I (new cuprophane phase) to 264 ± 67 before the sixth dialysis with cuprophane ($P \leq 0.002$). As seen in Figure 1, when the same patients were initiated on dialysis with PMMA, the levels rose to 1,066 ± 142 pg/ml before the sixth dialysis with PMMA ($P < 0.001$ compared with the start of Phase II but not significant (NS) compared with initial level), only to decline further to 439 ± 109 pg/mL predialysis at the end of the third phase (new cuprophane membrane). Thus, there was a significant change in the response of PBMNC within 2 wk (six dialyses) of dialysis with each membrane.

There was no significant difference in TNF-α levels between the beginning and end of any particular dialysis session, independent of the dialysis membrane. Predialysis levels at the beginning of the first cuprophane phase and levels at the end of the second (PMMA) phase were not statistically different from those of normal controls but were significantly higher than the TNF-α response of PBMNC harvested from uremic nondialysis patients (788 ± 122 pg/mL; $P = 0.03$) (Table 2).

IL-1

As seen in Figure 2 and Table 1, IL-1 levels measured in the same supernatants generally exhibited similar behavior, with a decline in the IL-1 response of predialysis PBMNC (activated with PHA) from 564 ± 76 pg/mL before the first cuprophane dialysis to 269 ± 61 pg/mL before the sixth dialysis with the new cuprophane membrane ($P \leq 0.01$); this was also evident during the third phase (new cuprophane dialysis) of the study when the IL-1 level decreased from 496 ± 90 to 374 ± 74 pg/mL before the sixth dialysis ($P \leq 0.001$). In contrast, during the PMMA phase, the levels of IL-1 rose from 396 ± 116 pg/mL at the first dialysis with PMMA to 602 ± 97 pg/mL. However, this was not statistically significant. In general, IL-1 levels did not change significantly from predialysis to postdialysis samples, independent of the membrane used.

There was close correlation between the ability of PBMNC to release IL-1β and TNF-α in response to PHA. This is shown in Figure 3, which plots all values of IL-1β and TNF-α in all phases of the study ($r = 0.76$; $P < 0.001$).

IL-2

Levels of IL-2 released by cultures of PBMNC obtained from normal controls and activated by PHA were 1,067 ± 110 pg/mL. At the beginning of the chronic dialysis with new cuprophane membranes, the predialysis IL-2 response by PBMNC was 1,037 ± 132 pg/mL, not statistically different from normal controls, but declined to 743 ± 116 pg/mL postdialysis, as seen in Table 1 and Figure 4 ($P < 0.001$). After 2 wk of recurrent dialysis with the new cuprophane membrane, the IL-2 concentration in the supernatant was 733 ± 89 pg/mL ($P < 0.03$ compared to pre–first dialysis), and declined further postdialysis to 628 ± 93 pg/mL (P = NS compared to pre–sixth dialysis). The levels of IL-2 remained low at the beginning of the PMMA phase, with an IL-2 level of 760 ± 96 pg/mL, but increased postdialysis to 1,052 ± 170 pg/mL ($P < 0.03$) and remained elevated both predialysis (1,015 ± 139 pg/mL) and postdialysis (1,102 ± 152 pg/mL) at the sixth dialysis with PMMA. Again, the levels at the end of this phase (predialysis and post–sixth dialysis with PMMA) were not statistically different from those of normals. When the same patients were crossed over to new cuprophane membranes, the IL-2 response of PBMNC gradually declined, and by the sixth dialysis, both predialysis

TABLE 1. Cytokine concentrations released by PBMNC in response to mitogens (mean ± SD)

	1st Dialysis		6th Dialysis	
	Pre	Post	Pre	Post
Phase I (New Cuprophane Membrane)				
TNF (pg/mL)	913 ± 160	938 ± 136	264 ± 67[a,b]	369 ± 84[a,b]
IL-1 (pg/mL)	564 ± 75	451 ± 76	269 ± 61[a,b]	380 ± 72[a,b]
IL-2 (pg/mL)	1,037 ± 132	743 ± 112[a]	733 ± 89[a,b]	628 ± 93[a,b]
sIL-2R (IU)	3,866 ± 489	2,310 ± 466	2,723 ± 559[a,b]	1,466 ± 614[a,b]
Phase II (New PMMA Membrane)				
TNF (pg/mL)	340 ± 100	569 ± 147[a]	1,066 ± 142[a,b]	1,072 ± 142[a,b]
IL-1 (pg/mL)	396 ± 116	413 ± 103	602 ± 97[a,b]	527 ± 123[a,b]
IL-2 (pg/mL)	760 ± 96	1,052 ± 170	1,015 ± 139[a,b]	1,102 ± 152[a,b]
sIL-2R (IU)	1,588 ± 516	1,806 ± 607	3,244 ± 520[a,b]	3,605 ± 620[a,b]
Phase III (New Cuprophane Membrane)				
TNF (pg/mL)	953 ± 121	834 ± 147	439 ± 109[a,b]	530 ± 113[a,b]
IL-1 (pg/mL)	496 ± 90	472 ± 83	374 ± 74[a,b]	375 ± 73[a,b]
IL-2 (pg/mL)	972 ± 129	813 ± 122	664 ± 81[a,b]	696 ± 93[a,b]
sIL-2R (IU)	3,492 ± 685	2,575 ± 663	2,708 ± 528[a]	1,668 ± 586[a,b]

[a] Significantly different ($P < 0.01$) compared with pre-1st dialysis.
[b] Significantly different ($P < 0.01$) compared with post-1st dialysis.

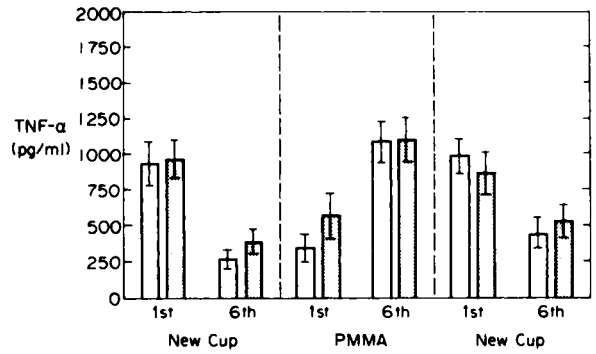

Figure 1. TNF-α levels before (open bars) and after (stippled bars) dialysis during each phase of the study. PBMNC were isolated at the beginning (1st) and end (6th) of each period of exposure to different dialysis membranes, and their response to PHA was assessed. Error bars represent SE of patient datum points. CUP, cuprophane.

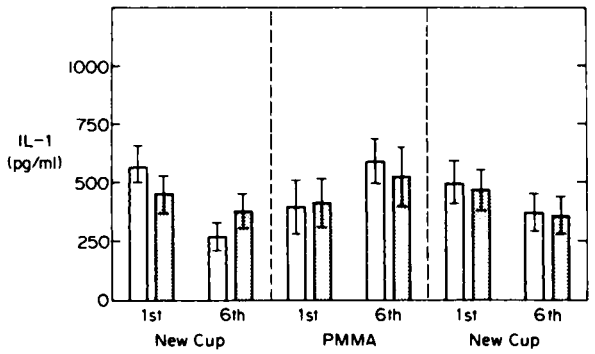

Figure 2. IL-1β levels. Notations are same as for Figure 1.

TABLE 2. Cytokine levels in normal and uremic patients after stimulation with PHA

	Controls	Uremic Nondialyzed
TNF (pg/mL)	1,125 ± 201	788 ± 122
IL-1β (pg/mL)	623 ± 140	586 ± 72
IL-2 (pg/mL)	1,167 ± 110	867 ± 103
IL-2Rα (IU)	3,674 ± 520	3,036 ± 649

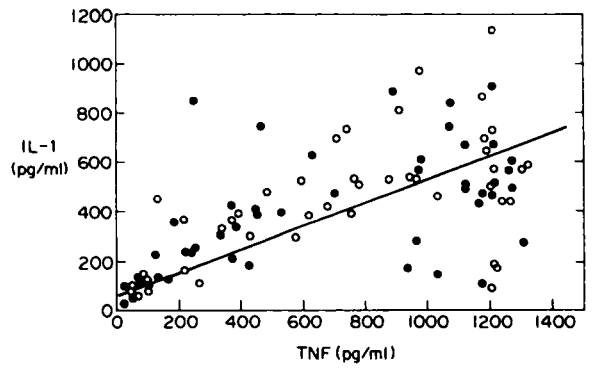

Figure 3. Correlation between levels of IL-1β and TNF-α for all phases of the study for predialysis ○ and postdialysis ●. Some datum points are superimposed ($r = 0.76$; $P < 0.01$).

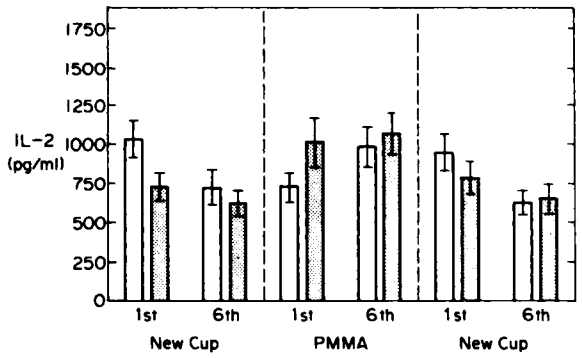

Figure 4. IL-2 levels. Notations are same as for Figure 1.

(664 ± 81 pg/mL) and postdialysis (696 ± 93 pg/mL) levels were significantly below levels obtained at the beginning of the study and at the end of the PMMA phase ($P \leq 0.01$).

Soluble IL-2Rα Release

Supernatants of PHA-stimulated cultures were also assayed for soluble IL-2Rα release. The data are summarized in Table 1. At the beginning of Phase I, PHA stimulation led to a marked release of IL-2Rα in the supernatant (comparable to normal cells): 3,866 ± 489 IU/mL, immediately before the initiation of patients on new cuprophane membrane dialysis. However, after the first dialysis with this membrane, there was a marked decrease in this release to 2,310 ± 466 IU/mL (60% of the predialysis value; $P < 0.01$). After 2 wk of dialysis with this membrane, there was a further decrease in the amount of PHA-induced sIL-2Rα release in the supernatants of cells harvested both predialysis (2,723 ± 559 IU/mL) and postdialysis (1,466 ± 614 IU/mL) ($P \leq 0.001$ compared with predialysis). These trends were reversed with the initiation of the PMMA dialysis period (Phase II), and by the sixth dialysis with the PMMA membrane, the release of sIL-2Rα after PHA stimulation was not different from normal values (3,244 ± 520 IU/mL) (P = NS). The second period of cuprophane dialyses (Phase III) reduced the release of this receptor subunit to the same extent as in Phase I.

DISCUSSION

Reported plasma levels of cytokines such as IL-1β and TNF-α in hemodialysis patients have been quite variable and may be influenced by factors such as the dialysate composition, endotoxin contamination, and membrane structure (14–16,19,20). We therefore chose to study the potential effects of the membrane in the same patients dialyzed with membranes of different complement-activating potential, using the cytokine response of their mononuclear cells to standard doses of mitogen.

The experimental conditions for the culture or stimulation of PBMNC are generally different for different cytokines. In this study, mitogen (PHA) stimulation was used in preference to endotoxin or heat-aggregated IgG because the latter agents maximally enhance the transcription as well as the translation of cytokines, and this may mask the potential influence of the dialysis membranes (32). In addition, studies have shown PHA to be a good mitogenic stimulus to discern differences in lymphokine production and proliferative response between PBMNC obtained from uremic patients and normal controls (33,34), whereas other studies have shown that intracellular and extracellular concentrations of IL-1β in PBMNC cultures equilibrate after 72 h of incubation (35,36). Finally, the crossover design of the study is such that identical culture conditions were used for all phases of the study, thus mitigating drawbacks associated with the use of mitogens or long-term culture for the study of IL-1 and TNF.

The results of the study suggests that chronic dialysis with new cuprophane membrane leads to the attenuation of the ability of mononuclear cells to release immune-related cytokines. This is reflected by the decreased elaboration of IL-1β, TNF-α, IL-2, and sIL-2R in supernatants of PBMNC when challenged with mitogen. In association with the previously documented decrease in the ability of lymphocytes to express high-affinity IL-2R during chronic dialysis with the cuprophane membrane, the studies suggest that the immune response in hemodialysis patients may be down-regulated by the chronic use of this membrane (37). The use of a biocompatible membrane in the same patient population reverses these findings to a large extent. These results have been recently confirmed by the findings of Freidlander *et al.*, who found decreased synthesis of IL-1β and TNF by mononuclear cells in response to endotoxin in patients chronically dialyzed with cellulose ester dialyzers, compared with patients on the biocompatible polysulfone dialyzers; the latter were not different from healthy controls (38). Thus, these results suggest that the interpretation of cytokines levels in plasma of hemodialysis patients must take into account the type and chronicity of exposure to a specific dialysis membrane before their measurement. Although these are not often specified, they may well be part of the explanation for the conflicting results of cytokine measurements in hemodialysis patients.

The pathophysiology of decreased cytokine release by PBMNC after chronic exposure to cellulosic membranes has not been defined in this study. However, several hypotheses can be considered. Although C5a has been shown to be capable of enhancing the transcription of IL-1 and TNF-α (*i.e.*, "priming of PBMNC") (10,39), it is possible that recurrent activation of

these long-lived cells may lead to the down-regulation of their ability to synthesize IL-1 and/or TNF, similar to the down-regulation of neutrophil and monocyte function with recurrent activation (40,41). Such a hypothesis is supported by studies that show high cytokine levels in plasma and supernatants of unstimulated mononuclear cells harvested from patients dialyzed with cuprophane membrane, but low levels in the same cells after stimulation (42–44). Alternatively, up-regulation of the synthesis of IL-1 receptor antagonist or TNF-binding protein may lead to the unavailability of IL-1 or TNF. Finally, recent data suggest that activated mononuclear cells may be transiently sequestered in the circulation and may no longer be part of the circulating pool of PBMNC (45). Such explanations remain speculative in the absence of further experimental data.

IL-2 is released by helper T cells in response to mitogens or antigens and requires the presence of IL-1 and/or TNF (34,46). Thus, the decreased IL-2 response by lymphocytes may result from decreased IL-1β and/or TNF-α synthesis by monocytes, as documented in this and other studies (44). This is in agreement with studies that suggest that the basic defect in the abnormal response by uremic cells resides in the monocytes (47) and that the defective production of IL-1 by the monocytes of hemodialysis patients could explain the low levels of IL-2 in PBMNC cultures (48). Our observations of a decreased IL-2 response in PBMNC harvested after 2 wk of exposure to the cuprophane membrane are also consistent with the observation of Gerez *et al.*, who showed a lack of inducibility of IL-2 mRNA in hemodialysis (but not peritoneal) patients. All of their patients were on a nonreuse cuprophane membrane (49). Although our observations were limited to a 2-wk period for each membrane, it is possible to speculate that the continued exposure of patients to cellulosic membranes may eventually lead to a (transient) loss of inducibility of IL-2 mRNA. Finally, this study is also in agreement with the results of Degiannis *et al.*, who found a normal proliferative response and IL-2 elaboration by PBMNC harvested from polysulfone biocompatible membrane–treated dialysis patients but not in cuprophane membrane–treated patients (50).

Release of the soluble p55 component of the IL-2R follows the autocrine activation of the high-affinity IL-2R by IL-2 and is a plasma marker of lymphocyte activation (2,51). The decreased concentration of sIL-2R with chronic exposure to complement-activating membranes seen in this study is consistent with our other findings of decreased IL-2R expression in patients dialyzed chronically with complement-activating membranes. Paradoxically, hemodialysis patients have been reported to have increased levels of sIL-2R (p55) in plasma (33,52,53). Our results are not inconsistent with these findings, because there appears to be substantial renal clearance of IL-2Rα (33); thus, even a modest release of sIL-2Rα may lead to substantial accumulation in patients without residual renal function.

It is possible that these observations may have clinical relevance. IL-1 has been identified as the "endogenous pyrogen" and is responsible for the development of fever. Decreased elaboration of IL-1 or an abnormal functional relationship between IL-1 and IL-2 in response to mitogenic stimuli may well be the explanation for the decreased febrile response of some dialysis patients in response to infections (7). Our observations of decreased IL-2 production after chronic dialysis with the cuprophane membrane may also be a factor in the defective cellular immune response in hemodialysis patients, such as delayed cutaneous hypersensitivity and decreased response to vaccines (3,54–56). These observations are also consistent with the findings by Meuer *et al.* that the response of chronic hemodialysis patients to hepatitis vaccine is significantly improved after the infusion of low-dose IL-2 (57). The presence of high concentrations of sIL-2R in uremic plasma may further attenuate the availability of IL-2 by binding to it and may further contribute to the well-documented immune defects in uremia *in vivo* (2,58).

In summary, chronic dialysis with the cuprophane membrane, which is associated with the activation of several pathways of the inflammatory response, leads to an attenuated response of mononuclear cells to mitogens, as shown by a decrease in the elaboration of IL-1β, TNF-α, and IL-2. Although this study did not investigate the role of uremic plasma on these defects (59), our conclusion is that cytokine production and, potentially, the immune response of hemodialysis patients are modulated by the type and chronicity of membrane used during dialysis.

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REFERENCES

1. **Kunkel SL, Strieter RM:** Cytokine networking in lung inflammation. Hosp Pract 1990; October:63–76.
2. **Rubin LA, Nelson DL:** The soluble interleukin-2 receptor: Biology, function, and clinical application. Ann Intern Med 1990;113:619–627.
3. **Chatenoud L, Herbelin A, Beaurain G, Descamps-Latscha B:** Immune deficiency of the uremic patient. Adv Nephrol 1990;19:259–274.
4. **Henderson LW, Koch KM, Dinarello CA, Shaldon S:** Hemodialysis hypotension: The interleukin-1 hypothesis. Blood Purif 1983;1:3–8.
5. **Dinarello C:** Cytokines: Agents provocateurs in hemodialysis? Kidney Int 1992;41:683–694.
6. **Platanias LC, Vogelzang NJ:** Interleukin-1: Bi-

ology, pathophysiology and clinical prospects. Am J Med 1990;89:621–629.
7. **Kimmel PL, Phillips TM, Phillips E, Bosch JP:** Effect of renal replacement therapy on cellular cytokine production in patients with renal disease. Kidney Int 1990;38:129–135.
8. **Lonneman G, Koch K, Shaldon S, Dinarello C:** Induction of interleukin-1 (IL-1) from human monocytes adhering to hemodialysis (HD) membranes. Kidney Int 1987;31:238.
9. **Roccatello D, D'Alfonso S, Peruccio D, *et al.*:** Induction of mRNA for tumor necrosis factor α in hemodialysis. Kidney Int 1993;43[Suppl 39]: s144–S148.
10. **Schindler R, Lonnemann G, Shaldon S, Koch KM, Dinarello CA:** Transcriptional activation but not synthesis of interleukin-1 beta and tumor necrosis factor by complement. Kidney Int 1990;37:85–93.
11. **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 1990;38:1089–1094.
12. **Haeffner-Cavaillon N, Cavaillon JM, Ciancioni C, Bacle F, Delons S, Kazatchkine MD:** In vivo induction of interleukin-1 during hemodialysis. Kidney Int 1989;35:1212–1218.
13. **Blumenstein M, Ziegler-Heitbrock H, Schiller B, Schmidt B, Ward R, Gurland H:** Differential activation of monocytes in haemodialysis patients exposed to different types of membranes. Scand J Immunol 1990;31:183–190.
14. **Ghysen J, DePlaen J, van Ypersele de Strihou C:** The effect of membrane characteristics on tumor necrosis factor kinetics during haemodialysis. Nephrol Dial Transplant 1990;5: 270–274.
15. **Herbelin A, Nguyen AT, Zingraff J, Urena P, Descamps-Latscha B:** Influence of uremia and hemodialysis on circulating interleukin-1 and tumor necrosis factor a. Kidney Int 1990;37: 116–125.
16. **Herbelin A, Urena P, Nguyen AT, Zingraff J, Descamps-Latscha B:** Elevated circulating levels of interleukin-6 in patients with chronic renal failure. Kidney Int 1991;39:954–960.
17. **Herbelin A, Urena P, Nguyen AT, Zingraff J, Descamps-Latscha B:** Influence of first and long-term dialysis on uraemia-associated increased basal production of interleukin-1 and tumour necrosis factor alpha by circulating monocytes. Nephrol Dial Transplant 1991;6: 349–357.
18. **Honkanen E, Gronhagen RC, Teppo AM, Maury CP, Meri S:** Acute-phase proteins during hemodialysis: Correlations with serum interleukin-1 beta levels and different dialysis membranes. Nephron 1991;57:283–287.
19. **Chollet-Martin S, Stamatakis G, Bailly S, Mery J, Gougerot-Pocidalo M:** Induction of tumor necrosis factor-alpha during haemodialysis influence of the membrane type. Clin Exp Immunol 1991;83:329–332.
20. **Davenport A, Crabtree J, Androjna C, Will E, Davison A:** Tumor necrosis factor does not increase during routine cuprophane haemodialysis in healthy well-nourished patients. Nephrol Dial Transplant 1991;6:435–439.
21. **Powell AC, Bland L, Oettiger CW, McAllister S, Oliver JC, Arduino M:** Plasma interleukin-1 beta and tumor necrosis factor alpha do not increase during unfavorable hemodialysis conditions [Abstract]. J Am Soc Nephrol 1990;1: 373.
22. **Hertel J, Kimmel P, Phillips T, Bosch J:** Eosinophilia and cellular cytokine responsiveness in hemodialysis patients. J Am Soc Nephrol 1992; 3:1244–1252.
23. **Pereira B, Poutsioka D, King A, *et al.*:** In vitro production of interleukin-1 receptor antagonist in chronic renal failure, CAPD and HD. Kidney Int 1992;42:1419–1424.
24. **Hakim RM, Fearon DJ, Lazarus JM:** Biocompatibility of dialysis membranes: Effects of chronic complement activation. Kidney Int 1984;26:194–200.
25. **Chenoweth DE, Cheung AK, Henderson LW:** Anaphylatoxin formation during hemodialysis: Effects of different dialyzer membranes. Kidney Int 1983;24:764–769.
26. **Carotenuto P, Pontecelli O, Camber JC, Hayward AR:** Desferoxamine blocks IL-2 receptor expression on human T lymphocytes. J Immunol 1986;136:2342–2347.
27. **Tabata T, Shoji T, Kikunami K, *et al.*:** In vivo effect of 1α-hydroxyvitamin D_3 on interleukin-2 production in hemodialysis patients. Nephron 1988;50:295–298.
28. **Degiannis D, Czarnecki M, Hornung N, Raskova J, Raska K Jr:** Mixed lymphocyte reaction-induced release of soluble IL-2 receptor. Transplantation 1991;51:518–523.
29. **Pockley A, Bolton A:** Placental protein 14 (PP14) inhibits the synthesis of interleukin-2 and the release of soluble interleukin-2 receptors from phytohaemagglutinin-stimulated lymphocytes. Clin Exp Immunol 1989;77:252–256.
30. **Cantrell D, Collins M, Crumpton M:** Autocrine regulation of T-lymphocyte proliferation: differential induction of IL-2 and IL-2 receptor. Immunology 1988;65:343–349.
31. **Froelich C, Burkett J, Guiffaut S, Kingsland R, Brauner D:** Phytohemagglutinin induced proliferation by aged lymphocytes: Reduced expression of high affinity interleukin-2 receptors and interleukin-2 secretion. Life Sci 1988;43: 1583–1590.
32. **Luger A, Kovarik J, Stummvoll HK, Urbanska A, Luger TA:** Blood-membrane interaction in hemodialysis leads to increased cytokine production. Kidney Int 1987;32:84–88.
33. **Donati D, Degiannis D, Homer L, Gastaldi L, Raskova J, Raska K Jr:** Immune deficiency in uremia: Interleukin-2 production and responsiveness and interleukin-2 receptor expression and release. Nephron 1991;58:268–275.
34. **Nelson D, Rubin L, Kurman C, Fritz M, Boutin B:** An analysis of the cellular requirements for the production of soluble interleukin-2 receptors in vitro. J Clin Immunol 1986;6:114–120.
35. **Donati D, Degiannis D, Homer L, Raska K Jr, Raskova J:** Production and kinetics of interleukin-1 in hemodialysis. In vivo and in vitro study. Am J Nephrol 1991;11:451–458.
36. **Donati D, Degiannis D, Homer L, Raska K, Raskova J:** Interleukin-1 kinetics in hemodialysis. Am Soc Artif Intern Organs 1991;37: M391–M392.
37. **Zaoui P, Green W, Hakim RM:** Hemodialysis

with cuprophane membrane modulates interleukin-2 receptor expression. Kidney Int 1991;39: 1020–1026.
38. **Freidlander MA, Hilbert CM, Wu YC, Rich EA:** Role of dialysis modality in responses of blood monocytes and peritoneal macrophages to endotoxin stimulation. Am J Kidney Dis 1993;22: 11–23.
39. **Okusawa S, Yancey K, Van Der Meer J, *et al.*:** C5a stimulates secretion of tumor necrosis factor from human mononuclear cells in vitro: Comparison with secretion of interleukin 1 beta and interleukin 1 alfa. J Exp Med 1988;168: 443–448.
40. **Roccatello D, Mazzucco G, Coppo R, *et al.*:** Functional changes of monocytes due to dialysis membranes. Kidney Int 1989;35:622–631.
41. **Himmelfarb J, Lazarus JM, Hakim RM:** Reactive oxygen species production by monocytes and polymorphonuclear leukocytes during dialysis. Am J Kidney Dis 1991;17:271–276.
42. **Memoli B, Libetta C, Rampino T, *et al.*:** Hemodialysis related induction of interleukin-6 production by peripheral blood mononuclear cells. Kidney Int 1992;42:320–326.
43. **Pertosa G, Gesualdo L, Tarantino E, Ranieri E, Bottalico D, Schena F:** Influence of hemodialysis on interleukin-6 production and gene expression by peripheral blood mononuclear cells. Kidney Int 1993;43[Suppl 39]:s149–s153.
44. **Hart P, Jones C, Jones K, Finlay-Jones J:** Reduced secretion of IL-1 B by peritoneal cells from patients on continuous ambulatory peritoneal dialysis. Immunol Cell Biol 1993;71:99–107.
45. **Schindler R, Linnenweber S, Schulze M, *et al.*:** Gene expression of interleukin-1 β during hemodialysis. Kidney Int 1993;43:712–721.
46. **Beaurain G, Naret C, Marcon L, *et al.*:** In vivo T cell activation in chronic uremic hemodialyzed and non-hemodialyzed patients. Kidney Int 1989;36:636–644.
47. **Tsakolos ND, Theoharides TH, Mendler ED, Goffinet J, Dwyer JM, Whisler RL:** Immune defects in chronic renal impairment: Evidence for defective regulation of lymphocyte response by macrophages from patients with chronic renal impairment on hemodialysis. Clin Exp Immunol 1986;63:218–227.
48. **Meuer SC, Hauer M, Kurz P, Meuer Zum Buschelfelde KH, Kohler H:** Selective blockade of the antigen-receptor-mediated pathway of T cell activation in patients with impaired primary immune responses. J Clin Invest 1987;80: 743–749.
49. **Gerez L, Madar L, Shkolnik T, *et al.*:** Regulation of interleukin-2 and interferon-γ gene expression in renal failure. Kidney Int 1991;40: 266–272.
50. **Degiannis D, Czarnecki M, Donati D, *et al.*:** Normal T lymphocyte function in patients with end stage renal disease hemodialyzed with high-flux polysulfone membranes. Am J Nephrol 1990;10:276–282.
51. **Rubin L, Kurman C, Fritz M, *et al.*:** Soluble interleukin-2 receptors are released from actiated human lymphoid cells in vitro. J Immunol 1985;135:3172–3177.
52. **Colvin RB, Fuller TC, MacKeen L:** Plasma interleukin-2 receptor levels in renal allograft recipients. Clin Immunol Immunopathol 1987;43: 273–276.
53. **Walz G, Kunzendorf U, Josimovic-Alasevic O, *et al.*:** Soluble interleukin 2 receptor and tissue polypeptide antigen serum concentrations in end stage renal failure. Nephron 1990;56:157–161.
54. **Dumann H, Meuer SC, Kohler H:** Uremic serum inhibits monocyte-dependent, but not interleukin-2 dependent steps of T-cell proliferation. Nephron 1990;56:162–165.
55. **Kirpatrich CH, Wilson WEC, Talmage DW:** Immunologic studies in human organ transplantation: I. Observation and characterization of suppressed cutaneous reactivity in uremia. J Exp Med 1964;119:727–742.
56. **Langhoff A, Lodefoged J, Olgaard K, Madsen S:** Restoration of uremic lymphocyte resonses in vitro by interleukin-2. Eur Dial Transplant Assoc 1985;22:932–936.
57. **Meuer S, Dumann H, Zum Buschenfelde KHM, Kohler H:** Low-dose interleukin-2 induces systemic immune response against HBsAg in immunodeficient non-responders to hepatitis B vaccination. Lancet 1989;15–18.
58. **Rubin LA, Jay G, Nelson DL:** The released interleukin 2 receptor binds interleukin 2 efficiently. J Immunol 1986;137:3841–3844.
59. **Donati D, Degiannis D, Raskova J, Raska K Jr:** Uremic serum effects on peripheral blood mononuclear cell and purified T lymphocyte responses. Kidney Int 1992;42:681–689.