Intradialytic Granulocyte Reactive Oxygen Species Production: A Prospective, Crossover Trial¹,²

Jonathan Himmelfarb,³ Kenneth A. Ault, Diane Holbrook, Donald A. Leebet, and Raymond M. Hakim.

J. Himmelfarb, D.A. Leebet, Division of Nephrology, Maine Medical Center, Portland, ME
K.A. Ault, D. Holbrook, Maine Medical Center Research Institute, South Portland, ME
R.M. Hakim, Division of Nephrology, Vanderbilt University School of Medicine, Nashville, TN


ABSTRACT
By the use of flow cytometric techniques, this prospective, randomized crossover study was designed to analyze intradialytic granulocyte reactive oxygen species (ROS) formation in whole blood with complement-activating and noncomplement-activating hollow fiber membranes. Dialysis with a complement-activating membrane resulted in a 6.5-fold increase in granulocyte hydrogen peroxide production 15 min after dialysis initiation and remained significantly elevated (P < 0.01) through the first 30 min with this membrane in comparison to both predialysis values and simultaneous values with a noncomplement-activating membrane. Further studies demonstrated that blood obtained at 15 min with a complement-activating membrane generated significantly less granulocyte ROS production in response to Staphylococcus aureus incubation than blood obtained either predialysis or at the same time in dialysis with a noncomplement-activating membrane. Both complement-activating and noncomplement-activating dialysis membranes caused slightly decreased granulocyte responsiveness to phorbol myristate acetate. It was concluded that hemodialysis with complement-activating membranes results in increased granulocyte ROS production during the dialysis procedure. These results document the potential role of ROS in hemodialysis-associated pathology and susceptibility to infection.

Key Words: Hemodialysis, biocompatibility, granulocytes, reactive oxygen species, cuprophane

Hemodialysis with cellulose membranes results in the development of transient leukopenia, followed by rebound leukocytosis (1). In this setting, neutrophils undergo modulation of important cell surface receptor proteins [CR1 (CD35) [2], MAC-1 (CD11b–CD18) [3], and L-selectin [LAM-1, LECCAM-1 [4,5]] and release of enzymes including elastase, myeloperoxidase, and lactoferrin (6). Although these changes have generally been ascribed to activation of the alternative pathway of complement by cellulosic membranes (7), other investigators have not found such correlations in specific markers of granulocyte activation. Horl et al., in comparing dialysis with cuprophane and polymethyl-methacrylate (PMMA), have found that the release of granulocyte elastase does not correlate with the well-known differences in complement activation (6). Because of these discrepancies, further controlled studies of granulocyte activation with both complement-activating and noncomplement-activating hollow fiber dialysis membranes were undertaken.

A major function of activated phagocytic cells in host defense is the production of reactive oxygen species (ROS) via the respiratory burst, which is crucial for the killing of adherent or adjacent microorganisms. To date, studies of ROS production during dialysis have been limited because of available methodology. Most studies have relied upon luminol-enhanced chemiluminescence to measure ROS production in hemodialysis (7–10). However, recent data have demonstrated that luminol-enhanced chemiluminescence measures primarily myeloperoxidase-catalyzed oxidation rather than the respiratory burst (11–15). Thus, luminol-enhanced chemiluminescence, although useful as a general measure of granulocyte activation, involves both ROS formation and degranulation and is not specific for either process.

Recently, Bass et al., developed an alternative assay for the measurement of intracellular ROS using flow cytometry (16). This method involves the use of 2′,7′ dichlorofluorescin diacetate (DCFH-DA), which rapidly diffuses across cell membranes and is
trapped within the cell by hydrolysis. In the presence of hydrogen peroxide and other peroxidases, this compound is oxidized to 2′,7′ dibromo fluorescein (DCF), which is highly fluorescent. The degree of intracellular fluorescence is proportional to ROS production.

In an earlier study, we were able to demonstrate increased intracellular ROS production in isolated phagocytic cells harvested during cuprophane dialysis (17). However, the measured DCF fluorescence in that study may not have represented the true extent of ROS production, because of the length of time necessary to isolate polymorphonuclear leukocytes and the potential confounding effect of granulocyte activation by isolation techniques. We have recently described a modification of the original method of Bass et al. that allows for the measurement of intracellular granulocyte ROS production in whole blood (18). This method can be performed rapidly, is highly reproducible, and has the potential advantage of eliminating artifactual granulocyte activation occurring during cell isolation procedures. This assay therefore reflects more accurately the in vivo granulocyte activation.

In this study, we have conducted a prospective crossover trial comparing granulocyte intracellular ROS production using a highly complement-activating (cuprophane) and a relatively low complement-activating (PMMA) dialysis membrane and have analyzed the functional capacity of granulocytes harvested during dialysis with each membrane to further stimulation.

**MATERIALS AND METHODS**

**Patient Characteristics**

Ten patients on chronic maintenance hemodialysis were selected for study. Informed consent was obtained from the subjects, and the project was approved by the respective Institutional Review Board. The mean age of the patients was 61.5 yr (range, 31 to 80), with a mean time on dialysis of 3.9 yr (range, 6 months to 14 yr). Five were men, and five were women. None of these patients had clinical evidence of infection at the time of the study. Before the time of the study, all patients were on a hemodialysis reuse program that used 0.66% formaldehyde and 0.25% bleach, with incubation of dialyzers at 40°C for 24 h.

**Hemodialysis Studies**

All patients were studied in a random crossover fashion with first-use cuprophane hemodialysis membranes and first-use PMMA membranes. A bicarbonate-based dialysate (Na, 140 mEq/L; K, 2 mEq/L; Ca, 3.5 mEq/L; HCO₃, 30 mEq/L; acetate, 3 mEq/L) was used in all cases. Blood samples were drawn predialysis and then subsequently from both the afferent and efferent lines to the dialyzer at 15, 30, and 90 min. and at the end of the dialysis. Anti-coagulation was maintained with heparin at an initial bolus of 100 U/kg.

**Preparation of Whole Blood Samples for Flow Cytometric Analysis**

Blood was drawn into heparinized vacutainer tubes from dialysis lines at specified times. Three 100-μL aliquots were removed—one for baseline ROS production and the other two for the measurement of ROS production after activation with phorbol myristate acetate (PMA; Sigma Chemical Co., St. Louis, MO) or heat fixed *Staphylococcus aureus* Cowan strain I (Cal Biochem, San Diego, CA). Phosphate-buffered saline (850 μL) and 5 mM 2′,7′ dibromo fluorescein diacetate (8 μL; DCFH-DA; Eastman Kodak, Rochester, NY) were added to each tube. The tubes were then capped, gently shaken, and placed in a water bath at 37°C for 15 min. In the appropriate tubes, 100 nM PMA or 10⁹ heat-fixed *S. aureus* organisms were added, mixed, and incubated at 37°C for an additional 30 min. At the end of the incubation, 30 μL of a 1.67 mg/ml solution of LDS-751 (Exiton, Inc., Dayton, OH) was added. The samples were fixed with a 1:1 vol of 2% paraformaldehyde and were analyzed within 2 h by flow cytometry.

**Flow Cytometric Analysis of Granulocyte ROS Production**

All samples were analyzed on a Becton-Dickinson FACScan flow cytometer (San Jose, CA). The instrument was set up to measure the linear forward light scatter (FSC), a measure of particle size; linear 90-degree light scatter (SSC), a measure of cell granularity; green (fluorescein) 535 nm fluorescence; and red (LDS-751) 620 nm fluorescence. Fluorescent parameters were collected by the use of 4-decade logarithmic amplification. LDS-751 (Styryl18; Exiton, Inc.) was used to label the nuclei of living cells and permit their identification in whole blood (18). This dye does not require the cells to be permabilized and has an additional advantage in that it is excited by a 488-nm argon ion laser light and emits at 700 nm with no significant emission at 535 or 580 nm. It thus permits the flow cytometer to be triggered on the LDS-751 signal and thus to make measurements on nucleated cells in whole blood without interference from erythrocytes.

Further gating on the granulocyte population was achieved with a combination of FSC and SSC (Figure 1). Platelets, erythrocytes, monocytes, and lymphocytes were excluded from analysis by an analysis of
only the bitmap with size and granularity characteristics of granulocytes.

The data were collected in list mode files and were analyzed. The instrument was calibrated with beads of known diameter (2 and 10 μm; Dukes Scientific, Palo Alto, CA) for the FSC parameter and with Calibrite beads (Becton-Dickinson) for the fluorescence parameters.

Statistical Methods

Statistical analysis was performed to compare results during the dialysis procedure with predialysis values with the same dialyzer and to compare afferent with efferent values at the same time point with the same dialyzer. Additional comparisons were made between cuprophane and PMMA at the same time points. All analysis was done by the use of multivariate analysis of variance with time, membrane, and patient as variables. Post-hoc two-tailed t tests were applied when the analysis of variance indicated the presence of a statistical significance.

RESULTS

Analysis of Granulocyte Intracellular ROS Production During Dialysis

Intracellular granulocyte DCF fluorescence was measured in whole blood from afferent and efferent dialyzer lines of the two dialyzer membranes. At 15 min after the initiation of dialysis with cellulosic membranes, there was a dramatic increase in granulocyte fluorescence representing ROS formation (Figure 2). Mean channel fluorescence had increased in the afferent samples from 103 ± 15 fluorescent channels predialysis to 216 ± 20 units at 15 min into dialysis (P < 0.0001). Because this is a logarithmic scale, this represents a 650% increase in intracellular fluorescence. Similarly, at 30 min after the initiation of cellulosic hemodialysis, mean channel fluorescence remained elevated compared with predialysis values at 167 ± 22 fluorescent channels (P < 0.01). However, 90 min after dialysis initiation and at the end of dialysis, intracellular granulocyte fluorescence had returned to approximate predialysis levels.

Dialysis with PMMA membranes was associated with only a slight increase in granulocyte intracellular ROS fluorescence at 15 and 30 min after the initiation in dialyzer afferent and efferent samples but was statistically significant at 15 and 30 min (P < 0.01) for the afferent samples and at 15 and 30 min for the efferent samples (115 ± 9 mean fluorescence channels at 15 min compared with 87 ± 10 fluorescent channels predialysis [P < 0.01]). No other times during PMMA dialysis with afferent samples were associated with a change in granulocyte ROS production in comparison to predialysis values.

A direct comparison of ROS production values revealed statistically significant increases in fluorescence at 15 (P < 0.0001) and 30 min (P < 0.0001) for dialysis with cuprophane versus PMMA membranes in afferent samples.

Samples of whole blood obtained from the efferent line during dialysis for analysis of intracellular granulocyte ROS production revealed a pattern of increased intracellular ROS production similar to that obtained from afferent samples (Figure 3). Thus, at 15 min after dialysis initiation with cellulosic mem-
branes, mean fluorescence had increased from $103 \pm 15$ channels predialysis to $236 \pm 27$ channels ($P < 0.0001$ versus predialysis). Thirty minutes after the initiation of cellulose hmodialysis, intracellular granulocye ROS was still markedly elevated at $186 \pm 22$ channels compared with predialysis levels ($P < 0.01$). By 90 min after the initiation of cellulose hmodialysis, levels of intracellular ROS production were not significantly different from baseline. A direct comparison of ROS production revealed statistically significant increases in fluorescence at 15 ($P < 0.0001$) and 30 min ($P < 0.0002$) for dialysis with cupophane versus PMMA membranes in efferent samples.

A comparison of changes in granulocyte intracellular ROS production from afferent to efferent samples or "across the dialyzer" with cupophane hmodialysis was performed. This revealed that there was a small, although statistically insignificant, increase in intracellular granulocyte ROS production at both the 15- and 30-min time points during cellulose hmodialysis.

**Intradialytic Changes in ROS Production in Response to S. aureus**

Blood obtained during both cellulose and PMMA hmodialysis was incubated with heat-fixed S. aureus organisms and analyzed for intracellular granulocyte ROS production by the measurement of DCF fluorescence (Figure 4). In contrast to the increase in spontaneous ROS production by granulocytes during cellulose hmodialysis, there was a marked decrease in ROS in response to challenge with S. aureus organisms. Thus, although predialysis samples were able to achieve a mean fluorescence of $528 \pm 25$ channels in response to S. aureus challenge, blood obtained from the afferent line 15 min into cellulose hmodialysis had a mean channel fluorescence of only $412 \pm 25$ channels ($P < 0.01$ compared with predialysis). Under the same conditions, the response of blood samples obtained 30 min after the initiation of cellulose hmodialysis was not statistically different from the response at baseline ($495$ mean fluorescence versus $528$). In contrast to results obtained with cellulose hmodialysis, dialysis with PMMA membranes was not associated at any time point with any changes in granulocyte ROS production in response to S. aureus compared with predialysis values. A direct comparison of S. aureus--induced ROS production revealed a statistically significant decrease in fluorescence at 15 min ($P < 0.0001$) for dialysis with cupophane versus PMMA membranes in both afferent and efferent samples.

The response of granulocytes to S. aureus incubation in whole blood drawn from the efferent line is shown in Figure 5. Fifteen minutes after the initiation of cellulose hmodialysis, there was a statistically significant decrease in intracellular ROS in response to S. aureus challenge ($454 \pm 25$ mean fluorescence versus $528 \pm 25$ mean fluorescence channels predialysis $P < 0.05$). At other times during cellulose hmodialysis and throughout the PMMA
Figure 5. S. aureus-stimulated whole blood granulocyte ROS production from efferent blood lines during hemodialysis. Whole blood was obtained at specified times with either cuprophane (closed squares) or PMMA (open squares) dialysis membranes, loaded with DCFH-DA, and incubated for 15 min at 37°C with 10^6 heat-fixed S. aureus organisms/ml. Results are expressed as the log mean fluorescence of granulocytes ± SE (N = 10 patients). * P < 0.05 compared with predialysis; ** P < 0.01 compared with predialysis; † † P < 0.002, cuprophane compared with PMMA at the same time.

hemodialysis, there was no statistically significant change in granulocyte ROS production in response to S. aureus challenge.

Intracellular Granulocyte ROS Response to Phorbol Myristate Acetate

In these experiments, whole blood was obtained from both afferent and efferent dialyzer lines at many times during both cellulose and PMMA dialysis and was incubated with a dose of PMA that leads to near maximal granulocyte stimulation. Intracellular granulocyte ROS production was then analyzed in an effort to determine whether the maximal capacity of granulocytes to elaborate ROS was affected by the hemodialysis process (Figures 6 and 7). During cellulose hemodialysis, blood sampled from afferent lines 15 min after the initiation of dialysis showed a decreased ability to respond to PMA (predialysis 422 ± 15 mean fluorescence channels versus 343 ± 18 mean fluorescence channels at 15 min [P < 0.01]). Significant decreases in granulocyte ROS response to PMA were also observed at 30 min [predialysis 422 ± 15 versus 376 ± 14 mean fluorescence channels [P < 0.01]]. Blood samples obtained from the afferent dialyzer line during PMMA dialysis showed statistically significant decreases at 15 min [457 ± 16 fluorescence channels versus 488 ± 20 fluorescence channels predialysis [P < 0.02]], at 30 min [448 ± 15 fluorescence channels [P < 0.01]], and at the end of dialysis [418 ± 13 fluorescence channels [P < 0.05]].

Figure 6. PMA-stimulated whole blood granulocyte ROS production from afferent blood lines during hemodialysis. Whole blood was obtained at specified times with either cuprophane (closed squares) or PMMA (open squares) dialysis membranes, loaded with DCFH-DA, and incubated for 15 min at 37°C with 100 nM PMA organisms/ml. Results are expressed as the log mean fluorescence of granulocytes ± SE (N = 10 patients). * P < 0.05 compared with predialysis; ** P < 0.01 compared with predialysis.

Figure 7 represents results obtained from analysis of whole blood incubation with PMA obtained from the efferent dialyzer line. Blood obtained 15 min after the initiation of cellulose hemodialysis showed a statistically significant decrease in fluorescence response to PMA (358 ± 17 fluorescence channels versus 422 ± 15 fluorescence channels predialysis [P < 0.02]). Blood obtained 30, 90, or 240 min after the initiation of cellulose hemodialysis and incubated with PMA did not differ significantly from predialysis samples. Blood samples obtained from the efferent dialyzer line with PMMA dialysis showed significant differences in intracellular ROS production in response to PMA at 15 min, at 30 min (435 ± 20 fluorescence channels predialysis [P < 0.02]) and at the end of dialysis (419 ± 8 fluorescence channels [P < 0.05]) compared with predialysis samples.

DISCUSSION

Kaplow and Goffinet demonstrated in 1968 that hemodialysis with cellulose membranes results in the development of a transient granulocytopenia followed by rebound granulocytosis (1). The pioneering work of Hammerschmidt, Craddock, and others established that complement activation can cause leukocytopenia and pulmonary capillary granulocyte sequestration and may be responsible for the transient granulocytopenia during hemodialysis (19). Cheung, Chenoweth, and Henderson established in the early 1980s that the activation of the alternative pathway of complement by cellulose dialysis membranes played a major etiologic role in the develop-
the bevel of complement activation.

signs and symptoms that hemodialysis patients ex-
vich et al. 

ment of granulocytopenia. Hakim et al. (20), Ivano-
vich et al. (21), and others correlated many of the 
signs and symptoms that hemodialysis patients ex-
perience during first-use cellulosic hemodialysis with 
the level of complement activation.

A major function of activated granulocytes is the 
production of bactericidal products via the respira-
tory burst. The reduction of molecular oxygen results 
in the formation of highly reactive oxidizing agents, 
consisting primarily of superoxide anion \( \text{O}_2^- \) and 
hydrogen peroxide \( \text{H}_2\text{O}_2 \). In this study, we have used 
a recently developed flow cytometric assay capable 
of measuring granulocyte intracellular ROS production 
in whole blood (18). We have demonstrated in a 
crossover trial comparing hemodialysis with cellul-
sic membranes to a low complement-activating 
membrane (PMMA) that hemodialysis with cellulosic 
membranes is associated with a marked increase in 
granulocyte ROS production through the first 30 min, 
with increases up to 650% of predialysis values. The 
increase in granulocyte ROS production was found in 
both afferent (blood sampled before the dialyzer) 
and efferent (blood sampled after the dialyzer) sam-
ples. This demonstrated that the process of intradia-
lytic granulocyte activation with cellulosic mem-
branes is a systemic inflammatory reaction and is 
not limited to blood traveling through the dialyzer. 
The lack of "step up" across the dialyzer between 
afferent and efferent lines is also not unexpected, 
because \textit{in vitro} experiments have demonstrated that 
several minutes are required to maximally generate 
intracellular ROS after activation (18), which would 
exceed transit time through the dialyzer. In contrast,
dialysis with PMMA membranes caused only a min-
imal increase in granulocyte ROS production.

The results of this study extend and amplify pre-
vious observations that we have made using a similar 
assay, but with isolated polymorphonuclear leuko-
cyte preparations and with cuprophane dialysis 
membranes only (17). In that study, we were only 
able to demonstrate a slight increase in granulocyte 
ROS production 15 min after the initiation of di-
alysis. The whole blood method used in the study 
presented here has proved much more sensitive in 
demonstrating dialysis-induced ROS production. The 
whole blood method allows blood sampled from dia-
lysis lines to be immediately incubated with DCFH-
DA, so that intracellular hydrogen peroxide can oxi-
dize this compound to the fluorescent product DCF. 
We believe that the time required for cell isolation in 
our previous work may result in the degradation of 
ROS by intracellular enzymes such as superoxide 
dismutase and catalase.

Previous studies of ROS production during hemo-
dialysis have relied upon luminol-enhanced chemi-
luminescence, a measure of the ability of stimulated 
granulocytes to emit light. However, luminol-
enhanced chemiluminescence depends on both res-
piratory burst activity and on the release of myelo-
peroxidase by degranulation (11–15). The assay used 
in this study is dependent only on respiratory burst 
activity and thus is preferable for measuring changes 
in ROS production.

Nguyen et al. (7) and Descamps-Latscha et al. (8) 
demonstrated increased chemiluminescence with 
cellulosic dialysis membranes, but not with polyacry-
lonitrile. These and other studies (9,10) have dem-
strated increased inradialytic responsiveness to 
yzmosan, F-met-leu-phe (an analog of bacterial cell 
walls), and PMA with cellulosic membranes. Al-
though a few studies have come to different conclu-
sions (22), these studies collectively support the find-
ings in this study that cellulosic hemodialysis causes 
increased ROS formation \textit{in vitro}, but decreased gran-
ulocyte responsiveness to challenges such as \textit{S. au-
reus}, F-met-leu-phe, or C5a. Significant inradialytic 
changes have not been seen with either polyacrylon-
itrile or PMMA dialysis membranes.

It is currently believed that oxidant-induced injury 
to the endothelium is an important mechanism in 
causing vascular damage (23,24). Interactions be-
tween activated granulocytes and endothelial cells 
have been shown to produce endothelial cell lysis and 
damage (25,26). Hydrogen peroxide appears to be a 
particularly important mediator in producing endo-
thelial cell injury and increasing vascular permeabil-
ity (27–29). The close approximation of granulocytes 
and endothelial cells, as would occur during the early 
phase of dialysis with cellulosic membranes, has 
been shown to dramatically increase \( \text{H}_2\text{O}_2 \) release 
from activated granulocytes. Thus the increased

Figure 7. PMA-stimulated whole blood granulocyte ROS pro-
duction from efferent blood lines during hemodialysis. 
Whole blood was obtained at specified times with either 
cuprophane (closed squares) or PMMA (open squares) 
dialysis membranes, loaded with DCFH-DA, and incubated 
for 15 min at 37°C with 100 nM PMA organisms/mL. Results 
are expressed as the log mean fluorescence of granulo-
cytes \( \pm \text{SE} \) (\( N = 10 \) patients). * \( P < 0.05 \) compared with 
predialysis; ** \( P < 0.01 \) compared with predialysis.
granulocyte H₂O₂ production detected during the first 30 min of cellulosic hemodialysis in this study may be an indicator of oxidant-induced endothelial injury during cellulosic hemodialysis. Richard et al. have recently demonstrated decreased intracellular antioxidant capability in chronic hemodialysis patients, which would support this concept (30). However, at present, data directly demonstrating endothelial injury during analysis have not been demonstrated. Although dialysis-induced oxidant injury to the endothelium remains speculative at present, recent data from animal models establish the importance of this concept. In a rat model, Schulman et al. demonstrated that an infusion of blood incubated with cuprophane dialysis membranes delayed the resolution of acute renal failure (31). The use of deferoxamine as an iron chelator, leading to a decrease in hydroxyl ion production, ameliorated the cuprophane-induced effects on delayed recovery of renal function.

Although this prospective crossover study demonstrates a clear correlation between intradialytic granulocyte activation and the use of complement-activating membranes, not all investigators have found a similar correlation. In particular, Horl et al., measuring granulocyte elastase release, found that both PMMA and cuprophane caused significant intradialytic elastase release, with PMMA proving to be a slightly greater secretagogue than cuprophane (6). Although it is difficult to fully reconcile these findings with those of this study, it is possible that granulocyte activation pathways for enzyme release and ROS formation may differ.

In this study, we also demonstrated decreased granulocyte intracellular ROS production in response to either S. aureus or PMA challenge. This defect is most pronounced at the 15 min point after the initiation of cellulosic dialysis in response to S. aureus. In our previous study, we observed similar results using both C5a and F-met-leu-phe as exogenous agonist during dialysis. These results indicate that during the early part of the dialysis procedure, at a time when there is potentially increased exposure to pathogens from dialysis needle placement, dialysis with cellulosic membranes may result in granulocytes being less bactericidal than they are predialysis. These data correlate well with a recent study by Vanholder et al., who prospectively studied granulocyte glycolytic metabolism in the hexose monophosphate shunt in patients dialyzed with either cuprophane or polysulfone dialysis (32). After 2 wk of dialysis therapy, only cuprophane-dialyzed patients had a decline in phagocytosis-induced glycolytic metabolism.

Infection remains a leading cause of morbidity and mortality in hemodialysis patients (33,34). Most of these infections are due to catalase-producing bacteria such as staphylococci and enterobacter, rather than to opportunistic organisms. This acquired defect in immune function closely resembles defects in patients with chronic granulomatus disease, who have a known inability to make ROS in response to infection challenge (35). The decreased intradialytic responsiveness to S. aureus in patients dialyzed with cuprophane membranes demonstrated in this study may contribute to this morbidity.

Although the defective response to S. aureus challenge induced by cuprophane membranes is transient, the repetitive nature of chronic dialysis may exacerbate this defect. Two recent retrospective clinical studies comparing outcomes with high-flux hemodialysis versus hemodialysis with cellulosic membranes have confirmed a difference in infectious morbidity and mortality, suggesting the validity of our hypothesis. Hornberger et al. have recently demonstrated that the hospital admission rates for infection dropped from 0.021 to 0.011 admissions per patient per month when patients were converted from conventional to high-flux hemodialysis (36). Levin et al., in a preliminary report, also confirm an enhanced rate of infection in patients on cuprophane hemodialysis compared with high-flux hemodialysis (37). These clinical studies support the contention that changes in leukocyte function related to biocompatibility may contribute to infectious morbidity and mortality.

In summary, we have used a new method to analyze granulocyte ROS production during hemodialysis in a prospective crossover trial with highly complement-activating and low complement-activating membranes. This method is superior to previous methods because it is specific for ROS production and can be performed in whole blood, thus minimizing artifacts from cell isolation.

We demonstrated that hemodialysis with cellulosic membranes causes increased intracellular production of ROS in granulocytes during the first 30 min of dialysis. Furthermore, the responsiveness of granulocytes to S. aureus is attenuated with cellulosic dialysis membranes during this same period. In contrast, dialysis with PMMA membranes caused a minimal increase in granulocyte ROS production and no change in responsiveness to S. aureus. The repetitive stimulation of granulocytes during cellulosic hemodialysis may contribute both to the high rate of infections in dialysis patients and to morbidity from endothelial injury.

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