Primed Peripheral Polymorphonuclear Leukocyte: A Culprit Underlying Chronic Low-Grade Inflammation and Systemic Oxidative Stress in Chronic Kidney Disease

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This study characterizes the causal relationship between peripheral polymorphonuclear leukocyte (PMNL) priming, systemic oxidative stress (OS), and inflammation in patients with varying degrees of renal insufficiency (chronic kidney disease [CKD] not on renal replacement therapy [RRT]: continuous ambulatory peritoneal dialysis or hemodialysis [HD]) and healthy control subjects. Rate of superoxide release was measured after stimulation of PMNL with phorbol 12-myristate 13-acetate or zymosan. Priming was estimated by the rate of superoxide release after phorbol 12-myristate 13-acetate stimulation. Systemic OS was related to PMNL priming and intracellular myeloperoxidase activity. Inflammation was linked to peripheral white blood cells and PMNL counts, PMNL apoptosis, and PMNL ex vivo survival in autologous and heterologous sera. PMNL priming and counts were related to the severity of renal failure in CKD not on RRT. Compared with control subjects, PMNL from all CKD patients showed increased priming, highest in HD, with a significant decrease in their response to zymosan. PMNL myeloperoxidase activity and apoptosis were increased in all renal failure patients. Decreased ex vivo cell survival and elevated leukocyte counts were found in all patients, highest in HD. Both PMNL priming and counts correlated negatively with the GFR. A positive significant correlation was shown between PMNL counts and their priming in all groups, suggesting that the increased PMNL count in peripheral blood is an adaptive response to PMNL priming. Hence, PMNL priming is a key mediator of low-grade inflammation and OS associated with renal failure, occurring before the onset of RRT and further augmented in chronic HD.

The polymorphonuclear leukocyte (PMNL), one of the main inflammatory cell types, exists in the blood stream in one of three functional states: Quiescent, primed, or activated (1). Under noninfectious conditions, the PMNL are quiescent, exhibiting little or no release of reactive oxygen species (ROS). Studies have led to the concept of a two-stage activation process: PMNL first encounter a stimulus that leaves the cells in a “primed” state. Upon encountering a second stimulus, PMNL proceed to the second state of full activation, releasing ROS, granule contents, and inflammatory mediators (1–3). Ward and McLeish (4–6) reported that PMNL from patients with chronic kidney disease (CKD) both before and while on renal replacement therapy (RRT) are primed. Our studies have also shown that PMNL are in a primed state in both continuous ambulatory peritoneal dialysis (CAPD) and hemodialysis (HD) patients (7,8). In addition to CKD patients, we have shown PMNL priming as a common denominator in other clinical states, such as hypertension, diabetes, and cigarette smoking, that are known to be associated with endothelial dysfunction, accelerated atherosclerosis, and increased prevalence of cardiovascular morbidity and mortality (9–11). In all of these clinical states, it was apparent that primed peripheral PMNL contribute concomitantly to chronic systemic oxidative stress (OS) and inflammatory processes and that PMNL priming was associated with a significant increase in peripheral white blood cells (WBC) and PMNL counts, although still in the upper quadrant of the normal range (7–11). Recently, epidemiologic studies have suggested that elevated WBC and neutrophil counts constitute a mortality predictor in HD patients (12,13) and are a risk factor for developing CKD in U.S. adults (14). We suggest that the elevation in peripheral PMNL counts is a feature of systemic low-grade inflammation derived from PMNL priming. Therefore, we designed a prospective, cross-sectional study aimed to characterize PMNL priming in relation to PMNL counts and the severity of renal failure in CKD patients before RRT was commenced and in patients who are on CAPD and HD treatment.

Materials and Methods

Patients

A total of 120 participants, 90 CKD patients and 30 healthy, normal control subjects (NC), were enrolled in this cross-sectional study after giving informed consent for blood sampling. Patients with evidence of
acute or chronic infection or malignancy or who had received a blood transfusion within 3 mo before blood sampling were excluded. This study was approved by the institutional committee in accordance with the Helsinki declaration.

The patients were divided into three groups: No RRT, CAPD, and HD (Table 1):

1. 30 stage 2 to 5 CKD patients who were not receiving RRT and had estimated GFR ranging between 8 and 73 ml/min per 1.73 m²; GFR was calculated according to the Modification of Diet in Renal Disease (MDRD) formula (15).

2. 30 CAPD patients with mean duration of dialysis treatment of 20 ± 4 mo (range 3 to 55 mo). All patients underwent dialysis with 8 L/d in four exchanges (three isotonic 1.36% and one hypertonic 3.86% glucose solutions).

3. 30 patients who were undergoing HD and had a mean duration of dialysis treatment of 47 ± 5 mo (range 9 to 90 mo). All patients underwent HD thrice weekly; each dialysis treatment lasted 4 h and was carried out with low-flux polysulfone membranes (F8; Fresenius Medical Care, Bad Homburg, Germany) using bicarbonate dialysate with an average single pool Kt/V of 1.2 ± 0.2. The water for dialysis met the standards of the Association for the Advancement of Medical Instrumentation.

**PMNL and Sera Separation**

Blood was drawn in the morning after an overnight fast from all patients and NC for the determination of biochemical and hematologic parameters and for PMNL isolation. Blood from HD patients was always drawn immediately before a dialysis session. PMNL isolation was carried out from a 20-ml heparinized blood sample as described previously (10). The separated PMNL (>98% pure, approximately 10⁷ cells per isolation) were resuspended in PBS that contained 0.1% glucose solutions.

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**PMNL-Mediated OS**

**Rate of Superoxide Release.** The measurements of the rate of superoxide release are based on superoxide dismutase inhibitable reduction of 80 μM cytochrome C (Sigma, St. Louis, MO) to its ferric form (16). The rate of superoxide release was monitored from 10⁶ separated PMNL: (1) under resting conditions, at 22 and 37°C, for up to 90 min, without any stimulant; (2) after stimulation with 0.32 × 10⁻⁹ M phorbol 12-myristate 13-acetate (PMA; Sigma), at 22 and 37°C for 50 min; and (3) after stimulation with zymosan (Sigma) at 37°C as described previously (10). Briefly, zymosan (4 mg; Sigma) was opsonized within 1 wk of the experiment, in 1 ml of pooled human sera of 10 healthy donors for 30 min at 37°C. This solution was washed twice with 154 mM NaCl, resuspended in 154 mM NaCl at a concentration of 10 mg/ml, and stored at −80°C until used. The particle-to-PMNL ratio was 15. PMNL priming was determined by the rate of superoxide released from PMA-stimulated 10⁶ PMNL at 22°C in 10 min.

**PMNL Myeloperoxidase Activity.** The method is a combination of the methods of Suzuki et al. (17) and Bradley et al. (18), measuring myeloperoxidase (MPO) activity after solubilization of cell membrane. Briefly, each frozen pellet of 10⁶ PMNL in 50 mM potassium phosphate (KPH) buffer was thawed and centrifuged at 20,000 × g at 4°C for 20 min, then resuspended in 0.1 M KPH buffer (pH 6) that contained 0.5% hexadecyltrimethyl ammonium bromide and 0.5 M EDTA and homogenized. KPH buffer (80 mM; pH 5.4) that contained 0.5% hexadecyltrimethyl ammonium bromide and 16 mM 3,3,5,5-tetramethyl benzidine was added to this suspension. The reaction was initiated by adding 0.001% H₂O₂ and stopped at 30-s intervals for 90 s by adding 0.01 mg/ml catalase (specific activity 13,600 U/mg solid; Sigma) at 4°C. The absorbance was detected at 650 nm, and the activity was expressed as ΔOD/min.

**PMNL-Derived Inflammation**

**WBC and PMNL Counts.** Counts of WBC and PMNL from blood drawn in EDTA were performed by an automated cell counter (Coulter STKS, Miami, FL) and used as a measure of inflammation.

| Table 1. Clinical and biochemical characteristics of CKD patients and normal control subjects* |
|-----------------|------------------|-------------------|------------------|
|                | NC (n = 30)      | CKD Patients (no RRT) (n = 30) | CAPD Patients (n = 30) | HD Patients (n = 30) |
| Age (yr)       | 44 ± 8.3         | 48 ± 11           | 50 ± 12           | 53 ± 11             |
| Gender (M/F)   | 16/14            | 16/14             | 16/14             | 16/14               |
| Underlying diseases |                |                   |                   |                   |
| hypertensive nephrosclerosis | —               | 10                | 6                 | 8                 |
| diabetic nephropathy       | 10              | 7                | 6                 |                   |
| polycystic kidney         | 2               | 5                | 5                 |                   |
| glomerulonephritis         | 2               | 4                | 1                 |                   |
| other                     | —               | 6                | 8                 | 10                |
| MAP (mmHg)      | 92 ± 12          | 113 ± 14          | 109 ± 7           | 100 ± 19           |
| Serum glucose (mg/dl) | 91.5 ± 10       | 127 ± 13          | 135 ± 57          | 129 ± 38           |
| Serum creatinine (mg/dl) | 0.97 ± 0.03 (0.77 to 1.2) | 4.3 ± 0.4 (2.4 to 8) | 9.0 ± 1.3 (6.1 to 12) | 8.2 ± 0.4 (4.8 to 13.5) |
| GFR (ml/min per 1.73 m²) | 90.5 ± 0.7 (80.7 to 114.6) | 16.7 ± 2.4 (8.0 to 73.0) | —                 | —                 |
| Serum albumin (g/dl) | 4.5 ± 0.05       | 4.0 ± 0.1        | 3.7 ± 0.2         | 3.7 ± 0.06         |
| Serum CRP (mg/dl) | 1.5 ± 0.1        | 11.9 ± 2.9        | 12.9 ± 2.8        | 23.3 ± 4.3         |
| Serum IL-6 (pg/ml) | 2.1 ± 0.4        | 4.7 ± 1.2        | 8.7 ± 1.1         | 7.8 ± 0.9          |
| Serum cholesterol (mg/dl) | 194 ± 30       | 190 ± 48         | 199 ± 84          | 192 ± 46           |
| Serum triglycerides (mg/dl) | 98 ± 60         | 163 ± 90         | 217 ± 43          | 222 ± 80           |

*Values are means ± SEM. NC, normal control subjects; CKD, chronic kidney disease; RRT, renal replacement therapy, CAPD, continuous ambulatory peritoneal dialysis; MAP, mean arterial pressure; CRP, C-reactive protein. P < 0.05; *versus NC; †versus HD patients; ‡versus CAPD patients.
PMNL Survival Ex Vivo. Separated PMNL, from 10 age- and gender-matched patients, from each group (10 CKD individuals, serum creatinine 3.7 ± 1.04 mg/dl, range 2.1 to 5.4; 10 CAPD; 10 HD, and 10 NC), were used at a concentration of 10^6 cell/ml. Duplicate samples of PMNL were incubated with either autologous or heterologous pooled sera (25% vol/vol diluted with Hank’s balanced salt solution) at 37°C and counted before and after 90 min of incubation. Cell viability was confirmed by trypan blue (0.1% wt/vol) exclusion. PMNL survival was expressed as the ratio of cell counts after 90 min of incubation to their counts before incubation (%).

Analysis of Apoptotic PMNL. Apoptosis was analyzed in whole blood from 20 patients and control subjects of each group by flow cytometry according to Kuypers et al. (19). Blood samples were assayed for apoptosis after lysis of red blood cells by Q prep (Beckman Coulter, Fullerton, California) and incubated with FITC-labeled mAb using the Annexin V kit (Bender MedSystems, Vienna, Austria). PMNL were defined by forward scatter/side scatter and by R-phycoerythrin-labeled monoclonal anti-CD16.

Statistical Analyses
Data are expressed as mean ± SEM. Differences in mean values were tested by two-way ANOVA and by the Bonferroni multiple comparison test, using Prism version 3.0 statistical software (GraphPad Software, San Diego, CA). Correlations between different study parameters were performed using Pearson correlation coefficients. P < 0.05 was considered significant.

Results
Study Population
Table 1 summarizes the clinical and biochemical characteristics of the participants. All studied groups of patients showed similar mean values of BP, serum cholesterol, triglycerides, and glucose. Serum creatinine levels were increased as expected. Reduced serum albumin and increased serum CRP and IL-6 were found in all three renal failure groups of patients, as compared with NC, with significantly higher CRP levels in HD.

PMNL-Mediated OS
Rate of Superoxide Release.
Effect of Temperature. The assay was performed at 22 and 37°C with resting or PMA-stimulated, separated PMNL (Figure 1). PMA stimulation caused an elevation in superoxide release in HD and in NC PMNL. After 10 min at 22°C, the reduction of cytochrome C by superoxide released from PMA-stimulated HD PMNL was significantly faster than by NC PMNL. At 37°C, the two cell populations (HD and NC) released superoxide at much faster rates than at 22°C, and the significant difference between these two cell populations was abolished at 10 min. The rate of superoxide release was negligible in the resting state compared with PMA-stimulated cells, for both cell populations and temperatures studied. However, although NS, resting HD compared with resting NC PMNL showed a tendency for higher rates of superoxide release at both temperatures. Altogether, to emphasize the differences between HD and NC PMNL, all further superoxide release experiments were performed at 22°C after PMA stimulation for 10 min (Figure 1).

Effect of Stimulant. Significantly faster rates of superoxide release from PMA-stimulated PMNL were found in the three renal failure groups as compared with NC (Figure 2A), reflecting a higher priming state in all groups versus NC. In PMNL from HD patients, the rate of superoxide release was highest and significantly higher than CKD (Figure 2A). For ruling out
the effect of hypertension or diabetes on the rate of superoxide release, intercomparison among three main subgroups of CKD patients before RRT, according to their underlying diseases (diabetes, hypertension, and others), was performed (n = 10 in each subgroup). No significant differences in the rates of superoxide release from PMNL of these subgroups were observed (data not shown).

In contrast to PMA, challenging the same cell with zymosan showed a significant decrease in the rate of superoxide release in PMNL from each of the three groups of renal failure patients as compared with NC (Figure 2B). No differences in zymosan-stimulated superoxide release among the three renal failure groups (Figure 2B) could be demonstrated.

**PMNL MPO Activity.** MPO activity from PMNL lysates of the three renal failure groups was similar but significantly higher than MPO activity in NC PMNL lysate (Table 2).

**PMNL-Derived Inflammation**

**WBC and PMNL Counts.** CKD, CAPD, and HD patients had significantly higher numbers of WBC and PMNL, as compared with NC (Table 2), although all values fell within the upper quartile of the normal range. WBC from CAPD and HD patients were significantly higher than those from CKD patients. PMNL counts from HD patients were significantly higher than those from CKD patients.

**Percentage of Apoptotic PMNL.** The percentage of apoptotic PMNL, assayed immediately after blood withdrawal in whole blood, was significantly higher in all three groups of renal failure patients as compared with NC (Table 2).

**PMNL Survival Ex Vivo.** PMNL that were isolated from peripheral blood of CKD, CAPD, and HD patients and from NC were incubated in their autologous sera for 90 min and counted by Coulter counter before and after 90 min of incubation. Figure 3 shows that PMNL from CKD, CAPD, and HD patients exhibit a significant lower survival versus PMNL from NC, with the lowest significant survival of PMNL from HD patients. Cross-incubation studies of cells from each group with NC sera were performed to clarify further whether the decreased survival is influenced by humoral factors. NC sera significantly promoted cell survival of PMNL from CKD, CAPD, and HD, with the smallest recovery, although significant, in HD PMNL (Figure 3).

**Relationship between PMNL Priming and Peripheral Counts**

A positive significant correlation was found between the rate of superoxide released by $10^6$ cells from each individual and PMNL counts in all participants ($r = 0.33, P = 0.0002; n = 120$; Figure 4).

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**Table 2.** PMNL-derived oxidative and inflammatory markers of CKD patients and NC.

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>CKD</th>
<th>CAPD</th>
<th>HD</th>
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<tbody>
<tr>
<td>WBC count ($10^9$/L)</td>
<td>6.7 ± 0.2</td>
<td>7.2 ± 0.4$^b$</td>
<td>7.7 ± 0.2$^{c,e}$</td>
<td>8.0 ± 0.4$^{d,f}$</td>
</tr>
<tr>
<td>PMNL count ($10^9$/L)</td>
<td>3.9 ± 0.2</td>
<td>4.3 ± 0.6$^b$</td>
<td>4.6 ± 0.4$^e$</td>
<td>5.6 ± 0.3$^{d,f}$</td>
</tr>
<tr>
<td>MPO activity</td>
<td>0.47 ± 0.03</td>
<td>0.68 ± 0.04$^b$</td>
<td>0.65 ± 0.1$^c$</td>
<td>0.71 ± 0.1$^g$</td>
</tr>
<tr>
<td>(ΔOD 650 nm/min per 10^6 cells)</td>
<td></td>
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<tr>
<td>Apoptosis (%)</td>
<td>7.7 ± 0.4</td>
<td>17.2 ± 5.4$^b$</td>
<td>14.7 ± 0.7$^e$</td>
<td>18.9 ± 0.2$^d$</td>
</tr>
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$^a$Values are means ± SEM. PMNL, polymorphonuclear leukocytes; WBC, white blood cells; MPO, myeloperoxidase.

$^bP < 0.05$, CKD versus NC.

$^cP < 0.05$, CAPD versus NC.

$^dP < 0.002$, HD versus NC.

$^eP < 0.05$, CKD versus CAPD.

$^fP < 0.05$, CKD versus HD.

$^gP < 0.05$, HD versus NC.
PMNL priming in relation to GFR

PMNL priming expressed by the rate of superoxide release in NC and CKD patients not on RRT was negatively correlated with the calculated GFR (by MDRD: \( r = -0.35, P = 0.0089; n = 60 \); Figure 5A); the lower the kidney function, the higher the superoxide release. The peripheral PMNL counts from CKD patients and NC were also negatively correlated with the values of calculated GFR (\( r = -0.55, P < 0.0001; n = 60 \); Figure 5B); the lower the GFR, the higher the number of PMNL.

Systemic inflammatory markers in relation to GFR

Albumin, CRP, and IL-6, the widely accepted systemic inflammation markers, determined in NC and in CKD patients not on RRT were correlated with the calculated GFR (MDRD). Serum CRP levels negatively correlated with GFR (\( r = -0.44, P = 0.0077; n = 25 \); Figure 6A). IL-6 levels negatively correlated with GFR (\( r = -0.46, P = 0.011; n = 32 \); Figure 6A). Albumin levels positively correlated with GFR (\( r = 0.72, P < 0.0001; n = 60 \); Figure 6B).

Systemic inflammatory markers in relation to PMNL priming

No correlation could be found between the rates of superoxide release from separated PMNL and either serum CRP levels \( (r = 0.07, P = 0.68) \) or IL-6 levels \( (r = 0.06, P = 0.71) \). No correlation could be found between peripheral PMNL counts with either serum CRP levels \( (r = 0.0003, P = 1.0) \) or IL-6 levels \( (r = -0.13, P = 0.45) \). However, albumin levels negatively correlated with both PMNL priming parameters: The rates of superoxide release from separated PMNL \( (r = -0.22, P = 0.04) \) and with the peripheral PMNL counts \( (r = -0.26, P = 0.007) \).

Discussion

The results of this study implicate PMNL priming in the cause of systemic OS and low-grade inflammation associated with renal failure. The augmented superoxide release together with the increased intracellular MPO activity are known contributors to systemic OS. The extent of PMNL priming correlates positively with the severity of kidney disease and is intensified by RRT, especially by HD. The notion that the increased rate of superoxide release is related directly to the severity of renal failure in CKD patients who are not on RRT is supported by others (4). The likelihood that hypertension (9) or diabetes (10) per se is a contributor to PMNL priming in this study was ruled out, because no significant differences could be seen among the subgroups of the enrolled CKD patients. Recently, Agarwal (20) reached a similar conclusion, reporting that CKD is associated with OS independent of hypertension.

In our findings, the priming of PMNL from CAPD patients did not differ significantly from CKD patients who were not on RRT, although Tarng and colleagues (21,22) observed increased PMNL priming in CAPD patients. The significantly enhanced priming of HD PMNL versus CKD not on RRT occurred despite...
blood withdrawal before HD session and may reflect accumulating nondialyzed uremic toxins as well as possible accumulating side effects of the extracorporeal treatment (23–25). From this and other studies (6), it seems that uremia per se is a major contributor to PMNL priming: PMNL priming was almost twofold higher in CKD patients who were not on RRT, compared with NC, whereas hemodialysis further increased the priming of PMNL by only 25%. The polysulfone low-flux membrane used in this study is probably not the main cause of PMNL priming. Rao et al. (26) supported this notion, reporting that superoxide release from PMNL is similar for both low- and high-flux polysulfone membranes. Uremic toxins present in the uremic milieu (6,22,27) are well-established factors and may carry leukoclastic activity, as suggested by our ex vivo cross-incubation studies.

The clear distinction among the different studied groups in PMNL priming was achieved by the in vitro use of lower assay temperatures, 22 instead of 37°C. Thus, solely for the purpose of slowing the reactions to emphasize the differences between the groups, we used nonphysiologic temperature, 22°C. Paul et al. (27) showed that the resting rates of superoxide released from both HD and NC PMNL were similar at 37°C. In our study, we have shown for the first time that at physiologic 37°C, the rate of superoxide release from resting HD PMNL is faster than NC, a phenomenon that is supported by the observations at 22°C. These findings suggest that in HD patients, the vascular wall is chronically and continuously exposed to ROS generated from resting PMNL. ROS generated near the vascular wall, when improperly scavenged, may be the cause for endothelial dysfunction found in these patients.

Stimulation of PMNL with different stimulants resulted in different, even opposite, effects: PMA stimulation of PMNL caused a faster release of superoxide, whereas stimulation with zymosan induced a slower release in all three renal failure groups, compared with NC. Zymosan, a physiologic stimulant that differs in mechanism of action compared with PMA, is used to assess the phagocytic potential of PMNL. Although we did not look at phagocytosis directly, the slower rates of superoxide released extracellularly, compared with NC, suggests that the overall response to this ingested particle is reduced in PMNL from renal failure patients. The increased percentage of apoptotic PMNL in the circulation of renal failure patients

Figure 6. C-reactive protein (CRP), IL-6, and albumin levels related to the severity of kidney disease. (A) Correlation between serum CRP or IL-6 levels and calculated GFR of NC and CKD patients not receiving RRT ($n = 32$ and 25, respectively). (B) Correlation between serum albumin levels and calculated GFR of NC and CKD patients not receiving RRT ($n = 60$).
demonstrated in this study, together with the decreased phagocytic-like function, reflects a decline in innate immunity. Altogether, these observations can explain, at least in part, the reported high prevalence of infectious complications, a major cause of morbidity and mortality, in these patients (28,29). It should be emphasized that in this study, the increased apoptosis of PMNL was determined immediately after withdrawal in whole blood. Other studies have also shown increased apoptosis in CKD patients; however, to the best of our knowledge, the determination of apoptosis in the unmanipulated samples is novel and probably reflects best in vivo conditions, because these PMNL were not affected by either separation (30) or ex vivo long incubations (31).

OS, the well-documented observation in CKD patients before and while on RRT (4,6,20–25), can originate from PMNL priming, followed by chronic release of ROS and increased MPO activity (32). This study indicates that the activity of MPO is higher in PMNL that were obtained from all renal failure patients as compared with NC. The increased MPO activity in primed PMNL in this study is similar to the MPO enrichment reported in primed macrophages under inflammatory conditions (33). Augmented intracellular MPO activity, associated with chronic release of ROS and increased degranulation (34), can explain the higher plasma MPO activity reported by Chen et al. (35). Because MPO recently became a predictor of cardiovascular disease (36), the increased MPO activity in PMNL from CKD patients may constitute a link between circulating PMNL and the risk for developing cardiovascular complications in these patients. PMNL priming, a source of chronic superoxide and MPO released near the vascular wall, may initiate and propagate the development of atherosclerosis, a common long-term complication of renal failure.

A new interesting correlation was observed between the rates of superoxide released from $10^6$ cells and peripheral PMNL numbers: The faster the rate, the more PMNL are found in the circulation. This increased PMNL number in the circulation is probably an adaptive response to superoxide chemoattraction (34) resulting in an elevation in peripheral PMNL counts. Hence, we propose that PMNL priming can serve as a new measure of systemic low-grade inflammation, involved in the deterioration of kidney function. This PMNL-mediated low-grade inflammation can explain the epidemiologic studies showing increase in WBC counts as a mortality predictor in HD patients (12,13) and as a predictor for developing CKD (14).

We show that systemic inflammatory markers such as CRP, IL-6, and albumin correlate with GFR. The low-grade inflammation derived from PMNL priming correlates significantly with GFR and albumin but does not correlate with IL-6 and CRP. These interesting findings suggest that different processes are involved in inflammation, which need to be clarified further.

In conclusion, our data suggest that PMNL priming is a key mediator in inducing a vicious cycle of systemic OS and inflammation in CKD patients. The characteristics of the priming agents remain to be elucidated.

Acknowledgments

We thank Prof. J. Kopple for the critical review of the manuscript. The assistance of E. Ron and Dr. M. Furmanov is gratefully acknowledged.

References

15. Vervoort G, Willems HL, Werzels JF: Assessment of glo-


