Kidney International, Vol. 61 (2002), pp. S109–S114

Oxidative stress in hemodialysis patients: Is NADPH oxidase complex the culprit?

MARION MORENA, JEAN-PAUL CRISTOL, LYNNE SENÉCAL, HELENE LERAY-MORAGUES, DETLEF KRIETER, and BERNARD CANAUD

New England Medical Center, Division of Nephrology, Boston, Massachusetts, USA; Biochemistry Laboratory, Department of Nephrology, Renal Research and Training Institute, Lapeyronie University Hospital, Montpellier, France

Oxidative stress in hemodialysis patients: Is NADPH oxidase complex the culprit?

Oxidative stress results from an imbalance between oxidant production, including reactive oxygen species (ROS), reactive nitrogen species (RNS), chlorinated compounds, and antioxidant defense mechanisms. Most reports prove that oxidative stress is present in ESRD patients. Several studies tend to accreditate the hypothesis by which oxidative stress is a strong co-factor for the development of complications related to long-term HD such as atherosclerosis, amyloidosis, malnutrition, anemia, and infection.

In order to evaluate the rationale for curative action against oxidative damage in chronic renal failure patients, we reviewed the putative factors involved in this process. Antioxidant systems are severely impaired in uremic patients and gradually altered with the degree of renal failure. Moreover, the inflammatory state caused by the hemoincompatibility of the dialysis system plays a critical role in the activation of NADPH oxidase, aggravating the pro-oxidant status of uremic patients.

Prevention of ROS overproduction by improvement of dialysis biocompatibility, an important component of adequate dialysis, might be completed by antioxidant supplementation.

To date, oxidative stress, which occurs when there is excessive oxidant production and/or low antioxidant levels, has emerged as a strong pathogenesis co-factor implicated in the development of long-term complications such as atherosclerosis, amyloidosis, malnutrition, anemia, and infection observed in long-term HD patients. There is an increasing body of evidence attesting to the multifactorial origin of oxidative stress. Uremia per se is largely incriminated in its genesis but renal replacement therapy contributes to a large extent to exacerbating and perpetuating this phenomenon.

This article updates our knowledge of oxidative stress in chronic renal failure (CRF) and HD patients. Therapeutic alternatives to restore the oxidative balance and prevent further tissue injury in HD patients will be discussed.

Key words: oxidative stress, hemodialysis, malnutrition, inflammation, atherosclerosis, NADPH oxidase.

OXIDATIVE STRESS: A COMMON FEATURE OF HD PATIENTS OR A MARKER OF CO-MORBID CONDITION?

The imbalance between antioxidant defense mechanisms and overproduction of oxidants including reactive oxygen species (ROS), reactive nitrogen species (RNS), and chlorinated compounds is now well established in HD patients [1, 2]. Several markers of oxidative stress concerning lipid, protein, carbohydrate, and nucleotide metabolism have been identified. Lipid peroxidation products, namely malonyldialdehyde (MDA), 4-hydroxynonenal (HNE) and F2-isoprostanes, enzymatically produced by free radical-catalyzed peroxidation of arachidonoyl lipids, have been consistently reported elevated in HD patients [3, 4]. Advanced glycation products (AGEs) formed from proteins and peptides by non-enzymatic glycation and/or glycoxidation have been identified as a marker of oxidative stress in the anemic population [5]. More recently, Witko-Sarsat et al identified in uremic patients the presence of oxidatively modified proteins, referred to as advanced oxidation protein products (AOPPs) [6], recognized as novel markers of oxidative stress and, more interestingly, as potent mediators of inflammation [7]. To date, 8 hydroxy 2'-deoxyguanosine (8-OH dG) resulting from leukocyte DNA damage has been identified as a surrogate marker of oxidative stress in the HD population [8]. Taken together, these findings lead to the conclusion that oxidative stress is present and may play a major role in CRF and in HD patients.

Recognizing the pathogenic role of oxidative stress, can one speculate that the degree of oxidative stress is the same for all HD patients? In other words, to what extent is oxidative stress a constant feature of HD patients? To elucidate that aspect, we evaluated in a cross-sectionnal study oxidative stress in two cohorts of HD

patients: home HD and center HD. MDA, AOPP, erythrocyte glutathione (erGSH), erythrocyte (er), and plasma (pl) vitamin E (vit E) levels were determined in the predialysis sera. Results clearly indicated significant differences in center versus home HD patients (MDA: 1.83 ± 0.08 vs. 1.60 ± 0.10 mmol/L; AOPP: 90.9 ± 4.4 vs. 66.9 ± 2.9 μmol/L; er GSH: 6.5 ± 0.3 vs. 5.0 ± 0.7 mmol/mg Hb; er vit E: 0.94 ± 0.07 vs. 0.40 ± 0.06 mg/L pellet, and pl vit E: 17.0 ± 1.0 vs. 13.0 ± 1.1 mg/L). Such original findings could in part explain the differences reported in the literature regarding the ex vivo LDL oxidizability, being normal by some studies [9] or increased by others [10, 11] when HD patients were compared with normal subjects. At this stage, one must regard that oxidative stress is not uniformly distributed in the HD population. Moreover, we hypothesized that oxidative stress is a marker of co-morbid state in ESRD patients as well as malnutrition, inflammation, or atherosclerosis [12]. Stenvinkel et al first established a link between nutritional, inflammatory markers and cardiovascular diseases, which they described as "MIA syndrome" [13]. Interestingly, the author found significant differences in terms of inflammation, atherosclerosis, and oxidative stress according to the nutritional state of patients. Malnourished patients had significantly increased intima-media thickness, elevated CRP and fibrinogen levels, and lower plasma vitamin E levels compared with well-nourished patients.

With respect to these results, it can be concluded that presence of pro-oxidant state is not a constant pattern of HD patients but is strongly linked to the co-morbid conditions of patients.

OXIDATIVE STRESS IN HD PATIENTS: A COMPONENT OF THE MICROINFLAMMATION STATE RELATED TO BIOINCOMPATIBILITY?

Overproduction of oxidants in HD patients is facilitated by the uremic state. Uremia-associated metabolic abnormalities are triggered by the bioincompatibility of the dialysis procedure.

It is now well documented that uremia-associated metabolic abnormalities include complex alterations of ROS production [14]. Indeed, Ward et al clearly showed that polymorphonuclear cells (PMNs) are in a "primed" state in patients with CRF [15, 16]. PMN "priming" refers in this case to a process whereby the response of PMNs to a stimulus is amplified by prior exposure to a priming agent [17]. Among functional consequences of neutrophil priming, enhancement of the respiratory burst resulting in a ROS production is being considered as an appropriate response. Full activation of the respiratory burst activity in the neutrophil results from translocation and assembly of the cytosolic components of the NADPH oxidase enzyme system ($p47^{phox}$, $p67^{phox}$, and p21rac) with the membrane-associated flavocytochrome, cytochrome b_{558} (Fig. 1). This translocation is dependent on the co-operative action of the various components involving multiple $p47^{phox}$-directed phosphorylation events [18]. The activated NADPH oxidase complex catalyzes the reduction of molecular oxygen O_2 into superoxide anion $O_2^{\circ-}$ that then rapidly dismutates to form hydrogen peroxide H_2O_2, a reaction catalyzed by superoxide dismutase (SOD). Thereafter, under the action of MPO, an enzyme present in azurophilic granules of leukocytes, H_2O_2 interacts with halides to produce hypochlorous acids; the latter could generate long-lived oxidants such as chloramines. Such reactions triggered by the NADPH oxidase, beneficial for bacterial killing, represents a deleterious source of ROS production in CRF. The mechanisms implied are therefore unidentified, but uremic toxins are suspected to be "provocateurs."

Hemoincompatibility of the dialysis system resulting in chronic inflammation may also play a critical role in neutrophil priming resulting in overproduction of oxidants. HD-related oxidative stress relies on two major components of the dialysis system: One is the dialyzer membrane [19], the other the microbial contamination and/or the pyrogen content of the dialysate. Contribution of the bioimcompatible dialyzer membrane to the production of free oxygen radicals has been shown in acute HD conditions by comparing cellulosic (cuprophane) to synthetic (polysulfone) membranes [20, 21]. More recently, Chen et al have shown that basal blood levels of superoxide anion were higher in chronic HD patients compared with healthy subjects and further increased by HD sessions [16]. Indirect evidences also exist suggesting that trace amounts of endotoxin (LPS) in dialysate are potent triggers of ROS production via the activation of PMNs. DeLeo et al showed that neutrophils harvested from normal human volunteers exposed to bacterial LPS up-regulated NADPH oxidase assembly [22]. We also showed that LPS was able to prime respiratory burst of phagocytes using two in vitro models, THP-1-derived monocytes and whole blood. LPS pretreatment strongly increased $O_2^{\circ-}$ production in THP-1-derived monocytes (24-h incubation) and whole blood in response to phorbol myristate acetate activation (activation percentage: 210 ± 46% and 212 ± 22%, respectively; $P < 0.05$) (Fig. 2). These results suggest that endotoxins and fragments of LPS act as priming mediators capable of amplifying the oxidant production by monocytes. One can conclude that endotoxin-contaminated dialysate synergistically amplify ROS production and cytokine-inducing ability by activating phagocytes [23, 24]. To date, it has been shown that the presence of LPS in the dialysate may activate blood monocyte/macrophage through the dialyzer membrane contributing to IL-1, IL-6 and TNF-α induction [25, 26] and the up-regulation of NADPH oxidase complex [27, 28]. In addition to this cytokine effect

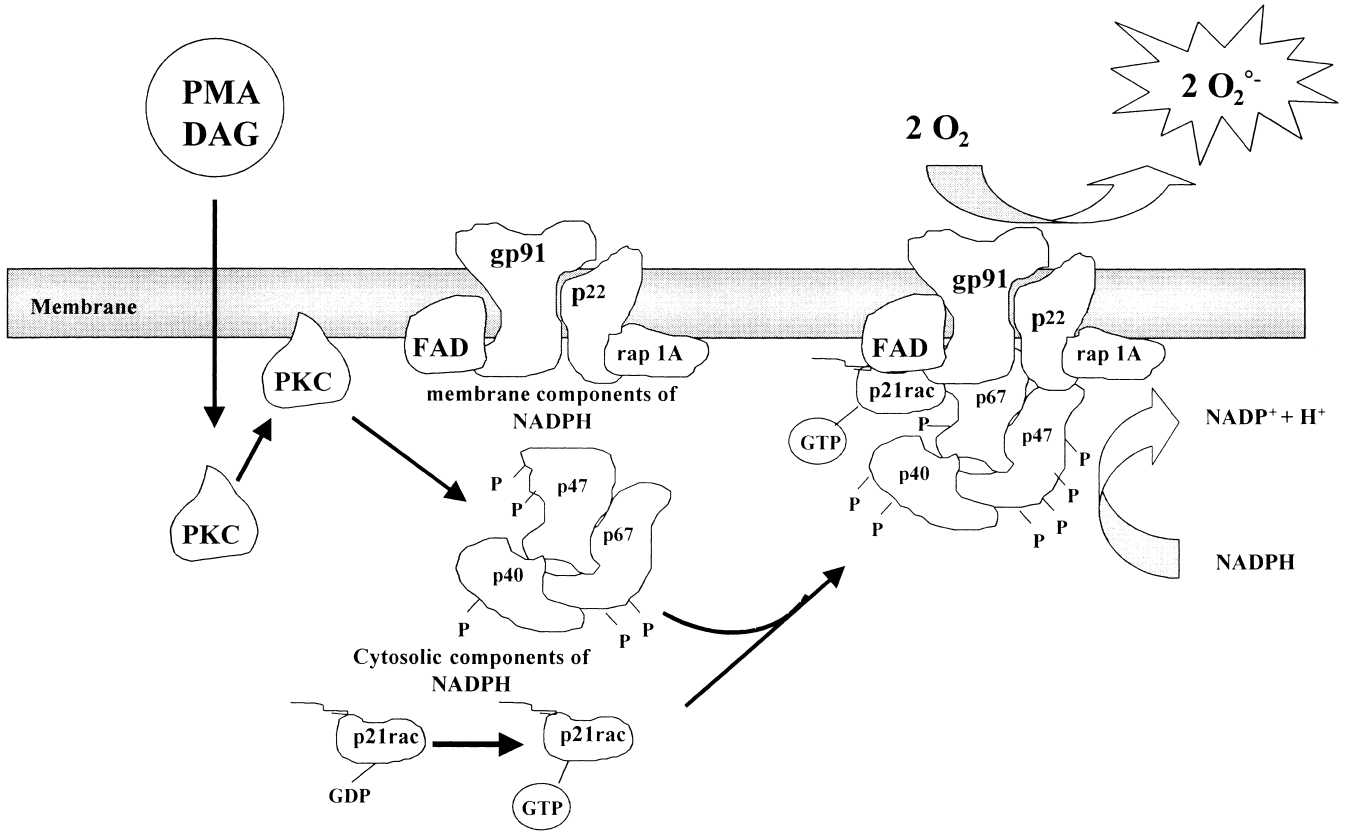

Fig. 1. "Respiratory burst" of phagocytes. Regulation of NADPH oxidase enzymatic complex. Translocation and assembly of the cytosolic components of the NADPH oxidase enzyme system ($p47^{phox}$, $p67^{phox}$, p^{21rac}) with the membrane-associated flavocytochrome, cytochrome, and further multiple $p47^{phox}$ phosphorylation events lead to activated NADPH oxidase which in turn catalyzes the reduction of molecular oxygen O_2 into $O_2^{\circ-}$.

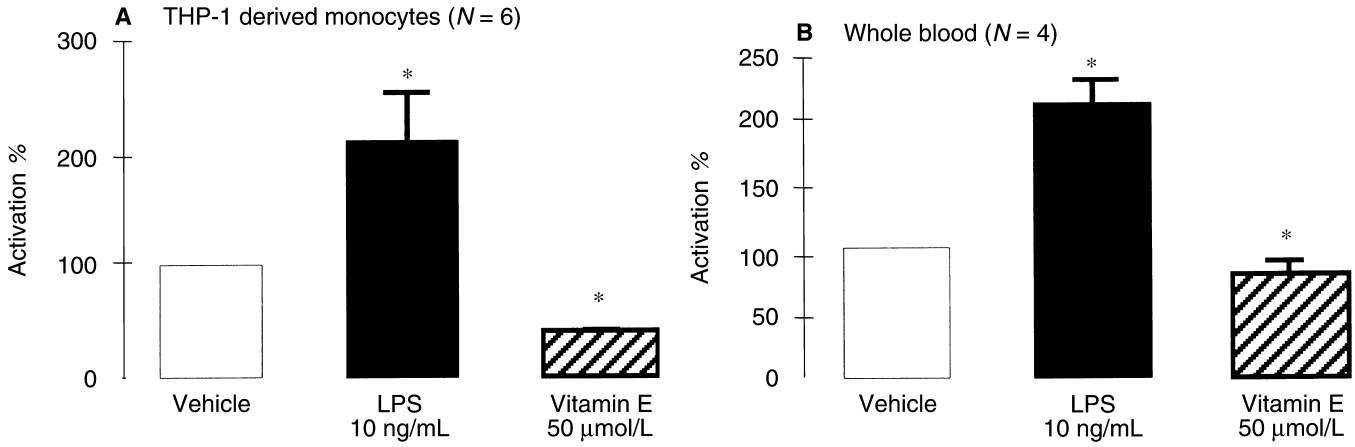

Fig. 2. Effect of LPS *(A)* and vitamin E *(B)* on superoxide production by THP-1-derived monocytes and whole blood. THP-1-derived monocytes or whole blood was incubated alternatively with or without LPS (10 ng/mL) for 24 hours and 90 minutes, respectively, and with or without vitamin E (50 μM) for 30 and 60 minutes, respectively. PMA-induced superoxide production was thereafter measured by chemiluminescence analysis. Values were considered statistically significant at $P < 0.05$ (*). Vehicle (□); LPS (■); vitamin E (▨).

on ROS production, it is important to note that ROS modulates cytokine release via transcription factors including NF-κB and AP-1 [29]. Endotoxin-contaminated dialysate, by contributing to the release of pro-inflammatory cytokines associated with excessive deleterious ROS production, underlies an amplification loop through which NADPH oxidase and transcription factors are of key importance (Fig. 3). Evidence is now provided that

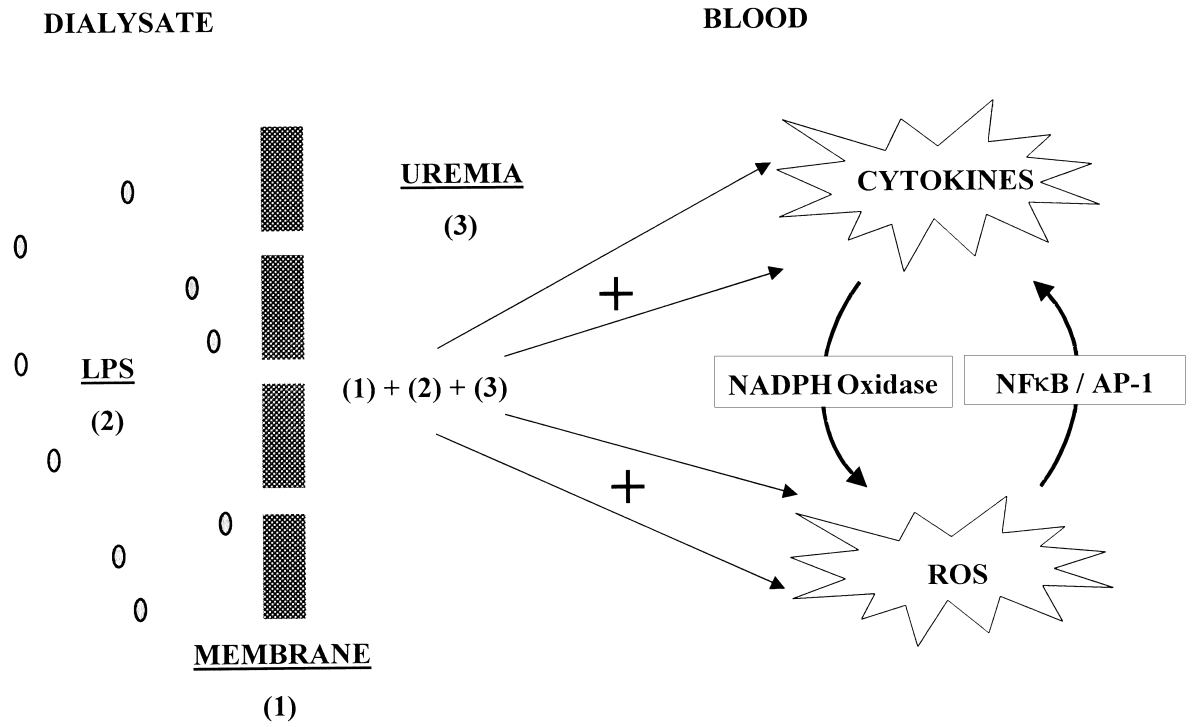

Fig. 3. Possible pathway of an auto-modulatory process responsible for the microinflammatory state present in HD patients.

NADPH oxidase may play an ambivalent role in the inflammatory response: as an "effector" (by ROS release) as well as a "modulator" (in regulating transcription factors) agent of the chronic micro-inflammatory process now well recognized in HD patients [30].

THERAPEUTIC INSIGHTS OF OXIDATIVE STRESS IN HD PATIENTS

Prevention and/or reduction of oxidative stress mechanisms in HD patients should include improving the hemocompatibility of the dialysis system to minimize oxidant generation during HD sessions and to supplement patients with antioxidant compounds to correct nutritionnal deficiencies, and to compensate dialytic losses and consumption from ROS overproduction. These therapeutic insights aimed at preventing long-term complications associated with dialysis could be achieved with either oral or intravenous supplementation or through the extraracorporeal circuit.

Several agents (including selenium) administered intravenously have confirmed to be effective and well tolerated clinically [31]. Interestingly, plasma and erythrocyte selenium content as well as glutathione peroxidase activity tended to return toward normal levels [32]. Beneficial effects of alpha tocopherol (500 mg/day during 6 mo) supplementation [33] included (*1*) oxidative state improvement, (*2*) anemia correction, and (*3*) atherosclerosis prevention. LDL oxidability observed in HD patients has been reduced significantly after oral vitamine E supplementation [34]. More interestingly, a significant reduction in composite cardiovascular disease end points and myocardial infarction has been reported in the SPACE study consisting of vitamin E supplementation in high-risk patients. Such interventions confirm the beneficial effect of antioxidants on cardiovascular mortality [35]. Two new therapeutic options based on the extracorporeal circuit have recently been introduced.One is vitamin E-bound hemodialyzers [36], and the other is hemolipodialysis, which consists of loading HD patients with vitamins E and C via liposomes in the extracorporeal circuit [37]. These two dialytic approaches provide a direct interaction of vitamin E on adherent phagocytes. Of particular interest, this action appears crucial when one considers the scavenging effect of vitamin E on oxidant release by phagocytes observed during in vitro experiments. Indeed, we have evaluated the in vitro effect of alpha tocopherol (50 uM) on $O_2^{\circ-}$ production both in THP-1-derived monocytes (30 min incubation) and whole blood (60 min incubation). Interestingly, we observed that vitamin E had a strong inhibitory effect on $O_2^{\circ-}$ production in both models (activation percentage: $56 \pm 1\%$ and $20.5 \pm 8.3\%$ in THP-1-derived monocytes and whole blood, respectively; $P < 0.05$) (Fig. 2). The explanation for this phenomenon came afterward, when alpha tocopherol was shown to inhibit the assembly of

NADPH oxidase by preventing the phosphorylation and the membrane translocation of $p47^{phox}$ [38]. Alternatively, anti-cytokine therapies using anti-TNF-α antibodies, soluble TNF-α receptors, or IL-1 receptor antagonist are of particular interest to limit oxidative stress, but prospective studies are needed to investigate the beneficial effect of such therapies on cardiovascular and nutritional status in HD patients.

CONCLUSION

Oxidative stress, resulting from an imbalance between pro- and antioxidative mechanisms, is a recognized phenomenon in HD patients that contributes to long-term complications. The question is still raised of why this process is not uniformly distributed in the whole HD population. Nevertheless, it is strongly associated to a degree of co-morbidity as well as malnutrition, inflammation, or atherosclerosis. This pro-oxidative state implies several factors related to the patient's condition, uremia state, and hemodialysis system. When exploring the mechanisms underlying oxidant overproduction in HD, the NADPH oxidase complex emerges as a key target to focus on. Thereby, modulation of this enzymatic complex appears to be a basic requisite to prevent complications in long-term dialysis patients associated with oxidative stress. Inhibition of $p47^{phox}$ phosphorylation by vitamin E constitutes one of the possible regulating pathways. Anti-cytokine therapies also emerge as interesting modalities to reduce ROS overproduction. Finally, it is of interest to speculate on preventive actions by investigating genetic polymorphisms of NADPH oxidase and cytokines.

Reprint requests to Dr. Bernard Canaud, Division of Nephrology, Lapeyronie University Hospital, 371 Avenue Doyen Gaston Giraud, 34295 Montpellier, France.
E-mail: b-canaud@chu-montpellier.fr

REFERENCES

1. Loughrey CM, Young IS, Lightbody JH, *et al*: Oxidative stress in haemodialysis. *Q J Med* 87:679–683, 1994
2. Canaud B, Cristol JP, Morena M, *et al*: Imbalance of oxidants and antioxidants in haemodialysis patients. *Blood Purif* 17:99–106, 1999.
3. Sommerburg O, Grune T, Hampl H, *et al*: Does long-term treatment of renal anaemia with recombinant erythropoietin influence oxidative stress in haemodialysed patients? *Nephrol Dial Transplant* 13:2583–2587, 1998
4. Handelman GJ, Walter MF, Adhikarla R, *et al*: Elevated plasma F2-isoprostanes in patients on long-term hemodialysis. *Kidney Int* 59:1960–1966, 2001
5. Vlassara H: Serum advanced glycosylation end products: A new class of uremic toxins? *Blood Purif* 12:54–59, 1994
6. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, *et al*: Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* 49:1304–1313, 1996
7. Witko-Sarsat V, Friedlander M, Nguyen Khoa T, *et al*: Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol* 161:2524–2532, 1998
8. Tarng DC, Huang TP, Liu TY, *et al*: Effect of vitamin E-bonded membrane on the 8-hydroxy 2′-deoxyguanosine level in leukocyte DNA of hemodialysis patients. *Kidney Int* 58:790–799, 2000
9. Sutherland W, Walker R, Ball M, *et al*: Oxidation of low density lipoproteins from patients with renal failure or renal transplants. *Kidney Int* 48:227–236, 1995
10. Maggi E, Bellazzi R, Falaschi F, *et al*: Enhanced LDL oxidation in uremic patients: An additional mechanism for accelerated atherosclerosis? *Kidney Int* 45:876–883, 1994
11. Morena M, Cristol JP, Dantoine T, *et al*: Protective effects of high-density lipoprotein against oxidative stress are impaired in haemodialysis patients. *Nephrol Dial Transplant* 15:389–395, 2000
12. Stenvinkel P, Heimburger O, Lindholm B, *et al*: Are there two types of malnutrition in chronic renal failure? Evidence for relationships between malnutrition, inflammation and atherosclerosis (MIA syndrome). *Nephrol Dial Transplant* 15:953–960, 2000
13. Stenvinkel P, Heimburger O, Paultre F, *et al*: Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. *Kidney Int* 55:1899–1911, 1999
14. Hasselwander O, Young I: Oxidative stress in chronic renal failure. *Free Radic Res* 29:1–11, 1998
15. Ward R, MCLeish K: Polymorphonuclear leukocyte oxidative burst is enhanced in patients with chronic renal insufficiency. *J Am Soc Nephrol* 5:1697–1702, 1994
16. Chen MF, Chang CL, Liou SY: Increase in resting levels of superoxide anion in the whole blood of uremic patients on chronic hemodialysis. *Blood Purif* 16:290–300, 1998
17. Condliffe AM, Hawkins PT, Stephens LR, *et al*: Priming of human neutrophil superoxide generation by tumour necrosis factor-alpha is signalled by enhanced phosphatidylinositol 3,4,5-trisphosphate but not inositol 1,4,5-trisphosphate accumulation. *FEBS Lett* 439:147–151, 1998.
18. el Benna J, Faust LP, Babior BM: The phosphorylation of the respiratory burst oxidase component p47phox during neutrophil activation. Phosphorylation of sites recognized by protein kinase C and by proline-directed kinases. *J Biol Chem* 269:23431–23436, 1994
19. Ward R, McLeish K: Hemodialysis with cellulose membranes primes the neutrophil oxidative burst. *Artif Organs* 19:801–807, 1995
20. Cristol JP, Canaud B, Rabesandratana H, *et al*: Enhancement of reactive oxygen species production and cell surface markers expression during hemodialysis. *Nephrol Dial Transplant* 9:389–394, 1994
21. Descamps-Latscha B, Goldfarb B, Nguyen A, *et al*: Establishing the relationship between complement activation and stimulation of phagocyte oxidative metabolism in hemodialyzed patients: a randomized prospective study. *Nephron* 59:279–285, 1991
22. DeLeo F, Renee J, Cormick SM, *et al*: Neutrophils exposed to bacterial lipopolysaccharide upregulate NADPH oxidase assembly. *J Clin Invest* 101:455–463, 1998
23. Dinarello C, Lonnemann G, Bingel M, *et al*: Biological consequences of monocyte activation during hemodialysis. *Contrib Nephrol* 59:1–9, 1987
24. Balakrishnans V, Jaber B, Natov S, *et al*: Interleukin-1 receptor antagonist synthesis by peripheral blood mononuclear cells in hemodialysis patients. *Kidney Int* 54:2106–2112, 1998
25. Knudsen P, Leon J, Ng A, *et al*: Hemodialysis-related induction of beta-2microglobulin and interleukin-1 synthesis and release by mononuclear phagocytes. *Nephron* 53:188–193, 1989
26. Kumano K, Yokota S, Nanbu M, Sakai T: Do cytokine-inducing substances penetrate through dialysis membranes and stimulate monocytes? *Kidney Int* 43(Suppl 41):S205–S208, 1993
27. Niwa Y, Ozaki Y, Kanoh T, *et al*: Role of cytokines, tyrosine kinase, and protein kinase C on production of superoxide and induction of scavenging enzymes in human leukocytes. *Clin Immunol Immunopathol* 79:303–313, 1996
28. Serfilippi G, Ferro T, Johnson A: Activation of protein kinase C mediates altered pulmonary vasoreactivity induced by tumor necrosis factor-alpha. *Am J Physiol* 267:L282–L290, 1994
29. Lander H: An essential role for free radicals and derived species in signal transduction. *FASEB J* 11:118–124, 1997
30. Lonnemann G: Chronic Inflammation in hemodialysis: The role of contaminated dialysate. *Blood Purif* 18:214–223, 2000
31. Koenig J, Fischer M, Bulant E, *et al*: Antioxidant status in patients

on chronic hemodialysis therapy: Impact of parenteral selenium supplementation. *Wien Klin Wochenschr* 109:13–19, 1997
32. Temple KA, Smith AM, Cockram DB: Selenate-supplemented nutritional formula increases plasma selenium in hemodialysis patients. *J Ren Nutr* 10:16–23, 2000
33. Cristol JP, Bosc JY, Badiou S, *et al*: Erythropoietin and oxidative stress in haemodialysis: Beneficial effects of vitamin E supplementation. *Nephrol Dial Transplant* 12:2312–2317, 1997
34. Islam KN, O'Byrne D, Devaraj S, *et al*: Alpha-tocopherol supplementation decreases the oxidative susceptibility of LDL in renal failure patients on dialysis therapy. *Atherosclerosis* 150:217–224, 2000
35. Boaz M, Smetana S, Weinstein T, *et al*: Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. *Lancet* 356:1213–1218, 2000
36. Buoncristiani U, Galli F, Rovidati S, *et al*: Oxidative damage during hemodialysis using a vitamin-E-modified dialysis membrane: A preliminary characterization. *Nephron* 77:57–61, 1997
37. Wratten ML, Navino C, Tetta C, Verzetti G: Haemolipodialysis. *Blood Purif* 17:127–133, 1999
38. Cachia O, Benna J, Pedruzzi E, *et al*: Alpha tocopherol inhibits the respiratory burst in human monocytes: Attenuation of p47phox membrane translocation and phosphorylation. *J Biol Chem* 273: 32801–32805, 1998