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Forum Review

Evolution of Oxidative Stress and Inflammation During Hemodialysis and Their Contribution to Cardiovascular Disease

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ABSTRACT

End-stage renal disease patients have increased cardiovascular morbidity and mortality. These patients have many unique risk factors, such as an accumulation of uremic toxins, electrolyte imbalances, metabolic disturbances, anemia, chronic inflammation, and thrombogenic disturbances. Oxidative stress has been implicated in many of these disturbances. This review will focus on some of the factors that may accelerate cardiovascular disease in uremic patients, with an emphasis on mechanisms and interactions of various components of oxidative stress and inflammation. Understanding the mechanisms of these pathways may be useful in developing effective prevention and treatment strategies. *Antioxid. Redox Signal.* 4, 935–944.

INTRODUCTION

End-Stage Renal Disease (ESRD) patients have a high incidence of cardiovascular morbidity and mortality, with cardiovascular disease contributing to ~45% of the overall mortality (4, 43, 47, 57, 65, 85). Although uremic patients have many risk factors associated with cardiovascular disease, they also have unique metabolic, inflammatory, and immune dysregulations that probably contribute as well. This has provoked a strong interest in determining the underlying mechanisms that contribute to cardiovascular mortality, with the aim of better identifying high-risk patients and the development of preventive strategies and treatments.

Traditional risk factors for cardiovascular disease are fairly common among chronic renal failure patients. These include hypertension, a high frequency of diabetes, hypertriglyceridemia, hyperhomocysteinemia, old age, and low physical activity. The presence of these risk factors, however, does not explain the high cardiovascular mortality in younger patients and patients without many of the traditional high-risk factors.

Uremic patients also have an accumulation of several hundred uremic toxins, electrolyte imbalances, fluid overload, as well as higher concentrations of cytokines, inflammatory mediators, acute-phase proteins, and thrombogenic factors. In addition, ESRD patients regularly undergo substitutive therapies, such as hemodialysis, and take pharmacologic agents aimed at correcting anemia and various metabolic imbalances. These therapies have the potential for diminishing some aspects of the inflammatory process by removing excess toxins, or correcting various metabolic disorders, but they may also provoke inflammatory reactions by virtue of the bioincompatibility of extracorporeal circuit components and introduction of inflammatory stimulators, such as endotoxin from the dialysate.

The question arises as to whether uremia plays a role in accelerated atherosclerosis and, perhaps more importantly, whether specific therapies can positively or negatively influence outcome. Chronic inflammation has recently emerged as one of the common links between many disorders associated with uremia. It is linked with malnutrition, immune suppression, coagulation disorders, anemia, and various secondary diseases, such as amyloidosis and accelerated atherosclerosis. Oxidative stress appears to be a key component of many reactions associated with chronic inflammation in uremic patients, as well as atherosclerosis in general (Fig. 1). Theoretically, if it were possible to significantly diminish oxidative

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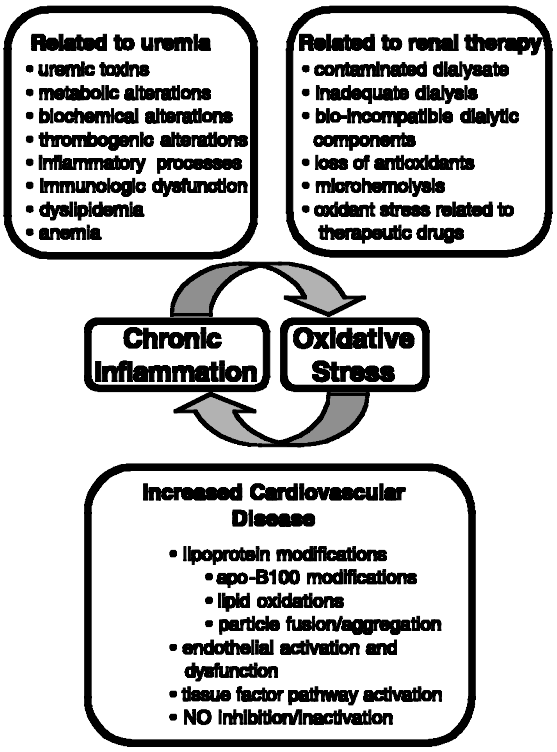

FIG. 1. Various factors that may play a role in chronic inflammation and oxidative stress in hemodialysis patients. ESRD patients have both intrinsic factors related to the loss of kidney function and external factors that are related to the renal replacement therapy. Both of these can lead to increased cardiovascular disease. Preventive strategies can be aimed at restoring normal physiologic function and reducing dialytic/pharmacologic complications.

stress and the subsequent inflammatory response, it should be possible to correct many of the pathologies associated with chronic renal disease that lead to cardiovascular disease.

Inflammation is a complex process that is both an epiphenomenon and an integral process in the evolution of ESRD and uremia. Multiple oxidative processes play a critical role in inflammation and act on various intra- and extracellular pathways. Although it may be possible under certain circumstances to have a "systemic rampage" of free radicals and diminished antioxidant defenses, a more likely scenario includes specific mediators in conjunction with free radicals that amplify inflammatory reactions at specific sites.

The localized aspect and activation of specific cellular pathways often make evaluation of free radical-mediated damage difficult to assess. This is particularly true in complex disease processes, such as atherosclerosis, where many of the cellular and biochemical changes occur in obscure microenvironments. This review will focus on some of factors that may be important in accelerated cardiovascular disease in uremic patients, with an emphasis on mechanisms and interactions of various components of the complex inflammatory process and oxidative stress. Understanding how these pathways operate and integrate is a central challenge to evaluating new prevention and treatment strategies.

DYSLIPIDEMIA

ESRD patients show a secondary form of complex dyslipidemia consisting of both quantitative and qualitative abnormalities in serum lipoproteins (38, 63, 77). These are thought to result from alterations in lipoprotein metabolism and composition. The prominent features of uremic dyslipidemia are an increase in serum triglyceride levels due to elevated very-low-density lipoprotein (VLDL) remnants and intermediate-density lipoprotein and low high-density lipoprotein (HDL) cholesterol. Low-density lipoprotein (LDL) cholesterol is often normal, but the aberrant deposition of cholesterol may originate from the atherogenic small and dense LDL subclass (64).

Release of lipoprotein lipase from extrahepatic vascular surfaces during heparin administration may also be an important factor in the change in triglyceride metabolism in uremic patients. Repeated administration of heparin can lead to a lipoprotein lipase depletion, leading to decreased VLDL metabolism. Nasstrom *et al.* (53) observed the kinetics of lipoprotein lipase after heparin injection in 10 healthy control subjects. They found that, after an initial peak, the activity dropped by almost 80% of its initial value, then leveled to a plateau of only ~15% of the initial activity. Administration of a second bolus of heparin increased lipoprotein lipase activity; however, enzyme activity remained at only 35% of its initial peak level (53).

Administration of heparin to hemodialysis patients may also modify many of the properties of LDL. Aggregation and fusion of LDL are thought to be important in the early events of atherogenesis. Recent evidence suggests that the glycosaminoglycan (GAG) chains of extracellular proteoglycans bind to both LDL and phospholipase A2 (PLA2). Both heparin-treated and heparin-bound lipolyzed LDL increase the tendency for the LDL particles to fuse (25).

Oxidation of LDL could be one of the key features in the development of atherosclerosis, and several authors have reported increased levels of oxidized LDL (ox-LDL) in uremic patients (5, 32, 86). These modifications occur at the level of the apolipoprotein B (apoB)-100, surface phospholipids, or lipids within the core region. Ox-LDL has a plethora of components that are not present in native LDL, and their presence and quantity depend on the nature, type, and extent of oxidation. Once modified, ox-LDL can impose an array of changes in cytokine and growth factor expression and/or release by a wide variety of cell types (10), and increase endothelial cell activation/dysfunction, affecting the production of important mediators, such as nitric oxide (NO). Although it is relatively easy to modify LDL *in vitro*, there is still a great debate over which modifications actually occur *in vivo* and where these modifications take place.

LDL are the main carriers of cholesterol and make up a heterogeneous class of particles that can vary in size, composition, and structure. They have a single copy of apoB-100. The surface monolayer of nonmodified LDL is made up of approximately 700 phospholipid molecules, whereas the core contains about 1,600 cholesterol ester and 170 triglyceride molecules. In addition, LDL particles contain about 600 molecules of unesterified cholesterol that is located mostly on the particle surface. In addition to approximately six molecules

of α-tocopherol (vitamin E), LDL also contains γ-tocopherol, carotenoids, oxycarotenoids, and ubiquinone (30).

The subset of small dense LDL, also known as LDLIII, represents a potential risk factor for cardiovascular disease (17), possibly because this subpopulation is particularly susceptible to oxidation (69). This subclass accumulates preferentially in hypertriglyceridemic diabetic patients with nephropathy or on hemodialysis (77).

Both plasma triglyceride levels and hepatic lipase activity are independent predictors of LDLIII concentration (17). These particles contain less phospholipids and unesterified cholesterol than large LDL, and the apoB-100 appears to occupy a more extensive area at its surface (66). It has been suggested by Hurt-Camejo *et al.* that human type IIa secretory phospholipase (sPLA2) may be important in the formation of LDLIII (34). Circulating levels of sPLA2-IIA appear to be an independent risk factor for coronary artery disease and a predictor of cardiovascular events. Circulating sPLA2 is therefore considered to be an acute-phase reactant (16, 39) whose levels can be modified during hemodialysis (67). The presence of sPLA2 greatly enhances the accumulation of hydro(pero)-xides of all major classes of LDL's lipids. Hydroperoxides of free fatty acids accumulate exclusively as enzymic products with kinetics reflecting both the formation of free fatty acids and the initial "build-up" of α-tocopheroxyl radical. In contrast, hydroperoxides of cholesteryl esters and phosphatidylcholine accumulate linearly over comparatively longer periods (54). As incubation with PLA2 increases the propensity of LDLIII to associate with proteoglycans, it has been hypothesized that the build-up of hydroperoxides causes the apoB-100 to undergo conformational changes leading to increased exposure of proteoglycan binding regions. This may encourage entrapment in the intima extracellular matrix, leading to an increased susceptibility of oxidation (17, 34, 39).

An oxidatively modified plasma LDL, referred to as LDL−, is found largely among the dense LDL fractions and has been isolated from plasma of hemodialysis patients (86, 87). LDL− and dense LDL particles contain greater amounts of lipid peroxides compared with total LDL or the more buoyant LDL fractions. This LDL species is characterized by its greater electronegativity and is one of the few modified forms of LDL isolated from patient plasma. The content of LDL− in dense LDL particles is positively associated with copper- or heme-induced oxidative susceptibility, and may be attributable to peroxide levels (69). Oxidation with hypochlorous acid (HOCl) also produces LDL− and a more negative population of particles, LDL(2−). Strong oxidation leads to the preferential formation of LDL(2−), whereas brief treatments with low HOCl concentrations yield primarily LDL− (20). Similar modification of LDL to LDL− takes place following incubation with hemoglobin and hydrogen peroxide (H_2O_2). In this case, the production of lipid peroxides is greater than with HOCl, but there is considerable oxidation of apoB-100 with pronounced oxidation of sulfur-containing and aromatic amino acids such as tyrosine (87). Tyrosine residues are converted to hydroxytyrosines and bityrosine, and the latter appear to mediate intramolecular covalent bonds in apoB-100 and intermolecular bonding with hemoglobin moieties (87). The LDL− produced by these reactions contains hemoglobin and has prooxidant properties similar to other hemoproteins, rendering LDL to a rapid propagation of lipid peroxidation after addition of metal catalysts.

Evaluation of the oxidative susceptibility of LDL from a variety of subjects has shown that, beyond a threshold LDL− content of ~2%, there is a significant increase in the oxidative susceptibility of normal LDL (nLDL) particles (*i.e.,* purified LDL that is free of LDL−), and this susceptibility becomes more pronounced as the LDL− content increases (69). Thus, regardless of the mechanism(s) by which LDL− is produced in humans, a common characteristic is the remarkable susceptibility to undergo oxidation and stimulate oxidation in nLDL particles.

LDL PROTEIN MODIFICATIONS

ApoB-100 is one of the largest monomeric proteins and originates from the VLDL particle. In uremic individuals, the apoB-containing portion of the lipoprotein may undergo several modifications [enzymatic and advanced glycation end-product (AGE)-peptide modification, oxidation, glycosylation, or carbamylation] (21), as well as depletion of triglycerides, that change its conformation and binding characteristics.

It should be noted that not all oxidized or modified LDL behave in a similar manner. Much of the literature regarding LDL oxidation and modifications reports on effects obtained from *in vitro* modifications. This is often done with strong oxidizing conditions, such as copper-induced oxidation, or under conditions that promote aggregation or fusion. A recent study by Parasassi *et al.* showed that, although there is a generalized unfolding during *in vitro* copper oxidation of LDL, the apoB-100 remains at the hydrophilic surface of the LDL particle (56). This contrasts with the modified LDL isolated from plasma in which the apoB-100 sinks into the LDL lipid core. Similar changes have been described for apoB-100 conformation and localization after treatment with PLA2 (30) and underscore the concept that the physical properties of ox-LDL can differ based on the type or nature of oxidative modification.

Numerous hydrolytic enzymes, such as lysosomal proteases, matrix metalloproteinases, and prooxidative agents, such as myeloperoxidase and 15-lipoxygenase, are present in the arterial intima. Treatment of LDL particles with either proteolytic, lipolytic, or oxidative enzymes will eventually induce aggregation and fusion of the particles. Treatment with proteases (such as plasmin, kallikrein, and thrombin) generates fragmentation of apoB-100 and fusion of LDL particles. Fusion requires not only fragmentation of apoB-100, but also loss of fragments (25).

Recent studies have identified reactive nitrogen species generated by monocytes as a novel mechanism for converting LDL into particles (NO_2-LDL) that are rapidly incorporated by macrophages. The reaction is proposed to be catalyzed by the myeloperoxidase–hydrogen peroxide–nitrite ($MPO–H_2O_2–NO_2^-$) system (60). The scavenger receptor CD36 appears to be at least one of the major receptors responsible for high-affinity and saturable cellular recognition of NO_2-LDL by murine and human macrophages (61). This mechanism for modification highlights the importance of NO production and its conversion to reactive nitrogen

species by vascular cells and indicates that LDL modification *in vivo* may be mediated by the generation of reactive oxygen and reactive nitrogen species. The presence of hemoprotein catalysts that mediate conversion of NO or nitrite to reactive nitrogen species represents an important component for localized inflammation and oxidative stress. While LDL particles undergo a vicious cycle of accumulation and modification, reverse cholesterol transport is also impaired due to low lecithin:cholesterol acyltransferase and paraoxonase activity. Uremic patients with dsylipidemia also have HDL with structural alterations that may impair hepatic cholesterol clearance. The composition of HDL may also be altered during states of inflammation (64).

ENDOTHELIAL CELL ACTIVATION AND DYSFUNCTION

In addition to disorders related to dyslipidemia, endothelial cell function is profoundly altered in uremic individuals. Some of these changes are invariably due to responses to modified or oxidized lipoproteins, but there are many other important factors. These include shear stress, cell production of inflammatory mediators and cytokines, biochemical and enzymatic changes that promote inflammation and proliferation, as well as inhibitors and compounds that interfere with NO (Fig. 2). A crucial role of the healthy endothelium is to produce a finely tuned balance of appropriate anticlotting, antiadhesive, and vasodilatory mediators. The endothelium contributes to the prevention of atherosclerosis in medium to large arteries by inhibiting platelet activation and maintaining a nonproliferative and biochemically quiescent intima. It is a dynamic organ that can respond to a wide array of agonists and environmental challenges that range from specific cytokines to physical forces such as shear stress (11).

Pober described endothelial activation as occurring in two distinct stages (59). The first stage occurs in seconds and involves endothelial cell stimulation. It does not require *de novo* protein synthesis of gene regulation. Endothelial cells in this stage can be observed to retract from other endothelial cells, express P-selectin, and release von Willebrand factor. The second stage involves gene transcription and protein synthesis. Cells in this stage express E-selectin, intracellular cell adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) and release interleukin-6 (IL-6), as well as other proinflammatory cytokines. Activation can be distinguished from endothelial dysfunction or injury in that it occurs earlier and participates directly in the inflammatory process. This type of activation is associated with gene expression, protein synthesis, and cell proliferation, whereas endothelial dysfunction generally involves long-term or cumulative effects that produce apoptosis and endothelial denudation. This suggests that there may be a continuous process by which irregularities in endothelial cell organization are often found overlying early fatty streaks, whereas overt endothelial denudation is seen only in the late stages of the disease.

Although some of the early literature reported that ox-LDL had primarily a negative effect on cell function (cell growth–growth arrest, injury, and toxicity), recent studies suggest that sublethal levels of ox-LDL cause some, but not all, cells to proliferate. Smooth muscle cells and monocyte-macrophages proliferate after exposure to ox-LDL; whereas there is conflicting literature regarding endothelial cells (10, 27, 29). The growth-promoting and activating constituents are largely thought to be due to lysophosphatidylcholine (lysoPC) and other bioactive lipid products. These components can induce expression and release of growth factors, as well as apoptotic factors (10), to which various vascular cells respond differently. Whether endothelial cells are injured may depend on the type of stimulus and the milieu of factors present during the stimulus. For instance, tumor necrosis factor usually acts as an activator of endothelial cells; however, in the presence of lipopolysaccharide with tumor necrosis factor, endothelial cells are lethally injured (59). The activation of the transcription factor, nuclear factor-κB (NFκB), appears to be a key element in endothelial cell response. Incubation of endothelial cells with ox-LDL has been reported to both activate (12) and inhibit NF-κB activity (27). The activation was observed after binding of ox-LDL to the ox-LDL receptor, LOX-1, and the activation was attributed to increased super-

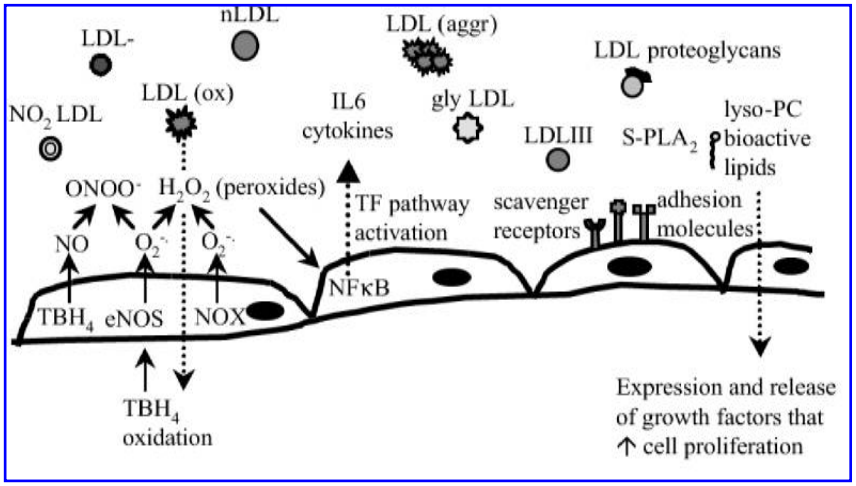

FIG. 2. Modified LDL and endothelial cells undergo complex interactions generating the release of cytokines, inflammatory mediators, growth factors, and reactive oxygen species. These in turn can further modify native LDL. See Abbreviations for definitions of terms.

oxide production. Cominacini *et al.* found that the effect could be attenuated by an anti-LOX-1 monoclonal antibody (13). In contrast, Heermeier *et al.* observed that ox-LDL and lysoPC dose-dependently inhibited NF-κB activity and induced apoptosis. They found that ox-LDL sensitized endothelial cells to apoptotic trigger by interfering with the induction of A20, an antiapoptotic gene regulated by NF-κB (27).

Some of the effects on endothelial cell proliferation and activation appear to be moderated by oxidative pathways. For instance, Heinloth *et al.* found that ox-LDL stimulates NADPH oxidase and induces proliferation (29). The proliferation could be reversed by the addition of superoxide dismutase or catalase. There is also a strong correlation between NADPH activity, atherosclerotic risk factors, and endothelial dysfunction. Both reduced endothelial vasorelaxation and increased NADPH oxidase activity are associated with increased clinical risk factors for atherosclerosis (24). Identification of various NADPH oxidase homologues (referred to as NOX) that are cell-specific and generate superoxide and H_2O_2 at different rates and at specific sites (3) suggests that subtle regulation of oxidative pathways may regulate cell growth and specific cell functions.

Tissue factor (TF) pathway activity is also implicated in increased atherosclerosis risk (58). TF is a procoagulant membrane-associated protein that is a pivotal regulator of blood coagulation. Penn *et al.* also recently observed that ox-LDL, but not native LDL exposure, increased surface TF pathway activity, whereas antioxidants were inhibitory (58). The authors suggested that oxidative stress activates the TF pathway by means of preexisting TF protein on the cell surface that, before oxidant stress, could not participate in an active complex formation. They proposed that TF exists in a latent form on the cell surface that can be activated by oxidized lipids (58). A recent study by Serradell *et al.* observed increased expression of TF antigen on endothelial cells exposed to uremic medium (68). These cells showed abnormal cell morphology and signs of accelerated growth, increased cell detachment, and platelet deposition. Incubation of the extracellular matrix with an antibody to human TF prevented the increase in platelet deposition, suggesting that the presence of TF in the extracellular matrix could be responsible for the enhanced platelet deposition (68).

Interestingly, hemodialysis patients also have increased levels of a TF pathway inhibitor (45, 46). The inhibitor works by neutralizing the catalytic activity of factor Xa and by feedback inhibition of the factor VIIa–TF complex in the presence of factor X (45). The levels of TF pathway inhibitor increase two to three times during hemodialysis and correlate with postdialysis heparin concentrations. Whether this catalytic activity is modulated by ox-LDL or abrogated by antioxidants has yet to be demonstrated.

Other circulating inhibitors of endothelial function, and in particular inhibitors of nitric oxide synthase (NOS), have also been implicated in the pathogenesis of vascular disease in chronic renal failure (50). Although hemodialysis can reduce the concentration of circulating inhibitors and increase flow-mediated dilation (15), concentrations of various NOS inhibitors are often increased in uremic patients. Asymmetric dimethylarginine is one such inhibitor that is increased, but other inhibitors have also been proposed to be important in endothelial derived relaxing factor (EDRF) inhibition (9, 40, 50). Ox-LDL induces a concentration-dependent inhibition of EDRF activity that directly impairs authentic NO-induced stimulation of cyclic GMP accumulation in detector cells. This appears to be related to the lipid component as extracted lipids from ox-LDL block NO-stimulated cyclic GMP accumulation to about the same extent as intact ox-LDL, whereas the protein component of ox-LDL does not inhibit the cyclic GMP response (9, 40).

Although NO synthesis can be modified by the cell interaction with oxidized proteins or lipids, it also appears to be sensitive to compounds such as urea. Two recent studies showed that urea can inhibit inducible NOS (48, 62). Moeslinger *et al.* found that concentrations of urea typically found in uremic patients increased proliferation and suppressed apoptosis (48). This may lead to an accumulation of macrophages and an enhancement of NF-κB-dependent expression of VCAM-1, macrophage-colony stimulating factor (MCSF), and monocyte chemoattractant protein-1 expression.

Cominacini *et al.* proposed a mechanism by which ox-LDL can reduce NO production by the LOX receptor (13). LOX binding produces superoxide, which in turn inactivates NO (13). This may operate as part of a modulating system for induction of NO synthesis by reactive oxygen species, where it has been recently shown that physiological concentrations of H_2O_2 stimulate Akt/phosphatidylinositol 3-kinase-mediated phosphorylation and acute activation of endothelial NOS (eNOS) (7). Under conditions where a large flux of superoxide is induced (as in the case of NADPH oxidase activation), eNOS may become uncoupled and thereby inactivated in terms of NO production, generating superoxide as an alternate product (36). Production of H_2O_2 by eNOS can be further aggravated in hemodialysis patients who often have low intracellular L-arginine concentrations or defects in transport (83) and can predispose NOS to generate superoxide by uncoupled O_2 reduction at its catalytic heme site (72). Moreover, superoxide anion ($O_2^{\cdot -}$) can interact directly with NO in diffusion-controlled rates, producing peroxynitrite ($ONOO^-$) (35). In this way, increased rates of $O_2^{\cdot -}$ production may divert NO from its physiological action toward pathological effects mediated by $ONOO^-$. Indeed, increased levels of proteins containing nitrosylated tyrosine residues—potential markers of $ONOO^-$ action—have been detected in the plasma of hemodialysis patients (Galaris, unpublished results).

Another interesting mechanism for the role of ox-LDL in NOS inhibition was proposed by Uittenbogaard *et al.* (75). These authors suggested that ox-LDL depletes the caveolae of cholesterol and causes the displacement of eNOS. This is thought to be mediated by class B scavenger receptors because they cofractionate and precipitate with caveolin-1. Blocking of the CD36 receptor prevents the redistribution of eNOS. This could also be prevented by HDL, which may maintain the cholesterol concentration of the caveolae (75).

Scavenger receptors are a group of diverse receptors that have an affinity for a broad array of ligands (73). They have been proposed to be important in the development of atherosclerosis as some scavenger receptors recognize modified forms of LDL and can mediate an excessive uptake of cholesterol and lipids leading to foam cell formation. These receptors are also important in processes such as adhesion, differen-

tiation, host defense (aiding clearance of gram-negative and gram-positive bacteria), and phagocytosis of damaged cells.

Uremic serum can dose-dependently enhance scavenger receptor activity, primarily by increasing the amount of receptor protein (2). Ando *et al.* found that although ultrafiltrate from uremic serum had no effect on scavenger receptor activity, heat-inactivated uremic serum had a stimulatory effect in an *in vitro* study using U937 cells. This was primarily attributed to MCSF because pretreatment of uremic serum with a neutralizing antibody to MCSF abolished the effect of uremic serum on scavenger receptor activity. It should also be noted that serum levels of MCSF are up to fourfold higher in uremic patients. The expression of scavenger receptor type I has been reported to be up-regulated in monocytes allowed to differentiate into macrophages isolated from hemodialysis patients (1).

PREVENTION AND TREATMENT STRATEGIES

Hemodialysis patients have many biochemical, immune, and inflammatory alterations that can lead to an increased risk for cardiovascular disease. The two major factors affecting these disorders are (a) metabolic, biochemical, immune, or inflammatory alterations due to the uremic syndrome *per se* and (b) alterations due to the therapeutic treatments of uremia.

Intrinsic factors related to uremia can be considered as components that both aggravate and amplify the inflammatory response, as well as compensatory factors that are produced to minimize inflammation. Many hemodialysis patients have high levels of inflammatory mediators or cytokines, but the biological activity of these mediators is low due to the simultaneous presence of inhibitors. The treatment of uremia, by renal replacement therapies and pharmacologic interventions, can both add to the complications of chronic inflammation and play a role in reducing factors that lead to chronic inflammation. Thus, some therapies may be able to effectively reduce some of the aberrant intrinsic factors related to uremia, whereas others may contribute to their formation.

Vast improvements have been made in renal replacement therapies. Modern dialytic techniques are increasingly oriented toward "global biocompatibility." This includes the use of nonactivating dialytic membranes, ultrapure water to avoid blood contamination by endotoxin or cytokine-inducing substances, products that are free of releasable contaminants, and the use of state-of-the-art machines that aid in correct dialytic delivery. However, there is still much debate over treatment approaches that will ultimately influence treatment outcomes. For instance: Which of the hundreds of uremic toxins are most important in affecting patient outcome? Is it better to use a higher permeable membrane to remove uremic toxins with the possible loss of serum albumin? How "clean" does the dialysate really need to be? What dialytic factors modify the acute-phase response? Do "biocompatible membranes" really improve treatment outcome? In addition, as technology advances, it becomes increasingly easier to improve dialytic treatments; however, on a practical level, factors such as limited economic and medical resources continue to be driving forces in treatment choices. One of the great challenges in developing new dialytic strategies is to prevent or correct the tendency to develop long-term chronic conditions, such as amyloidosis or cardiovascular disease. Oxidant stress appears to be an important factor in many long-term dialytic complications. Many authors have reported increased oxidant stress and an overall decrease in many important antioxidants. Uremic patients also have increased amounts of damaged or modified proteins (31, 78, 81, 82), as well as increased levels of several markers of oxidant stress (26, 55, 74). The question remains, however, as to whether reducing inflammation or oxidant stress is sufficient to reverse the pathological processes leading to cardiovascular disease in renal failure patients.

Several large clinical trials, such as the CHAOS, CARET, or the GISSI prevention trial, were unsuccessful in demonstrating that supplementation with antioxidants could reduce cardiovascular disease or atherosclerosis prevention. This may have been due to several reasons, including lack of bioavailability at specific locations where oxidative stress occurs. Additionally, many oxidative reactions occur at a localized cellular level, such as the cytoplasm, nucleus, or interstitial space. Once free radicals are produced, they may initiate an amplified inflammatory response at localized sites of inflammation, causing further production of other inflammatory mediators. Supplementation with oral antioxidants usually has only a moderate effect on plasma antioxidant levels, and in most cases, the antioxidant status of well nourished humans is adequate and antioxidant supplementation is unlikely to provide a significant increase in oxidant defenses. However, there may be populations with compromised antioxidant activities or levels as indicated by the SPACE trial, which showed a protective effect for vitamin E in hemodialysis patients (6).

Prevention of chronic microinflammation in renal replacement patients must incorporate a globalized strategy to reduce as many factors affecting inflammation as possible. These include a thorough evaluation of treatment modalities, material choices (dialyzers, lines), diet, avoidance of bacterial contaminants from the dialysate, adjustment of vitamin deficiencies, anemia correction, correct fluid balance, and efficient elimination of uremic toxins. Chronic inflammation in these patients has a complex multifactorial base that must be taken into consideration.

Hemodialysis patients today have a wide choice of materials and treatment modalities. A recent trend has been toward higher permeable dialyzers, treatments incorporating increased convection, and the use of adsorbents (8, 18, 19, 41, 42, 70, 76, 79). The rationale behind these treatments has been to better remove "larger uremic toxins" such as AGE-modified proteins, β2-microglobulin, oxidized proteins, factor D, and other toxins that are often not adequately removed by conventional hemodialysis. Although there is some concern that "beneficial molecules" may be lost during high-efficiency treatments, other factors need also to be taken into consideration. Albumin is a molecule that is of particular concern because many hemodialysis patients have low serum albumin levels and hypoalbuminemia is associated with increased morbidity and mortality (37, 49, 71). Its loss is of understandable concern because 60–70% of hemodialysis patients have low serum albumin levels; however, as it is a negative acute-phase protein, the increased removal of other

proinflammatory toxins may actually be of greater benefit to reducing the acute-phase response than the loss of a small percentage of albumin. It should also be noted that circulating albumin in hemodialysis patients may be compromised by the loss of its antioxidant properties (due to oxidation of its sulfhydryl group) or by saturation with uremic toxins. In this case, small losses of albumin may actually turn out to be beneficial in stimulating new protein synthesis.

The advent of new renal replacement treatments, such as on-line hemodialfiltration and the use of higher permeable membranes, also underscores the need for ultrapure dialysate. Endotoxin and cytokine-inducing substances can increase the acute-phase response, activate inflammatory cells, and create a generalized microinflammatory state (44). In addition, endotoxin can also activate coagulation by increasing monocyte TF expression and may possibly play a prothrombotic role in atherosclerosis (52).

Treatments with antioxidant supplements or renal replacement therapies that incorporate antioxidants can be another way of preventing oxidant stress. Many authors have reported antioxidant deficiencies in hemodialysis patients; including lower levels of vitamin C (28, 33), decreased serum scavenging activity against the hydroxyl radical (51), and reduced superoxide dismutase and glutathione peroxidase activity (84). Although vitamin E levels have been reported to be normal in hemodialysis patients, supplementation has been shown to reduce lipid peroxidation, as well as have other beneficial effects (14, 23). Vitamin C supplementation has also been suggested to benefit hemodialysis patients, particularly considering its low predialysis levels and diffusive loss during hemodialysis (28, 33).

As the hemodialysis procedure *per se* can be one of the risk factors associated with cell activation and microinflammation (due to bacterial contamination, bioincompatibility reactions), as well as loss of water-soluble antioxidants, two dialytic procedures have been developed to protect against oxidant stress. The first method, hemolipodialysis, uses vitamin E-loaded polyunsaturated liposomes plus ascorbate added to the dialysate (80, 81). The liposomes, due to their amphophilic nature, are able to remove various hydrophobic inflammatory mediators, such as platelet activating factor, and reduce oxidant stress due to the synergistic action of vitamin C and vitamin E. The second method incorporates vitamin E grafted onto a cellulose membrane. This technique shows increased antioxidant protection, as well as good biocompatiblity (22).

Although many of the new treatments and techniques appear promising, real reduction of cardiovascular morbidity and mortality is still a distant goal. Much more basic science is needed to better define mechanisms involved in the pathogenesis of the precocious development of cardiovascular disease in renal failure patients.

ABBREVIATIONS

AGE, advanced glycation end-product; apoB, apolipoprotein B; EDRF, endothelial derived relaxing factor; eNOS, endothelial nitric oxide synthase; ESRD, end-stage renal disease; gly LDL, glycated LDL; HDL, high-density lipoprotein; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; IL-6, interleukin-6; LDL, low-density lipoprotein; LDL−, oxidatively modified plasma LDL with greater electronegativity; LDL (aggr), aggregated LDL; LDLIII, subset of small dense LDL; LOX-1, oxidized LDL receptor; lysoPC, lysophosphatidylcholine; MCSF, macrophage-colony stimulating factor; NF-κB, nuclear factor-κB; nLDL, normal LDL; NO, nitric oxide; NO_2-LDL, LDL modified by reactive nitrogen species; NOS, nitric oxide synthase; NOX, NADPH oxidase homologues; $O_2^{\cdot -}$, superoxide anion; $ONOO^-$, peroxynitrite; ox-LDL, oxidized low-density lipoprotein; PLA2, phospholipase A2; sPLA2-IIA/sPLA2, human type IIa secretory phospholipase; TF, tissue factor; VCAM-1, vascular cell adhesion molecule-1; VLDL, very-low-density lipoprotein.

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