The Microinflammatory State in Uremia: Causes and Potential Consequences

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Abstract. Mortality is markedly elevated in patients with end-stage renal disease. The leading cause of death is cardiovascular disease. Lipoprotein levels are only slightly elevated in dialysis patients, and cardiovascular risk is inversely correlated with serum cholesterol, suggesting that a process other than hyperlipidemia plays a role in the incidence of cardiovascular disease. Hypoalbuminemia, ascribed to malnutrition, has been one of the most powerful risk factors that predict all-cause and cardiovascular mortality in dialysis patients. The presence of inflammation, as evidenced by increased levels of specific cytokines (interleukin-6 and tumor necrosis factor α) or acute-phase proteins (C-reactive protein and serum amyloid A), however, has been found to be associated with vascular disease in the general population as well as in dialysis patients. The process of inflammation, also called the acute-phase response, additionally causes loss of muscle mass and changes in plasma composition—decreases in serum albumin, prealbumin, and transferrin levels, also associated with malnutrition. Inflammation alters lipoprotein structure and function as well as endothelial structure and function to favor atherogenesis and increases the concentration of atherogenic proteins in serum, such as fibrinogen and lipoprotein (a). Inflammation in dialysis patients is episodic. The causes are likely to be multifactorial and include vascular access infection, less-than-sterile dialysate, dialysate back leak, and nonbiocompatible membranes in addition to clinically apparent infection. In addition, proinflammatory compounds, such as advanced glycation end products, accumulate in renal failure, and defense mechanisms against oxidative injury are reduced, contributing to inflammation and to its effect on the vascular endothelium.

Morbidity and mortality rates remain quite high for patients with end-stage renal disease (ESRD) (1). The major cause of death is cardiovascular disease (1). This is somewhat surprising when it is considered that lipid abnormalities are not severe in this population, being primarily characterized by a slight increase in serum triglyceride levels, a reduction in HDL levels, and a small increase in LDL levels (2). Although it clearly presents some risk, this pattern should not be associated with the massive increase in risk that is observed. This increase is even more remarkable when it is noted that mortality rates are inversely associated with cholesterol levels (3).

A number of risk factors that provide a rationale for the remarkable prevalence of vascular disease in the dialysis patient population were recently identified. Among these, inflammation has been identified as an epidemiologically important risk factor for cardiovascular disease in the general population (4). Inflammation strongly predicts all-cause (5,6) and cardiovascular (6) mortality rates among the dialysis patient population. Additionally, levels of homocysteine, a factor that is known to promote atherogenesis in patients with normal renal function (7), are markedly increased among patients with renal failure (8). Oxidative stress (9) and postsynthetic modification of proteins, leading to accumulation of advanced glycation end products (AGE) (10), are additional risk factors that put patients with renal failure at increased risk of vascular injury and may play a role in the initiation of the inflammatory response.

It has been widely accepted by the nephrology community that malnutrition, as defined by reduced levels of serum albumin (1,3) or prealbumin (11) or by anthropometric markers of malnutrition, predicts poor outcomes. The relationship between malnutrition and vascular disease was initially obscure. It is now recognized, however, that inflammation is responsible for serologic or anthropometric evidence of malnutrition in this population (12). The biochemical basis for the apparent malnutrition among patients with inflammation is the fact that most of the serum proteins whose concentrations are decreased in response to protein malnutrition, i.e., prealbumin (13), transferrin (14), retinol-binding protein (15), and albumin, are negative acute-phase proteins (16). Their synthetic rates are decreased in response to inflammation, making their use as unambiguous markers of nutritional status problematic. Indeed, serum albumin concentrations vary inversely with those of positive acute-phase proteins, such as C-reactive protein (CRP), serum amyloid A (SAA) (17), and fibrinogen (18), and those of cytokines that modulate the synthesis of all of these proteins (19).
Malnutrition in Renal Disease

As the GFR decreases below approximately 20 ml/min, signs of nutritional deterioration develop (20). Serum albumin and transferrin concentrations and the arm muscle circumference decrease (20). One component of these decreases is likely to be the spontaneous reduction in dietary calorie intake that occurs; however, this reduction cannot explain why this same malnourished subgroup of the predialysis population exhibits significantly increased levels of the positive acute-phase proteins CRP and fibrinogen, nor can it explain the relationship between nutritional parameters (either Subjective Global Assessment scores or serum albumin concentrations) and the levels of CRP in this population (12). Of interest, the malnourished patient population described by Stenvinkel et al. (12) also exhibited evidence of vascular disease, suggesting either that inflammation causes vascular disease in this population or that vascular disease is itself responsible for the inflamed/malnourished state. Unfortunately, no markers of inflammation were measured for the Modification of Diet in Renal Disease Study patient population (20). Therefore, two independent processes, i.e., reduced protein and calorie intake and (unmeasured) inflammation, may well have been operative, each contributing separately to nutritional outcomes, as described by Stenvinkel et al. (12) for the pre-ESRD patient population.

The same relationships between nutritional markers and inflammation remain when renal function deteriorates, necessitating dialysis therapy. At that point, however, levels of cytokines (19) and acute-phase proteins increase, as does the fraction of the population that exhibits evidence of inflammation (21).

Causes of Inflammation

Inflammation in dialysis patients may be related to processes associated with renal failure itself, may be a consequence of the treatment for renal failure (dialysis-related), or may be unrelated to either renal failure or the dialysis process specifically. Because 15% of the deaths among patients with ESRD are attributable to infectious causes, these must be strongly considered, especially because they may be amenable to direct therapy.

Causes of Inflammation Related to Renal Failure

Postsynthetic Modification of Proteins. Renal failure itself may possibly contribute to inflammation as a result of the accumulation of proinflammatory compounds or products of metabolism. AGE such as pentosidine are normally associated with diabetes mellitus (22), and levels of advanced lipoxidation end products are also increased in the plasma of patients with renal failure, irrespective of their blood glucose levels. Levels of these substances are increased in diabetes mellitus, generally as a consequence of prolonged hyperglycemia. They result from the reaction of glucose and other carbohydrates with protein, specifically with lysine, via nonenzymatic glycation and oxidation (glycoxidation) of proteins. In diabetes mellitus, these substances are thought to play a role in the progression of renal disease (23).

The kidney normally plays an important role in the metabolism of AGE, which are processed by glomerular filtration followed by tubular uptake and metabolism (24). Because most of the pentosidine in blood is bound to high-molecular weight proteins such as albumin, dialysis has little effect on AGE concentrations (25). AGE may play a role in activating mononuclear cells, thus directly inciting an inflammatory response (24). Inflammation, however, can also play a role in the production of AGE (26). Although AGE and related compounds clearly can modify proteins, including the vascular endothelium (27) and lipoproteins (28), and thus could play a significant role in the development of vascular disease in patients with renal failure, it is not clear why the increased concentrations of these compounds that are observed for all dialysis patients would lead to activation of the acute-phase response or to hypoalbuminemia in only a fraction of dialysis patients.

Although oxidative stress and other postsynthetic modifications of proteins that occur in parallel with decreasing GFR may well explain a component of the inflammation observed for dialysis patients, they do not explain the large skews in the frequencies of increased levels of interleukin-6 (IL-6) (10) or acute-phase proteins (17,19) that were observed among the dialysis patient population (Figure 1A) or the wide temporal variability that we observed (Figure 1B) (29). These observations are more consistent with an episodic process either driving inflammation for this population as a whole or superimposed on a low level of inflammation.

Oxidative injury and protein glycation have also been suggested to play a role, although neither of these processes has been demonstrated to directly increase either acute-phase protein levels or cytokine levels in dialysis patients. However, levels of advanced oxidation products increase as GFR decreases (30) and are closely related to levels of the AGE pentosidine (30). There is a correlation between these products and cytokines, specifically IL-1 receptor antagonist, tumor necrosis factor α (TNF-α), TNF soluble receptor 55, and TNF soluble receptor 75, and these products are associated with evidence of monocyte activation (30), providing at least a theoretical link between advancing loss of renal function and the onset of an inflammatory response.

Oxidative Stress. Renal failure reduces plasma antioxidant activity (31). The increase in oxidative stress is identified by an increase in malondialdehyde levels in red blood cell membranes and a reduction in levels of the reduced forms of glutathione. The loss of antioxidants (32), zinc, selenium, and vitamins C and E (33) occurs during renal failure or as a consequence of dialysis and may contribute to susceptibility to oxidative injury. Plasma glutathione peroxidase activity is also reduced in renal failure (34). Superoxide dismutase and glutathione peroxidase activities are decreased in plasma and in erythrocytes. Increased oxidative stress occurs even before dialysis is instituted. After initiation of dialysis, the opportunity for oxidative injury of circulating blood elements is also present (35). Dialysis does not reduce oxidative injury and may actually exacerbate oxidative stress (36). Increased oxidative stress leads to lipid peroxidation (37) and oxidative alteration of lipoproteins. Cell membranes are also subject to oxidative

Causes of Inflammation Related to Renal Failure

Postsynthetic Modification of Proteins.
The majority of dialysis patients exhibit no evidence of activation of the inflammatory response, despite the fact that all patients are exposed to dialysis membranes and dialysates. The highly skewed distributions of CRP and SAA (Figure 1A) predicted serum IL-6 levels in their patients. Activation of cytokines occurred after dialysis using cellulose membranes (47), in contrast to biocompatible membranes (48).

Other investigators have suggested that even dialysis with biocompatible membranes may pose risks for activation of the acute-phase response. Honkanen et al. (41) observed very rapid and similar increases in the levels of IL-1β and the acute-phase protein SAA during 240 min of dialysis with cellulose, cellulose acetate, or polymethylmethacrylate membranes; the latter two types are biocompatible membranes. Cytokine production during in vitro dialysis of whole blood has been demonstrated (49), suggesting that interactions of circulating nuclear cells directly stimulate cytokine production. Dialysis also alters mononuclear cells so that they respond more vigorously to subsequent exposure to endotoxin (50). Therefore, dialysis with a variety of membranes is a potential source of inflammation, although most other investigators, including our group, failed to find convincing evidence of activation of the acute-phase response during dialysis with membranes other than cellulose membranes.

Reuse techniques and the number of times of reuse also may contribute to the interaction of blood with the dialyzer (51), leading to changes in protein loss and possibly changes in the acute-phase response. Pyrogenic reactions in the absence of septicemia are closely associated with reuse (52).

Dialysate Quality. The quality of water used to prepare the dialysate may also contribute to inflammation (53). Mounting evidence suggests that the use of less-than-sterile dialysate or back-leakage of lipopolysaccharide through the dialysis membranes can cause dialysis-related inflammation (54). Several groups recently prepared ultrapure, endotoxin-free water by membrane filtration of the dialysate and observed reduced levels of cytokines (55), which suggests either that monocytes may be activated by endotoxin that remains on the dialysate side of the membrane or that endotoxin can directly cross the dialysis membrane. This latter route may be of increased importance with the use of highly permeable membranes (56).

Neither the exposure of blood to dialysis membranes nor the use of less-than-ultrapure water in the dialysate can explain the inflammation variability observed within the dialysis population (Figure 1A). Furthermore, the acute-phase response also plays an important role in establishing albumin levels in patients undergoing continuous ambulatory peritoneal dialysis (57), a population exposed to neither lipopolysaccharide-containing dialysates nor dialysis membranes. Indeed, in our own patient population, CRP and SAA levels were significantly greater among patients undergoing peritoneal dialysis than among patients undergoing hemodialysis (57). Peritoneal dialysis may, however, carry its own special risk of exposure to mediators of inflammation. Exposure of the peritoneal membrane to plasticizers found in dialysates may be one source of inflammation among this population. Another potential source is transperitoneal access.

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Inflammatory response in these patients (29). The variance in CRP levels was 2 orders of magnitude greater than that for albumin or transferrin (29). Therefore, factors other than dialysis, which are specific to individual patients, might also be responsible for initiating the inflammatory response. It is unclear what specific process is responsible for activation of the acute-phase response in dialysis patients; however, the process does not occur at all times in all dialysis patients.

**Infectious Causes of Inflammation**

Infections occur commonly among hemodialysis patients. This population is at increased risk of infection as a consequence of impaired humoral and cellular immunity (59) and vascular access, as discussed below.

In a longitudinal cohort study of the incidence of and risk factors for hospitalized cases of septicemia among diabetic and nondiabetic hemodialysis patients, using baseline data from the United States Renal Data System, it was reported that, during a 7-yr period, 11.1% of nondiabetic patients and 12.5% of diabetic patients experienced at least one episode of septicemia (60). Risk factors that predicted septicemia were low serum albumin levels and advancing age.

Clinical infections from actively infected grafts, as well as old clotted grafts that do not exhibit signs of active inflammation (61), are also infectious sources of inflammation and may play a very significant role in individual cases. The detection of graft infections may require $^{111}$In white blood cell scans (61) or other procedures.

The prevalence of tuberculosis is increased among dialysis patients (62). Dialysis patients are also subject to other sources of infection, especially because of the prevalence of diabetes mellitus among this population. Indeed, diabetes mellitus was found to be a risk factor for inflammation in a cross-sectional study of 128 hemodialysis patients (58). Diabetes mellitus poses the risk of peripheral vascular disease and neuropathy, which are both risk factors for foot infections. Peritonitis presents an additional risk of infection for peritoneal dialysis patients (63). It was recently hypothesized that vascular disease itself may be infectious in origin (64). This is reviewed below.

**Inflammation as a Cause of Malnutrition**

Unfortunately, malnutrition has been defined in the renal literature on the basis of nutritional outcomes, specifically the levels of plasma proteins such as albumin, prealbumin, and retinol-binding protein, or anthropometric or functional measures of nutritional status, rather than measures of nutritional intake. Because inflammation causes the same changes in concentrations of the same proteins as does malnutrition (inadequate intake) and also increases muscle protein catabolism (65), the literature has become confused by the consideration of inflammation and malnutrition together. When healthy volunteers were subjected to semistarvation (1500 kcal/24 h) for 2 wk, serum albumin levels decreased only moderately (from 4.28 to 3.86 g/dl), despite marked reductions in body weight (23% reduction, from 69.3 to 53.6 kg) and muscle mass (66). This suggests that, when serum albumin concentrations are reduced to very low levels, processes in addition to malnutrition, such as inflammation and direct loss into the urine or dialysate, contribute to the very low albumin concentrations. It is difficult to decrease serum albumin concentrations to <3.0 g/dl in the absence of both inflammation and malnutrition.

**Inflammation as a Cause of Anemia**

The presence of inflammation contributes to anemia (67) and causes erythropoietin resistance (68) and thus may complicate the diagnosis of iron deficiency. Ferritin synthesis and transferrin receptor synthesis are reciprocally regulated by intracellular iron. Ferritin levels are thus elevated in iron-replete individuals. Ferritin secretion is also increased directly by cytokines (69). Therefore, ferritin levels are increased in the presence of inflammation (70). Serum iron levels are reduced, as is transferrin saturation, further obscuring the diagnosis of iron deficiency.

**Relationship between Inflammation and Vascular Injury**

Possibly the most significant process that is correlated with inflammation is vascular disease. The relationship between these two processes is complex. Endothelial injury involving attachment to and subsequent invasion of the endothelium by macrophages plays an important role in the development of atherosclerosis. Atherosclerotic burden is therefore one potential cause of inflammation. It has been proposed that infections with *Chlamydia pneumoniae* or *Helicobacter pylori* may cause both inflammation and atherosclerosis (64). This connection has not yet been verified and, even if it later is, there is no clear connection between this infectious process and the accelerated atherosclerosis in renal failure. It is more likely that inflammation causes both the anthropometric and serologic changes...
that are attributed to malnutrition in dialysis patients and cause the vascular disease as well.

It was recently established, in large cross-sectional studies, that even small increases in the levels of either cytokines or acute-phase proteins predicted vascular disease among populations of otherwise healthy-appearing adults (4). This observation was based on the assumption, although it was not explicitly stated, that CRP and IL-6 levels are characteristic for specific patients, as LDL levels are. The finding that even small increases in the levels of these markers of inflammation predicted future vascular events generated the term “microinflammatory state.” Markers of inflammation have the same predictive values for dialysis patients (5,6); however, for such patients we know that inflammation is not characteristic for each patient but instead varies with time (29). Elevations in the levels of acute-phase proteins are not subtle, and the incidence of vascular disease is greatly increased. The relationship between inflammation and vascular disease in this population is strong enough to obscure other well known risk factors. Although total cholesterol (and LDL cholesterol) levels are powerful predictors of vascular disease in the general population, the inverse is true for dialysis patients (3).

The relationship between the variability of the acute-phase response and vascular disease in the dialysis patient population remains unknown. Several possibilities present themselves. One is that even a short period of inflammation may result in future vascular events. Inflammation clearly alters the plasma protein composition and both endothelial and lipoprotein structure and function to favor vascular injury, and the time between inflammation and vascular occlusive disease may not be short. After vascular injury occurs, it may be self-propagating, and the inflammatory cells (monocytes) involved in vascular injury may contribute to a chronic increase in the levels of acute-phase proteins and cytokines. We observed, however, that both cytokine and acute-phase protein levels decreased to baseline values after episodes of acute inflammation (29).

There are several ways in which inflammation can promote vascular injury, i.e., through alterations in lipoprotein structure and function, changes in the composition of plasma proteins, alterations in the vascular endothelium, and changes in the expression of specific ligands on the surfaces of platelets, neutrophils, and mononuclear cells.

Several acute-phase proteins are directly associated with vascular disease. One of these is fibrinogen (71). Fibrinogen levels are increased in the subset of patients with inflammation/malnutrition (12), and the levels of this protein, like those of CRP and other acute-phase proteins, vary with time among dialysis patients. Therefore, a specific episode of inflammation not only promotes alterations in the vascular adhesion of mononuclear cells but also increases the concentration of this coagulation factor in plasma. Levels of lipoprotein(a) are also increased among patients with ESRD and are correlated with both vascular injury (carotid artery intimal thickening) and inflammation/malnutrition (12), suggesting that this protein may also be regulated as part of the acute-phase response. Although the concentrations of lipoprotein(a) are generally genetically controlled, the levels of this lipoprotein increase in patients with renal failure (72), in conjunction with increased levels of acute-phase proteins; this increase is in turn associated with increased mortality rates for the hemodialysis patient population (73). This lipoprotein is highly atherogenic when its levels are genotypically increased. The increase in the dialysis patient population may be a component of the acute-phase response and may also contribute to vascular injury.

Apolipoprotein A-I is a negative acute-phase protein, and its synthetic rate is decreased during inflammation (74). SAA displaces apolipoprotein A-I from HDL and alters HDL structure and function (Figure 2), causing HDL to become adherent to the vascular endothelial surface (75). Paraoxonase activity is reduced, and HDL is then no longer capable of reducing oxidized LDL. Furthermore, HDL that has been thus altered acts as a chemoattractant to macrophages, serving as a bridge between them and the endothelial surface (Figure 2) (76).

Inflammation also alters the expression of soluble vascular cell adhesion molecule-1 (77) and soluble intercellular adhesion molecule-1, i.e., adhesion molecules that promote monocyte binding to endothelial cells and are associated with vascular disease (78).

P-selectin levels are increased during acute inflammation, increasing the adherence of platelets to the endothelium. P-selectin (platelets) and E-selectin (endothelial cells) are surface glycoproteins that mediate leukocyte rolling on the endothelial surface, a step that immediately precedes adhesion. P-selectin is able to initiate the cascade of events that increases cell adherence and leukocyte infiltration into injured tissues, by first promoting leukocyte rolling along the vascular endothelium (79). P-selectin levels are increased by hemodynamic stress (80) and are regulated by TNF-α during inflammation (81). Therefore, inflammation also alters the expression of endothelial adhesion molecules that favor the attachment of mononuclear cells and neutrophils, which then initiate oxidative burst activity and further the inflammatory response, contributing to increased oxidative modification of lipids and proteins. Patients with renal failure have a decreased antioxidant reserve, as described above, and thus are less likely than others to be able to prevent this process from becoming self-perpetuating.

**Effects of Inflammation and Oxidation of Lipoproteins**

During inflammation, SAA is incorporated into HDL and displaces apolipoprotein A-I. The triglyceride content of HDL is thus increased (Figure 2). Both paraoxonase and platelet-activating factor-acetyl hydrolase are thought to play a role in preventing the peroxidation of other lipoproteins, including LDL. The activities of both paraoxonase and platelet-activating factor-acetyl hydrolase are reduced. Paraoxonase activity remains suppressed long after levels of the acute-phase proteins have returned to normal after a single episode of minor surgical stress (82). Paraoxonase plays an important role in preventing the oxidation of HDL. Oxidative modification of LDL has been implicated in atherogenesis (83). This interaction between acute inflammation and lipoprotein structure (specifically HDL structure) provides one link between accelerated vascular disease and inflammation among patients with ESRD.
tion itself can directly yield lipoprotein oxidation, in addition to removing potential defense mechanisms against lipoprotein oxidation.

The leukocyte enzyme myeloperoxidase oxidatively modifies LDL via the oxidative nitration of tyrosine and other modifications of the tyrosine residue (Figure 2) (84). Both ascorbate and probucol inhibit the myeloperoxidase-catalyzed oxidation of LDL, providing the possibility for therapeutic intervention. Ceruloplasmin carries copper and is an acute-phase protein whose concentrations increase during inflammation. It also acts as an oxidant for lipoproteins during acute inflammation.

In the absence of inflammation, the antioxidant activity of HDL is usually normal in hemodialysis patients (85), although total HDL levels are reduced. Reductions in HDL particle size are linked to insulin resistance and accompany reductions in LDL particle size and hypertriglyceridemia.

The predictors of death resulting from cardiovascular causes are low HDL cholesterol levels and high triglyceride/HDL ratios. Indeed, patients with the lowest total cholesterol levels, not those with the highest LDL levels, succumb to vascular disease (3). The combined effects of inflammation and oxidative injury on lipoprotein structure and function may provide some insight into this apparent contradiction.

Inflammation among patients with renal failure is common and may present with markers of malnutrition, e.g., reduced albumin or prealbumin levels, decreased muscle mass, or high Subjective Global Assessment scores. Inflammation contributes to both anemia and erythropoietin resistance. Inflammation predicts death, including death resulting from cardiovascular causes, and is likely to explain the relationship between malnutrition and death resulting from cardiovascular causes among this population. The cause of inflammation is likely to be multifactorial and, although it may reflect underlying vascular disease, inflammation is also a potent factor causing vascular injury. This effect is most likely mediated by changes in lipoprotein structure and function, alterations in endothelial function, adhesion to mononuclear cells and platelets, and alteration of the composition of plasma proteins to promote hemostasis and improve host defenses, with the unwanted consequences being vascular injury and death.

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