Hemodialysis Membranes: Interleukins, Biocompatibility, and Middle Molecules

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Abstract. Maintenance hemodialysis patients display evidence of elevated interleukin-1 (IL-1) and tumor necrosis factor alpha release after stimulation either by contaminated dialysate, bioincompatible membrane material, or both. This release is followed by the stimulated secretion of a large number of other interleukins, particularly IL-6, the cytokine principally responsible for acute-phase protein synthesis. It has been shown that high levels of the circulating proinflammatory cytokines IL-1, tumor necrosis factor alpha, IL-6, and IL-13 are associated with mortality in hemodialysis patients. Essential functions of polymorphonuclear leukocytes—that is, phagocytosis, oxygen species production, upregulation of specific cell surface receptor proteins, or apoptosis—are disturbed in patients with end-stage renal disease. These are further altered as a result of complement activation by the hemodialysis procedure, particularly if bioincompatible dialyzers are used. Polymorphonuclear leukocyte degranulation occurring during extracorporeal circulation does not depend on complement activation but rather on intracellular calcium and the presence or absence of the degranulation inhibitory proteins angiogenin and complement factor D. Clinical signs and symptoms of end-stage renal disease patients are at least in part related to the accumulation of middle molecules such as β2-microglobulin, parathyroid hormone, advanced glycation end products, advanced lipoxidation end products, advanced oxidation protein products (formed as a result of oxidative stress, carbonyl stress, or both), granulocyte inhibitory proteins, or leptin. Currently available membrane materials do not provide long-lasting, effective reduction of middle molecules in patients who require maintenance hemodialysis.

Hemodialysis therapy may induce activation of complement and blood cells such as polymorphonuclear leukocytes (PMNL), monocytes, or lymphocytes, depending on the membrane material of the dialyzer used. The clinical situation of the patient with end-stage renal disease (ESRD) is further complicated by the accumulation of uremic retention solutes and uremic toxins, which are often inadequately removed during the three-times-per-week dialysis procedure. The following overview focuses on interleukins, biocompatibility, and middle molecules and their relationships to different dialyzer membranes.

Mononuclear Cells

Effect of Uremia and Hemodialysis

Patients undergoing hemodialysis via routine regenerated cellulose membranes show evidence of elevated interleukin-1 (IL-1) and tumor necrosis factor (TNF) before a dialysis session and a further increase at the end of the procedure (1–5). Not all studies, however, confirm these findings. Cytokine production is not only related to hemodialysis with cellulosic membranes; it has also been shown that patients undergoing regular hemodialysis treatment with hemophane or polyamide dialyzers have comparable plasma concentrations of IL-1β, IL-6, and IL-10 predialysis at the end of the first treatment and after 48 treatments (4 mo) (6). Analysis of peripheral blood mononuclear cells (PBMC) is more likely to yield consistent results regarding increased cytokine production during hemodialysis treatment than circulating plasma concentrations (7). IL-1 is present in the mononuclear cells of patients undergoing regular hemodialysis (5,8–10), but not in mononuclear cells isolated from healthy subjects. PBMC from hemodialysis patients are “primed” to produce increased levels of IL-1β and TNF upon in vitro stimulation (4,5,11). In contrast to studies with isolated monocytes, Le Meur et al. (12) found that monocytes from hemodialysis patients in whole blood cultures are hyporesponsive to phytohemagglutinin or lipopolysaccharide (LPS). This finding might partly explain the immune defect in uremic and hemodialysis patients, if it reflects the in vivo situation (12). It is possible that the cell separation procedure affects the response of PBMC.

Mononuclear cells exiting the cuprophane dialyzer have initiated transcription to express large amounts of IL-1β and TNF-α, whereas cytokine genes are not activated by noncomplement-activating membranes during dialysis (13,14). The anaphylaxins C5a and C3a may stimulate cytokine synthesis (15), particularly in the presence of endotoxin (16). Concentrations of the monokine IL-1β in PBMC supernatants cultured for 24 h with 500 ng/ml LPS were significantly lower after dialysis in patients treated with polyamide when compared with those treated with hemophane (6). In the study of Memoli et al. (17), PBMC were cultured for 48 h with and without nonspecific mitogen stimulation. In unstimulated con-
ditions, PBMC harvested from patients who underwent dialysis with the cuprophane membrane showed higher IL-12 production than PBMC of healthy subjects, uremic patients who did not undergo dialysis, and patients who underwent dialysis with the biocompatible polymethylmethacrylate (PMMA) membrane. IL-12 production was correlated with C3a concentration. Under stimulation, PBMC interferon gamma release was lower in patients who underwent dialysis with cuprophane membranes as compared with PBMC obtained from patients who underwent dialysis with PMMA membranes, from uremic patients who did not undergo dialysis, or from healthy subjects. It was concluded that the altered release of interferon gamma could play a role in cell-mediated immunodeficiency of the uremic patients who underwent dialysis with cuprophane membranes (17).

Girndt et al. (18) measured cytokine induction in lymphocytes obtained from patients undergoing regular hemodialysis treatment with hemophane dialyzers. These patients were entered into a crossover study with a vitamin E–coated cuprophane (VE) dialyzer or a synthetic polyamide dialyzer. Four weeks of treatment with either VE or polyamide dialyzer enhanced in vitro proliferation of peripheral blood leukocytes as compared with treatment with the hemophane membranes used before study entry. The VE membrane reduced acute production of IL-6 during dialysis, whereas the regulatory cytokine IL-10 was not affected. It was concluded that the VE membrane, despite being based on a cuprophane backbone, is similar to the highly biocompatible polyamide dialyzer in terms of its effect on lymphocyte function (18).

LPS and LPS fragments containing the lipid A subunit bind to the LPS-binding protein (LBP). The LBP-LPS complex binds to the CD14 receptor on mononuclear cells resulting in de novo synthesis of cytokines. Neutrophils produce the bactericidal permeability inhibitory protein (BPI), which competes with LBP for the same binding domain on LPS. BPI inhibits LPS-dependent cytokine induction in mononuclear cells because the BPI-LPS complex does not bind to the CD14 receptor. The balance between BPI and LBP may determine LPS activity (19). Plasma BPI levels, however, are one order of magnitude lower than LBP levels, indicating no inhibitory effect of BPI on LPS in dialysis patients (20). The secretion of IL-1 and TNF-α is followed by the stimulated secretion of a large number of other interleukins, particularly IL-6, the cytokine principally responsible for acute-phase protein synthesis (e.g., stimulation of C-reactive protein and serum amyloid A, inhibition of albumin synthesis). Exotoxin A, actively secreted from microorganisms such as Pseudomonas aeruginosa, binds to the low-density receptor protein on monocytes and inhibits both cytokine and protein synthesis (21). Exotoxin A fragments, however, are able to induce cytokine production in mononuclear cells (19).

Dialysate contaminants of microbial origin, such as endotoxin, can unequivocally induce IL-1 and TNF synthesis and secretion. Endotoxin monomers, low molecular weight substances derived from dialysate-borne bacteria or certain LPS fragments, can permeate across dialysis membranes and stimulate cytokine production by monocytes. Activated monocytes may sequester in the lung and transit into tissue as macrophages with potential pathologic sequelae (22). LPS (molecular weight >100 kD), peptidoglycans (molecular weight >20 kD), or endotoxin A (molecular weight 71 kD) are not able to penetrate low-flux dialyzer membranes. In contrast, small fragments of bacterial products with cytokine-inducing activity present in contaminated dialysate may penetrate dialyzer membranes. Pyrogen permeability of dialyzer membranes depends on membrane thickness, hydrophobic domains, charge, and protein adsorption. Synthetic dialyzer membranes such as polysulfone and polyamide have reduced pyrogen permeability because of their thickness and their ability to adsorb pyrogens either by hydrophobic membrane domains or by plasma proteins coating the membrane surface (19).

IL-1 receptor antagonist (IL-1RA) can reduce or block the ability of IL-1 to activate cells, whereas soluble TNF receptor can reduce or block the activity of TNF (22). In this study, 32.6% of the hemodialysis patients were found to present with anti-IL-1α autoantibodies, but only 1.4% of healthy subjects did so. These autoantibodies have a neutralizing effect on IL-1α, at least in vitro. In contrast, IL-6 autoantibodies detectable in 18.2% of hemodialysis patients were not found to influence IL-6–dependent proliferation of B9 cells (23,24). The endogenous synthesis of IL-1RA is a reliable marker of the level of IL-1β synthesis in hemodialysis patients. On the other hand, the diminished endotoxin- or IgG-stimulated IL-1RA synthesis with increasing time on dialysis is possibly another sign of the impaired host defense system in patients on long-term hemodialysis (25).

Patient Outcomes

Hemodialysis hypotension has been linked to IL-1 production during hemodialysis treatment (26). A symptomatic fall in BP may be caused by a net ultrafiltration rate exceeding the vascular refilling rate or by the use of acetate resulting in a vasodilatory action on the microvascular resistance vessels. Furthermore, an autonomic nervous system neuropathy of the afferent limb of the carotid and aortic baroreflex arc that blunts or blocks the normal vasoconstrictor or cardioaccelerator response to vascular volume depletion may be responsible for hemodialysis associated hypotension. BP fall may also be caused by bioincompatibility of the cuprophane membrane, releasing the anaphylatoxins C3a and C5a. Finally, the lower the body temperature that occurs during treatment with hemodiafiltration, the better the hemodynamic stability. IL-1 is produced during hemodialysis by either or both of two mechanisms: stimulation of monocytes and induction of IL-1 secretion by the binding of C5a to monocyte surface, particularly during hemodialysis with bioincompatible membranes; and stimulation of monocytes adsorbed to the blood side of the dialysis membrane by dialysate-derived exogenous pyrogenic materials (26).

This suggestion, however, is based on experimental data. The findings of these animal studies should not automatically be extended to humans. The difference in incidence of symptomatic hypotension between hemofiltration and hemodialysis could be explained by the following: (1) the reduced capacity of the noncellulosic membranes used for hemofiltration to
activate complement; (2) the lack of exogenous pyrogenic substances in the dialysate of patients undergoing hemofiltration; and (3) the lower body temperature associated with low-monoctye-mediated IL-1 production during hemodiafiltration (because monocytes are believed to be sensitive to clinically relevant changes of temperature) (26).

Several studies have shown that lowering of temperature in the extracorporeal circuit causes BP stabilization. The lack of exogenous pyrogenic substances in the dialysate of hemofiltration patients is also an important factor reducing symptomatic hypotension.

Kimmel et al. (27) performed a prospective cross-sectional, observational multicenter study of 230 urban hemodialysis patients to determine the contribution of immunologic factors to patient survival. It was found that higher levels of circulating proinflammatory cytokines such as IL-1, TNF-α, IL-6, and IL-13 are associated with mortality, whereas IL-2, IL-4, IL-12, and immune parameters reflecting improved T cell function are associated with survival in patients with ESRD treated with hemodialysis, independent of other medical risk factors.

**Neutrophils**

There are numerous blood parameters that may be used to evaluate biocompatibility of dialysis membranes. The potential of different dialyzers to produce cytokines has been discussed above. The following discussion will focus on neutrophil parameters and their relevance to determine membrane biocompatibility.

**Complement Activation and Neutropenia**

Hemodialysis with cuprophane membranes produces a transient, severe neutropenia during the first half hour (28). *In vitro* and *in vivo* data have shown that this effect is dependent on complement activation via the alternative complement pathway (29,30). The activated neutrophils migrate to the lungs, resulting in mild pulmonary dysfunction during hemodialysis, particularly if dialysate containing acetate was used. Measurement of plasma C3a during routine hemodialysis with cuprophane membranes showed early (10 to 15 min) and striking (6000 ng/ml) elevations of this anaphylatoxin occurring in the venous line from the dialyzer. There is a strong correlation between hemodialysis leukopenia and complement activation as measured by the release of C3a during the first 30 min of treatment (31). Complement activation has been shown to increase the adhesiveness of granulocytes for a variety of foreign surfaces (32–34). Furthermore, both activated granulocytes and platelets share the ability to aggregate, arriving in the lung by microembolization (35). Less complement-activating membranes such as polysulfone (36), polyacrylonitrile (37) or cellulose acetate (38) are associated with less neutropenia and less hypoxemia. Reused dialyzers also lead to less complement activation than do fresh ones as a result of fixation of plasma proteins to the dialyzer membrane (39,40).

Reversal of granulocytopenia during hemodialysis with cellular membranes has been attributed to some or all of the following: selective downregulation of C5a complement receptors on PMNL; internalization of C5a complement receptors on granulocytes (41); and reduction of plasma C5a concentration below the critical threshold needed for granulocyte-endothelial cell adhesion.

Adhesion of neutrophils stimulated by C5a complement to endothelial cells is transient (42). Himmelfarb et al. (43) found, however, that the C5a receptor number does not significantly change during cuprophane hemodialysis, suggesting that this mechanism is not important. Complement activation by the dialysis membrane may cause an increase in the expression of the β2-integrin receptors (42,44–47). These activated cells will aggregate and adhere to the pulmonary endothelium (48). Synthetic membranes generally induce markedly less complement activation and also reduce the upregulation of adhesion receptors compared with cuprophane membranes (49).

**Phagocyte Function and Oxygen Species Production**

Phagocyte function is suppressed during dialysis with complement-activating cuprophane (50,51). In contrast, PMNL activity is unaltered during hemodialysis with other, non-complement-activating dialyzier membranes. In prospective studies, Vanholder et al. (52) and Vanholder and Ringoir (53) found that during the first 12 wk after starting hemodialysis, the glycolytic response and reactive oxygen production were substantially lower in patients treated with cuprophane when compared with those who underwent dialysis with polysulfone membranes. It was concluded that complement activation by cuprophane suppresses phagocytic response both acutely and chronically. Himmelfarb et al. (43,54,55) found less granulocyte oxygen species production in response to *Staphylococcus aureus* during hemodialysis with complement-activating membranes compared with non–complement-activating membranes. Rosenkranz et al. (56) observed significantly higher production of reactive oxygen intermediates by PMNL during hemodialysis with cuprophane as compared with polysulfone membranes. There are reports from several other groups that show both suppression and enhancement of neutrophil oxygen radical production (57,58).

**Upregulation of Specific Cell Surface Receptor Proteins**

Upregulation of specific cell surface receptor proteins such as CR1 (59) and Mac-1 (60) and downmodulation of the selectin LAM-1 (61) and sialophorin (CD43) have been reported during hemodialysis with cellulosic membranes (62). In contrast, hemodialysis treatment of the same patients with biocompatible non–complement-activation membranes results in only mild granulocytopenia and no significant changes in receptor expression. Complement split products are responsible for the up- and downregulation of neutrophil integrins and selectins (61).

Tielemans et al. (63) evaluated the expression of CD11b (Mac-1, CR3, or C3bi receptor), CD11a (leukocyte function antigen 1 or LFA-1, or gp 180/95), CD54 (intracellular adhesion molecule 1 or ICAM-1), and CD45 (leukocyte common antigen) on circulating leukocytes. Hemodialysis treatment with cuprophane or cellulose acetate membranes was associated with upregulation of CD11b and CD45, confirming the previous data of Arnaout et al. (60). The magnitude of CD11b
expression was similar to *in vitro* expression induced by incubation with C5a desarg. In contrast, hemodialysis treatment with reused cuprophane membranes is not associated with complement activation and did not upregulate CD11b on PMNL (60). Increased Mac-1 expression after 15 min of hemodialysis with a cuprophane membrane has also been reported by Thylén et al. (47). Expression of Mac-1 remained stable during polysulfone hemodialysis (64). Surface expression of L-selectins of granulocytes was found to decrease rapidly during the initial phase of cuprophane hemodialysis and then recover after 3 h to levels observed before starting hemodialysis. In contrast, the surface expression of L-selectins on granulocytes during the first 15 min of polysulfone hemodialysis was unchanged (64). A study by Mrowka et al. (65) suggested that the level of circulating adhesion molecules does not serve as an appropriate marker of membrane biocompatibility.

Selectins are responsible for the initial “rolling” interaction of leukocytes with the vascular endothelium (66), whereas firm adhesion of leukocytes to endothelial cells is dependent on the \( \beta_2 \)-integrins (67). Low L-selectin and high Mac-1 expression on granulocytes occurring during cuprophane hemodialysis lead to a decreased ratio between L-selectin and Mac-1 on granulocytes. This could impair the host defense of hemodialysis patients, particularly when cellular dissociation membranes are used (64).

Rosenkranz et al. (68) evaluated the contribution of reactive oxygen intermediate formation for receptor modulation by the cuprophane membrane on neutrophils. In patients who underwent dialysis with cuprophane, CD11b and CD66b upregulation of neutrophils and L-selectin downregulation was seen. The expression of CD11a remained unaltered. Hemodialysis with polysulfone did not change the surface receptor expression. Similar results were obtained during *in vitro* incubation of isolated neutrophils with cuprophane or polysulfone in the presence of serum. Generation of reactive oxygen intermediates by cuprophane occurred in the absence of serum. A novel C5-dependent neutrophil-activating mechanism, probably attributable to the release of oxygen radicals in addition to the expected complement activation, was found (68).

**Degranulation**

Neutrophil degranulation occurs during hemodialysis (69). This process, however, is not dependent on complement activation. For example, hemodialysis therapy with PMMA membranes results in only mild complement activation compared with cuprophane. Degranulation of neutrophils, however, is as pronounced with PMMA as with cuprophane (70). Reuse of cuprophane membranes considerably reduces complement levels but fails to affect degranulation of secondary granules (71). It has been shown that low doses of heparin may result in clot formation within the dialyzer and enhance complement formation, but neutrophil degranulation does not increase (72). Degranulation of neutrophils during hemodialysis depends on intracellular calcium and the presence or absence of degranulation-inhibiting proteins. Calcium channel blockers reduce cytosolic calcium and neutrophil degranulation during dialysis (73) and during aortocoronary bypass operation (74), even in the presence of complement activation. Extracorporeal calcium depletion during regional citrate anticoagulation abolishes lactoferrin (75) or myeloperoxidase (76) release. The mechanisms for the change in intracellular calcium that leads to degranulation are unclear.

Two neutrophil degranulation-inhibiting proteins identified as angiogenin (77) and complement factor D (78) have been described. Both proteins are markedly elevated in plasma of patients with ESRD. During hemodialysis with low-flux dialysis membranes, neither plasma levels of angiogenin nor complement factor D are altered. Under these conditions, there is only a mild release of lactoferrin from neutrophils. Surprisingly, hemodialysis with PMMA or polyacrylonitrile results in marked lactoferrin release (73,79). This is explained by the fact that both high-flux dialyzers cause a striking reduction of both plasma angiogenin (80) and complement factor D (81,82). This is a novel mechanism explaining neutrophil degranulation during extracorporeal circulation (80). Both PMN degranulation inhibitors are markedly elevated in the plasma of patients with ESRD.

Table 1 shows the differences between polysulfone and polyacrylonitrile with respect to angiogenin and complement factor D reduction. AN69 reduced plasma angiogenin level to 34.4% of the predialysis values and factor D level to 72.5% during hemodialysis, whereas polysulfone reduced plasma angiogenin to 64.1% and did not change complement factor D level. These data demonstrate that endogenous PMNL inhibitors such as angiogenin and complement factor D must be considered if neutrophil activation parameters were measured to investigate bioincompatibility of dialyzer membrane materials. However, there must be more to this process than just removal of degranulation inhibitory proteins; otherwise, there would not be degranulation with low-flux cellulose membranes.

**Apoptosis**

Cendoroglo et al. (83) investigated the contribution of apoptosis to neutrophil dysfunction in uremia. Compared with

<table>
<thead>
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<th>Parameter</th>
<th>Time (h)</th>
<th>F60</th>
<th>AN69</th>
</tr>
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<tr>
<td>Angiogenin</td>
<td>0</td>
<td>745.8±137.8</td>
<td>790.5±89.7</td>
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<tr>
<td></td>
<td>2</td>
<td>504.3±64.7</td>
<td>385.7±51.5 (( P &lt; 0.0002 ))</td>
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<tr>
<td></td>
<td>4</td>
<td>478.3±70.4</td>
<td>271.9±57.3 (( P &lt; 4.4 \times 10^{-6} ))</td>
</tr>
<tr>
<td>Factor D</td>
<td>0</td>
<td>93.2±14.6</td>
<td>76.2±39.5 (( P &lt; 0.02 ))</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>95.9±15.4</td>
<td>52.2±11.9 (( P &lt; 0.02 ))</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>96.2±15.7</td>
<td>55.3±16.3 (( P &lt; 0.04 ))</td>
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*Mean values ± SEM from 12 patients; significances were calculated versus 0 h.*
normal neutrophils, uremic neutrophils demonstrated greater apoptosis in the presence of autologous plasma as well as 10% fetal calf serum. Furthermore, compared with normal neutrophils exposed to heterologous normal plasma, those exposed to heterologous uremic plasma exhibited higher apoptosis rates, lower N-formyl methionyl-leucyl-phenylalanine–stimulated superoxide production, and a lower phagocytosis index. Apoptosis correlated inversely with N-formyl methionyl-leucyl-phenylalanine–stimulated superoxide production. It was concluded that uremic neutrophils undergo accelerated apoptosis in vitro. The authors also demonstrated that uremic plasma accelerates apoptosis of normal neutrophils, resulting in a dysfunctional pattern that is similar to that observed in uremia (83).

Martin-Malo et al. (84) evaluated the effect of both uremia per se and hemodialysis membranes on the induction of apoptosis. Cell apoptosis was higher in hemophone than the other hemodialysis membranes. Moreover, apoptosis decreased in hemodialysis patients after they switched from hemophone to polysulfone. Mononuclear cell circulation through minidialyzers made of different types of membranes (cuprophane, hemophane, cellulose acetate, polyacrylonitrile, and polysulfone) produced a significant increase in apoptosis when hemophone or cuprophane were used, as compared with the other three membranes. Uremia and membrane characteristics might independently affect the mononuclear cell apoptosis (84).

Cohen et al. (85) found that PMNL apoptosis increases in the presence of glucose-modified serum proteins. Early glycation of proteins, as analyzed by boronate chromatography and the fructosamine assay, is responsible for the increase of PMNL apoptosis. In addition, early glycated proteins, isolated by preparative boronate chromatography from the effluent of chronic ambulatory peritoneal dialysis patients, increased PMNL apoptosis (85). Reduction of glycated proteins by high-flux dialyzers either via adsorption or convection may improve PMNL apoptosis in vivo.

Recently Cohen et al. (86) investigated the influence of isolated free Ig light chains (IgLC) and of commercially available IgLC on spontaneous PMNL apoptosis. IgLC of both kappa and lambda type were able to increase the percentage of viable PMNL by inhibiting spontaneous apoptosis in a concentration-dependent manner. IgLC, elevated in plasma of patients with ESRD, could interfere with the normal resolution of inflammation and thereby contribute to the chronic inflammatory state found in dialysis patients.

**Middle Molecules**

Clinical signs and symptoms of patients with ESRD are at least in part related to the accumulation of uremic retention solutes and uremic toxins. Middle molecules accumulate as a result of decreased renal function and are the result of oxidative stress, microinflammation, or uremia per se, leading to protein modifications that may cause new biologic functions. Middle molecules include the following: parathyroid hormone; \( \beta_2 \)-microglobulin; advanced glycation end products (AGE); advanced lipoxidation end products; advanced oxidation protein products; granulocyte inhibitory proteins; and leptin.

**Parathyroid Hormone**

Parathyroid hormone has been identified as an uremic toxin. Parathyroid hormone increases in patients with ESRD as a result of enhanced glandular secretion and not as a result of decreased renal function. Excess parathyroid hormone causes a rise in intracellular calcium, disturbing the functions of virtually every organ system, including bone mineralization, pancreatic response to metabolic and hormonal changes, erythropoiesis, the immune system, the heart, the liver, or the brain (87). Increased cellular calcium results in PMNL deactivation (88,89), which can be normalized by calcium channel blockers (90).

**\( \beta_2 \)-Microglobulin**

Dialysis-related amyloid is to a large extent composed of \( \beta_2 \)-microglobulin (\( \beta_2 \)M). The clinical picture of dialysis-related amyloidosis (DRA) include (91) the following: carpal tunnel syndrome; chronic invalidating arthralgias; multiple bone cysts; bone fractures; spinal cord compressions; and gastrointestinal complications (bleeding, perforation). A third of the patients are affected by DRA before 4 yr, and more than 90% are affected after more than 7 yr on hemodialysis treatment. Risk factors for the development of DRA include the following: contamination of the dialysate; age of the patient at onset of dialysis; type of hemodialysis membrane; and time on dialysis.

Protective effect of high-flux membranes such as AN69 or polysulfone with respect to DRA results mainly from the effective reduction of \( \beta_2 \)M via adsorption, convection, or both. Residual renal function may also play a role in \( \beta_2 \)M removal. The lowest incidence of DRA has been observed when ultra-pure dialysate is used (92).

Numerous studies have been performed demonstrating lower manifestation of DRA in patients on long-term treatment with biocompatible membrane materials as compared with cuprophane. For example, van Ypersele et al. (93), who used carefully defined criteria for radiologic amyloid bone cysts, found significantly more amyloid bone cysts in cuprophane-treated patients as compared with patients undergoing regular hemodialysis treatment with the AN69 membrane. Hakim et al. (94) randomly assigned 159 patients starting hemodialysis to either a low-flux biocompatible cellulosic membrane or a low-flux biocompatible PMMA membrane. Sixty-six patients completed the 18-mo study. Despite a similar progressive reduction of residual renal function in both groups, serum \( \beta_2 \)M level increased by an average of 11.8 mg/L in the cellulosic group (\( P < 0.0001 \)). There was, however, no significant increase of \( \beta_2 \)M in the PMMA group during the study period of 18 mo. Bioincompatibility of the cuprophane membrane causes either stimulation of synthesis or release \( \beta_2 \)M (89). Data of Matsuo et al. (95) provided the first evidence for the presence of TGF-\( \beta \) in DRA tissue, as well as the stimulatory action of AGE-\( \beta_2 \)M on tissue macrophages.

**Advanced AGE**

Irreversible nonenzymatic modification of proteins occurs in chronic renal failure. Proteins exposed to glucose or other
carbohydrates form advanced AGE. AGE accumulate as a result of impaired renal excretion. Elevated AGE concentrations have been reported in the tissues and plasma of patients with diabetes and patients with chronic renal failure (96,97). These end products account for numerous biologic responses (98) and also promote the release of cytokines (99). For example, Miyata et al. (100) provided evidence that β2M modified by advanced AGE is a dominant constituent of these amyloid deposits and increases the secretion of TNF-α and IL-1β from macrophages, whereas normal β2M had no effect (101). High-flux hemodialysis markedly reduced low molecular weight AGE-modified molecules (by 47.9% in patients with diabetes and 60.6% in dialysis patients without diabetes), but concentrations returned to the pretreatment range within 3 h (102).

Specific methods have been developed to quantify AGE such as pentosidine or carboxymethyllysylsine (103,104). It has been shown that pentosidine and carboxymethyllysylsine are significantly higher in plasma and tissue proteins of hemodialysis patients compared with healthy subjects (105,106). In uremia, however, neither of these AGE correlate with fructoselysine, suggesting that factors other than hyperglycemia are responsible for the rate of AGE formation.

**Advanced Lipoxidation End Products**

Malondialdehyde-modified proteins also accumulate in the plasma of patients with ESRD. Malondialdehyde is derived from the oxidation of polyunsaturated fatty acids. The malondialdehyde and other lipid modified proteins are called “advanced lipoxidation end products” (107).

Formation of AGE and advanced lipoxidation end products occurs between proteins and reactive carbonyl compounds derived from carbohydrates and lipids; their production in uremia is mainly the result of oxidative stress and decreased renal clearance (108). Residual renal function influences the pentosidine level in uremic patients (109,110). It has been suggested that the “carbonyl stress” is associated with long-term complications of patients with ESRD, such as dialysis-related amyloidosis or atherosclerosis (108).

**Advanced Oxidation Protein Products**

Changes in cell surface receptor expression occur in uremia by activated phagocytes or interaction of white blood cells with bioincompatible dialysis membranes (45–47,57,111). In addition, uremia causes oxidant-mediated protein alterations (112–114). Furthermore, these patients have a severe defect in antioxidant enzyme activity, antioxidant enzyme cofactors, and antioxidant vitamins.

Protein damage mediated by reactive oxygen species results in oxidation of amino acid residues such as tyrosine. When di-tyrosine is formed, protein aggregation, cross-linking, and fragmentation occurs. These di-tyrosine-containing, cross-linked protein products were designated “advanced oxidation protein products” (AOPP) (112,113). AOPP were found in very high concentrations in the plasma of dialysis patients. Because patients treated with peritoneal dialysis and patients experiencing preterminal renal failure also have significantly higher AOPP levels than healthy subjects, it was concluded that uremia per se induces a significant oxidative stress (112,113). The accumulation of AOPP starts at an early stage of chronic renal failure and gradually rises with the progression of renal failure. There is an inverse relationship between AOPP levels and creatinine clearance. A close relationship between AOPP and AGE-pentosidine has been observed. Human serum albumin exposed to chlorinated oxidants (HOCI) resulted in AOPP–human serum albumin formation. Myeloperoxidase released during hemodialysis is a major factor contributing to AOPP formation. Myeloperoxidase and chlorinated oxidant–induced lipoprotein by-products are present in atherosclerotic lesions (115,116). These data suggest that AOPP may play a role in the development of atherosclerosis (117). It has been shown that regional citrate anticoagulation reduces PMNL degranulation (75) and myeloperoxidase release (76). Therefore, it would be interesting to investigate whether regional citrate anticoagulation reduces AOPP formation.

**Granulocyte Inhibitory Proteins**

Low molecular weight uremic toxins that inhibit neutrophil functions include p-cresol (118) and several guanidino compounds (119). It has been shown that uremic neutrophils undergo accelerated apoptosis (83,84). AGE-modified serum proteins have been identified as uremic toxins that accelerate PMNL apoptosis, at least in vitro (85), whereas light chain proteins inhibit PMNL apoptosis (86).

Several peptides accumulate in renal failure and interfere with specific cellular functions, as follows:

- Granulocyte inhibitory protein (GIP I), showing homology with light-chain proteins, inhibits the uptake of deoxyglucose, chemotaxis, oxidative metabolism, and intracellular bacterial killing by PMNL (120).
- Granulocyte inhibitory protein (GIP II), showing homology with β2M, inhibits O2– production and glucose uptake by PMNL (121).
- Degranulation inhibiting protein I (DIP I) inhibits PMNL degranulation and is identical to angiogenin (77).
- Degranulation inhibiting protein II (DIP II) inhibits PMNL degranulation and is identified as complement factor D (78).
- Kappa and lambda light chain protein monomers and dimers inhibit in vitro glucose uptake and chemotaxis of PMNL (122).
- Chemotaxis-inhibiting peptide with homology to ubiquitin is responsible for inhibition of chemotaxis (intact ubiquitin is not responsible for this effect but rather a modified form) (123).

The presence of granulocyte inhibitory proteins of middle molecular weight in the plasma of patients with ESRD holds promise of explaining the common occurrence of hospitalization and death by infection in this population.

**Leptin**

Uremic patients have elevated serum leptin that accumulates with the decrease of renal function. Renal transplantation normalizes plasma leptin levels (124). Men with normal body-
mass index and low plasma insulin levels, however, have normal or even low leptin levels despite renal insufficiency (125). It has been shown that nonrenal tissues such as splanch- nic organs contribute substantially to the removal of leptin (126). Ob gene expression in uremic patients is lower than in controls (127). Leptin mRNA expression is stimulated by increased body fat mass and hyperinsulinemia in nonuremic subjects and in uremic patients. Molecular studies of leptin for renal disease patients and the possible link between leptin and BP have been reviewed (128,129).

Conclusion
Enhanced production of proinflammatory cytokines by dialysate pyrogens, by complement activation, or both, as well as inhibition of anti-inflammatory cytokine secretion, may contribute to the cell-mediated immunosuppression seen in patients with ESRD. Furthermore, multiple alterations of neutrophils occur as a result of uremia per se and bioincompatible dialyzer membranes. The presence of low and high molecular weight inhibitors of neutrophils in the plasma of uremic patients may explain, at least in part, why infections are the most common cause of hospitalization and the second most common cause of death in patients with ESRD. Other middle molecules accumulate, and proteins are modified by oxidative stress, carbonyl stress, or both, contributing to the uremic syndrome. To lower these toxic substances, either their generation should be reduced or strategies for effective removal should be developed.

References


