Causes and therapy of microinflammation in renal failure

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Abstract

Microinflammation in renal failure has been the subject of numerous studies, but the causes of the inflammatory response in these patients are not clear. There are several potential causes and possible therapies for microinflammation, and they are discussed in this review with regard to uraemia and acidosis, heart failure and volume overload, oxidative stress and iron therapy, and bioincompatibility, especially regarding dialysis membranes. In addition, issues regarding dialysate contamination and access site infection are examined, followed by a discussion of possible drug therapy for microinflammation with angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, statins, aspirin, and antioxidants, such as vitamin E.

Keywords: cardiac failure; dialysis; drug therapy; microinflammation; oxidative stress; uraemia

Uraemia

Patients with renal insufficiency who have not yet begun dialysis have elevated levels of CRP that rise further after starting regular HD treatment and, therefore, the dialysis procedure may only be partially responsible for inflammation in patients with renal disease [5]. Stenvinkel summarized the prevalence of elevated CRP levels from several studies and concluded that ~35% of patients with renal failure who had not yet begun dialysis had elevated levels of CRP, compared with >50% of patients receiving HD [6]. Thus, in patients with uraemia, inflammation starts long before renal replacement therapy. Accumulation of mediators in renal failure [pro-inflammatory cytokines and advanced glycation end-products (AGEs)] may contribute to inflammation. Reduced elimination of mediators in renal failure leads to an accumulation of factor D, the rate-limiting step in complement activation, and subsequent amplification of C3 activation [7]. The kidney is the major site for elimination of many cytokines, as shown in studies investigating IL-1 and tumour necrosis factor (TNF) clearance in nephrectomized rats [8] as well as in pharmacokinetic studies of injected recombinant IL-10 in humans with different degrees of renal dysfunction [9]. Descamps-Latscha et al. demonstrated that plasma levels of IL-1 receptor antagonist (IL-1Ra) increased significantly from the earliest stage of renal failure. Plasma levels of TNF-α and soluble TNF receptors rose with the severity of renal failure and correlated with the glomerular filtration rate [10].

Both pro- and anti-inflammatory cytokines and mediators accumulate in patients with renal failure, and it has been questioned whether the overall result is inflammation, or whether a balance is reached between pro-inflammatory mediators and their inhibitors. When cytokine production was investigated in whole blood (which best simulates the in vivo situation), however, we observed that spontaneous and lipopolysaccharide (LPS)-induced production of IL-1 and IL-6 in whole blood from HD patients is almost doubled compared with normal individuals (Figure 1) [11].
Thus, the net effect of the accumulation of mediators appears to be pro-inflammatory. Among the numerous other products that accumulate in renal failure are AGEs [12] and advanced oxidation protein products (AOPPs), which may also contribute to a pro-inflammatory state [13].

Acidosis is a well-known complication of uraemia and could possibly contribute to inflammation. An established consequence of acidosis in chronic renal failure is protein catabolism and loss of lean body mass [14]. In animal models, consumption of ammonium chloride (NH₄Cl) suppresses growth in normal rats and increases their excretion of urea nitrogen [15]. Correction of acidosis, on the other hand, has beneficial effects on body weight and muscle mass in patients receiving peritoneal dialysis [16]. Pickering et al. recently reported similar results in eight patients receiving chronic ambulatory peritoneal dialysis (CAPD) and observed a decrease in muscle ubiquitin mRNA as well as in TNF plasma levels after correction of acidosis [17]. Pro-inflammatory cytokines are probably involved in protein catabolism, as TNF injection into rats is associated with muscle protein and branched chain amino acid degradation [18]. We recently observed that acidosis augments the production of IL-6 and the chemokine RANTES from smooth muscle cells in vitro [19], indicating that acidosis may contribute to the inflammatory state in uraemia. The possible causes of microinflammation in uraemia are summarized in Table 1.

Heart failure and volume overload

Overhydration is frequent in patients with renal failure and may contribute to inflammation. Nibauer et al. [20] reported elevated plasma levels of endotoxin in oedematous patients with chronic heart failure compared with stable patients with chronic heart failure. Oedematous patients had the highest concentrations of several cytokines. After treatment with diuretics, endotoxin concentrations decreased significantly, suggesting that overhydration and/or heart failure leads to increased endotoxin levels, possibly triggering activation of the immune response. There are also reports on negative correlations between excess extracellular fluid volume and serum albumin concentrations in dialysis patients; the greater the excess fluid, the lower the concentration of albumin [21]. Thus, overhydration may lead to increases in endotoxin and cytokine levels, reducing the synthesis of albumin.

Upregulation of cytokines has also been reported in heart failure, as shown by elevated levels of IL-6 and

Table 1. Possible causes of microinflammation in uraemia

<table>
<thead>
<tr>
<th>Cause</th>
<th>Possible therapies</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Uraemia</td>
<td>Preserve renal function</td>
<td>[7,9,10]</td>
</tr>
<tr>
<td>Acidosis</td>
<td>Increase dialysis dose?</td>
<td></td>
</tr>
<tr>
<td>Volume overload/heart failure</td>
<td>Prevent/treat acidosis</td>
<td>[17,19]</td>
</tr>
<tr>
<td></td>
<td>Continuously adjust ‘dry weight’</td>
<td>[22,64,65]</td>
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<tr>
<td></td>
<td>Use of ACE inhibitors/angiotensin receptor blockers?</td>
<td></td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Preserve renal function, increase dialysis dose?</td>
<td>[26,29–31,66]</td>
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<td></td>
<td>Antioxidant therapies?</td>
<td></td>
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<tr>
<td></td>
<td>Avoid iron overload</td>
<td></td>
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<tr>
<td>Bioincompatibility</td>
<td>Use of non-complement activating membranes</td>
<td>[11,35,36]</td>
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<td></td>
<td>Use of high-flux membranes?</td>
<td></td>
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<tr>
<td>Dialysate contamination</td>
<td>Use of ultrapure dialysate and adequately designed water treatment systems</td>
<td>[45–47,67,68]</td>
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<tr>
<td>Access infection</td>
<td>Vigilant search for access infection, avoid venous catheters and</td>
<td>[50,51,69]</td>
</tr>
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<td></td>
<td>polytetrafluoroethylene grafts</td>
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TNF in patients with heart failure [22]. TNF is not expressed in normal myocardium, but myocardial cells can express TNF mRNA and produce TNF in response to increased left ventricular pressure or volume overload [23]. Elevated sympathetic activity in heart failure may contribute to enhanced cytokine response because chronic β-adrenergic stimulation induces myocardial expression of TNF and IL-6 [24]. In addition, treatment with β-blockers leads to a reduction in the plasma levels of TNF [25]. Thus, every effort should be made to search continuously for the correct ‘dry weight’ of patients receiving HD to avoid overhydration and subsequent heart failure, which are additional causes of inflammation.

**Oxidative stress**

Neutrophils and monocytes produce reactive oxygen species (ROS) as part of the host’s defence against microorganisms. Neutrophils obtained from patients receiving HD exhibit a higher rate of spontaneous production of ROS than those from healthy individuals [26]. Furthermore, neutrophils from patients receiving HD are primed for an enhanced respiratory burst following additional stimuli. The enhanced phagocytosis-stimulated hydrogen peroxide (H₂O₂) production is conferred by uraemic plasma because it is observed not only in neutrophils from uraemic patients, but also in neutrophils from normal individuals incubated in plasma from uraemic patients [27]. In addition, a single high-flux HD session leads to a decrease and almost normalization in hydrogen peroxide production by neutrophils, regardless of the membrane used [28].

Consistent with an increased production of ROS, proteins and lipids from patients receiving HD exhibit higher levels of oxidation, as shown by an increase in protein carbonyl groups and AOPPs [29]. As renal failure progresses, the accumulation of uraemic solutes increases and they can serve as targets for increased oxidation [13]. Thus, some authors concluded that the primary stimulus for oxidative stress in renal failure patients is uraemia per se and not the dialysis procedure [30].

An additional factor contributing to oxidative stress in uraemia may be intravenous (i.v.) iron therapy. I.v. iron can release free iron that may react with hydrogen peroxide to produce the strong ‘oxidant’ hydroxyl radical. Druke et al. reported that in patients receiving HD, AOPP levels correlate with serum ferritin and the dose of i.v. iron administered [31]. Furthermore, early signs of atherosclerosis (wall to lumen ratio) were associated with plasma AOPP, serum ferritin and the annual administered i.v. iron dose [31]. Thus, iron overload should be avoided.

**Bioincompatibility**

According to Medline®, there have been 610 publications on biocompatibility and HD within the last 20 years. The term ‘biocompatibility’ involves coagulation, thrombocytes, leukocytes, complement activation, and cytokine and bradykinin production, each of which may affect inflammation. Craddock et al. described the activation of the alternative complement pathway with cuprophan in 1977 [32]. Activated complement factors, such as C3a and C5a, increase during HD and reach maximal levels after 15–30 min. At the same time, leukopenia is also maximal. There are large differences between the membranes in complement activation and, usually, cellulosic membranes, in which polar hydroxyl groups are substituted with acetyl or diethylylaminoethyl (DEAE) groups, or synthetic membranes activate less complement. Complement factors, C3a and C5a, activate granulocytes and also monocytes, which subsequently become ‘primed’ to produce cytokines. Cells which leave the cuprophan dialyser express large amounts of mRNA for IL-1β and IL-6, while HD with non-complement-activating membranes results in the expression of far less mRNA for these cytokines [33]. When mRNA-expressing cells are stimulated subsequently with endotoxin, they are sensitized to produce considerably more cytokines than non-activated cells. During the course of granulocyte activation, these cells release their granular products (elastase, myeloperoxidase and lactoferrin) and express surface markers, such as CD11b, Mac-1 or CD66b [34]. While the latter process is also mainly dependent on complement activation, degranulation is not complement dependent, but it is observed when re-used cuprophan membranes that activate small amounts of complement are used.

**In vivo** studies underline the clinical relevance of these observations and support membrane differences regarding induction of inflammatory processes. For instance, Tayeb et al. switched patients receiving HD from cuprammonium membranes to polysulfone [35]. Serum albumin levels increased significantly in patients both with and without diabetes mellitus. Memoli et al. dialysed the same patients with cuprophan, synthetically modified cellulosic membranes and cellulose diacetate and observed significant differences in the plasma levels of CRP, IL-6 and albumin depending on the types of membrane used [36]. During dialysis with cuprophan, higher levels of CRP and IL-6 and lower levels of albumin were observed. In a randomized, crossover study, 18 patients receiving HD were treated subsequently with dialysers containing polyamide, polycarbonate or cuprophan for 8 weeks on each dialyser [11]. CRP levels were lower when patients were dialysed with polyamide compared with the levels when the same patients were dialysed with cuprophan. The whole blood content of IL-1Ra was higher when patients were dialysed with cuprophan compared with the same patients receiving dialysis with polyamide or on polycarbonate. Thus, the degree of inflammation in patients receiving HD is affected by the choice of the dialyser.

It should be noted that it is not only the type of membrane, but also its flux and the extent of convective transport which may influence inflammation. Cytokine
inducing activity of cytokine-inducing substances from the dialysate and direct blood–membrane interactions (Figure 2). With high-flux membranes and convective therapies, one would expect that more pro-inflammatory products (e.g. C3a, C5a, AGEs and AOPPs) would be removed, while the passage of bacterial products is hindered, resulting in less inflammation on the blood side. Clinical data on the effect of convective therapies on inflammation are, however, scarce.

**Dialysate contamination**

Since the introduction of HD into clinical practice, there has been concern about the transfer of bacterial cytokine-inducing substances (CIS) from the dialysate into the blood compartment and the subsequent deleterious effects on the patient. A number of bacterial products (Figure 2), such as LPS, exotoxins and peptidoglycans, share the ability to induce cytokines and are known activators of immune functions. A number of in vitro and in vivo studies have been performed investigating the permeation of these substances through dialysis membranes. In many studies, the biological test of cytokine induction in peripheral blood mononuclear cells (PBMCs) has been used to detect these substances. This test detects all biologically relevant bacterial substances, whereas the Limulus test (LAL) detects only LPS-derived substances. Moreover, only LPS fragments >8 kDa are reactive in the LAL test, but smaller fragments may still be pyrogenic [37]. The exact chemical nature of bacterial CIS is not completely understood, and, most probably, CIS consist of a mixture of bacterial products. LPS is not the only product in dialysate that induces cytokines. This is supported by the observation that the cytokine-inducing activity of *Pseudomonas* products that appear on the blood side of dialysers cannot be blocked entirely by polymyxin B [38] and are negative in the LAL test [39].

New pyrogenic candidates that may pass through the dialyser membranes are bacterial-derived short DNA fragments. It has been reported that PBMCs ingest bacterial DNA [40]. Immunosimulation by oligonucleotides (ODNs) requires an unmethylated cytosine–guanosine (CG) core for the stimulation of mammalian cells [41]. Mammalian DNA shows extensive suppression of CG sequences and they are frequently methylated; only in bacterial DNA can unmethylated CG motifs (CpG) be found. This unmethylated CpG distinguishes bacterial DNA from mammalian DNA and allows phagocytic cells to recognize and be activated by bacterial DNA. CpG ODNs of 15–20 bp are able to induce natural killer cell activity and induce interferon-γ, TNF-α and IL-6 from PBMCs [42,43]. When injected intraperitoneally (i.p.), CpG ODNs induce IL-6, TNF and IL-12 production in mice and may even lead to septic shock [44]. The signalling pathways by which CpG ODNs activate cells currently are being characterized and involve Toll-like receptor 9 (TLR9). Macrophages and dendritic cells from TLR9-deficient mice do not respond to ODNs [44]. Cytokine-inducing ODN are of a sufficiently small size (10 bp, ~2500 Da) to pass through dialyser membranes (R. Schindler, submitted). Thus, bacterial ODNs may be a factor contributing to cytokine induction during HD, which are not easily removed by conventional ultrafilters.

Several studies investigated the permeability of low- and high-flux dialyser membranes for CIS. Most of these studies demonstrated prompt transfer of CIS through low-flux cellulose membranes, but no or less transfer of CIS through high-flux polysulfone or polyamide membranes [45–47]. It was concluded that the sponge-like structure of these high-flux membranes adsorbs bacterial products; this feature even enabled the use of polysulfone and polyamide as ultrafilters to remove CIS efficiently from aqueous solutions [48]. High-flux membranes were considered to be a safe barrier against possible bacterial products in the dialysate. However, this may not be true for all high-flux membranes. We observed recently that there are large differences between high-flux membranes regarding their permeability to cytokine-inducing substances from *Escherichia coli* as well as for LPS derived from *E. coli* and *Stenotrophomonas maltophilia* [49].

Ultrapure dialysate is not yet the standard of dialysate quality in most dialysis centres. Although the consequences of inflammation in dialysis patients are not fully understood, preventing the penetration of bacterial products from the dialysate seems prudent. When using high-flux membranes that are possibly permeable for bacterial CIS, the use of CIS-free dialysate is essential. To remove all CIS completely, including bacterial DNA from the dialysate, supplementary measures in addition to ultrafiltration may be required.
Access site infection

Infection of venous access sites is a frequent and often overlooked cause of inflammation. Sites involving foreign materials are especially liable to infection and may be a source for bacteremia. Ayus and Sheikh Hamad reported a series of infected, old, non-functioning grafts in patients receiving HD [50]. There are not always any physical signs of graft infection, so diagnosis requires a high index of suspicion. Venous catheters are associated with increased rates of infection compared with other forms of vascular access, including bacteremia, osteomyelitis and endocarditis [51]. Recently, Pastan et al. [52] reported that medium-term, all-cause mortality and mortality caused by infection are correlated with the use of venous catheters (cuffed or non-cuffed).

Possible drug therapy for microinflammation

Several drugs have been reported to reduce inflammation assessed by CRP and cytokine levels (Table 2). Angiotensin-converting enzyme (ACE) inhibitors reduce the synthesis of IL-1 and TNF in vitro [53], and IL-6 levels in patients with heart failure in vivo [54]. In patients receiving HD, the use of ACE inhibitors is associated with lower levels of CRP and TNF [55]. Angiotensin receptor antagonists (e.g. candesartan) also decrease levels of IL-6 and TNF in patients with heart failure [56]. Whether this effect is direct or caused by amelioration of heart failure is not known.

The CRP-lowering effect of statins is well described for the general population [57,58] as well as for patients with renal failure [59]. All statins including atorvastatin, simvastatin and cerivastatin seem to lower CRP. This effect appears to be dose dependent [57], but lowering levels of CRP does not correlate with the decrease in cholesterol [58].

The effect of aspirin and antioxidants, such as vitamin E, on inflammation is more controversial. One study reported reductions in both CRP and IL-6 with aspirin in patients with coronary artery disease [60], but this effect may be dose dependent and may not be observed with the usual daily dose of 100 mg aspirin [61]. Devaraj and Jialal reported that supplementation with α-tocopherol lowered CRP levels and IL-6 release from monocytes in 47 patients with diabetes and in normal volunteers [62]. In contrast, Bruunsgaard et al. failed to observe an effect of α-tocopherol and vitamin C on CRP levels in 55 healthy individuals [63].

The authors concluded that long-term combined supplemental α-tocopherol and vitamin C in reasonable doses have no detectable systemic anti-inflammatory effects in healthy men. The effect of vitamin E and aspirin on inflammation in dialysis patients needs to be clarified by further trials.

Thus, at the present time, therapy with blockers of the renin–angiotensin system and statins can be recommended to ameliorate inflammation in uraemic patients, while the use of antioxidants for this purpose awaits further confirmation. Targeting the inflammatory response in uraemic patients most probably requires a multifactorial approach involving all of the described pathogenic aspects.

Conflict of interest statement. None declared.

References


Table 2. Drugs which possibly interfere with inflammation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitors/AT1-receptor antagonists</td>
<td>[53,56]</td>
</tr>
<tr>
<td>Statins</td>
<td>[57–59]</td>
</tr>
<tr>
<td>Aspirin</td>
<td>[60,61]</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>[62,63]</td>
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