

Immunological basis of inflammation in dialysis

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Abstract

Several studies indicate that chronic inflammation is the major determinant of the 'dialysis syndrome' characterized by malnutrition, cachexia and vasculopathy and responsible for the high mortality and morbidity rate in dialysed patients. Uraemia together with repeated contact with the dialytic devices are considered the most important factors eliciting the immune system response resulting in inflammation. Dialysis may also contribute by means of bacterial contamination of dialysis fluids or acetate-containing dialysis buffers. Chronic infections (*Chlamydia pneumoniae*) in combination with a defective immune system and favourable genetic background can also be involved. The chronic inflammation of long-term dialysed uraemic patients induces an increased production of reactive oxygen species which cannot be counterbalanced due to defective antioxidant capability typical of uraemia: the resulting altered redox state is responsible for the accelerated senescence characteristic of the dialysis syndrome. The most important immunological mechanisms underlining the development of the dialysis syndrome are reviewed.

Keywords: carbonyl stress; cytokines; immune system; nitric oxide; oxidant stress; uraemia

Introduction

In spite of the impressive improvement in clinical and technological treatment of end-stage renal disease (ESRD) patients by renal replacement therapy (RRT), an insidious pitfall of dialysis is an enhanced risk of complications, particularly chronic inflammatory diseases such as vasculopathy [1,2].

The chronic inflammatory state of long-term dialysed patients depends upon two factors: a defective

immune system function and a continuous non-specific immune cell stimulation by dialytic devices.

Uraemic patients have a paradoxical immune system (extremely active concerning the response to foreign material used in the extracorporeal circuit, but defective as far as the normal immune response is concerned) and they have many similarities to patients with autoimmune disease, such as systemic lupus erythematosus (SLE). Moreover, uraemic toxins completely abrogate several granulocyte and monocyte functions.

The blood contact with foreign surfaces during haemodialysis produces a variety of complex and often interrelated events in the immune system. Activation of mononuclear cells, complement fixation, cytokine synthesis and release, reactive oxygen species (ROS), carbonyl stress and nitric oxide (NO) production result in an acute/chronic inflammatory response. This response consists of modulation of positive [such as C-reactive protein (CRP), serum amyloid A, fibrinogen and haptoglobin] or negative (such as hypoalbuminaemia and transferrin) acute-phase reactants (APRs).

Great interest has been shown recently in the role of APRs in the pathogenesis of dialysis complications, reported cardiovascular diseases and related co-morbid complications as the first cause of death in patients undergoing RRT.

The aim of this review is to describe the immunological mechanisms regulating the pathogenesis of chronic inflammation during chronic haemodialysis (Figure 1).

Complement activation

The complement fraction C3 covalently binds to nucleophilic surfaces, to cell wall constituents or synthetic polymer including those of the extracorporeal circuits. This interaction leads to activation of the alternative complement cascade resulting in the generation of the terminal complement complex (TCC). The critical step in dialysed patients is the formation of the C3 convertase complex, either in the extracorporeal circuit or within the patient's vessels [3].

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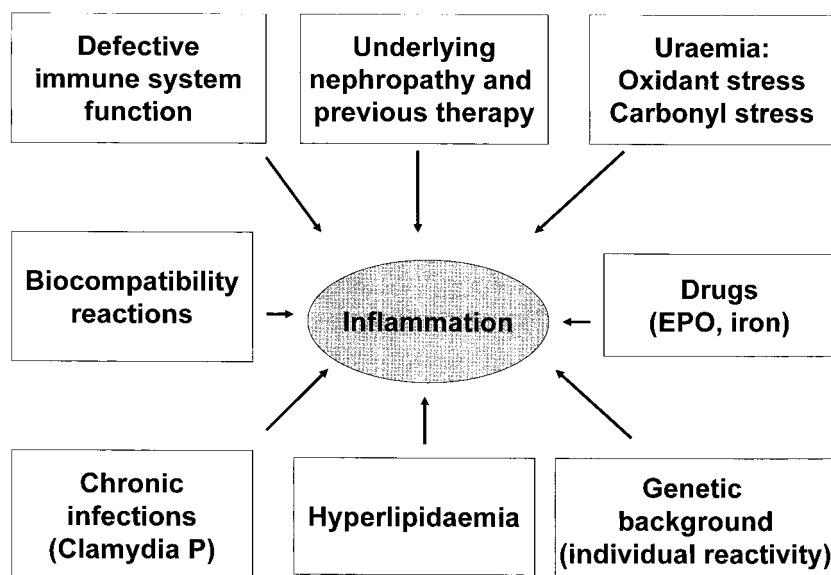


Fig. 1. Pathogenetic factors involved in the development of inflammation in long-term dialysed patients.

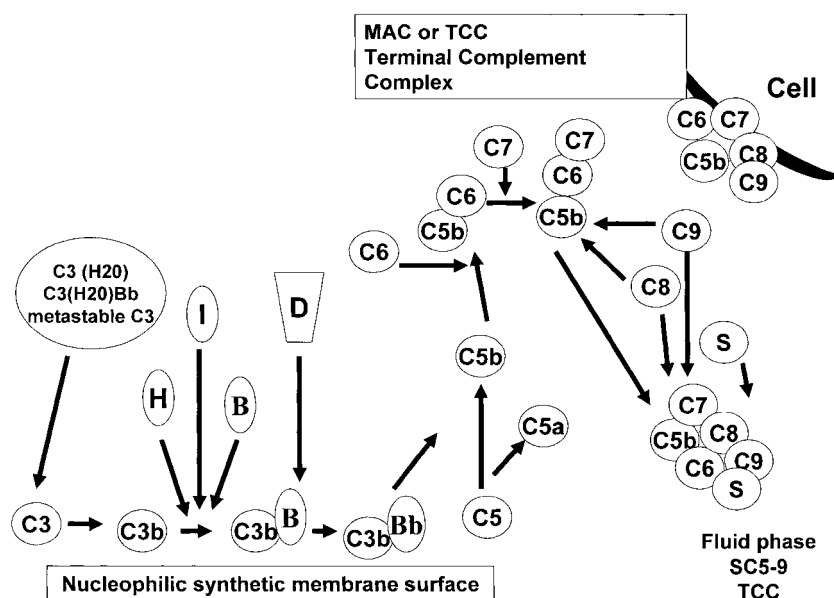


Fig. 2. Alternative pathway of complement activation by artificial membrane surfaces. Generation of the terminal complement complex (TCC) from C3 activation.

The formation of C3 convertase is regulated by factor D, a serine protease produced by the adipocytes, which accumulate in uraemia because of diminished renal excretion. Factor D cleaves factor B in the C3bB complex to form the active convertase C3bBb; in the presence of excess C3, additional C3b molecules are bound, forming the C3bBb–C3b complex, which displays C5 convertase activity with final activation of C5. The latter initiates the terminal pathway of the alternative complement system [3–5] (Figure 2). The formation of the anaphylatoxins C3a and C5a, and the factors Ba, C3b and TCC induce cytotoxic action on monocytes, polymorphonuclear cells (PMNs)

and platelets, and this effect is parallel to cytokine gene transcription and translation [6]. Moreover, high TCC levels positively correlate with intracellular calcium signalling in PMNs, with release of elastase and oxidative stress products [7].

The complement pathway-activating factors C3, factor B and mannose-binding protein are acute-phase response elements (APREs) and their concentrations are elevated in patients undergoing dialysis [8].

Cellulosic membranes providing nucleophilic sites, expressing OH residues, are strong complement activators, inducing a C3 turnover of up to 70%, while polymeric microdomain surfaces, based on a polymer

blend of polyarylethersulfone, polyamide and polyvinylpyrrolidone, are less active in this regard [9].

Another effect due to complement activation during haemodialysis is the neo-expression of adhesion molecules expressed on leukocyte surfaces, particularly CD11b/CD18, rendering them extremely adhesive to the endothelial cell layer, already activated as demonstrated by the high levels of plasmin-antiplasmin complex or soluble factor VIII (von Willebrandt factor) and E-selectin at the end of the dialysis session [10]. Simultaneously with white cell activation, highly complement-activating cellulosic dialysis membranes induces the neo-synthesis of transforming growth factor- β (TGF- β), a regulatory molecule in the pathogenesis of vessel sclerosis.

The activation of the complement cascade is magnified by the non-enzymatic glycation of CD59 (a physiological down-regulator of the TCC), secondary to carbonyl and oxidative stress. This leads to an inability to block TCC functions, leading to increased release of growth factors from endothelial cells [11].

Besides poorly biocompatible cellulosic membranes, other methods of complement activation are represented by oxidized lipids, lipopolysaccharides (LPS) and other bacterial wall products due to water or dialysate contamination.

Chronic infections can perpetuate a state of inflammation; in fact, the APR C-reactive protein (CRP) forms complexes with polysaccharidic bacterial wall products, and these complexes are able to activate complement, amplifying tissue damage [12].

Peritoneal dialysis patients have lower concentrations of CRP, suggesting that high CRP levels detected in haemodialysis patients could be due mostly to chronic bioincompatible reactions enhanced by the extracorporeal circuit [13,14].

The inflammatory role of complement in the development of vascular lesions has been demonstrated further by the detection of the TCC in the intima of the vessel walls [15]. Moreover, lipids extracted from fibrous atherosclerotic plaques are potent complement activators [16]. The presence of the TCC in the intima, close to the vascular smooth muscle cells (VSMCs) that do not express CD59, leads to a continuous stimulation of VSMCs with production of chemoattractants, including monocyte chemoattractant protein 1 (MCP-1), a strong signal for recruitment of monocytes [17]. The presence of CRP, C3 and C4, together with the TCC in the intima overlying sclerotic vessel lesions points toward the role of complement activation in the inflammatory disease during RRT.

The identification of a significant involvement of complement in vascular inflammation during chronic dialysis allows us to speculate on manipulations aiming to reduce the effects of chronic complement activation, including use of complement-regulating proteins, decay-accelerating factors (DAFs), CD59, soluble CR1 or blockade of TCC assembly [18].

Another approach may be the down-modulation of factors D and Ba by haemofiltration or high flux dialysis, resulting in reduced TCC production [19].

C-reactive protein (CRP)

Human CRP is constituted by five identical covalently bound non-glycosylated and non-phosphorylated subunits, encoded by a gene on chromosome 1 [20,21]. Each subunit consists of 206 polypeptides with an intrachain disulfide bond [22]. CRP is a multifunctional protein: it binds to pneumococcal C-polysaccharide, has several calcium-dependent binding specificities and acts as an aspecific host defence factor. Moreover, CRP is an opsonin for bacteria, parasites and immune complexes, and it activates the classic complement pathway [23].

This immunomodulatory protein has either pro- or anti-inflammatory properties. For example, it is worth mentioning that CRP binds to chromatin, histones and ribonucleoproteins, preventing the initiation of nuclear antigen-specific autoimmunity in necrotic tissues, explaining the need for high levels of CRP and SAA (circulating pentaxins) sharing similar binding activities during inflammation [24].

Even if several factors initially released at the site of inflammation by activated phagocyte mononuclear cells and lymphocytes [including interleukin (IL)-1 β , tumour necrosis factor (TNF)- α , TGF- β , leukaemia inhibitory factor, interferon (IFN)- γ , steroid, IL-11, oncostatin and retinoic acid] [25–27] are able to enhance the transcription of the CRP gene in the hepatocyte; the most powerful factor is IL-6 [28]. Not only does IL-6 dramatically increase the transcription of CRP mRNA but its final protein synthesis is also regulated by translational and post-translational mechanisms. IL-1 β significantly amplifies the stimulatory effect of IL-6 [28]. The promoter of the human CRP gene contains two APREs. APRE1 has a binding site for the liver-specific transcription factor HNF1, and APRE2 has an HNF1-binding site (β site) and an IL-6-binding site (α site) [29]. Another transcription factor is NF-IL-6, activated by a protein kinase C-dependent mechanism: IL-6 activates NF-IL-6 which in turn activates the α site and enhances binding of HNF1 to the β site, resulting in the cooperative liver-specific induction of CRP [30] (Figure 3). Post-transcriptional events in the endoplasmic reticulum further enhance the release of CRP.

In normal conditions, an acute-phase response lasts a few days, while in the case of chronic or periodically recurring inflammations, as in the case of repeated bioincompatible reactions, the acute-phase response may participate in the development of tissue damage leading to further complications, such as cardiovascular disease or reactive amyloidosis. The predictive value of CRP for myocardial infarction and ischaemic stroke in apparently healthy men has been demonstrated recently in a prospective study [31]. The mechanisms that relate the levels of CRP to vascular damage are unclear.

The role of CRP in the genesis of cardiovascular disease in dialysed patients is controversial, and CRP has often been considered an epiphenomenon rather than a pathogenetic mechanism. However, CRP and

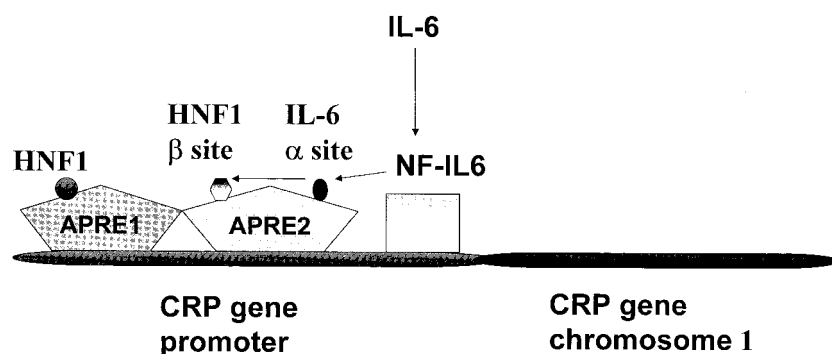


Fig. 3. Regulation of CRP gene transcription.

activated complement are localized in inflamed tissue [32], including atherosclerotic vessels and infarcted myocardium, suggesting a pathogenetic role for both factors.

Lagrand *et al.* postulated the flip-flop hypothesis, starting from the observation that membrane phospholipids may constitute ligands for CRP [33]. The inner and outer leaflet of the cell membrane differ in their composition of phospholipids: sphingomyelin and phosphatidylcholine are present in the outer leaflet, while phosphatidylserine and phosphatidylethanolamine are present in the inner leaflet. During ischaemia or apoptosis, the symmetry is lost, with an exchange of phospholipids between the two sites of the membrane (flip-flop) [33]. The flip-flop of the membrane generates ligands for CRP during activation of the classic complement pathway, with amplification of the inflammation [33].

A strict correlation has been found between CRP levels and the cross-sectional calculated intima media thickness and the mean luminal diameter [34].

In conclusion, CRP expresses an enhanced hepatic synthesis of proteins activated by a condition of chronic or repeated immune challenge. While its role as a marker of immune system activation is clear, its pathogenetic role in the development of vessel damage is still under investigation. The CRP binding to C1q leading to complement fixation and activation suggests a likely pathogenetic role.

T-cell, B-cell and monocyte function

The cells of the immune system are influenced by the toxic effects of uraemia and by different dialytic procedures. It is known that patients on RRT are at high risk for infectious complications. B cells are able to recognize polysaccharide antigens directly, while they need specific T-cell help for recognition of other antigens. It appears that in uraemia, B-cell function is normal [35], while there is a defect in T-cell function [35,36].

The leukocyte number in dialysed patients is normal, while there is a relative lymphopenia with an impressive monocytois. The $CD4^+/CD8^+$ ratio is

normal [37], but *in vitro* a clear defect in lymphocyte proliferation with a strongly reduced synthesis of $IFN-\gamma$ and IL-2 [38] is observed. Experimental studies suggest that in dialysed patients the defect does not involve the T cell itself but rather the co-stimulatory mechanisms of the antigen-presenting cells (APCs) [39]. Accordingly, the B cells have a normal functional activity in uraemia in the absence of APCs. In haemodialysis patients, the expression of co-stimulatory molecules, such as B7-2 (CD86) which binds to CD28 on T cells, is significantly reduced, while the expression of B7-1 (CD80) and the antigen class II is not affected. B7-2 transmits signals that directly induce the synthesis of IL-2, which is significantly reduced in haemodialysed patients [36]. Since the addition of IL-2 to uraemic T cells *in vitro* restores their proliferative capacity, a chronic unspecific activation has been hypothesized.

$CD4^+$ T cells can differentiate into the Th1 or Th2 T subset: Th1 lymphocytes produce high amounts of $IFN-\gamma$ and are effectors of cellular immunity, while Th2 cells mainly secrete IL-4, promoting B-cell function. In uraemia, both Th populations are reduced due to the defective synthesis of IL-2, the regulatory cytokine in the expansion of either Th population. In haemodialysis, a relative prevalence of Th1 $CD4^+$ T cells has been reported, probably related to the monocytic overexpression of IL-12, a cytokine driver in Th1 differentiation [40].

During the interdialytic interval, the cytokine production from monocytes is normal, even though these cells release large amounts of pro-inflammatory cytokines (IL- 1β , IL-6 or TNF- α) under stimulation, in comparison with healthy controls. This phenomenon is known as 'pre-activation' of monocytes and indicates a state of chronic intermittent inflammatory activation. This condition fits very well with a clinical immune defect. A key molecule in this complicated mechanism is IL-10, also produced by monocytes as a counter-regulatory mechanism aimed to limit the pro-inflammatory response [41]. IL-6 and IL-10 production by monocytes is inversely correlated, explaining the role of IL-10 in relating inflammatory parameters with the clinical immune defect. Uraemia, haemodialysis and the consequent continuous complement activation induce the synthesis and release of IL-1 and IL-6,

which have a physiological role in the immune defence. However, in the absence of specific pathogens, this response results in immune failure. High levels of IL-1 and IL-6 are followed by secretion of IL-10, in an attempt to limit the inflammatory response [42]. IL-10 gene polymorphism that detects high or low producers of IL-10 explains the differences in the individual immune system function in haemodialysed patients [43].

Cytokine production

More than 15 years ago, Henderson *et al.* proposed the 'interleukin hypothesis' considering the secretion of IL-1 produced during dialysis as the factor enhancing acute and chronic inflammatory responses [44].

Several aspects, including morbidity and mortality of dialysed patients, are related to the production of cytokines by peripheral blood mononuclear cells (PBMCs). IL-1, IL-6, IL-8, TNF- α and MCP-1 are the cytokines and chemokines most involved in the bioincompatibility reactions during haemodialysis [45–49]. Cytokine production during haemodialysis results from: (i) a direct contact of PBMCs with the dialytic membrane; (ii) complement activation with active fragments (C3a, C5a and C5–9) during extracorporeal circulation; and (iii) backtransport of bacterially derived material (i.e. LPS) from the dialysate to the blood compartment.

An important role is played by the dialysis membrane using favourable monocyte adherence, which leads to specific and selective mRNA expression for monocyte mediators and proto-oncogenes [50]. Additionally, the active complement fractions, including the TCC, are able to stimulate cytokine gene expression and secretion by monocytes. High levels of TCC are a potent stimulator of monocytes to produce TNF- α and IL-6 [45–49]. Finally, contaminant LPS is *per se* able to induce release of TNF- α and IL-6 [45–49].

However, the abundant literature on cytokines in haemodialysis is controversial. It has been shown that stimulation of cytokine gene transcription is not always followed by message translation and protein secretion. Moreover, the matter is complicated further by high levels of circulating soluble receptors during dialysis (i.e. TNFr) rendering the role of cytokines in haemodialysis-induced inflammation a complex and still debated issue [46–53].

Oxidant stress

A perfect balance between oxidative and antioxidative mechanisms represents a '*conditio sine qua non*' for human well-being. Oxidants result from the production of a large variety of dangerous ROS originating from the superoxide anion, a product of the respiratory chain in the internal mitochondrial membrane. Increased production of superoxide anions results from

other enzymatic processes such as activation of NADPH oxidase during PMN and monocyte stimulation, xanthine oxidase in ischaemic injury and cyclo-oxygenase during prostaglandin synthesis. The contemporary presence of other free radicals such as NO or transition metals leads to production of hydroxyl or peroxy radicals, the most deleterious peroxidative agents.

Individual detoxifying antioxidant ability results from a complex system of molecules (tocopherol, carotenoids, ascorbic acid, selenium and others) and enzymes (such as catalase, superoxide dismutase and glutathione peroxidase) acting as free radical scavengers.

The oxidative stress acts by inducing peroxidation of membranes and circulating lipids, damaging proteins and nucleic acids and interacting with cytokines and NO.

It has been well established that ESRD patients undergoing haemodialysis present an imbalance in oxidative equilibrium, essentially characterized by a reduction in oxygen radical scavenger activity and an enhanced production of ROS. This results from different factors: uraemia-associated metabolic abnormalities, haemodialysis *per se* and drugs [54–58].

During chronic renal failure, an enhanced expression of receptor-mediated oxidative burst has been described, and the inability of TNF to increase the oxidative burst further suggests that uraemic PMNs are in a primed state [59].

Concomitantly with an increased production of ROS, the activity of the scavenger system is severely impaired in uraemia, with a reduction in both enzymatic pathways (glutathione peroxidase) and concentration of scavenger molecules such as selenium and vitamin C: the consequence is an increased peroxidative activity [60].

Markers of lipid oxidation, such as malonyl-dialdehyde (MDA) [60], and of protein oxidation, such as advanced oxidative protein products (AOPPs), are elevated in ESRD [58]. Another marker of oxidative stress in uraemia is the enhanced LDL oxidation, an indirect index of circulating lipid peroxidation [61]. The oxidized LDL assumes antigenicity, and the related synthesis of antibodies against oxidized LDL is characteristic of uraemic patients. It is at present well known that these antibodies play a pathogenetic role in the development of vascular sclerosis in patients undergoing dialysis, leading to *in situ* activation of monocytes/macrophages, formation of foam cells and proliferation of VSMCs [61].

The levels of AOPPs increase during the progression of chronic renal failure and even more during haemodialysis [62,63]. Moreover, AOPPs are in a close, direct relationship with advanced glycation end-products (AGEs) and monocyte activation markers during dialysis. Both AOPPs and AGEs are strong activators of the respiratory burst in monocytes.

The bioincompatibility of dialysis is an amplifying factor that worsens the pro-oxidant state of uraemic patients [58]. Moreover, dialysis *per se* could be a

mechanism responsible for loss of scavenger molecules, such as vitamin C [64] and selenium [65]; these losses increase further by improving the dialysis efficacy and enlarging the spectrum of solute removal by convective clearance. A reduction in activity of selenium glutathione peroxidase activity has been reported recently [65].

Not only haemodialysis devices but also drugs used to restore metabolic abnormalities and correct the uraemic syndrome are involved in the production of ROS: among others, iron and erythropoietin (EPO) are most frequently evoked [66]. Iron, as the transition metal in the Fenton reaction, is one of the major inducers of hydroxyl radical formation, a reaction involving hydrogen peroxide and the superoxide anion [67,68]. EPO itself enhances the superoxide anion of FMLP [*N*-formyl-methionyl-leucyl-phenylalanine]-stimulated PMNs [67,68].

Carbonyl stress

Under the oxidative stress that chronically develops in uraemic patients undergoing dialysis, proteins are modified directly by ROS with formation of oxidized amino acids. The proteins are also modified indirectly by reactive carbonyl compounds formed by auto-oxidation of carbohydrates and lipids, leading to the eventual formation of AGEs and advanced lipoxidation end-products (ALEs) [69]. AGEs, pentosidine and carboxymethyllysine (CML), and the ALE, MDA-lysine, as well as precursor carbonyl compounds derived from carbohydrates and lipids are present at high levels in plasma and tissues of uraemic patients [70–72].

A common structural feature of carbonyl compounds is a carbonyl group(s) that reacts with protein amino groups, leading to the formation of Schiff base adducts and, eventually, AGEs such as pentosidine or CML [73]. In the presence of oxidants, the formation of AGEs is accelerated and amplified by the Namiki's pathway. Other carbonyl compounds, such as 3-deoxyglucosone, glyoxal and methylglyoxal, are produced by non-oxidative mechanisms and react with proteins to form AGEs.

The increased levels of AGEs and ALEs are not a direct effect of glucose or loss of glomerular clearance. In uraemia, the increased levels of these molecules depend upon an accumulation of low molecular weight AGE or ALE precursor carbonyl compounds (such as glyoxal, methylglyoxal, 3-deoxyglucosone and MDA) [70,71].

The pathogenesis of carbonyl stress in uraemia is not completely clear; however, it follows an increased oxidative stress as demonstrated by a significant correlation between serum levels of pentosidine and AOPPs [64] and oxidized ascorbate [74], markers of oxidative stress.

The dialysis bioincompatibility, by enhancing the oxidative stress during each session, is one of the major

causes of increased 'carbonyl stress'. Since AGEs and ALEs are bound to proteins, a more significant reduction of carbonyl stress could be achieved by using more biocompatible membranes than with a better removal, with high flux techniques [17].

Carbonyl stress compounds may have a direct or indirect pathogenetic role in the appearance of the most common dialysis inflammatory complications, such as atherosclerosis or amyloidosis. They can directly modify the circulating and structural proteins and lipids: AGEs, CML, pentosidine, ALEs, MDA-lysine and hydroxynonenal (HNE)-protein adducts have been identified immunohistochemically in the amyloid deposits and in the thickened neointima of dialysed patients. Moreover, carbonyl compounds, AGEs and ALEs may directly, or in a receptor-mediated way, modify the reactivity of immune or non-immune cells. It has been demonstrated that they induce monocyte chemotaxis [75], macrophage secretion of inflammatory cytokines [75], VSMC proliferation and phosphorylation of tyrosine residues of several intracellular proteins.

Our group demonstrated that two of the major carbonyl compounds, glyoxal and methylglyoxal, are able, *via* NF- κ B activation, to induce the neo-transcription and translation of two molecules involved in the apoptotic pathway, the tumour suppressor protein p53 and the inducible isoform of cyclo-oxygenase (COX-2). The apoptotic effect on endothelial cells of glyoxal and methylglyoxal was blunted by co-incubation with antioxidants, such as vitamin C [76].

Nitric oxide (NO)

NO, an uncharged, diatomic, free radical neutral gas, is one of the most rapidly transmitted messenger molecules. The NO signal is proximity limited, since it decomposes very rapidly by reacting with oxygen and haem proteins. Its half-life in the body is ~10–30 s. NO is synthesized from L-arginine by nitric oxide synthase (NOS). Three different genes, sharing 50–60% homology, are well known and identified. Two of them are constitutively expressed in endothelial cells (eNOS) and neuronal cells (nNOS). The third isoform of NOS is inducible by immunological stimuli in virtually all nucleated mammalian cells (iNOS). The eNOS and nNOS isoforms produce NO in small puffs in response to transient elevation in intracellular calcium. The output of NO from iNOS in response to pro-inflammatory cytokines (such as IL-1 β and TNF- α) or bacterial products (LPS) is ~1000 times that of the constitutive isoforms, and is important to the body's defence against pathogens. Excessive production of NO from iNOS can be detrimental, if not deadly, and is a common feature in many inflammation-related pathologies [77].

NO is a powerful vasorelaxant and its activity is related to the activation of guanylate cyclase in

VSMCs. Elevated cGMP levels, in turn, activate a series of reactions that produce a stabilized form of myosin, resulting in VSMC relaxation. NO is also a potent inhibitor of platelet activation, acting synergistically with prostacyclin to inhibit platelet aggregation and induce the disaggregation of platelets [77]. Additionally, NO is a cytotoxic effector molecule: it can interact with ROS such as superoxide to produce peroxynitrite, a deadly effector molecule that induces severe oxidative damage to lipids, DNA and protein [77]. Also, peroxynitrite-induced breaks in the DNA molecule can activate the DNA repair enzyme poly(ADP)-ribosyl transferase and the tumour suppressor protein p53, starting the apoptotic pathway [77,78].

These features of NO suggested an involvement in dialysis complications, initially on the vascular tone in intradialytic hypotension. The 'interleukin hypothesis' strongly supported the involvement of NO in intradialytic hypotension [79,80]. Another suggestive factor for the involvement of NO in dialysis hypotension is dimethyl arginine (ADMA), an endogenous iNOS inhibitor, which is elevated in uraemia; the removal of ADMA by the haemodialysis procedure correlated with the occurrence of hypotension [81].

Over the last years, we have been focusing on NO release because we began thinking of a relationship between bioincompatibility reactions and an involvement of NO in long-term dialysis complications, particularly dialytic vasculopathy. The initiating event in the pathogenesis of vessel sclerosis is thought to be an altered endothelial cell function. Following this observation, we focused our attention on the possible role played by the reactivity of the endothelial cell layer during bioincompatibility reactions in patients undergoing repeated dialysis treatment. The blood flowing out of the dialysis devices first comes into contact with the endothelial cells, which are able to synthesize a large number of growth factors, cytokines, chemokines and vasoactive mediators [82].

In order to verify our hypothesis of an endothelial NO generation following the interaction of blood with dialysis devices, we used an *in vitro* model of dialysis with blood from healthy donors (excluding the component 'uraemia') which, after recirculation, was incubated with cultured endothelial cells (ECs). The circulation of blood on cuprophane membranes induced in ECs a progressive increase in NOS activity and iNOS mRNA expression, while PMMA and AN69 membranes failed to induce any modification. In plasma samples obtained at different time points during the *in vitro* dialysis on PMMA, AN69 and cuprophane, TNF- α and IL-1 β were undetectable, while only in lymphomonocytes dialysed on cuprophane were TNF- α and IL-1 β significantly increased at both the transcriptional and translational levels. NOS activity and TNF- α and IL-1 β synthesis were significantly correlated. Our data allowed us to conclude that dialysis *per se* could stimulate NO synthesis by the patients' ECs and that chronic haemodialysis patients are exposed to periodic stimulation of

endothelial iNOS, whose final product, NO, might be involved in the development of inflammation initiating and perpetuating the dialytic vasculopathy [82].

Besides the role of poorly biocompatible membranes in enhancing NO production, we recently demonstrated that acetate-containing buffers, even in the low amounts present in the bicarbonate dialysate, can enhance NOS activity (iNOS mRNA and enzymatic activity), while acetate-free dialysate failed to modify the EC basal NOS activity [83]. Acetate, like propionate and malonate, enters the Krebs cycle and is able to conjugate CoA to produce acetyl-CoA, a reaction that activates ATP with production of AMP and adenosine. AMP can condense into cAMP, ATP, AMP and ADP, through adenylate cyclase, are interconvertible, with final production of cAMP, which is one of the most potent stimulators of NOS.

The high levels of NO produced following these mechanisms are toxic for EC function and integrity, eliciting the process of vessel damage. Oxidative agents provoke apoptosis, and scavengers such as superoxide dismutase or glutathione prevent apoptotic death. NO carries an uncoupled electron and acts as a free radical. NO apparently induces apoptosis by promoting expression of the tumour suppressor protein, p53 [78]. The unpaired electron of NO modifies the intracellular redox state and thereby stimulates p53 expression; the translated p53 protein modulates the expression of the WAF1/CIP1 gene, which in turn induces the synthesis of the regulator protein p21. Finally, p21 inhibits the activity of cyclin E. Apoptosis ensues upon the consequent inhibition of the DNA polymerase δ isoform, the most active enzyme involved in DNA replication [78]. The nuclear expression of p53 protein we have observed in response to incubation of ECs with acetate or blood recirculated on cuprophane was blunted by co-incubation with either of two inhibitors of iNOS activity (L-NAME or aminoguanidine) or by the protein synthesis inhibitor cycloheximide. The effects of L-NAME or aminoguanidine on the synthesis of p53 lend further support to a causal role for NO in the apoptotic response. We speculated that the endothelial denudation of the vessel wall due to NO-dependent apoptosis is related to bioincompatibility reactions to the dialytic device. In this way, exposure of the underlining smooth muscle cell to unfamiliar molecules could represent an additional pathogenetic mechanisms of vessel inflammation during dialysis [77].

Concluding remarks

The coincidence of altered immune system functions due to uraemia with the repeated immune system stimulation by poorly biocompatible dialysis membranes, acetate-containing dialysis buffers and dialysis fluids with bacterial contamination results in a state of inflammation that accounts for several dialytic complications, first of all the vessel sclerotic damage.

Different therapeutical interventions can prevent or at least limit these events. Among these are: (i) an adequate treatment with discrete Kt/V could in part restore the immune system functionality by removing the molecules that accumulated in uraemia which could interfere with an adequate immune system function; (ii) the use of biocompatible membranes could limit the complement activation and cytokine release; (iii) the use of recombinant DAF, CD59, soluble CR1 or blockade of TCC assembly would reduce complement activation; (iv) high flux dialysis or haemofiltration would reduce the circulating levels of complement-activating molecules such as factor D and Ba to limit the inflammatory effects of complement activation; (v) the accurate use of sterile water. All these measures represent the basic principles to reduce the chronic state of inflammation during long-term haemodialysis. Almost all the activated noxious mechanisms result in oxidative stress. The latter represents the tip of the iceberg of the bioincompatible reactions during haemodialysis and in the initiating event of endothelial dysfunction leading to vascular sclerosis. Any attempt to limit oxidative stress seems to offer significant advantage to the patient.

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