Invited Comment

Inflammation modifies lipid-mediated renal injury

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Introduction

Clinical findings in humans suggest, and experimental studies in animal models demonstrate, that dyslipidaemia can lead to disease progression and glomerulosclerosis [1,2]. Thus, it has been established that cholesterol supplementation of the diets of several animal species leads to focal and segmental glomerulosclerosis (FSGS) [3–7] and foam cells and lipid deposits are found in focal segmental sclerosis in human renal biopsies [8]. Many of the features of progressive glomerular and tubulo-interstitial diseases share biological mechanisms with those of atherosclerosis [9,10], which affects both large and medium sized arteries and the microvasculature, as well as in progressive renal disease. A consequence of this shared pathology has led to the use of lipid-lowering drugs to assess the contribution of hyperlipidaemia to the progression of renal damage [11].

Although many animal models of diet-induced hyperlipidaemia support the hypothesis that lipid abnormalities contribute to renal injury, there are some notable exceptions to this rule. The Watanabe heritable hyperlipidaemic rabbit model, which is characterized by a deficiency of low-density lipoprotein (LDL) receptors and hypercholesterolaemia, develops atherosclerosis but not renal lesions [12]. There is also no evidence of renal disease in the Nagase analbuminaemic rat model, which is also hypercholesterolaemia [13]. In humans, familial hypercholesterolaemia is not usually associated with renal failure and renal disease rarely occurs in patients with primary hyperlipidaemias. Exceptions are massive obesity in man [14] and inherited lecithin:cholesterol acyltransferase (L:CAT) deficiency with abnormal LDL, which causes renal failure in some families [15].

Interestingly, while atherosclerosis regresses with reduction of serum cholesterol, human renal disease does not [16]. In other words, the plasma level of cholesterol per se does not correlate with glomerulosclerosis. Therefore, in the absence of experimental and clinical evidence that hyperlipidaemia alone causes kidney damage de novo in patients with normal renal function, the involvement of additional factors such as intra-renal hypertension, and inflammation are necessary for the induction and progression of lipid-induced renal dysfunction.

Cardiovascular problems are the most important cause of morbidity and mortality at all stages of progressive renal disease [17,18]. Atherosclerotic renal artery stenosis in particular contributes to end-stage renal disease (ESRD) and is especially significant in elderly patients starting renal replacement therapy [19]. Since the dyslipidaemia of established chronic renal diseases is thought to contribute to the progression of both renal and cardiovascular disease, it is doubly beneficial to regulate atherosclerosis risk factors. Large-scale primary and secondary prevention studies have demonstrated the therapeutic value of lowering lipid levels. However, lipid-lowering drugs alone do not prevent the progression of atherosclerosis or progression of renal disease in many individuals at risk. Fifty percent of patients with cardiovascular disease do not have hypercholestero-laemia and therefore, it is important to consider other factors. Multiple risk factors for atherosclerosis include homocysteine, fibrinogen, impaired fibrinolysis, increased platelet reactivity, hypercoagulability, lipoprotein (a), small dense LDL cholesterol and inflammatory-infectious markers [20]; this explains why drugs that affect only one risk factor may not arrest disease progression. In this review, we discuss the evidence associating inflammation with the pathogenesis of atherosclerosis and how inflammation may modify lipid-mediated renal injury.

Atherosclerosis as an inflammatory disease

Immunohistochemical studies have confirmed Virchow’s suggestion that atherosclerosis has features
Ross contributed to the inflammation model by developing the response to injury hypothesis; he established the idea that atherosclerosis is an inflammatory disease [22–24], the first manifestations of which are the recruitment to the subendothelial space of monocytes, monocyte-derived macrophages, and T cells, with accumulation of lipids intracellularly in foam cells and extracellularly in the matrix to form fatty streak lesions. A key role for monocyte recruitment and macrophage differentiation in atherogenesis was demonstrated in osteopetrotic op/op mice, which have reduced numbers of monocytes and macrophages because of a naturally occurring mutation for the gene coding for monocyte-macrophage colony stimulating factor (M-CSF) [25]. Atherosclerosis is reduced by 70–90% when these animals breed to apoE deficient mice, despite an increase in cholesterol levels [26]. In addition to the above, the atherosclerotic plaque contains large numbers of proliferating vascular smooth muscle cells (VSMC) and extracellular matrix that includes sulphated glycosaminoglycans, and collagen. Some of the VSMCs also contain cholesteryl ester droplets that resemble those of the macrophage foam cells [27]. This complex lesion occurs even in human fetal aortas and is greatly enhanced by maternal hypercholesterolaemia, with subendothelial accumulation of LDL, the in situ oxidation of which precedes monocyte recruitment into atherosclerotic lesions [28].

Because the inflammatory response is complex and multifactorial, it is difficult to describe the sequence of events or assign them to hierarchical positions. Perhaps LDL itself has the potential to generate pro-inflammatory signals that generate a cascade of events: the evidence suggests that oxidative products of LDL including apoB, fatty acids, oxysterols and lysophosphatidylcholine might act as pro-inflammatory mediators when trapped in the intima [29–31]. Studies in mice and humans suggest a strong correlation between the plasma levels of auto-antibodies against oxidized LDL and the extent of atherosclerosis [32]. Although there is compelling evidence that oxidative modification of LDL is the major event in transforming LDL to a pro-inflammatory mediator, Bhakdi et al. [33] proposed an alternative hypothesis that non-oxidative, enzymatic modification of LDL transforms these molecules to an atherogenic moiety. Enzymatically modified LDL activates complement through the alternative pathway and is recognized by scavenger receptors; it also induces monocyte chemotactic protein (MCP-1).

Atherosclerosis also has an association with autoimmune-mediated and infectious disease. Many viruses, bacteria and even parasites are claimed to affect atherosclerotic plaque deposition [34] and systemic lupus erythematosus is commonly associated with early onset cardiovascular disease and hyperlipidaemia in young women, an otherwise low-risk population for atherosclerotic disease. Numerous case reports and series have documented the occurrence of complications of premature atherosclerosis in adults and children with SLE in whom the frequency of clinically recognizable coronary artery disease (CAD) has been reported to be ~9%. Subclinical CAD is considerably higher in adults, approaching 40–45% [35]. In certain genetically susceptible individuals, infection with very common organisms, such as Chlamydia pneumoniae or cytomegalovirus, may lead to localized infection and a chronic inflammatory state. Cytomegalovirus may play a role in atherosclerosis in transplanted hearts, and this virus, together with tumour suppressor protein p53, can be found in re-stenosis lesions following angioplasty. Low-grade infections may also be one of the causes of the inflammatory reaction observed in atherosclerotic lesions and acute ischaemic symptoms, reflected in elevated levels of C-reactive protein (CRP) [36]. The increased incidence of death in older people from myocardial infarction in winter months in comparison with summer months is due to increased incidence of respiratory infections.

Cardiovascular complications caused by accelerated atherosclerotic disease represent the largest single cause of mortality in chronic renal failure patients. The rapidly developing atherosclerosis of the uraemic syndrome appears to be caused by the synergistic action of several different mechanisms, including malnutrition, oxidative stress and genetic factors. Chronic inflammation as evidenced by increased levels of pro-inflammatory cytokines and CRP is a common feature in ESRD [37]. Elevated serum levels of plasma CRP are associated with an increased risk of myocardial infarction and sudden cardiac death in apparently healthy subjects. In patients affected by pre-dialysis renal failure, increased levels of CRP and interleukin (IL)-6 were recorded in 25% of the population; CRP and IL-6 were inversely related to renal function. The data suggest the activation—even in the pre-dialysis phase of renal failure—of mechanisms known to contribute to the enhanced cardiovascular morbidity and mortality of the uraemic syndrome [38]. Torzewski et al. [39] demonstrated CRP deposits in the human coronary artery wall by immunohistochemical staining, along with terminal components of complement. Furthermore, the majority of subendothelial foam cells showed positive staining for CRP. These results suggest that CRP is not an innocent marker of inflammation but is involved in atherosclerotic lesion formation by activating complement and being involved in foam cell formation [40]. The acute-phase serum amyloid A proteins (A-SAA) are multifunctional apolipoproteins which are involved in cholesterol transport and metabolism, and in modulating numerous immunological responses during inflammation and the acute-phase response to infection, trauma or stress. During the acute-phase response the hepatic biosynthesis of A-SAA is up-regulated by pro-inflammatory cytokines, and circulating concentrations can increase by up to 1000-fold. In the later stages of the acute-phase response, A-SAA expression is effectively down-regulated via
the increased production of cytokine antagonists such as the IL-1 receptor antagonist (IL-1Ra) and of soluble cytokine receptors, resulting in less signal transduction driven by pro-inflammatory cytokines [41]. These markers may reflect vascular inflammation and their measurement could pinpoint the mechanisms by which cholesterol-lowering therapy and other interventions could confer benefits.

**Inflammation modifying lipid-mediated renal injury**

**Lipid-mediated inflammatory signals**

Lipoproteins might act as pro-inflammatory mediators. At certain concentrations cholesterol-rich lipoprotein (LDL) and triglyceride-rich lipoprotein (VLDL, IDL) enhanced the secretion of inflammatory cytokines, such as IL-6, platelet-derived growth factor (PDGF), and transforming growth factor beta (TGFβ) by human mesangial cells (HMCs), whereas tumour necrosis factor alpha (TNFα) secretion was stimulated by oxidized LDL (Ox-LDL) [42]. Ruan et al. [43] have shown that minimally modified LDL led to TNFα induction in rat mesangial cells. Gröne et al. [44] also showed that exposure of HMCs to LDL resulted in a transient elevation of PDGF mRNA. Studies have also investigated the role of glomerular macrophages together with hyperlipidaemia in promoting glomerulosclerosis following an initial glomerular injury [45]. In addition to its role in the recruitment of circulating monocytes [46] modified LDL induces smooth muscle cells and/or endothelial cells to produce chemotactic and adhesive factors such as MCP-1 [47], monocyte m-CSF [48], IL-1β [49], and other adhesion molecules [50,51]. Modified LDL may also inhibit the motility of resident monocytes once they have differentiated into macrophages within the intima [46]. Furthermore, Ding et al. [52] demonstrated that glomerular macrophages obtained from hypercholesterolaemic animals displayed higher expressions of TGF-β mRNA by comparison with controls. Another study demonstrated that oxidized LDL inactivated endothelial cell nitric oxide [53]. These results suggest that lipoprotein and its oxidative products might act as pro-inflammatory mediators. Lipoprotein-mediated cytokine production may cause recruitment of monocytes, lipid-mediated cell proliferation, and matrix production, thus contributing to glomerulosclerosis.

**Inflammation may also accelerate lipid-mediated renal injury by affecting cholesterol metabolism**

A number of cytokines including TNFα and the interferons increase serum triglyceride levels due to an early increase in hepatic VLDL secretion, while the late increase may be due to a variety of factors including increased hepatic production of VLDL or delayed clearance secondary to a decrease in lipoprotein lipase activity and/or apolipoprotein E levels on VLDL.

Cytokines increase hepatic cholesterol synthesis by stimulating HMG CoA reductase gene expression and decrease hepatic cholesterol catabolism by inhibiting cholesterol 7 alpha-hydroxylase, the key enzyme in bile acid synthesis [54]. Cytokines also decrease HDL cholesterol levels and induce alterations in its composition—apolipoprotein A1 and the cholesterol ester content may decrease, while free cholesterol increases. Additionally, key proteins involved in HDL metabolism are altered by cytokines: LCAT activity, hepatic lipase activity, and CETP levels fall. Thus, cytokines induce marked changes in lipid metabolism that lead to hyperlipidaemia [54]. Inflammatory mediators, including TNFα and IL-1 may induce oxygen radical production by mesangial cells [55], which may then promote oxidation of lipoprotein [56,57]. Ox-LDL is demonstrably more cytotoxic than unmodified native LDL [58]. Oxidized LDL binds preferentially to the glomerulus when injected intraarterially in the rat and binds to mesangial cells in vitro [59].

**Inflammation accelerates lipid-mediated renal injury by affecting cholesterol homeostasis at the cellular level**

Glomerular atherosclerosis is characterized by the presence of lipid-loaded cells derived from macrophages and mesangial cells, hence the latter, which share many properties of VSMC and take up both unaltered and altered LDL, should be considered in the context of lipid-mediated renal injury. Inflammatory cytokines can affect lipid uptake, so an important question is whether inflammation and inflammatory mediators can modify cholesterol homeostasis in mesangial cells and transform them into foam cells. Others have found that pre-exposure to endothelin-1 and PDGF, doubled the uptake of LDL by HMCs [44]. Several lipoprotein receptors may be involved in cellular lipid uptake, including LDL receptor, scavenger receptor(s), VLDL receptor, and LDL receptor-related protein/α2-macroglobulin receptor. Clearly no single receptor pathway is solely responsible for increased lipid uptake in lesion cells but several redundant mechanisms may contribute to the uptake and degradation of lipoproteins in atherosclerotic lesions. For example, Ruan et al. [60] demonstrated that inflammatory cytokines induced type A scavenger receptors (Scr) in HMCs. Anami et al. [61] and Quaschning et al. [62] demonstrated that human mesangial cells express VLDL receptors and VLDL also induces foam cell formation in these cells. Furthermore, we have demonstrated that inflammatory cytokines TNFα and IL-1β accelerate VLDL-induced foam cell formation (unpublished data). Ruan et al. [63,64] have reported that inflammatory cytokines can modify cholesterol homeostasis through the dysregulation of the LDL receptor. In this study, using native LDL as a ligand they showed that inflammatory cytokines could overcome sterol-induced suppression of LDL receptor and make mesangial foam cells. This process
is mediated through activation of SREBP by increasing cleavage activation protein (SCAP) expression. In the kidney, this process may contribute to progressive renal disease and chronic renal transplant dysfunction, while the same process may result in atherosclerosis in arteries. The implication of these findings is that inflammatory cytokines are important risk factors for atherogenesis and progression of renal disease.

Since intracellular lipid content is governed by both influx and efflux mechanisms, the balance between lipid uptake by the lipoprotein receptor and cholesterol efflux mechanisms, becomes important. We have observed that ATP binding cassette A1 (ABCA1) gene expression in HMCs mediates cholesterol efflux and that inflammatory cytokine IL-1β inhibits both cholesterol efflux and ABCA1 gene expression. Ettinger et al. [65] also showed that human recombinant TNFα, IL-1β and IL-6 resulted in a dose-related reduction in the concentrations of apolipoprotein (apo) A-I, apoB, and L:CAT activity in HepG2 cells. Cytokines decreased the concentration of cellular apoA-I mRNA and the hepatic synthesis and/or secretion of apolipoproteins in a dose-related fashion, which may explain, in part, the acquired hypocholesterolaemia seen during acute inflammation [65]. In this regard it is interesting that in a retrospective study of 12 nephrotic patients with progressive renal disease, heavy proteinuria continued throughout the time course of renal disease progression, but serum cholesterol gradually fell to normal levels as patients approached ESRD. No important dietary or drug changes were made during the course of deterioration in these patients [66].

Protective measures against inflammation

Lipid-lowering drugs have also been used to assess the contribution of hyperlipidaemia to the progression of renal damage. A range of drugs including clofibrate acid, cholestyramine, HMG-CoA reductase inhibitors and fish oils have been used in several animal models such as the obese Zucker rat model, the PAN nephrosis model and the renal ablation model [67–69]. Most of these studies demonstrated that reducing serum lipids ameliorated the degree of glomerular injury and had a positive effect on renal function without significantly altering glomerular hemodynamics. An essential fatty acid-free diet significantly reduced TNF and IL-1 positive glomerular cells and total deprivation of lipid mediators through essential fatty acid-deficient diets has also shown to prevent progression in glomerulonephritis in rats [70]. Lipids and immune complexes acted synergistically in lupus nephritis in mice, and fish oil rich in eicosapentaenoic acid prolongs the life of the animals [71].

There is no strong evidence from short-term controlled trials to suggest that lipid lowering slows the progression of renal disease in patients and it is difficult to demonstrate a beneficial effect of lipid lowering in conditions such as nephritic syndrome [72]. However, we have recently demonstrated that PPARα agonist bezafibrate and PPARγ agonist PGJ2 significantly increase cholesterol efflux and ABCA1 gene expression even in the presence of inflammatory cytokine IL-1β, suggesting that PPAR agonists may prevent the reduction of cholesterol efflux induced by inflammatory cytokines. Hence, new strategies for therapy should include lipid lowering, and anti-oxidant and anti-inflammatory agents.

Conclusion

Studies done in many laboratories over the last 10 years have shown an association between markers of inflammation and atherosclerosis with exacerbation of the inflammatory process during acute myocardial ischaemia and reperfusion [73] suggesting that atherosclerosis is an inflammatory process [22]. Chronic renal disease is considered to be an inflammatory disease because many markers of inflammation have been identified with this condition and the inflammatory state is augmented by components of renal replacement therapy such as the use of bio-incompatible materials for dialysis therapy [74–76]. Therefore, it is logical to extend the lipid nephrotoxicity hypothesis [1] to include the influence of inflammation. Systemic or local inflammation could be an additional factor, which influences both intracellular and extracellular cholesterol homeostasis through the regulation of lipoprotein receptors. In this respect the regulation of lipoprotein receptor centrally in the hepatic tissue and peripherally in the vascular bed is important.

References

Inflammation modifies lipid-mediated renal injury


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