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REVIEW

Role of cytokines in the pathogenesis of acute and chronic kidney disease, glomerulonephritis, and end-stage kidney disease

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Abstract: Cytokines are soluble mediators that are released from sites of local injury exposed to an inflammatory environment. In kidney diseases, cytokines can be released by circulating leukocytes and/or from activated or injured kidney cells, which in turn attract and activate leukocytes to specific sites of injury. Therefore, cytokines may act in a systemic, paracrine, or an autocrine fashion. Different patterns of pro-inflammatory and anti-inflammatory cytokines expression and activation characterize acute kidney injury (AKI), glomerulonephritis (GMN), and end-stage kidney disease (ESKD). Moreover, plasma levels of certain cytokines and gene polymorphisms for certain cytokines may have predictive value in these different clinical scenarios. The present review will compile information regarding the role of different types of cytokines in the pathogenesis of AKI, GMN, and ESKD. Both clinical data and experimental models of injury will be discussed.

Keywords: cytokines, acute kidney injury, glomerulonephritis, hemodialysis, peritoneal dialysis, end-stage kidney disease, inflammation

Introduction

Cytokines are soluble messenger proteins produced by a whole range of different cell types which exert their actions within a local environment or in a systemic manner to modify and regulate immunological and inflammatory reactions as part of their response. Under normal circumstances, cytokines are usually present at low concentrations and may not even be detectable in body fluids or tissues. Cytokine-mediated inflammatory processes have been implicated in the pathogenesis of acute kidney injury (AKI)[1] and chronic kidney disease (CKD), where endothelial and tissue injuries are associated with the release of specific mediators that may initiate the inflammatory cascade.[2] Besides having a role in disease pathogenesis, cytokines may have a predictive role. In fact, specific allelic polymorphisms can influence outcomes not only in AKI or CKD, but also in several glomerulonephritis (GMN) as well as in end-stage kidney disease (ESKD) and in renal transplantation.

Increased cytokine production has been described in ESKD patients, although markedly divergent cytokine concentrations were reported. Most of the published works concentrate on a few cytokines that were measured in plasma, culture supernatants, or in association with circulating cells.[3] Assessment of cytokine release in dialysis patients is complicated by the fact that the dialysis procedure *per se* seems to further stimulate cytokine production.[4] These studies provided a sound body of information on the chronic inflammation of renal disease. However, they do not take into account a more complex phenomenon by which many cytokines are counterbalanced by specific cytokines inhibitors. The way we gained much of our knowledge on cytokine

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action, namely by incubating cells with cytokines *in vitro*, still guides our thinking. However, this may be misleading for two reasons. First, cytokines do not exhibit their effects as a single substance that takes action on a definite cell type. One might think of the cytokine network as a "spider web" in which movement of one knot leads to the movement of many others. The second misconception is that many of the effects of cytokines are local, not systemic. As a significant proportion of the effects are paracrine, and very hard to detect, the measurement of plasma cytokine may have limited implications.

CKD may be a valid model to illustrate the cytokine network hypothesis. Pro-inflammatory cytokines are counterbalanced at several levels. For example, the secretion of inter lukin (IL)-1β is linked to the secretion of the IL-1 receptor antagonist (IL-1RA), which binds the cytokine and prevents its actions.[5] The same mechanism applies to tumor necrosis factor (TNF-α), which is counterbalanced by soluble TNF receptors.[6] One might hypothesize that the production of active cytokines and specific inhibitors is a mechanism that allows the mediators to act locally while preventing them from acting systemically. As we know, cytokines are clustered into several classes and exert a pleiotropic effect with different targets and multiple physiological actions in an autocrine and apocrine fashion. There is also a functional balance between pro-inflammatory mediators and counter regulation mediated by anti-inflammatory cytokines, such as IL-10. All stimuli that induce pro-inflammatory activation in immunocompetent cells also induce IL-10, which ultimately limits the production of inflammatory cytokines. There is a tight regulatory circle between cytokines which leads to the negative correlation between the secretions of TNF-α and IL-10.[7] Evaluation of cytokine regulatory systems is complicated by the high inter-individual genetic variation of plasma cytokine levels. Finally, it should be mentioned that different binding proteins may serve as extracellular cytokine reservoirs and protective shields against degradation of cytokines.[8] In this review, we will provide a general comprehensive overview of the role of the main cytokine families in the setting of AKI, CKD, ESKD, GMN, and transplantation, and explore their relationship to clinical outcomes, mortality, and recovery of renal function.

Cytokines and acute kidney injury

AKI occurs in 5% to 7% of hospital patients[9] with the disturbing feature of an inability since the mid 1960s to substantially lower mortality rates in AKI.[10] The financial costs are estimated to be more than eight billion dollars per year.[11] Five percent to 20% of ICU patients experience an episode of AKI during the course of their illness, often accompanied by multiorgan failure.[12] Renal ischemia is the leading cause of AKI and delayed graft function. Emphasis has been placed on tubular epithelial cell injury playing a central role in ischemia. There is growing evidence that additional mechanisms, including renal vascular endothelial injury, and dysfunction, play an important part in extending renal tubular epithelial injury, contributing to the ongoing pathogenesis of ischemic AKI.[13] Given the recent recognition of the consequences of endothelial injury and dysfunction in a range of disease states such as sepsis, hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), diabetes, and hypertension, the concept of endothelium as an organ has become widely appreciated.[14]

Clinically, ischemic AKI has classically been divided into initiation, maintenance, recovery, and extension phases. During pre-renal azotemia, cellular integrity is maintained.

In the initiation phase there is decreased renal blood flow to a level resulting in severe cellular adenosine 5′-triphosphate (ATP) depletion which in turns leads to acute cellular injury and dysfunction. These cause alterations in renal proximal tubular epithelial cells that are directly related spatially and temporally with disruption of the normal framework of filamentous actin (F-actin) in the cell.[15] The activation of epithelial and possibly endothelial cells during the early initiation phase results in the upregulation of a variety of chemokines and cytokines, including but not limited to, IL-1, IL-6, IL-8, monocyte chemo attractant protein-1(MCP-1), and TNF-α.[16] Early upregulation and release of TNF-α and activation of nuclear factor-κB (NF-κB) have been observed in an animal model or renal ischemic injury.[17] What role these early cellular response play in further worsening renal function remains to be determined.

The extension phase is ushered by two major events: hypoxia and inflammatory response. Events are more pronounced in the corticomedullary junction. During this phase cells continue to undergo injury and death, with both necrosis and apoptosis being present predominantly in the outer medulla.[18] In contrast, the proximal tubule cells in the outer cortex, where blood flow has returned to near normal levels, actually undergo cellular repair and improve morphologically during this phase. There is also continued production and release of chemokines and cytokines that further enhance the inflammation cascade. Interrupting the amplification of this inflammatory cascade may have therapeutic implications. Inhibition of TNF-α has been shown to limit the decrease in glomerular filtration rate

(GFR) occurring in the renal artery clamp model.[19] Although the extension phase is probably the most likely phase for therapeutic intervention in the ischemic AKI, there is a short therapeutic window of opportunity since inflammatory cell infiltration in the outer medullary region of the kidney is significant by 24 hours following ischemia.[20] Leukocytes may begin to migrate in as early as two hours after ischemia.[21]

In the maintenance and recovery phases cells undergo repair, migration, and proliferation in an attempt to re-establish and maintain cellular and tubular integrity. During the recovery phase cellular differentiation continues, epithelial polarity is re-established, and normal cellular and organ function returns.[22]

Regarding the functional aspects of the endothelial–leukocyte interactions, the relative roles specific leukocytes play in the injury observed in models of ischemic AKI has been a point of uncertainty. A growing body of evidence suggests that T cells may play a pivotal part in this injury,[23] although a recent study adds to the controversy concerning the role of T cells in ischemic AKI.[24] On the other hand, endothelial-leukocyte interactions mediated through complementary adhesion molecules on endothelial–cells and leukocytes play a key role in the local accumulation of leukocytes. Ischemic injury has been demonstrated to increase expression of P- and E-selectin on the surface of endothelial cells.[25]

Rearrangements of the actin cytoskeleton are important in the rapid delivery of P-selectin from its storage location. Increased expression of intercellular adhesion molecule (ICAM)-1 by endothelial cells has been demonstrated also *in vitro* in response to oxidative injury.[26] Therefore, it is feasible to target endothelial-leukocyte interactions with agents such as anti-B7-1, anti-ICAM-1 antibodies (Ab), P-selectin antagonists, platelet activating factor (PAF) antagonists, adenosine 2A receptor agonists, phosphodiesterase type IV antagonists, and TNF-α binding protein.[27]

In AKI related to sepsis, induced by bacterial endotoxins, several mediators of inflammation have been assigned a key role, among them TNF-α,[28] which is released by macrophages and other cells into the circulation following bacterial endotoxin administration.[29] While experiments using TNF-blocking agents have shown protection against bacterial endotoxin-induced mortality in mice, earlier clinical trials of similar agents in humans failed to demonstrate any improvement in survival. TNF-α mediates endotoxin-induced AKI directly in the kidney. In addition, experiments in mice deficient in TNF receptor-1 (TNFR1) demonstrated that these mice are fully sensitive to bacterial endotoxin-induced mortality, but resistant to bacterial endotoxin AKI, further supporting a renal-specific role for TNF-α.[30] TNF can cause AKI by renal cell apoptosis and neutrophil infiltration through activation of renal TNFR1 and upregulation of adhesion molecules such as E- and P-selectin and ICAM-1. Also chemotactic molecules such as IFN-inducible protein-10 and macrophages inflammatory protein-2.[31] Prevention of renal failure in septic patients receiving anti-TNF Ab has been observed.[32]

In contrast to ischemia-reperfusion injury, there is relatively little information concerning the role of inflammation in nephrotoxic AKI. Cisplatin, for example, is a highly effective treatment of certain forms of cancer, but causes AKI in many patients. In bone marrow transplant recipients who received cisplatin, severe renal dysfunction developed in 21% and the mortality rate increased 5-fold.[33] Studies have demonstrated an upregulation of TNF-α and ICAM-1 expression in the kidney in response to cisplatin.[34] In animal models of cisplatin nephrotoxicity, blocking of ICAM-1 reduces the severity of cisplatin-induced renal injury.[35] As previously mentioned, many of the cytotoxic and pro-inflammatory actions of TNF-α are mediated by TNFR1. TNF-receptor 2 (TNFR2) is thought to cooperate with TNFR1 by passing ligands to TNFR1 or forming heterocomplex with TNFR1 mediating TNF-α actions in cisplatin nephrotoxicity.[36] TNFR1-deficient mice developed several renal failure following injection with cisplatin, with severe tubular injury reflected by cast formation, loss of brush border membranes, sloughing of tubular epithelial cells, and dilatation of tubules throughout the cortex and outer medulla. In contrast, TNFR2-deficient mice had better preservation of function and the histological findings were less severe. Inhibition of TNF-α production limits cisplatin toxicity, probably through inhibition of NFκB and its upstream activator IκB kinase β, as it can be achieved with high doses of salicilates.[37] Additional studies are required to define the mechanisms of TNF-α production in response to cisplatin and the mechanisms whereby TNF-α produces renal failure.[38]

There is growing evidence of the presence of allelic polymorphisms in the promoter regions of cytokine genes that regulate the expression of cytokines.[39] Polymorphisms involving the promoter (5′-flanking) region of the TNF-α and IL-10 genes may influence outcomes in critically ill patients.[1] Several studies have evaluated the relationship between TNF-α promoter gene polymorphism and outcome in sepsis. The presence, among septic patients, of a TNF-α high producer (–308 A allele carrier) genotype was associated with a 3.7-fold increase risk of death after adjustment for severity of disease.[40] The interleukin-10 intermediate/high producer genotype was associated with a lower risk of death

upon adjustment for the multiorgan failure score, consistent with its anti-inflammatory role. Paradoxically, elevated levels of circulating IL-10 in sepsis have been shown to correlate with adverse outcomes.[41] This is not inconsistent with the anti-inflammatory role of IL-10, which is normally produced in response to TNF-α and acts to diminish transcription and production of TNF-α, and other pro-inflammatory cytokines.[42]

Studies based on differences in cytokine genotypes need to be reconciled with studies using cytokine levels, with the caveat that the latter are more subject to variability.

Genetic studies on polymorphism at position −308 and −1082 of the TNF-α and IL-10 genes have yielded no ethnic differences among Caucasians and African-Americans in a healthy US population.[43] There is considerable utility in the identification of genetic susceptibility markers that may help identify patients with AKI at greater risk of adverse outcomes, and help determine who may benefit from therapeutic interventions.

Regarding the relationship between cytokines and survival outcomes in the renal failure milieu, AKI affects 5% to 20% of ICU patients, often accompanied by multiorgan failure.[12] Severe illness of almost any etiology is accompanied by a generalized host inflammatory response.[44] Central to this process is the release of a cascade of potent inflammatory mediators into the systemic circulation, including TNF-α, IL-1β, and IL-6. It is now recognized that this intense pro-inflammatory reaction is often followed temporally by a compensatory anti-inflammatory response syndrome (CARS), during which time anti-inflammatory cytokines, including IL-10, are liberated into the bloodstream.[45] Several studies have examined the prognostic role of plasma levels of the various inflammatory mediators in predicting outcomes in critically ill patients.[46] Pro-inflammatory cytokines IL-6 and IL-8, as well as the anti-inflammatory cytokine IL-10, were significantly higher in nonsurvivors than in those patients who survived hospital discharge. The association between cytokine concentrations and in-hospital mortality was maintained even after adjusting for demographics (age, race, sex) and sepsis status.

Pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) are able to induce each other in a series of cascade events, thereby resulting in a synergistic potentiation of pathobiologic effects. High concentrations of pro-inflammatory cytokines have been reported to correlate with the prognosis of sepsis and the development of multiorgan dysfunction syndrome.[47] In many sepsis studies, the pro-inflammatory cytokines IL-1β and TNF-α predict mortality, while IL-6 and IL-8, cytokines more distal in inflammatory cascade, are significant predictors of survival. Renal failure may in and of itself confer an altered cytokine profile, even in the context of critical illness. Again, discrepancies in these studies could emerge due to the timing of cytokine determination in the overall course of illness. Also, elevated plasma levels of IL-10 in sepsis syndrome are associated with poor survival.[48] Interleukin-10 can directly inhibit the monocyte inflammatory response to endotoxin and other stimuli, and a number of studies have demonstrated that monocyte lack of responsiveness in sepsis and multiorgan dysfunction syndrome in critically ill patients is also strongly associated with mortality.[49]

Dysregulation of pro-inflammatory and anti-inflammatory cytokine networks may proceed in parallel and the overall degree of cytokine network disruption may be an important prognostic indicator. Plasma cytokine levels have a large degree of interpatient variability that could be influenced by different parameters that interplay in the critically ill patient in general. It is unlikely that GFR *per se* has a significant impact on cytokine levels in critically ill AKI patients. Instead, volume overload associated with diminished urine ouput may itself be a significant mediator of plasma cytokine elevation in AKI, similar to that observed in patients with congestive heart failure, and may account for the loss of significance between cytokine concentrations and mortality when adjusted for the impact of urine output.[50] Moreover, circulating cytokines have short half-lives, and there may be considerable intrapatient variability overtime. Several investigators have suggested that plasma cytokine levels may not reflect monocyte responsiveness to pro-inflammatory stimuli and thus, may not accurately reflect the physiologic state of the immune mechanisms at the time that they are assayed.[51]

Cytokines in nephropathies and glomerulonephritis

The recruitment of T cells into the kidney is a hallmark of human and experimental GMN and is closely correlated with renal function.[52] Infiltrating T cells can induce tissue injury, either directly by cytotoxic functions and cytokine secretion or indirectly by activating macrophages.[53] In particular, infiltrating effector T cells of the Th1 type are supposed to initiate and perpetuate renal tissue damage in crescentic and proliferative forms of GMN, which eventually leads to progressive loss of renal function.[54] Renal T cell and monocyte recruitment and subsequent tissue damage are attenuated in mice with genetic deletion of Th1 cytokines such as IL-12 p40[55] and interferon IFNγ and by blocking of Th1 cytokines

with inhibitory antibodies including anti-IL-12 p40(p35) and anti-IFNγ.[56] Furthermore, exogenous administration of IL-12 augments Th1 response and crescentic GMN. Also, elevated serum levels of IL-10 ameliorate acute and chronic renal inflammation.[57] Th2 cells, however, produce IL-4, IL-5, and IL-13 and are of central importance to IgE production and to the immunopathogenesis of allergic diseases. Their role in glomerular inflammation is less well characterized. IL-17 and TNF-α synergistically induce chemokine mRNA and protein production in mesangial cells, which are known to play an important role in renal leukocyte recruitment.[58] Tubular cells also respond to IL-17 with increased protein production of different chemokines.[59]

Diabetic nephropathy is one of the most common causes of ESKD in the western world.[60] The characteristic early changes of diabetic kidney disease are increased renal size, glomerular volume, and hyperfiltration. Later on in the incipient stage an increase in albumin excretion rate (AER) is followed by development of mesangial proliferation, glomerular extracellular matrix (ECM) accumulation, and glomerular sclerosis. Overt diabetic nephropathy is clinically characterized by proteinuria, hypertension, and progressive renal insufficiency. Cytokines have attracted attention in various aspects of diabetes research including conceivable effects on functional and structural changes in the development of diabetic kidney disease.

The TGF-βs belong to a super family of several related proteins. The kidney is a site of TGF-β production and a target of TGF-β action, as both mRNA for TGF-β isoforms and receptors and the active TGF-β proteins have been shown in all cell types of the glomerulus[61] and proximal tubular cells, including localization in both nuclei and cytoplasm. TGF-β has been shown to modulate ECM production, and notably both glomerular mesangial and epithelial cells increase synthesis of ECM proteins including proteoglycans, fibronectin, type IV collagen, and laminin in response to TGF-β.[62] In addition, TGF-β inhibits the synthesis of collagenases and stimulates tissue production of metalloproteinase inhibitors.[63] Both these mechanisms could lead to a reduced degradation of ECM and possibly contribute to matrix accumulation. In addition, there is *in vitro* evidence for TGF-β playing a part in the development of diabetic nephropathy.

High glucose concentrations increase TGF-β1 mRNA expression in both cultured mesangial cells and proximal tubular cells *in vitro*.[64] Furthermore, increased glucose concentrations *in vitro* stimulate TGF-β m-RNA expression and bioactivity, cellular hypertrophy, and collagen transcription in proximal tubules in addition to increased mesangial production of heparin sulphate proteoglycan.[65] In a study in streptozocin (STZ)-diabetic rats, an increase in glomerular TGF-β-1 mRNA was reported as early as 24 hours after the onset of hyperglycemia.[66] Intensive insulin treatment to restore blood glucose concentrations to normal attenuated the rise in glomerular TGF-β expression. Certain TGF-β types like RII protein (receptors) have been found to be increased during development of diabetes. This observation is of note because TGF-β type RII is a necessary receptor for signaling the fibrogenic response to the nucleus as described above.[67] It seems that glomerular TGF-β type RII is coordinately upregulated with the glomerular TGF-β2 isoform in diabetes suggesting that fibrogenic signaling is upregulated, which could result in the increased rate of fibrosis observed in the diabetic kidney.

The activation of the renal TGF-β system in diabetes is mediated through, apart from a direct stimulatory effect of hyperglycemia itself, an activation of the renin-angiotensin system because exposure of mesangial cells *in vitro* to angiotensin II stimulates the expression of TGF-β and ECM proteins.[68] In a porcine mesangial cell line, high-glucose concentrations increased TGF-β1 mRNA, TGF-type IR, and IIR protein receptor expressions and cellular hypertrophy whereas cellular mitogenesis and hypertrophy was inhibited by captopril, an angiotensin-converting enzyme inhibitor (ACEI), in a dose-dependent manner.[69] Enalapril, a similar agent, partially prevents the diabetes associated renal hyperthrophy and fully prevented the increase in urinary albumin excretion. Further, enalapril treatment decreased the glomerular concentrations of TGF-β type IR and TGF-β type IIIR isoforms to values below those of nondiabetic control and decreased the glomerular TGF-β type IIR to almost undetectable concentrations. The glomerular expression of the TGF-β isoforms were not greatly influenced by treatment.[70] These findings suggest that the TGF-β axis operating through a complex intrarenal system, are a statistically significant mediator of the renal changes observed in experimental diabetes. Moreover, ACE inhibition has pronounced inhibitory effects on the increased concentrations of the TGF-β receptors required for intracellular signaling through this growth factor system. Other factors that are also expressed in renal cells and have a pathogenic role in diabetic kidney disease, like insulin-like growth factors (IGFs) and vascular endothelial growth factor (VEGF), unfortunately do not have a tangible therapeutic option like that shown in the TGF-β system.

IL-6, IL-8, TNF-α, and IL-18 are also found in serum of patients with type 2 diabetes.[71] A possible explanation for this elevation is the presence of oxidative stress

mechanisms. Activation of NF-κB through oxidative stress induced by hyperglycemia increases concentrations of circulating pro-inflammatory cytokines.[72] These inflammatory mechanisms are mediated by macrophages and play an important role in the pathogenesis of diabetic nephropathy. ICAM-1 is regulated and mediates infiltration of macrophages in kidneys of patients with diabetic nephropathy and in diabetic animals.[73] ICAM-1-deficient mice are resistant to renal injuries after induction of diabetes, suggesting that inflammatory processes contribute to the development of diabetic nephropathy. IL-18 and TGF-α levels are significantly elevated in diabetic patients with microalbuminuria as compared with normoalbuminuria, whereas serum IL-6 levels were not elevated in diabetic patients with microalbuminuria. This might indicate that certain interleukins are involved in the pathogenesis of diabetic nephropathy through different mechanisms. For example, IL-18 is a potent pro-inflammatory cytokine that induces interferon-γ, which in turn induces functional chemokine receptor expressions in human mesangial cells.[74] This leads to production of other pro-inflammatory molecules, including IL-8, IL-1β, TNF-α, and intercellular adhesion molecule-1,[75] from mononuclear cells and macrophages. These molecules are known to increase in type 2 diabetes and may contribute to maintain micro inflammation in renal tissues of patients with type 2 diabetes.[76] Although the increase of certain urinary cytokines have been suggested as an early biomarker of diabetic kidney disease in experimental models, these studies remain to be validated in humans.

Minimal change disease (MCD) is the commonest cause of nephrotic syndrome in children and is also an important cause in adults.[77,78] For many years, it has been postulated that a disturbance of T cell function underlies minimal change nephropathy (MCN). This T-cell disorder is primary in nature and results in glomerular podocyte dysfunction. Immunologic triggering factors, such as viral infections or allergy, are associated with alterations in the permselectivity barrier of the glomerular capillary wall, resulting in proteinuria and nephrotic syndrome. There is also strong evidence that proteinuria in MCN is mediated by cytokines. IL-4 which is produced by T cells, and mast cells, is the key cytokine involved in the development of atopy, being absolutely required for class switching of B cells to IgE production and also promoting eosinophil chemotaxis and adherence. There is evidence for enhanced activity of IL-4 in MCN with increased serum levels, increased production by peripheral blood mononuclear cells *in vitro* and enhanced expression of IgE receptors (Fcε, CD23). Several studies have suggested that genetic polymorphisms at loci encoding IL-4 and IL-4 receptor influence the captivity of these genes or their products and are associated with genetic predisposition to atopy and/or elevated serum IgE.

Unfortunately, this association between polymorphisms in IL-4 or the IL-4 receptor and MCN has not been proven. IL-13 gene expression is also upregulated in both $CD4^+$ and $CD8^+$ T cells in children with steroid-sensitive nephrotic syndrome in relapse.[79] This was associated with increased intracytoplasmic IL-13 production by $CD\text{-}3^+$ cells,[80] as well as downregulation of gene expression on the monocyte pro-inflammatory cytokines IL-8 and IL-12. Receptors for IL-13 have been demonstrated in podocytes with the detection of mRNA expression of IL-4Rα, IL-13Rα1, and IL-13Rα2 in podocyte cultures and isolated glomeruli of human and rats.[81] Moreover stimulation of cultured monolayers of products with IL-13 resulted in decreased transepithelial electrical resistance, suggesting possible direct effects of this cytokine on podocytes. IL-13-transfected rats showed significant albuminuria worsening over time, with the development of frank nephrotic syndrome as defined by generalized swelling, significant increase in body weight, low serum albumin <30 g/L, and hypercholesterolemia. The heavy proteinuria was selective in nature consistent with MCN. The presence of IL-13 causes a decrease in the expression of nephrin, podocin, and dystroglycan at both the transcriptional and the translational levels causing an increased urinary excretion of albuminuria with foot process effacement, suggesting that these proteins are essential in maintaining the filtration barrier, thus controlling glomerular permeability. Increased IL-13Rα2 gene expression localized to the podocyte has been found in most of the glomeruli of nephrotic rats.[79] The appearance of proteinuria in mice could be targeted to the induction of CD80 through IL-13 binding, causing foot-process fusion.[82] Urinary CD80 levels are increased in patients with MCD during relapse and return to normal after remission.[83] MCD might be considered a two-step disease in which there is initial stimulation of CD80 on podocytes by IL-13 or other cytokines followed by inadequate silencing of CD80 by insufficient release of soluble CTLA-4(cytotoxic T-Lymphocyte antigen-4) which acts as an inhibitor in the expression of CD80 at the level of the podocyte.

IgA nephropathy (IgAN) is an immune-mediated disorder and the most frequent type of GMN in humans.[84] Its clinical course is variable. About 10% to 20% of patients progress to ESKD within 10 years. Several microbial or dietary antigens participate in the pathogenesis of IgAN in susceptible persons and genetic factors are likely to contribute to progression of

the disease.[85] The development of glomerular inflammation in IgAN has been associated with various cytokines. Cytokines produced during infections or other antigenic challenges may play a role in the pathogenesis of IgAN. Patients with IgAN have an increased memory repertoire of IgA1-producing B cells in their bone marrow together with high plasma levels of IgA1. Major effects of the cytokine IL-1 and TNF-α include T and B cell help, with IL-1Ra (receptor antagonist) blocking the activity of IL-1, while IL-6 affects B cell differentiation and maturation. Genetic polymorphism of IL-1 (α and β), TNF (α and β) and IL-1Ra are implicated in several inflammatory diseases. IL-1Ra gene polymorphism in IgAN has been reported in previous studies.[86] Carriage rate of IL-1RN*2 and TNF2 are associated with gross hematuria in IgAN patients, and IL-1RN*2 increases the risk of IgAN progression twofold while carriage of TNF2 was found to be protective.

The impact of IL-1 and IL-6 gene polymorphism on IgAN has not been reported. It seems possible that both pro- and anti-inflammatory cytokines play a role in the development and progression of IgAN. IgA is defectively glycosylated and the deposition in the mesangium is followed by mesangial proliferation and chronic glomerular inflammation. IL-1 is expressed in the glomeruli of these patients, and it may be involved in mesangial cell proliferation and ECM production. IL-1Ra also plays a role as a natural blocking agent in the inflammation mediated by IL-1. IL-4 synthesis by peripheral blood mononuclear cells (PBMC) in patients with IgAN was greater than in healthy subjects and patients with non-IgA mesangioproliferative GMN, and has a definite role in the pathogenesis of IgAN, modulating B cell function especially immunoglobulin class switching.[87] The source of cytokines in the kidney has been assumed to be abundant in interstitial infiltrating mononuclear cells as well as residential renal cells such as mesangial cells. In IgAN patients, IFN-γ messages in PBMC showed a trend of association with renal expression of IL-α, β, TNF-α, and IFN-γ suggesting that IFN-γ produced by PBMC might induce the synthesis of inflammatory cytokines by cells in the kidney.

Furthermore, both IFN-γ transcriptions in PBMC and serum level showed a negative correlation with creatinine clearance in IgAN in some studies. IFN-γ produced by T or natural killer (NK) cells, remaining in circulation or infiltrating the kidneys from peripheral blood, might be one of the significant factors in the process of deterioration of the GFR in IgAN. It can be speculated that enhanced activities of T cells, NK cells, and macrophages are driven by IFN-γ/IL-2 interaction as a pathogenic mechanism existing specifically in IgAN. This is consistent with the immunologic nature of IgAN. The altered cellular immunity might drive abnormal production of cytokines such as IL-4, IFN-γ, and IL-12. The balance of interactions among these cytokines may play a critical role in the pathogenesis of IgAN.

Idiopathic membranous nephropathy (IMN) is characterized by a decrease in renal function, persistent nephrotic range proteinuria (>3.5 g of protein in a 24-hour urine collection), and interstitial cell infiltration.[88] Severe proteinuria usually is associated with faster progression, and this correlation is even stronger for patients with nephritic range proteinuria. A highly significant correlation between baseline urinary protein excretion and subsequent glomerular filtration rate decline has been shown. A number of studies in the last ten years have indicated that in human glomerular diseases the severity of proteinuria correlated with tubulointerstitial infiltrates and the degree of tubulointerstitial injury predicts renal function decline better than the severity of glomerular damage. Abnormal glomerular permeability to proteins causes proximal tubular cell dysfunction and tubular activation with over expression of chemokines and fibrogenic cytokines.[89]

The mechanisms underlying the involvement of the tubulointerstitium in progressive GMN remains largely unknown. In the experimental model of Heymann nephritis (animal model that mimics IMN), osteopontin (OPN), and renal monocyte chemoattractant protein (MCP-1) was detected in proximal tubule cells congested with protein at the sites of interstitial inflammation. In addition to MCP-1 and OPN, a third powerful chemoattractant produced by activated tubular cells has been proposed: regulated on activation normal T cells expressed and secreted chemokines (RANTES). The presence of interstitial myofibroblasts α-smooth muscle actin-positive (α-SMA+) cells, which can be modulated by TGF-β and platelet-derived growth factor (PDGF), has been proposed to be the main component in the pathogenesis of tubulointerstitial injury. MCP-1, RANTES, and OPN are involved in the evolution of progressive IMN. A strong upregulation of MCP-1, RANTES (mRNA and protein), and OPN protein has been demonstrated, mainly in the cortical tubular epithelial cells in renal tissue sections of patients with a progressive disease, especially in patients with IMN. This local production of cytokines correlates with monocyte/macrophage interstitial infiltration. On the other hand, the expression of MCP-1, RANTES, and OPN was significantly milder in patients with a nonprogressive disease. The strong upregulation of these chemokines, mainly localized in the tubules, is consistent with the upregulation

of MCP-1 and OPN observed in rats with protein-overload proteinuria and with Heymann nephritis.[90] The increased intracellular trafficking of proteins in the tubules could activate the transcription factor NF-κB and consequently increase the expression of a variety of pro-inflammatory genes (MCP-1, RANTES, and OPN), inducing the recruitment of inflammatory cells. Also, a strong increase of tubuloepithelial cells expressing PDGF-BB (mRNA and protein) and TGF-β mRNA significantly correlates with the degree of tubulointerstitial damage and with the presence of interstitial myofibroblasts (α-SMA-positive cells). These myofibroblasts are the source of α1 (III) collagen. Despite all the above data, the role of growth factor in the progression of tubulointerstitial fibrosis remains poorly understood.

Focal segmental glomerulosclerosis (FSGS) is currently a leading cause of nephrotic syndrome in adults and an increasingly frequent cause of ESKD. There is a genetic polymorphism encoding key cytokine production. Increased TGF-β is an important regulator of cell proliferation and gene expression as well as podocyte apoptosis. Glomerulosclerosis has been detected in animal models of FSGS.[91] Urinary TGF-β excretion is elevated in patients with FSGS and correlates with the severity of interstitial fibrosis. Higher glomerular and tubulointerstitial TGF-β_1 expression has been observed in patients with FSGS compared with normal biopsies or minimal change disease. Administration of the potent pro-inflammatory cytokine TNF-α induces glomerular damage even in normal animals, and TNF-α neutralization has been shown to reduce glomerular injury. In patients with FSGS, elevated TNF-α serum levels and increase TNF-α release from white blood cells have been demonstrated. IL-6, a pleiotropic cytokine with both pro- and anti-inflammatory actions, is produced not only by infiltrating, but also by intrinsic renal cells.[92] There are no reports concerning the expression of IL-6 in FSGS at present. Although the role of cytokines in the development and progression of glomerular diseases is well established and TGF-β_1 gene Arg^{25}→Pro, TNF-α gene G-308A, and IL-6 G-174-C polymorphisms have been reported to influence the production of these cytokines, they appear to have no clinical relevance in FSGS. The role of cytokines in the pathogenesis and progression of renal injury are more complex than originally thought. Visibly, our understanding of the complex interactions in the network of cytokines involved in the pathogenesis and progression of FSGS is still incomplete.

HIV nephropathy (HIVAN) presents histologically with similar structural changes at the level of the glomerulus as idiopathic FSGS. HIV-1 infection of renal cells results in increased synthesis of TGF-β. This cytokine has been associated with fibrotic disease in other organs, and is linked to glomerulosclerosis in numerous models of experimental renal disease.[93] It possesses the remarkable property of stimulating its own synthesis, and has profound effects on the ECM-like synthesis of numerous matrix proteins, downregulates the synthesis of proteases acting on matrix proteins, and stimulates the expression of integrins, important in matrix formation, on the cells surface. TGF-β is significantly increased in glomeruli and the tubulointerstitium in kidney biopsies from patients with HIVAN compared to disease controls or normal kidneys. Incubation with TGF-β of human mesangial cells transfected with the HIV-1 leads to increased expression of viral genes. Renal infection with HIV-1 initiates accelerated production of TGF-β and possible other cytokines.[94] TGF-β, in addition to a positive feedback on its own synthesis, also stimulates viral replication. A vicious cycle is established whereby infection is rapidly increased within the kidney and ECM formation is driven by ever higher levels of TFG-β.

Such a scheme could account for the widespread glomerular and interstitial sclerosis seen in the kidneys of patients with HIVAN as well as the rapidly advancing renal failure which occurs as a result of it. TGF-β also causes hypertrophy of cultured rat mesangial cells, perhaps offering an experimental analog for the mesangial prominence seen in some patients with HIVAN,[95] participating as a candidate mediator of these effects after the infiltration of CD4-positive lymphocytes/macrophages and the initiation of the renal lesion at the level of the glomeruli.

In renal vasculitis, and particularly antineutrophil cytoplasmic antibodies (ANCA)-positive vasculitis, TNF-α, and IL-1β may stimulate neutrophils to present antigens of azurophilic granules on their surface making them reactive to ANCA. Consequently there is activation and degranulation of leukocytes and release of reactive oxygen species, including superoxide,[96] with subsequent damage to vascular endothelium. Increased expression of adhesion molecules (E-selectin, ICAM-1, and vascular cell adhesion molecule [VCAM-1] on the surface of endothelial cells induced by TNF-α and IL-1β may result in infiltration of glomeruli and renal interstitium by neutrophils and macrophages. Plasma levels of TFG-α in renal vasculitis were reported to be moderately or substantially increased, exclusively in ANCA-positive patients with glomerular crescents.[97] Increased plasma levels of TNF-α in renal vasculitis may reflect both increased expression of the gene for TNF-α in peripheral blood mononuclear cells and the effect of renal

insufficiency. Even though increased urinary and fractional excretion of IL-6 seems to reflect increased production by infiltrating macrophages and tubular cells, there is no definite confirmation of increased plasma levels.

In lupus nephritis there is some controversy concerning serum levels of IL-6 which were found to be either increased or normal. Serum levels of IL-6 have been suggested to be a marker for the activity of the disease and maybe specific for renal involvement in lupus. Serum and maybe urine levels of TNF-α are also increased in patients with lupus nephritis and correlate with activity of the disease and also antidouble-stranded DNA antibodies titers. Urinary excretion of IL-8 can be found in patients with proliferative forms of lupus nephritis and correlates with glomerular leukocyte infiltration and glomerular expression of IL-8. Soluble VCAM and E-selectin plasma levels have been also confirmed in patients and correlates with activity of the disease. Interestingly, in kidney biopsies from patients with proliferative lupus nephritis a podocyte specific induction of co stimulatory molecules such as B7-1 was observed.[82]

Goodpasture's GMN, is a glomerular disease characterized by loss of tolerance in humans of the noncollagenous domain of α3(IV)NC1 (alpha 3 chain of type IV collagen of the glomeruli basement membrane) resulting in antiglomerular basement membrane (anti-GBM) GMN.[98] Antibodies binding to the GBM can activate, complement, and recruit macrophages and neutrophils. Passive transfer of anti-GBM antibodies can induce disease in monkeys, rats, and mice.[99] Auto reactivity to α3(IV)NC1 is driven by IL-23 since it is detectable in the immune system in the course of the autoimmune response. In mice there is more hyporeactivity to α3(IV)NC1 in the absence of IL-23, including decreased B cell activation and proliferation, diminished autoantibody titers, less cytokine production, and fewer regulatory cells. The cellular autoimmune response against α3(IV)NC1, including T cell proliferation and activation, production of TH1, TH2, and TH17 cytokines, and delayed-type hypersensitivity (DTH) to α3(IV)NC1, was impaired in the absence of IL-23. All antigen-specific IgG sub-class titers are reduced, especially the IgG2b and IgG3 subclasses. In human anti-GBM disease, little is known about IL-23 having any pathogenic role.[100]

Glomerular crescent formation is a feature of the most severe forms of human GMN. This form of glomerular inflammation results from a DTH-like cell-mediated immune response.[101] Crescents are a feature of rapidly progressive GMN which is associated with poor prognosis and they consist of layers of cells within the Bowman's space, that after its rupture invade the glomeruli structure hampering glomerular filtration and causing renal failure. IL-1 has been demonstrated in human and experimental crescentic GMN, with infiltrating macrophages implicated as the main source of this cytokine. IL-1 promotes both glomerular, and particular interstitial, macrophages infiltration by upregulation of ICAM-1 expression.[102] In addition, IL-1 promotes glomerular cell proliferation. Although whether this is a direct action has yet to be determined. Blockade of IL-1 activity has a profound effect on the phenotype of crescents, demonstrating its effect in macrophage accumulation within Bowman's space during the progression of early to advanced fibrocellular crescentic formation. TNF-α has been described in human and experimental crescentic GMN. Blockade of TNF-α has been shown to suppress the induction of glomerular injury in rat anti-GBM GMN. IL-1 and TNF-α act through similar pathways in GMN through the transcription and activation of factor NF-κB *in vitro*. Interestingly there is no added benefit by attempting to block both cytokines simultaneously in clinical studies of GMN. Mesangial cells *in vitro* have been demonstrated to express IL-12 receptors and respond by increasing production of platelet activator factor and reactive oxygen species, consistent with an autocrine role for mesangial cell-derived IL-12 in crescentic GMN. Paracrine effects may be exerted on intraglomerular T cells, macrophages, or endothelial cells. IL-12 has also been shown to promote Th1 development and proliferation of cells with induction of IFN-γ production by resting and activated T cells. IFN-γ stimulates the release of MCP-1 and production of pro-inflammatory cytokines, including IL-12. One report suggests that the absence of the IFN-γ receptor does not alter crescent formation in anti-GBM nephritis.

There is scant data regarding anticytokine therapies targeted towards the proliferation of autoantibodies or the interaction of B and T-cells in humans. Most of it stems from case reports and new data will hopefully emerge in ongoing clinical trials in upcoming years. There are two ongoing multicenter clinical trials in lupus nephritis evaluating abatacept (fusion protein binding to CD80 and CD86 on antigen-presenting cells, blocking engagement of CD28 on T-cells) in combination with immunosuppressive therapy. Also depleting agents directed against B-cells targeted by anti-CD20 antibodies (rituximab) are currently under investigation in multicenter clinical trials. Initial results in lupus nephritis have been disappointing. Similar therapy with rituximab has been implemented in case series of steroid resistant MCD patients with poor results while a decrease

in proteinuria and viral titers were detected in patients with hepatitis C cryoglobulinemic GMN.

Cytokines and end-stage kidney disease

During hemodialysis, blood contact with a foreign surface, such as a complement-activating dialytic membrane, promotes a variety of complex and interrelated events, leading to an acute inflammatory response. In particular, activation of mononuclear cells and concomitant complement activation induce the release of an array of inflammatory mediators into the extracellular environment, including cytokines, reactive oxygen species (ROS), and nitric oxide (NO).[103] *In vitro* and *in vivo* data support the hypothesis that cytokine transcription and/or production during hemodialysis are mainly caused by direct contact of PBMCs with dialysis membrane, active complement fragments (C3a, C5a, C5b-9) generated during hemodialysis, and backtransport of bacterial-derived material,(lipopolysaccharide [LPS]), from the dialysate to the blood compartment. Cuprophane membranes stimulate IL-1 expression in monocytes in the absence of complement. On the other hand, cellulosic membranes can activate, through the alternative pathway, the complement cascade and can generate active fragments able to stimulate cytokine gene expression and secretion by monocytes. This can affect cell metabolism and constitute a potent signal for activation of monocytes to produce inflammatory mediators such as TNF-α and IL-6. The basal release of these cytokines during hemodialysis is independent of the biocompatibility features of the membrane used and is influenced more by the endotoxin content of the dialysate.[104] Molecular biology techniques have demonstrated that long-term hemodilaysis with synthetic high-flux membranes, such as polymethylmethacrylate (PMMA) and polyamide, downregulated cytokine gene expression and improved the ability of PBMCs to secrete cytokine in culture. Fever and chills commonly occur during or after dialysis in the presence of endotoxin-contaminated dialysate. This specific endotoxin-induced effect may be potentiated by the use of a bioincompatible complement-activating membranes. IL-1, IL-6, or TNF-α produced by PMBCs in the bloodstream may be recognized as pyrogenic signal by specific centers within the central nervous system.[105] They induced the synthesis of prostaglandins that represent the central mediators of the coordinate response leading to fever. For example, IL-1 may act directly on specific regulatory sites within the hypothalamus, turning up the set point of the physiologic thermostat and causing an increase in body temperature via the normal thermoregulatory pathways. This is corroborated by the presence of IL-1 receptors expressed in human hypothalamus.[106]

Sleep disorders have been reported to be high among uremic patients on hemodialysis treatment, likely contributing to the impaired quality of life experienced by many of these patients. The hypothesis stems from the theory that immune-active molecules, such as cytokines, may induce profound alterations in several neurotransmitters in the brain. IL-1β and TNF-α are involved in physiologic sleep regulation and may induce slow-wave sleep. There is a daily rhythm of TNF-α and IL-1β mRNA expression in the central nervous system, with the highest levels occurring during peak sleep periods. Moreover, IL-1β and TNF-α are part of a larger biochemical cascade involved in sleep regulation. An alteration in cytokine production may explain the altered sleep pattern observed in chronic hemodialyzed patients, particularly in those treated with bioincompatible membranes.[107]

The incidence of symptomatic reduction in blood pressure during hemodialysis ranges from 15% to 50% of dialysis sessions. Hypotension and cardiovascular instability are the most frequent side effects of dialysis, occurring both chronically in long-term hemodialysis patients and acutely during dialytic sessions, and have been related to induction of IL-1 and TNF-α synthesis in monocytes. Hypotensive responses to hemodyalisis may result from cytokine-induced synthesis of NO, a potent vasodilator *in vitro* and *in vivo*, within vascular smooth muscle cells and endothelial cells. This synthesis was demonstrated more in acetate based dialysate buffers coupled with the interaction of the IL-1β and TNF-α with bioincompatable complement-activating membranes and PBMCs.

Cytokines are well-known regulatory factors for erythropoiesis, especially in pathological conditions. Chronic inflammatory diseases characterized by high cytokine circulating levels, are often associated with anemia caused by hyporesponsiveness to erythropoietin. There is a direct correlation between erythropoietin dose and IL-6 and TNF-α production from stimulated and unstimulated cultured PBMCs. The erythropoietin effect on erythroid colony formation is clearly inhibited by these cytokines. The addition of specific anti-TNF-α and interferon-γ (IFN-γ) antibodies to this system almost completely restored erythropoietin response. There is an upregulation of genes encoding proteins known to suppress cytokine signaling, which can interfere with transcription factor like JAK-STAT involved in erythropoietin signaling. This family of genes is expressed in response to IL-1, TNF-α, or IL-6 in erythroid cells.

Patients with ESKD present with various debilitating forms of osteodytrophy characterized either by high or low bone turnover. Alterations in parathyroid hormone (PTH) and calcitriol production do not completely account for the observed abnormalities in bone resorption/formation. Cytokines can act as autocrine factors regulating osteoblast/osteoclast cell functions. IL-6 is physiologically produced by osteoblasts in response to PTH and, may induce osteoclastogenesis and bone resorption.[108] IL-1 and TNF-α may induce directly bone resorption by stimulating the development of osteoclast-like multinucleated cells and by increasing the bone-resorbing activity of formed osteoclasts.[109] These two cytokines may modulate the actions of calciotropic hormones on osteoblasts by inhibiting intracellular calcium release and inositol triphosphate production in a tyrosine kinase-dependent manner. Cytokine may modulate PTH production. Paratharoid cells express IL-8 type B receptor and respond to IL-8 incubation with marked increase in PTH expression. On the other hand, IL-1 can induce an upregulation of extracellular calcium-sensing receptors mRNA while inhibiting PTH secretion in cultured parathyroid tissue slices. Cellulosic membranes may indeed induce an increased synthesis of β_2-microglobulin via complement system activation and cytokine release. Particularly IL-1, TNF-α, and IL-6 have been shown to stimulate β_2-microglobulin release by leukocytes and endothelial cells.

Malnutrition is often present in hemodialized patients, and is a strong predictor of morbidity and mortality. Several studies suggest a role for cellulosic membranes in enhancing active catabolism, most likely through an increase release of pro-inflammatory cytokine. IL-1 and TNF-α, and particularly IL-6, appear to play a central role in both the loss of skeletal muscle proteins and the utilization of exogenously administered nutrients. IL-1 is well known to act directly on the hypothalamus, causing anorexia. The role of TNF-α in neoplasia-induced cachexia is well established, and there is now evidence of the involvement of TNF-α, formerly known as cachectin in uremia-associated malnutrition. In certain hemodialysis patients, high plasma levels of IL-6 correlated with lower albumin levels and a significantly higher weight loss over a three-year period than patients with low plasma levels of IL-6.[110] Malnutrition could depress cytokine production and potentially contribute to reduced immune responsiveness in patients on chronic hemodialysis.

There is an increasing body of evidence that uremic patients on dialysis present an increased susceptibility to infections. Particularly the use of cellulosic membranes is associated with dysfunction of phagocytic cells, NK cells, and other immunologic alterations including altered cytokine production and complement activation. Insufficient or delayed cytokine release may decrease the immune reaction and consequently increase the risk of infection. Contact of circulating mononuclear cells with complement-activating membranes results in increased gene expression for an array of different cytokines, including IL-6, MCP-1, and IL-8, without a corresponding increased translation into proteins.[111] This hypothesis is further supported by the observation of an impaired endotoxin-induced IL-1β, TNF-α, and IL-6 release from PBMCs of hemodialysis patients. The use of poor biocompatible membranes, by recurrently captivating mononuclear cells or altering Th1/Th2 balance, may contribute to downregulation of the synthesis and release of different immunoregulatory cytokines and may play a role in cell-mediated immunodeficiency of dialyzed uremic patients. In peritoneal dialysis patients, pro-inflammatory cytokines and sclerosing growth factors are actively released thought a period of at least six weeks despite apparent clinical remission from peritonitis. TGF-β modulates tissue repair cells differentiation and increased production of ECM through IL-1β. These cytokines are more likely to be released by peritoneal macrophages and mesothelial cells after continuous exposure to peritoneal dialysate with high glucose levels and acidic PH; release that is enhanced in peritonitis.

Finally, some studies have found that IL-6 levels are strong predictors of mortality in patients on hemodialysis as well as normal patients.[112] In some particular groups, mortality was associated with more frequent cardiovascular deaths, elevated C-reactive protein (CRP), low albumin, and an increased IL-6 levels. IL-6 may be the most reliable predictor of mortality in ESKD in comparison to several biochemical markers (CRP, TNF-α, and albumin).

Conclusion

There is a vast literature on cytokine derangement in patients with AKI, GMN, and ESKD. All studies are based on cytokine measurement from plasma, culture supernatants, or in circulating cells. These studies strongly support a role of inflammation in the pathogenesis of renal diseases. However, they do not take into account the complex interaction among cytokines and between cytokines and their natural inhibitors. Furthermore, they do not provide any evidence for a causative role of a given cytokine in disease pathogenesis and/or progression. In fact, although many observational studies on the role of cytokines in kidney disease are available, how effective interventions act through direct

modulation of cytokines production and/or activity remains to be established. Additionally, the complexity of the cytokines network makes it difficult to design a specific intervention and to time each intervention properly. Finally, interpretation of circulating systemic cytokines may have limited relevance to disease pathogenesis and progression since a significant proportion of cytokines effect are paracrine, and are therefore difficult to detect and monitor.

Disclosures

The authors report no conflicts of interest in this work.

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