Cytokines in Glomerulonephritis

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Summary: Cytokines play central roles in both innate and adaptive immune responses that lead to renal inflammation. They are involved systemically in cross-talk between antigen-presenting cells, leukocytes, and regulatory cells to initiate and modulate nephritogenic immunity. Within the kidney, cytokines play a central role in signaling between infiltrating leukocytes and intrinsic renal cells and orchestrate the effector responses that lead to renal damage. Glomerulonephritis (GN) is an important cause of renal inflammation leading to renal failure that results from adaptive responses targeted at the kidney. Animal models of GN have shown that cytokines play critical roles in initiation and modulation of renal inflammatory responses through their ability to modulate the T helper 1/T helper 2 balance of nephritogenic immune responses. Evidence from clinical studies is now confirming the importance of this paradigm in directing the inflammatory mechanisms, histologic patterns, and clinical consequences of human GN. Cytokines also have critical intrarenal effector roles in the development, perpetuation, and resolution of GN. The proinflammatory role of intrarenal cytokine production by leukocytes in GN is well recognized, but, more recently, the role of intrinsic renal cell cytokine production in amplifying renal inflammation has been shown in animal models of GN. Studies showing benefits of specific anticytokine therapies directed at tumor necrosis factor in human GN are now appearing.

Semin Nephrol 27:275-285

Keywords: *T cell, macrophage, intrinsic renal cell, interleukin, tumor necrosis factor, interferon*

Cytokines have diverse biological roles in growth and development, physiologic homeostasis, inflammation, repair, and fibrosis. This review focuses on the role of cytokines in directing inflammatory processes and patterns of injury in glomerulonephritis (GN). GN results in a substantial burden of renal disease and is a major cause of chronic renal failure requiring renal replacement therapies.

GN occurs predominantly in the context of pathogenic adaptive immune responses in which cytokines play important facilitatory, regulatory, and effector roles. These nephritogenic immune responses can involve systemic autoimmunity to nonrenal antigens (eg, neutrophil antigens in antineutrophil cytoplasmic antigen [ANCA]-associated vasculitis and nuclear antigens in lupus nephritis), organ-specific autoimmunity (eg, to glomerular basement membrane [GBM] antigens in anti-GBM GN), and protective immunity to a variety of exogenous antigens during which the kidney is injured as a passive bystander during the clearance of immune complexes.

Studies of the pathogenic mechanisms in GN are complicated by the fact that it is not a single disease, but occurs in response to many different antigens and has a variety of disease associations. The development of immunohistology and the widespread adoption of diagnostic renal biopsy in the 1960s revealed the frequent participation of immunoglobulin, complement, and leukocytes in GN. These findings underpin the now well-accepted concept that most forms of GN result from adaptive immune inflammation. The pattern of these immune participants has become important in the classification and diagnosis of the currently recognized forms of human GN. The development of immunologically induced experimental models of GN that produce similar patterns of injury to those ob-

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0270-9295/07/$ - see front matter
 doi:10.1016/j.semnephrol.2007.02.002

served in human GN gave strong support to the pathogenic role of adaptive responses and has allowed the initiating events and mechanisms of injury, including the involvement of cytokines, to be dissected. Studies using autoimmune-prone strains of mice[1,2] and the development of models of anti-GBM GN[3] confirmed the capacity of autoimmunity targeted either to renal or extrarenal antigens to induce renal injury similar to that seen in human GN.[4] Models of acute and chronic serum sickness confirmed that animals (and most likely human beings) could indirectly develop GN in the course of normal immunity to foreign antigens. Furthermore, individual differences in immune responses to the same antigen could lead to different pathologic outcomes, reflecting the spectrum of injury observed in human GN.[5] More recently, animal models of ANCA-associated vasculitis have been used to show that autoimmunity to neutrophil antigens can lead to renal injury with similar features to the human diseases.[6]

The identification and elimination of the causative antigens has the potential to stop the progression of glomerular injury and allow repair. This goal has been partially achieved by the prevention of exposure to many nephritogenic microbial antigens among Western populations. However, recognition of the wide diversity of antigens stimulating nephritogenic immune responses has lead to the realisation that a vaccination strategy directed against a limited number of microbial organisms will not alleviate the major burden of human GN. Research in GN has turned to the effector mechanisms of immune injury looking for common pathways and key molecules, including cytokines, which might allow targeted therapeutic intervention to halt and potentially reverse renal damage.

CYTOKINE MODULATION OF NEPHRITOGENIC IMMUNE RESPONSES

CD4+ T cells play a central role in the initiation of antigen-specific adaptive immune responses. Naive CD4+ cells can differentiate along a limited number of pathways that determine the effectors they recruit and the patterns of injury they induce. Cytokines direct T-cell differentiation along these pathways and also define their effector phenotype after differentiation. Specific intracellular signaling pathways regulate their differentiation and a number of critical checkpoints in each of these pathways have been identified.[7] Variable involvement of CD4+ T-cell differentiation pathways in the immune responses directing GN may explain the participation of distinct immune effectors, patterns of injury, and disease outcomes.[8] Knowledge of the predominant cytokines directing particular histologic patterns of injury may allow the development of selectively targeted therapeutic strategies.

The 2 major pathways of CD4+ T-cell differentiation relevant to GN are the T helper 1 (Th1) and T helper 2 (Th2) pathways. Factors determining this differentiation include the dose of antigen, the affinity of the T-cell receptor for the peptide/major histocompatibility complex (MHC) class II complex, and the cytokine milieu at the time of antigen presentation. Danger signals that indicate cell injury and/or sepsis are also major factors in directing differentiation pathways. Many microbial products initiate danger signals by binding to Toll-like receptors on antigen-presenting cells (APCs).[9] Toll-like receptors also bind products of necrotic cells[10] and damaged cells,[11] as well as hypomethylated endogenous DNA.[12] Signaling provided to APCs via these Toll-like–receptor interactions at the time of antigen presentation enhances the expression of costimulatory molecules and modulates the cytokine milieu in a way that promotes Th1-biased responses.

Th1 effector cells direct cell-mediated responses by activation of macrophages via production of interferon γ (IFNγ) and engagement of CD154 with macrophage CD40.[13] These activated macrophages have an enhanced capacity for killing obligate intracellular micro-organisms and are major effectors of delayed-type hypersensitivity responses. In responses to persistent antigen stimulation, they can promote local fibrin deposition (via tissue factor expression) and undergo epithelioid transformation and giant cell formation, which are typical features of granuloma formation.

Th1 CD4+ cells also provide help to B cells, which direct immunoglobulin class shifting

to strongly opsonizing and complement-fixing subclasses (immunoglobulin [Ig]G1, IgG3, and IgG2 in human beings; and IgG2a, IgG3, and IgG2b in mice[14]). Cytokines, including interleukin (IL)-12, IFNγ, IL-18, and IL-27, have been shown to play important roles in directing Th1 responses.[13] Th1 CD4+ cells express a characteristic profile of chemokine receptors, including CXCR3, CCR5, and CCR1,[15] and have a characteristic pattern of cytokine production (IFNγ, IL-2, tumor necrosis factor [TNF], and lymphotoxin α). Signals driving Th1 differentiation include STAT1 and the induction of T-bet, which induces the IL-12 Rβ2 chain.[16] IL-12–receptor signaling through STAT4 induces IL-18 and IFNγ production by Th1 cells.

IL-4 is the key cytokine that induces Th2 responses that direct humoral immunity characterized by production of IgG4 and IgE immunoglobulin subclasses in human beings and IgG1 and IgE in mice. The major cytokines produced by Th2 cells are IL-4, IL-5, IL-13, and IL-10 (in mice), and their chemokine-receptor profile includes CCR3, CCR4, and CCR8.[15] Th2 cells typically direct allergic responses that involve recruitment and activation of eosinophils. Th2 cytokines down-regulate Th1 differentiation (and vice versa) and are associated with induction of tolerance and T-regulatory cells. In the context of Th1-driven autoimmunity (or allograft rejection), Th2 deviation generally is seen as a beneficial (therapeutic) outcome. Th2 differentiation is signaled through the IL-4 receptor, which induces STAT6. This induces a key check point, involving induction of GATA-3, which results in IL-5, IL-13, and IL-15 expression and inhibition of Th1 signaling.[17]

Genetic disruption of IL-12 heterodimer in mice by deletion of the gene for its p40 chain has shown that Th1 cells induce common autoimmune diseases targeting the kidney directly (eg, anti-GBM)[18] or indirectly (eg, systemic lupus erythematosus).[19] The recognition that the p40 chain of IL-12 is shared by IL-23[20] led to a re-evaluation of the role of IL-12 by studies in mice with a genetic deletion of the unique components of each cytokine, the p19 chain for IL-23, and the p35 chain for IL-12. These studies suggest that IL-23–dependent, IL-12–independent pathways are important in models of common autoimmune diseases of human beings (eg, experimental allergic encephalomyelitis [EAE][21] and arthritis[22]). They provide a growing body of evidence for the existence of a third major pathway of T-cell differentiation (Th17) that is characterized by CD4+ T cells that produce IL-17 (IL-17 CD4 pathway).[23] IL-23 is not primarily required for commitment to this pathway but plays an important role in later stages of differentiation and persistence of Th17 immunity. IL-6 and transforming growth factor β are important cytokines in lineage commitment,[24] but the balance is regulated closely and changes in relative contribution can induce regulatory cells, suggesting this pathway pivots around powerful effector responses and tolerance. Th17-producing CD4+ T cells are now considered to be important in many autoimmune diseases that previously were thought to be Th1 dependent. There are currently no studies showing a role of Th17 cells in GN, but it seems likely these will soon be forthcoming.

TH1 AND TH2 CYTOKINES DEFINE PATTERNS AND OUTCOMES OF GN

Observations in human GN and interventions in relevant experimental models suggest that Th1 or Th2 predominance of nephritogenic immune responses in GN is a major determinant of the patterns and outcomes of renal injury and in many situations determines the histopathologic features that define major categories of human GN.[8]

The most accessible information is the pattern of immune effectors observed by immunohistologic examination of human renal biopsy specimens. Perhaps the 2 most clear-cut examples are crescentic and membranous GN. In crescentic GN, the prominence of delayed type hypersensitivity (DTH) effectors, fibrin, CD4+ T cells, and macrophages, together with Th1 immunoglobulin subclasses including IgG3 and IgG2a, strongly suggest that injury is induced by a Th1-predominant mechanism.[25] In membranous GN, few if any DTH effectors are encountered and the usually uncommon immunoglobulin isotype is greatly overrepresented, consistent with a Th2-driven response.[26,27] These are the most obvious situations of strongly polarized responses, in many other

cases mixed patterns of effectors suggests more balanced Th1 and Th2 involvement.

In some instances (eg, lupus nephritis), varying glomerular pathologies can be seen to result from a common autoimmune process. In type IV lupus nephritis, glomerular effectors suggest Th1 polarization of the autoimmune response,[28,29] whereas in type V lupus nephritis, response to the same autoantigens activates Th2-associated immune effectors, with a predominance of IgG4 and a few DTH effectors.[30] In lupus nephritis, Th2 predominance appears to be responsible for a less aggressive form of renal injury.

Directly assessing the Th1 or Th2 predominance of systemic nephritogenic immune responses is difficult because few target antigens have been clearly characterized and isolation of antigen-specific CD4+ T cells is difficult. However, studies of cytokines produced from activated peripheral blood mononuclear cells provide an indication of the Th1/Th2 balance of the nephritogenic responses. These patterns of cytokine production have been studied in patients with crescentic GN and show that peripheral blood mononuclear cells from patients with ANCA-associated GN[31] and anti-GBM[32] show Th1 cytokine profiles during active disease and Th2 profiles during remission.[33] Studies in patients with lupus nephritis also show correlations between Th1/Th2 cytokine profiles of blood mononuclear cells, glomerular patterns of effectors, and disease activity.[29,34,35]

Studies in experimental GN allow direct manipulation of Th1/Th2 differentiation. In experimental crescentic anti-GBM GN, a number of in vivo experiments manipulating key cytokines have explored the relative contributions of Th1- and Th2-differentiated CD4+ T cells to the development of renal injury. This model of GN is characterized by severe injury with prominent crescent formation and fibrin deposition, together with CD4+ T-cell and macrophage influx. Immunoneutralization experiments and studies using knockout mice confirm that blocking Th1 cytokines (IL-12,[18,36] IFNγ,[37,38] TNFα,[39] and granulocyte-macrophage colony-stimulating factor[40]) attenuates crescent formation, whereas administration of IL-12 augments this disease.[18] The deletion of IL-4[41] or IL-10[42] augments injury whereas the administration of these cytokines before[43] or after establishment of the disease[44] attenuates injury. Involvement of Th1 cytokines also has been shown in an autoimmune model of crescentic anti-GBM GN.[45] These studies provide strong evidence supporting the role of Th1 nephritogenic immunity in inducing injury through delayed-type hypersensitivity–like mechanisms. They raise the possibility of potential new therapeutic approaches of crescentic GN by targeting selected Th1 cytokines.

In crescentic autoimmune murine lupus GN, inhibition of IL-18 by vaccination with complementary DNA effectively reduced GN and improved survival[46] and IL-12 deficiency also provided protection from GN.[19] Protective effects of IFNγ in autoimmune anti-GBM GN[47] may be related to antiproliferative effects on autoreactive T cells in this model. In autoimmune lupus nephritis, the administration of IL-18[48] or IL-12[49] accelerates GN in lupus-prone mice. The observation that deficiency of IFNγ receptor in lupus-prone mice augments autoimmunity and GN[50] is consistent with an antiproliferative effect of IFNγ on nephritogenic autoreactive T cells. However, studies showing protection from lupus nephritis in IFNγ-receptor–deficient[51] and IFNγ-deficient mice[52] is inconsistent with this. When autoimmunity is established, the administration of soluble IFNγ receptor or anti-IFNγ decreases the severity of injury[53,54] and treatment with IFNγ aggravates GN, consistent with activation of Th1-effector pathways in murine lupus nephritis.

CYTOKINES IN THE EFFECTOR PHASE OF GN

The prototypic proinflammatory cytokines TNF and IL-1 have received the most attention regarding their role in renal inflammation, and specific therapies targeting TNF now are being used in the clinical setting. TNF is produced by a wide variety of leukocytes, including monocytes/macrophages, activated CD4+ T cells, natural killer cells, and mast cells, as well as by intrinsic renal cells (mesangial cells, podocytes, endothelial cells, and tubular epithelial cells). TNF expression is prominent in crescents, infiltrating macrophages, and tubular epithelial

cells of patients with proliferative human GN,[55] particularly in ANCA-associated GN with active glomerular lesions,[56,57] and also in membranous GN.[58] TNF infusion into rabbits results in glomerular damage with an accumulation of inflammatory cells and fibrin,[59] and in rats the administration of TNF augments endotoxin and anti-GBM antibody induced glomerular injury,[60] whereas genetic deficiency of TNF in mice provides protection against crescentic GN.[61,62] Several studies have shown beneficial effects from the inhibition of TNF in experimental models of GN.[63-68] In Wistar-Kyoto rats, treatment with anti-TNF antibodies both before the initiation of disease[66] or after crescentic injury has been established[67] has been shown to significantly attenuate crescentic glomerular injury. The inhibition of TNF using soluble p55 TNF receptor–Ig fusion protein also is effective in prevention and early treatment protocols.[64,65]

Evidence for the involvement of TNF in severe forms of crescentic GN and the clinical success of TNF-inhibiting therapies, such as anti-TNF antibodies (infliximab) and recombinant soluble TNF-receptor–Fc fusion protein (etanercept) in rheumatoid arthritis[69] and of infliximab in Crohn's disease, has stimulated interest in similar therapy for ANCA-associated crescentic GN.[70] Initial small studies suggested that positive outcomes in ANCA-associated vasculitis resulted from treatment with anti-TNF antibody[71-73] and soluble TNF receptors.[74,75] A larger study of these therapies has shown rapid responses associated with infliximab in ANCA-associated vasculitis but with a significant incidence of relapse and infection.[75] Although a high rate of sustained remissions has been reported in a randomized controlled trial of entanercept in Wegener's granulomatosis, no significant difference was observed between the patients treated with standard therapy and with or without entanercept.[76]

IL-1 has proinflammatory roles in innate and adaptive immunity in addition to its important physiologic roles in the brain and hypothalamic-pituitary-adrenal axis.[77] IL-1 has 2 biologically active isoforms: IL-1α, which is mainly cell-membrane associated, and IL-1β, which is a principal soluble product of monocytes and macrophages.[77] Other cellular sources of IL-1β include neutrophils, lymphocytes, fibroblasts, smooth muscle cells, endothelial cells, and intrinsic renal cells.[78,79] Cellular responses to IL-1 are mediated principally through the type I IL-1 receptor (IL-1RI), which is widely expressed on a variety of tissues and cells, including lymphocytes.[80,81]

Prominent IL-1 expression has been shown in glomeruli in crescentic forms of human[56,78,82] and in experimental GN.[83] Glomerular macrophages (in rabbits)[84] and mesangial cells (in rats)[83] have been shown to be important sources of renal IL-1 production. Studies in murine crescentic GN have indicated that IL-1β is the major proinflammatory isoform mediating glomerular injury.[39] The benefits of inhibiting IL-1 using anti–IL-1 antibodies,[63] soluble IL-1 receptors, or IL-1–receptor antagonist (IL-1ra)[65] have been shown in experimental GN. In crescentic anti-GBM GN, several studies have shown the benefits of treatment with IL-1ra, with reduction of renal injury and reduced expression of other inflammatory proteins in the kidney.[85-88] IL-1ra treatment also has been shown to be effective in halting the progression of established crescentic GN.[89] The infusion of transfected mesangial cells expressing IL-1ra can induce IL-1ra expression in glomeruli[90] and transplantation of mice with bone marrow cells transfected to express IL-1ra protected against injury induced by anti-GBM antibody.[91] Because recombinant human IL-1ra (anakinra) has been shown to be safe and effective in combination with methotrexate for patients with rheumatoid arthritis,[92] it may prove a clinically useful specific anticytokine therapy in glomerulonephritis.

CONTRIBUTION OF CYTOKINE FROM INTRINSIC RENAL CELLS TO INFLAMMATORY INJURY

Cytokine production by APCs and lymphocytes is well documented to play a central role in the initiation, differentiation, and amplification of adaptive immune responses and the role of leukocyte-derived cytokines as effectors of inflammation is well recognized. The contribution of cytokine production by parenchymal cells within organs to local inflammatory responses and repair is less clearly documented. Various

cells of the glomerulus and the tubular epithelium have been shown to both synthesize and respond to cytokines in vitro. In inflammatory renal injury, these cytokines can be produced both by infiltrating leukocytes and intrinsic renal cells themselves. Some progress is now being made to unravel the complex network of interactions within the kidney by which cytokines from leukocytes and intrinsic renal cells contribute to inflammatory renal injury.

The relative contributions of cytokine production by leukocytes and intrinsic renal cells to the development of injury in experimental crescentic GN have been explored using chimeric cytokine-deficient mice. These chimeras, in which cytokine production is restricted to either bone marrow–derived or non–bone marrow–derived cells, are created by the transplantation of cytokine-deficient bone marrow into normal mice or vice versa. Previous studies of rabbits with crescentic GN showed that infiltrating glomerular macrophages are an abundant source of TNF,[93] but were unable to address their contribution relative to other cells capable of producing TNF. Studies using TNF chimeric mice have shown a dominant role for TNF production by intrinsic renal cells in the development of crescentic GN[62] because the extent of histologic and functional attenuation of glomerular injury in mice with TNF-deficient kidneys and normal leukocytes was similar to that seen in mice with complete TNF deficiency. In contrast, the development of proliferative GN in chimeric mice with TNF-deficient leukocytes but intact intrinsic renal cell–derived TNF was similar to that in normal mice.

The relative contribution of leukocytes and intrinsic renal cells to IL-1 production in crescentic GN has been studied in IL-1β chimeric mice produced by bone marrow transplantation. These studies showed a dominant role for leukocytes as the source if IL-1β production in crescentic renal injury because histologic and functional protection afforded by selective absence of leukocyte IL-1β production was equivalent to the protection seen with complete IL-1β deficiency.[94] In addition, glomerular production of TNF was reduced significantly in the absence of leukocyte IL-1β, whereas crescentic injury and glomerular TNF expression were minimally affected in the absence of intrinsic renal cell IL-1β production. These studies suggest a minor overall contribution of renal-derived IL-1β to crescentic GN. Intrinsic renal cells appear to be the dominant target for IL-1 β because absent renal expression of this receptor provides similar protection to complete IL-1RI absence in murine crescentic GN.[94] These studies in IL-1β and IL-1RI chimeric mice, together with the studies using TNF chimeric mice,[62] suggest a sequence of the involvement of cytokines in the development of crescentic injury in which IL-1β from leukocytes acts principally via IL-1RI on intrinsic renal cells to stimulate renal TNF production and crescentic injury.

INTRARENAL ROLES FOR IMMUNOMODULATORY CYTOKINES

IL-12 and IFN-γ have well-established roles in the initiation of adaptive immune responses, particularly in promoting the development of Th1 cells, which drive cellular immunity and delayed-type hypersensitivity. IL-12 production by APCs promotes Th1 differentiation[95] and IFNγ production by T cells amplifies their IL-12 responses[96] and activates macrophages.[97] The contribution of these cytokines to the development of nephritogenic immune response has been studied in various experimental models of GN, but their local contribution within the kidney is defined less clearly.

IL-12 deficiency attenuates crescentic GN in mice whereas administration of IL-12 exacerbates injury[18] in murine anti-GBM GN and in murine autoimmune "lupus-like" models, absence of IL-12 also appears to provide protection from nephritis.[19,98] These effects of IL-12 appear to be mediated via effects on the Th1/Th2 balance of the nephritogenic immune response. The local role of IL-12 in renal inflammation is less well defined. There is limited evidence for increased renal IL-12 expression in human GN (in patients with class IV lupus nephritis)[30] but stronger experimental evidence for intrarenal involvement in experimental models of GN.[45,99] The in vivo contribution of IL-12 production by intrinsic renal cells to inflammatory renal injury has been shown using p40 IL-12–deficient chimeric mice.[100] These

studies have shown that deficiency of renal IL-12 production reduces crescentic GN without attenuation of the systemic immune response to the nephritogenic antigen,[100] indicating a proinflammatory role for renal-derived IL-12 in addition to the systemic role of IL-12 in the initiation and amplification of nephritogenic Th1 responses. Because the p40 subunit of IL-12 is also a component of IL-23, the potential contribution of IL-23 to these effects needs further evaluation.

IFNγ is produced predominantly by activated T cells and natural killer cells and augments Th1 responses by inducing IL-12–receptor expression on T cells and up-regulating MHC expression on macrophages.[101] Renal IFNγ expression has been detected in human[30,57,102] and experimental GN,[45,103] but its intrarenal effects in GN have not been as well studied as its effects on nephritogenic immune responses. IFNγ inhibits the proliferation of mesangial cells,[104] up-regulates their expression of FcR1,[105,106] MHC II,[105] and chemokines,[107] and also may inhibit intrarenal proliferation of macrophages in murine lupus.[108] By using IFNγ chimeric mice, a significant contribution of IFNγ production by both leukocytes and intrinsic renal cells was shown in crescentic GN.[109] Up-regulation of renal MHC II expression showed a greater dependence on intrinsic renal cell IFNγ production than leukocyte IFNγ production. IFNγ expression was identified on tubular cells, whereas CD8+ cells were the principal leukocyte producing IFNγ in nephritic kidneys. Although CD4+ cells in the spleen produced IFNγ, production by CD4+ cells in the kidney could not be detected. This suggests a key role for leukocyte-derived IFNγ from secondary lymphoid organs in directing nephritogenic Th1 responses, but a dominant role for intrinsic renal cells as the major source of IFNγ in nephritic kidneys.

SUMMARY

Cytokines play diverse roles in the inflammatory injury associated with GN. They have central roles in the modulation of nephritogenic immune responses, which direct different effector pathways and pathologic outcomes. Cytokines are potent effectors of injury within the kidney where they are produced by both infiltration leukocytes and intrinsic renal cells. This review has emphasized the proinflammatory roles of cytokines in GN. However, it also should be recognized that manipulation of the cytokine milieu can direct immune responses along pathways which activate less injurious effector mechanisms in GN. Therapies targeting specific cytokines show promise in the treatment of human GN.

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