

Functions of TNF and its Receptors in Renal Disease: Distinct Roles in Inflammatory Tissue Injury and Immune Regulation

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Summary: Tumor necrosis factor (TNF) α is a potent proinflammatory cytokine and important mediator of inflammatory tissue damage. In addition, it has important immune-regulatory functions. Many experimental studies and clinical observations support a role for TNF in the pathogenesis of acute and chronic renal disease. However, given its dual functions in inflammation and immune regulation, TNF may mediate both proinflammatory as well as immunosuppressive effects, particularly in chronic kidney diseases and systemic autoimmunity. Blockade of TNF in human rheumatoid arthritis or Crohn's disease led to the development of autoantibodies, lupus-like syndrome, and glomerulonephritis in some patients. These data raise concern about using TNF-blocking therapies in renal disease because the kidney may be especially vulnerable to the manifestation of autoimmune processes. Interestingly, recent experimental evidence suggests distinct roles for the 2 TNF receptors in mediating local inflammatory injury in the kidney and systemic immune-regulatory functions. In this review the biologic properties of TNF and its receptors, TNF receptors 1 and 2, relevant to kidney disease are summarized followed by a review of the available experimental and clinical data on the pathogenic role of the TNF system in nonimmune and immune renal diseases. Experimental evidence also is reviewed that supports a rationale for specifically blocking TNF receptor 2 versus anti-TNF therapies in some nephropathies, including immune complex-mediated glomerulonephritis.

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Cytokines are central mediators of both immune and nonimmune renal diseases. Tumor necrosis factor- α (TNF- α), a 26-kd type II transmembrane protein, is a prototypic proinflammatory cytokine. It is produced mostly by activated macrophages, although antigen-stim-

ulated T cells, natural killer cells, neutrophils, and mast cells also secrete TNF. Moreover, fibroblasts and intrinsic renal cells such as mesangial cells and glomerular and tubular epithelial cells produce TNF.¹⁻⁷ Many potentially noxious stimuli, physical, chemical, or immunologic, can rapidly induce TNF production and release from these cell types.^{8,9}

TNF was identified originally by its capacity to induce hemorrhagic necrosis in murine tumors,¹⁰ although its use as an anticancer agent failed in vivo as a result of severe side effects. Subsequent work identified TNF as a potent proinflammatory cytokine and key mediator of both innate and adaptive immune responses. Studies of TNF blockade in experimental models

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of arthritis and subsequent clinical trials established therapies targeting TNF as a highly effective treatment for rheumatoid arthritis.^{11,12} Today, anti-TNF therapy with specific antibodies or neutralizing receptor fusion proteins is approved for the treatment of a variety of chronic inflammatory diseases in human beings, including rheumatoid arthritis,^{12,13} Crohn's disease,^{14,15} ankylosing spondylitis,¹⁶ psoriasis,¹⁷ and psoriatic arthritis.¹⁸

However, there is increasing experimental evidence that TNF is not only an important mediator of innate inflammation but has additional functions in regulating adaptive immune responses. In regards to the latter, TNF showed both proinflammatory and immunosuppressive properties.¹⁹ The immunosuppressive activity of TNF is particularly important in protecting against potential autoimmune complications after exposure to self-antigens and has important clinical implications in patients with autoimmune disease who are subjected to TNF blocking therapies. Indeed, with its more widespread use, there is an increasing number of reports that TNF blockade induces autoimmune symptoms in some patients, including the development of autoantibodies, lupus-like syndromes, and even immune-complex glomerulonephritis (GN).²⁰⁻²⁶ Moreover, TNF blockade in multiple sclerosis caused immune activation and disease exacerbation.^{27,28}

In many experimental models of renal diseases, TNF has been reported to promote renal injury. Thus, TNF blockade appears to be a potential therapeutic strategy in human nephropathies.^{29,30} However, results of the few clinical trials reported have been controversial, with absence of any beneficial effect of TNF antagonism in some cases.^{29,31} Recent data, including from our own group, suggest independent roles for the 2 TNF receptors, TNF receptor 1 (TNFR1) and TNFR2, in mediating TNF's proinflammatory and systemic immunosuppressive TNF effects, at least in certain animal models of renal disease.

PROINFLAMMATORY AND IMMUNOSUPPRESSIVE ROLES OF TNF

TNF may coordinate the early response to injury and thus represents an important point

of regulation in inflammatory disease. The blockade of TNF with specific antibodies is associated with a reduction in the expression of other proinflammatory cytokines, such as interleukin (IL)-1 and IL-6, both *in vitro* and *in vivo*.^{32,33} TNF induces the expression of endothelial adhesion molecules and chemokines that attract inflammatory leukocytes to sites of tissue injury³⁴ and stimulates leukocytes and parenchymal cells to release additional chemokines and inflammatory cytokines such as IL-1, which facilitates the further local accumulation and subsequent activation of immunologic effector cells. Consistent with these TNF functions, TNF blockade in rheumatoid arthritis patients reduced the TNF-dependent cytokine response and diminished leukocyte recruitment to inflamed tissue.^{35,36} TNF has many additional proinflammatory functions in innate and adaptive immunity, including cell activation, proliferation, and migration, which have been reviewed in more detail elsewhere.^{8,9,37} TNF's proinflammatory actions are summarized in **Table 1**. TNF also shows important immune-regulatory properties. It is essential for the development of secondary lymphoid organ structures in lymph nodes, spleen, and Peyer's patches, and its deficiency results in the absence of germinal centers and follicular dendritic cells.³⁷ Moreover, TNF induces apoptotic cell death in both leukocytes and parenchymal cells.³⁸⁻⁴¹ This is important for the immune-regulative functions of TNF, but also may contribute substantially to organ-specific damage, as reported in some acute nonimmune nephropathies. The proinflammatory effects of TNF in the context of specific renal diseases is reviewed in subsequent sections of this article.

Recent experimental data and clinical observations have shown TNF's immunosuppressive functions, especially in chronic inflammatory and autoimmune diseases. Several cellular mechanisms could be responsible for the immunosuppressive actions of TNF. For example, chronic TNF exposure has been reported to attenuate T-cell-receptor signaling and to down-modulate T-cell proliferative responses and cytokine secretion *in vitro* and *in vivo*.⁴²⁻⁴⁴ This could prevent the development of autoreactive T cells at sites of TNF-induced inflamma-

Table 1. Proinflammatory Effects of TNF**Blood vessels**

Induces expression of adhesion molecules in endothelial cells via activation of NF- κ B
 Increases vascular permeability at sites of tissue injury
 Reduces anticoagulant properties of the endothelium
 Stimulates angiogenesis

Cells

Activates leukocytes and thrombocytes
 Induces expression of major histocompatibility complex class I and II molecules
 Increases affinity of adhesion receptors (eg, CD44)
 Mediates maturation and migration of dendritic cells into secondary lymphoid organs
 Mediates leukocyte positioning and germinal center reaction in secondary lymphoid organs
 Induces proliferation of fibroblasts and mesangial cells

Chemokines, cytokines, and other inflammatory mediators

Induces proinflammatory chemokines in leukocytes and parenchymal cells (eg, CCL2/monocyte chemoattractant protein-1 and CCL5/RANTES)
 Induces proinflammatory cytokines in leukocytes and parenchymal cells (eg, IL-1, IL-6, granulocyte-macrophage colony-stimulating factor)
 Up-regulates expression of matrix metalloproteinases
 Induces production of prostaglandins, leukotrienes, nitric oxide, and reactive oxygen species

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tion. In the mouse, TNF-dependent T-cell inhibition likely is mediated via TNFR1 because it is achieved by treatment with human TNF, which does not bind significantly to murine TNFR2.^{43,44} However, in primary human T cells, TNFR2, but not TNFR1, has been implicated in mediating this effect.⁴⁵ Importantly, TNF also mediates activation-induced apoptotic cell death in CD8⁺ T cells,⁴⁶⁻⁴⁸ providing a potent mechanism of terminating T-cell responses. Depending on the system investigated, TNFR1 or TNFR2 was implicated in this activity. In addition to the enhancing role of TNF and TNFR1 in exogenous or autoantigen-specific CD4⁺ T-cell priming,^{49,50} TNF also may stimulate regulatory T-cell responses that induce tolerance and suppress autoimmunity.⁵¹ In experimental autoimmune encephalomyelitis, a murine model of human multiple sclerosis, TNF deficiency prolonged expansion of myelin-specific autoreactive T cells, leading to exacerbated disease,⁴⁹ an effect that did not require TNFR1 activation. In contrast, TNFR1 was responsible for TNF-dependent priming of autoreactive T cells and local cerebral injury during the acute phase of

the disease.⁴⁹ Because suppression of autoimmune reactivity also was present in TNFR2-deficient mice, but not in double-deficient TNFR1 and TNFR2 mice, both receptors apparently can relay the immunosuppressive effects of TNF. These findings correlate with the observation of immune activation and disease enhancement in recent clinical trials with TNF blockade in multiple sclerosis patients.^{27,28}

An immunosuppressive role of TNF also has been shown in experimental models of systemic autoimmunity, particularly in models of systemic lupus erythematosus (SLE). Data obtained in the (NZB \times NZW)F1 mouse model of SLE indicated that the *TNF^Z* allele, which leads to decreased production of TNF, is a dominant contributor of susceptibility to autoimmunity in this model.⁵² Indeed, early administration of recombinant TNF or TNF-inducing cytokines attenuates autoimmune disease in these mice.^{53,54} When NZB mice were crossed with TNF-deficient B6/129 mice (NZB \times B6/129 *Tnf*^{-/-})F1, offspring with heterozygous deficiency for TNF developed autoimmunity and fatal lupus nephritis in an otherwise nonsuscep-

tible (NZB \times B6/129)F1 background.⁵⁵ The immunosuppressive function of TNF appears to be mediated by TNFR1. In B6/lpr mice, which develop a mild lupus-like syndrome, TNFR1 deficiency results in a greatly accelerated autoimmune disease with increased TNF levels, high mortality, severe lymphadenopathy, and lupus nephritis.⁵⁶ In contrast, a study in transgenic mice overexpressing the human TNFR2 provided proof for a proinflammatory role for TNFR2 because these mice spontaneously developed a severe systemic inflammatory syndrome.⁵⁷ Together, the data indicate that on the one hand, TNF suppresses autoimmunity (probably through activation of TNFR1), whereas on the other hand, its presence promotes organ injury (potentially through TNFR2 activation). The importance of systemic immunosuppressive functions of TNF also is suggested by clinical observations that patients subjected to anti-TNF therapies can develop lupus-related symptoms.²⁰⁻²⁶

BIOLOGY OF THE TNF RECEPTORS

TNF achieves its pleiotropic cellular and pathologic effects by binding to its 2 surface receptors: TNFR1 and TNFR2. These receptors also bind lymphotoxin- α (formerly TNF- β), but no other members of the TNF ligand superfamily. TNFR1 (p55, CD120a) and TNFR2 (p75, CD120b) are related structurally, but are functionally distinct receptors that are co-expressed on the surface of most cell types.^{58,59} They are single transmembrane glycoproteins with 28% homology mostly in their extracellular domain. In the human system, soluble TNF rapidly binds to TNFR1 with high affinity and a slow dissociation, leading to efficient activation of the receptor.⁵⁹ The dissociation kinetics of TNF from TNFR2 is 20- to 30-fold faster than from TNFR1,⁶⁰ although to date there is a lack of consensus on the affinity of TNF for TNFR2 compared with TNFR1.^{60,61} Generally, it is believed that soluble TNF leads to a greater activation of TNFR1 than TNFR2.⁵⁹ Although TNFR1 is stimulated equally well by soluble and membrane-bound TNF, TNFR2 is activated more efficiently by the membrane-bound form.⁶² Membrane-bound TNF and thus activation of both TNFR1 and TNFR2 may be highly relevant when TNF receptors are stimulated

through cell-cell interactions. Cooperation between these receptors may enhance cell signaling and may occur as a result of the formation of receptor heterocomplexes⁶³ or a process referred to as *ligand passing* in which TNFR2-bound TNF increases the local TNF concentration in the vicinity of TNFR1 receptors.⁶¹

Several mechanisms are in place to desensitize cells to TNF by down-regulating TNFR activity at the surface. Stimulation of both TNF receptors leads to internalization of the ligand-receptor complex through a coated-pit/coated-vesicle pathway that delivers the proteins to endosomal/lysosomal compartments.⁶⁴ Soluble forms of the 2 TNF receptors, which represent the extracellular portions of the membrane-associated receptors, are shed from the cell surface on cell activation and injury by the action of a metalloproteinase, probably identical to the TNF- α -converting enzyme TACE.^{65,66} The surface pool of TNFR1 may be replaced by the large reservoir of TNFR1 observed within the Golgi apparatus in many cell types, including renal glomerular and peritubular endothelial cells.^{64,67} In patients, the levels of soluble TNF receptors in serum and urine increase during acute bacterial sepsis and chronic inflammatory diseases, such as rheumatoid arthritis and SLE,^{68,69} and after TNF infusion.⁷⁰ The soluble receptors, which are capable of binding TNF, may attenuate TNF activity by competing for the ligand with the cell surface receptors.⁶⁶ TNFR shedding, as a mechanism for down-regulating inflammation, likely is functionally relevant in vivo. Knock-in mice expressing a mutated nonshedtable TNFR1 have an enhanced susceptibility to inflammatory diseases. These mice develop spontaneous hepatitis, exacerbated TNF-dependent arthritis, and experimental autoimmune encephalomyelitis.^{66,71} In human beings, structural mutations in TNFR1 that lead to a deficiency in receptor shedding result in TNFR-associated periodic syndrome, characterized by febrile episodes caused by overstimulation of TNF signaling.⁷²

SIGNALING MECHANISMS OF TNF RECEPTORS

Intracellular sequences of both TNF receptors are largely unrelated, suggesting that the 2 TNFRs

signal through distinct intracellular regions. Both receptors possess sequences that are capable of binding different intracellular adaptor proteins that link TNF-receptor stimulation to downstream signaling pathways (reviewed in MacEwan⁵⁹ and Ledgerwood et al⁷³). Ligand-occupied TNFR1 binds the TNF-receptor-associated death domain (TRADD) adapter protein through interaction of homologous regions, called *death domains*, expressed in both proteins.⁷⁴ TNFR1-bound TRADD can recruit the downstream adapter molecule fas-associated death domain (FADD) through death domain interactions, and thereby initiate apoptotic cell death through subsequent activation of caspases 8, 10, and effector caspases.

Many of the inflammatory effects of TNF, including the increase in expression of adhesion molecules and chemokines, are triggered by activation of gene transcription via the transcription factors nuclear factor κ B (NF- κ B) and activation protein-1 (AP-1). Two adapter molecules, the receptor interacting protein (RIP) and the TNF-receptor-associated factor 2 (TRAF2), mediate activation of NF- κ B and AP-1.^{59,75,76} RIP contains a death domain that allows binding to TNFR1-associated TRADD, whereas TRAF2 complexes with the N-terminal domain of TRADD (ie, outside the death domain).⁷⁷

In contrast to TNFR1, TNFR2 does not contain a death domain but binds TRAF2 directly.⁷⁸ The RIP adapter protein associates with TRAF2 in some cases, and thus with TNFR2.^{59,79} Consistent with the shared intracellular signaling targets of TNFR1 and TNFR2, TNFR2 also is capable of activating NF- κ B and AP-1 in a wide range of cell types.⁵⁹ Although TNFR1 is currently the primary receptor implicated in death induction through caspase mechanisms, TNFR2 may signal apoptosis directly.³⁸ Because RIP also can activate apoptotic caspase pathways, its variable, context-specific interaction with TRAF2 (and thus TNFR2) may be responsible for switching TNFR2 signaling between anti-apoptotic NF- κ B activation and death induction through caspase mechanisms.^{38,59,76,79-81}

RENAL EXPRESSION OF TNF

TNF messenger RNA (mRNA) or protein expression is barely detectable in normal kidney,

for example, by Northern blotting, reverse-transcription polymerase chain reaction, in situ hybridization, or immunohistology, with conflicting results reported in some biopsy studies of normal human kidney.⁸²⁻⁸⁴ With activated monocytes and macrophages being a principle source of TNF, it is not surprising that renal TNF expression increases with leukocyte infiltration in many renal diseases. For example, the primary renal source of TNF after acute aminonucleoside-induced nephropathy in rats is the infiltrating macrophage.⁸⁵ Renal TNF detected early in the course of nephrotoxic serum nephritis, a model of immune complex-mediated GN,^{86,87} is attributed primarily to infiltrating glomerular macrophages.^{88,89} However, recent work has shown that TNF expressed by intrinsic renal cells may be more relevant functionally in mediating renal inflammation in this model.⁹⁰ Intrinsic renal cells, including mesangial cells,¹⁻³ podocytes,^{4,5,91} and tubular epithelial cells,^{6,7} produce TNF. TNF production also has been shown in macrovascular endothelial cells,^{92,93} but its secretion by glomerular or interstitial endothelial cells has not been reported.⁹⁰

In most studies, unstimulated rodent and human mesangial cells do not secrete significant amounts of TNF, but release TNF when stimulated or injured.^{1,2,3,91} However, one report did note TNF expression in mesangial cells, in smooth muscle cells of renal arteries, and in the interstitium of normal human biopsy specimens.⁸⁴ In vitro, TNF expression in mesangial cells can be induced by lipopolysaccharide (LPS),¹ aggregated immunoglobulins (IgG),² IL-1, TNF, advanced glycosylation end products, and the membrane attack complex of complement.⁸² In vivo, glomerular mesangial cells produce TNF in response to LPS injection that models endotoxemia and sepsis.^{94,95} TNF is expressed in podocytes from rats with adriamycin nephropathy, a model of glomerular sclerosis induced by podocyte damage.⁹¹ Interestingly, in renal ischemia-reperfusion injury, TNF expression localizes predominantly to tubular epithelial cells.⁹⁶ Similarly, TNF production is found in tubular cells after renal obstruction, independent of inflammatory cell infiltration.⁹⁷ These data indicate that TNF production in intrinsic renal cells is largely confined to the pri-

marily injured compartment, with additional TNF contributed by renal leukocytes that accumulate during disease progression.

TNF generated in the kidney may be important in inducing the production of inflammatory mediators in mesangial cells such as monocyte colony stimulating factor,⁹⁸ CCL2/monocyte chemoattractant protein 1,⁹⁹ reactive oxygen metabolites,¹⁰⁰ and tissue factor expression,¹⁰¹ as well as promote mesangial cell proliferation or apoptosis.¹⁰² TNF also induces intercellular adhesion molecule 1 expression and enhances monocyte adhesion to mesangial cells.¹⁰³ TNF may promote the generation of IL-8 production in proximal epithelial cells,¹⁰⁴ but also can stimulate their apoptosis.¹⁰⁵⁻¹⁰⁷ These data not only show that TNF can be a regulator of proliferation and apoptosis in renal cells, but also has the capacity to elicit a local proinflammatory cytokine cascade that can promote renal injury.

There are several examples of renal TNF expression in human glomerular diseases. TNF is produced locally within inflamed glomeruli by mesangial cells and podocytes, as well as by infiltrating macrophages.^{108,109} The contribution of local versus leukocyte-derived TNF in human renal injury has not been defined precisely. Although TNF mRNA expression clearly has been localized to intrinsic cells of the human kidney, several studies have suggested that infiltrating leukocytes are the major source of TNF in human GN.¹⁰⁸ Mesangial TNF could be detected in biopsy specimens of IgA nephropathy with minor glomerular proliferation.⁸⁴ In contrast, TNF expression decreased in proliferative lesions of IgA nephropathy, whereas production of IL-10 was induced.⁸⁴ Increased mesangial expression of TNF also was found in biopsy specimens of minimal change disease and idiopathic membranous GN.⁸⁴ In membranous GN, TNF is expressed by glomerular podocytes, and localized along the capillary wall in association with the immune deposits.⁵ TNF also has been identified in mesangial cells in lupus nephritis,^{108,110} with prominent glomerular expression in proliferative lupus nephritis (World Health Organization classes III and IV) and membranous lupus nephritis (class V), but not mesangioproliferative disease (class II).⁸³ In patients with antineutrophil cytoplasmic anti-

body (ANCA)-associated crescentic GN, TNF expression can be detected in glomeruli, tubules, and the interstitium.¹⁰⁹ In patients with glomerular disease and nephrotic-range proteinuria, interstitial TNF expression correlated with the degree of renal fibrosis.⁸⁴ In renal transplant patients undergoing acute rejection, TNF was found in infiltrating leukocytes and in adjacent tubular cells.⁶⁷ Urinary TNF secretion is increased in patients with proliferative GN, although urinary TNF levels do not inevitably correlate with TNF expression in renal biopsy specimens. Of note, patients with progression of membranous GN have apparently greater urinary TNF excretion than patients with stable disease.¹¹¹

RENAL EXPRESSION OF TNF RECEPTORS

Renal expression of both TNF receptors is up-regulated in acute and chronic renal diseases, and soluble forms of the receptors are secreted into the urine. Because leukocytes express TNFR1 and TNFR2, leukocytic infiltrates may contribute substantially to renal receptor expression and urinary excretion in inflamed kidneys.⁶⁷ Interestingly, surface expression of both TNF receptors is lost in macrophages, but not T lymphocytes, that have infiltrated into kidneys of mice subjected to nephrotoxic serum nephritis.¹¹²

In contrast to TNF, only few reports localized expression of TNFR1 and TNFR2 in intrinsic renal cells in experimental animals or human biopsy specimens. In normal mouse kidney TNFR1 expression was found predominantly in cortical tubules by immunohistochemistry.^{83,113} A human biopsy study reported strong mRNA and protein expression of TNFR1 in the glomerular endothelium of normal kidney. In addition, glomerular podocytes were positive for TNFR1, and moderate staining for TNFR1 was evident at the luminal surface of endothelial cells of arterioles and peritubular capillaries.⁶⁷ Interestingly, immunogold electron microscopy of normal human kidney revealed that TNFR1 predominantly localized to the Golgi complex, but not to the surface of glomerular endothelial cells,⁶⁷ consistent with previous findings that TNFR1 localizes to the Golgi apparatus in cultured human

umbilical vein endothelial cells⁶⁴ and potentially could replace shed receptors on the cell surface.³⁴

In contrast, TNFR2 protein is not expressed in normal mouse kidney, as indicated by immunohistochemistry studies.¹¹² Consistently, Al Lamki et al⁶⁷ found no TNFR2 expression in normal human kidney tissue except a weak signal on few epithelial cells of the proximal tubules. However, another group reported glomerular expression of TNFR2, but not TNFR1 protein, in normal human kidney.⁸³ Differences in the sensitivity and specificity of applied antibodies may account for these differing results.

In diseased kidneys, TNF receptors are induced in a compartment- and injury-specific manner. For example, TNFR1 protein expression was increased strongly in human proliferative lupus nephritis (classes III and IV), but not in mesangioproliferative and membranous lupus nephritis or idiopathic membranous GN.⁸³ In this study, the glomerular staining for TNFR1 correlated with the histologic activity index of lupus nephritis.⁸³ In human renal transplants with acute cellular rejection, TNFR1 expression was lost in glomeruli, but TNFR1 was detected abundantly in infiltrating macrophages and T lymphocytes in the interstitium of allografts.⁶⁷ In murine nephrotoxic serum nephritis, TNFR2 expression was induced in glomerular cells and postcapillary venules of the interstitium. Confocal microscopy confirmed glomerular endothelial cells as the predominant TNFR2-positive cells in this model of immune complex GN.¹¹² In contrast, in kidneys with tubulointerstitial injury after unilateral ureteral obstruction (UUO), TNFR2 expression was confined to tubular epithelial cells.¹¹² In human renal transplants with acute cellular rejection increased TNFR2 protein expression was observed predominantly in epithelial cells of distal convoluted tubules near TNF-positive leukocytes, but not in glomerular cells.⁶⁷ In human lupus nephritis (classes II, III, IV, and V) and mesangioproliferative GN glomerular TNFR2 expression was observed, although in this study glomerular staining for TNFR2 in normal control kidneys was similar.⁸³

In summary, these data indicate that expression of the 2 TNF receptors, particularly

TNFR2, can be induced readily in intrinsic renal cells, with cell-specific up-regulation of a particular receptor depending on the type of disease and the primary compartment of renal injury. The pathophysiologic role of TNF and its receptors in renal disease, as well as available data suggesting relevance of these findings to human pathology, are discussed below.

INVOLVEMENT OF TNF AND TNF RECEPTORS IN ACUTE RENAL INJURY

Ischemia-Reperfusion Injury

TNF is increased in kidneys and in serum on experimental renal ischemia-reperfusion injury.^{96,114-116} Renal TNF production after ischemia-reperfusion peaked at 2 hours after reperfusion and localized predominantly to renal tubular epithelial cells.⁹⁶ Its production could be activated by reactive oxygen species released after reperfusion and subsequent activation of p38 mitogen-activated protein kinase and NF- κ B, leading to TNF synthesis.¹¹⁷ In a positive feedback loop, binding of TNF to its membrane receptors can reactivate NF- κ B and further augment TNF production in tubular cells, as well as the expression of inflammatory mediators such as chemokines and cytokines. In addition, TNF can induce tubular cell apoptosis (eg, in ischemic tubular cells), depending on the type of signaling pathways activated after TNFR1 and TNFR2 binding.¹⁰⁷

Interventional studies clearly show a role for TNF in mediating ischemic renal injury. Blocking TNF activity with a recombinant TNF binding protein reduced neutrophil infiltration and ameliorated ischemic injury in renal ischemia-reperfusion injury.¹¹⁴ A similar effect was reported when neutralizing anti-TNF antibodies were used.¹¹⁵ Functional studies on the role of TNFR1 and TNFR2 in ischemia-reperfusion injury have not yet been published. However, given the reported role of TNFR2 in other forms of acute renal failure with prominent tubular cell injury (eg, cisplatin nephropathy, see later) and its up-regulation in tubular epithelial cells during acute and chronic interstitial injury (obstructive nephropathy after UUO, acute cellular rejection), one could speculate that TNFR2 on

these cells plays an important role in mediating renal TNF effects.

Cisplatin-Induced Acute Renal Failure

Oxidant stress-induced activation of NF- κ B and subsequent TNF production also is present in cisplatin-induced renal injury. Renal expression of TNF peaks 48 hours after cisplatin injection into mice. Serum and urine levels of TNF also were increased by cisplatin.¹¹⁸ Blockade of TNF production or activity by TNF antagonists, pentoxifylline, or anti-TNF antibodies reduced the renal expression of proinflammatory chemokines and cytokines, and cisplatin-induced renal dysfunction, leukocyte infiltrates, and structural damage.^{118,119} Similar results were obtained when TNF-deficient mice were investigated.¹¹⁸

Expression of TNFR1 and TNFR2 is up-regulated in kidneys with cisplatin nephrotoxicity. Interestingly, the up-regulation of TNFR2, but not TNFR1, was blunted in TNF-deficient mice, indicating ligand-dependent up-regulation of TNFR2.¹²⁰ Importantly, TNFR2-deficient knockout mice developed less severe renal dysfunction and showed reduced renal injury, apoptosis, and leukocyte infiltration when compared with either TNFR1-deficient or wild-type mice. This was associated with lower renal TNF expression and serum TNF levels in TNFR2-deficient animals.¹²⁰ These data suggest a role for TNFR2, but not TNFR1, in TNF-mediated renal inflammation in cisplatin nephrotoxicity. Cell-type-specific TNFR2 expression and relative roles of renal cell-expressed versus leukocytic TNFR2 remain to be elucidated. Again, it is intriguing to speculate that tubular cell-expressed TNFR2, potentially activated in a paracrine fashion by TNF secreted from adjacent tubular cells, may mediate tubular apoptosis and/or inflammatory activation, ultimately leading to tubular injury, interstitial leukocyte influx, and acute renal failure.

Endotoxin-Induced Acute Renal Failure

Endotoxemia, caused by systemic release of LPS, provokes renal dysfunction and cellular injury, including tubular cell apoptosis.¹¹³ TNF is a key mediator of LPS-induced septic syndrome^{33,121} and is released into the circulation

predominantly by activated macrophages. Consistent with these findings, systemic administration of large doses of TNF causes circulatory shock and mimics the effects of LPS.¹²² In human beings, an increase in serum levels of TNF is detected early in endotoxemia,¹²³ and increased serum levels of soluble TNFR1 and TNFR2 were shown to be predictive factors for acute renal failure in septic shock.¹²⁴ When smaller than shock-inducing doses of TNF were infused into rabbits, glomerular endothelial damage, fibrin deposition, renal neutrophil infiltration, and renal failure occurred,¹²⁵ pointing to a pathogenic role of systemic TNF in endotoxin-induced acute renal failure.

In addition to its systemic release, renal expression of TNF mRNA and protein is strongly up-regulated in response to LPS, with a prominent protein expression in the glomerular, but not interstitial, compartment.^{113,114} Infiltrating renal leukocytes have been considered to be the main source of renal TNF. Locally produced TNF possibly in the membrane-bound form by either infiltrating leukocytes or glomerular mesangial cells may play an important role in the pathogenesis of endotoxin-induced acute renal failure.^{1,94,95,126} Importantly, Knotek et al¹²⁷ reported that pretreatment with a TNF-neutralizing recombinant TNF-receptor protein attenuated LPS-induced acute renal failure in mice. Moreover, pentoxifyllin administration protected against endotoxin-induced acute renal failure. This effect was associated with reduced TNF serum levels in treated mice and potentially is caused by the inhibitory effect of pentoxifyllin on the expression of several proinflammatory cytokines, including TNF.¹²⁸ Pentoxifylline-mediated protection also was associated with attenuation of the induction of serum IL-1 β , and nitric oxide, as well as renal inducible nitric oxide synthase and adhesion molecule expression.¹²⁸ These data indicate a TNF-mediated effect both on hemodynamic and inflammatory mechanisms of endotoxin-induced renal injury.

Activation of TNFR1 expressed in the kidney may play an essential role in endotoxin-induced acute renal failure. Mice deficient in TNFR1 were resistant to LPS-mediated renal failure, with less apoptosis in tubular cells and fewer neutrophils infiltrating the kidney.¹¹³ TNFR1-defi-

cient kidneys transplanted into TNFR1-expressing mice were protected from LPS-induced renal injury 10 days after transplantation, whereas TNFR1-positive kidneys transplanted into TNFR1-deficient animals were not.¹¹³ These elegant experiments showed that TNF acting directly on TNFR1 on intrinsic cells of the kidney mediates acute renal failure in endotoxemia. In contrast to other forms of acute renal failure,¹²⁰ functional studies on the role of TNFR2 in LPS-induced renal injury have not been reported, although increased endotoxin-induced serum TNF levels were reported in mice lacking TNFR2.¹²⁹ The latter data suggest an important role for TNFR2 in suppressing TNF-mediated systemic inflammatory responses induced by endotoxin.

TNF AND ITS RECEPTORS AS MEDIATORS OF NONIMMUNE RENAL DISEASE

Obstructive Nephropathy

It is now widely accepted that locally secreted cytokines and infiltrating immunocompetent cells are important humoral and cellular mediators of renal diseases that do not have a primary immune-mediated pathogenesis.¹³⁰ Among those, obstructive nephropathy, experimentally induced by UO, is a well-established model of progressive interstitial nephritis and renal fibrosis that mimics tubulointerstitial injury and sclerotic changes seen in end-stage renal disease independently of the initial insult. Obstructive nephropathy is characterized by apoptotic death of tubular epithelial cells, interstitial leukocyte infiltrates, and progressive renal fibrosis. After ureteral obstruction renal TNF mRNA and protein production is increased, and localize primarily to cortical tubular cells at early time points after UO when a significant renal leukocyte infiltrate is not yet present.^{97,131-133} At later time points accumulating renal leukocytes are thought to contribute to renal TNF production. Interestingly, in mice treated with an angiotensin converting enzyme inhibitor or with genetic deficiency for the angiotensin II receptor AT_{1a}, renal TNF production after UO was reduced, suggesting that angiotensin induces renal TNF expression.^{132,134} Blockade of TNF

with a pegylated form of soluble TNFR1 significantly reduced obstruction-induced TNF production and tubular cell apoptosis.¹³³ However, effects of TNF blockade on the extent of interstitial leukocyte infiltration or fibrosis were not investigated in this study.

When UO was performed in TNFR1, TNFR2, or TNFR1/TNFR2 double-deficient mice, interstitial matrix deposition, myofibroblast differentiation, and activation of NF- κ B was reduced, with effects through TNFR1 predominating.^{132,134} It is not clear whether these effects were mediated through TNF receptors expressed on intrinsic renal cells or infiltrating leukocytes. However, we recently reported a disease-specific induction of TNFR2 protein expression in tubular epithelial cells after UO,¹¹² which suggests that renal cell-expressed TNF receptors may contribute to tubular cell apoptosis and local NF- κ B-dependent inflammatory pathways. Together these data clearly show a role for renal TNF in mediating tubular cell apoptosis and interstitial fibrosis in obstructive nephropathy, and potentially other chronic kidney diseases with progressive fibrosis.

Hypertensive Nephropathy

Interventional studies in rat models of angiotensin II-induced hypertension and kidney damage indicate a role for TNF in mediating local renal injury. Double transgenic rats with expression of human renin and angiotensinogen develop hypertension, albuminuria, and infiltration of leukocytes. Blockade of TNF with etanercept, a soluble human TNFR2-IgG fusion protein that inhibits the biologic action of TNF, reduced albuminuria, maturation of renal dendritic cells, and leukocyte infiltrates, apparently without apparent effects on blood pressure.¹³⁵ A decreased NF- κ B activation in etanercept-treated rats suggested that TNF-dependent transcriptional activation of inflammatory genes in renal cells and infiltrating leukocytes may be an important mechanism of renal damage in these rats. In a model of salt-sensitive, angiotensin-induced hypertension, etanercept slowed blood pressure increases and decreased albuminuria, urinary excretion of inflammatory chemokines, and renal macrophage infiltrates.¹³⁶ Interestingly, TNF may inhibit renal production of epoxyeicosatrienoic acids,

which mediate renal vasodilatory effects and suppress endothelial cytokine activation.¹³⁶ As indicated previously, angiotensin II can stimulate renal expression of TNF directly.^{132,134} Thus, it remains to be determined if TNF blockade in angiotensin-independent forms of hypertension or in the presence of pharmacologic blockade of angiotensin II has beneficial effects on renal injury. Studies specifically addressing the role of TNF receptors in hypertension-induced renal injury have not been reported.

Diabetic Nephropathy

Early studies reported an increased glomerular expression of TNF mRNA in rats with streptozotocin-induced diabetic nephropathy.¹³⁷ Increased expression of TNF also was shown in proximal, but not distal, tubular cells isolated from diabetic rats.¹³⁸ The increase in renal expression of TNF and urinary TNF levels preceded the onset of albuminuria in streptozotocin-induced diabetic rats, and at later time points correlated with the degree of albuminuria.^{139,140} Administration of angiotensin-converting enzyme inhibitors or angiotensin type I-receptor blockers prevented enhanced expression of TNF, and decreased both urinary albumin and TNF secretion.^{140,141} These studies indicated that angiotensin II, through stimulation of the angiotensin II type 1 receptor, increases the renal content of TNF in diabetes. Thus, beyond their established effect on renal hemodynamics, inhibition of renal TNF production may contribute to the renoprotective function of angiotensin inhibitors in many renal diseases including hypertensive and diabetic nephropathy. Pentoxifylline, presumably through inhibition of TNF synthesis, prevented the increase in TNF mRNA and protein in proximal tubular cells in diabetic rats and reduced urinary TNF excretion.¹³⁸ Interestingly, pentoxifylline, which apparently inhibits TNF synthesis independently of angiotensin, has an additive anti-proteinuric effect in patients with diabetes who already are treated with angiotensin-receptor blockers.¹⁴²

An increasing number of clinical studies have indicated a pathophysiologic role for TNF in human diabetic nephropathy. Increased serum levels of TNF were found in diabetic patients

with microalbuminuria and clinical albuminuria, compared with diabetic patients without albuminuria or healthy controls.¹⁴³⁻¹⁴⁵ Another study found that patients with microalbuminuria or macroalbuminuria showed higher plasma levels of soluble TNFR1 and TNFR2 than patients without albuminuria.¹⁴⁶ Moreover, urinary TNF excretion independently correlated with clinical markers of glomerular and tubulointerstitial injury in type 2 diabetic patients (ie, urinary excretion of albumin and N-acetyl- β -glucosaminidase, respectively).^{143,145} However, no correlation between increased serum and urinary TNF levels was found.¹⁴³ The latter may indicate local production of TNF in the kidney readily secreted in the urine rather than in systemic circulation. Genetic variations of the TNF and TNFR2 gene were not found to be associated with diabetes or its microvascular complications in the general population, including diabetic nephropathy.^{146,147} Taken together, an increasing number of experimental and clinical studies have indicated that renal TNF expression is associated with diabetic nephropathy. Interventional animal studies or therapeutic trials with specific TNF or TNFR antagonists in diabetic nephropathy are needed to determine whether TNF plays an important role in the pathogenesis of the disease.

ROLE OF TNF AND ITS RECEPTORS IN GLOMERULONEPHRITIS

Immune-Complex-Mediated GN

TNF plays an important role in glomerular inflammation and scarring. As noted previously, renal expression of TNF is up-regulated significantly in experimental animals and patients with GN, including IgA nephropathy and membranous GN.^{5,83,84,108} Serum and urine levels of TNF increase in GN.^{108,148} Moreover, an increased glomerular expression of TNFR1 and TNFR2 has been found in animal and human biopsy studies of GN.^{83,112} The functional role of TNF in GN has been shown in animal models. Systemic administration of TNF exacerbated glomerular injury in nephrotoxic serum nephritis in rats.¹⁴⁹ TNF-deficient mice subjected to nephrotoxic serum nephritis showed a reduction in proteinuria, glomerular crescent

formation, infiltration of leukocytes, and expression of vascular adhesion molecules.¹⁵⁰ By using bone marrow chimeric mice, it was shown that intrinsic renal cells are the major source of TNF contributing to renal injury in nephrotoxic serum nephritis.⁹⁰ In rats, pentoxifylline treatment at the time of induction of anti-glomerular basement membrane antibody-mediated GN significantly suppressed renal mRNA expression of TNF. This was associated with reduced proteinuria, inhibition of macrophage and T-cell infiltration, and improvement of histologic damage including glomerular crescent formation.¹⁵¹ Pentoxifylline also had a beneficial effect, although to a lesser extent, in rats with established disease.¹⁵¹ Anti-TNF antibodies attenuated acute glomerular injury in anti-glomerular basement membrane GN in rats,⁸⁹ and administration of antibodies or soluble TNF receptors, which neutralize biological TNF activity, reduced glomerular lesions and prevented crescent formation in rats developing crescentic GN.¹⁵²⁻¹⁵⁴ Of note, when treatment was delayed until the peak of crescent formation in this model, TNF blockade still resulted in a significant reduction in tubulointerstitial scarring and preserved renal function.¹⁵⁴ These data suggest that TNF not only mediates inflammatory injury in the glomerulus, but also subsequent tubulointerstitial fibrosis.

We recently investigated the functional role of TNFR1 and TNFR2 in murine immune complex-mediated GN.¹¹² TNFR1-deficient mice subjected to nephrotoxic serum nephritis developed less albuminuria, glomerular injury, and renal leukocyte infiltrates at early time points after disease induction. This was associated with a reduced humoral immune response to the nephrotoxic rabbit IgG injected after subcutaneous immunization against rabbit IgG. However, albuminuria, renal pathology, and renal functional impairment were similar to wild-type controls at later time points, when a lack of TNFR1 resulted in excessive renal T-cell accumulation, possibly because of the observed reduction in apoptosis of infiltrating renal T cells.¹¹² TNFR1 is required for T-cell priming after subcutaneous injection of antigen,⁴⁹ and delayed-type hypersensitivity responses are impaired in TNFR1-deficient mice.¹⁵⁵ Thus, the

early protection from glomerular injury as well as the decreased humoral response in TNFR1-deficient mice may result from an impaired generation of sensitized T cells. Sensitized T cells not only provide B-cell help for the production of autologous anti-rabbit IgG antibodies in this model of nephrotoxic serum nephritis, but also are required for the development of GN independently of antibody production.¹⁵⁶ On the other hand, TNFR1 mediates apoptosis of activated T cells.⁴⁶⁻⁴⁸ The reduced apoptotic rate of these cells in nephritis kidneys may account for the excessive accumulation of renal T cells at later time points of the model, leading to an exacerbated disease course.

Surprisingly, TNFR2-deficient mice were completely protected from GN at all time points, despite an intact systemic immune response.¹¹² TNFR2 expression was induced on glomerular endothelial cells of nephritic kidneys and bone marrow transplantation experiments showed that TNFR2 expression on intrinsic renal cells, but not leukocytes, was essential for the development of GN and glomerular complement deposition.¹¹² This model of GN is complement dependent.¹⁵⁷ Interestingly, endothelial cells activated by TNF can promote complement deposition in areas of exposed subendothelial matrix *in vitro*, which is initiated by cytokine-induced retraction of these cells.¹⁵⁸ Whether this mechanism is TNFR2 dependent and operational in glomerular inflammation is not known. Nevertheless, these data clearly indicate an essential proinflammatory function of TNFR2 in GN. With humoral and cell-mediated adaptive immunity being intact in TNFR2-deficient mice,^{112,158} renal TNFR2 apparently is required in the effector phase to mediate the local tissue destruction in this model of immune-complex GN.

Lupus Nephritis

Both in experimental and human lupus nephritis, renal expression of TNF and its receptors is increased. Lupus-prone MRL/lpr mice express increased levels of TNF in nephritic kidneys and show high TNF serum levels, both of which correlated with disease activity.¹⁵⁹⁻¹⁶² Importantly, both macrophages and glomerular mesangial cells can be induced to produce

high levels of TNF by immune complexes, which are abundant in the circulation and are deposited in large amounts in the glomeruli during SLE.^{2,163-165} In turn, augmented expression of renal TNF increased local macrophage accumulation in MRL/lpr mice developing lupus nephritis.¹⁶⁶ In human beings, TNF is highly expressed in glomeruli in lupus nephritis,^{108,110,167} and the degree of renal TNF expression correlates with renal inflammatory activity.¹⁶⁸ Moreover, serum levels of TNF and soluble TNFR receptors are increased in human SLE, and parallel clinical disease and autoimmune activity.¹⁶⁹⁻¹⁷¹ A recent meta-analysis showed that the A/A and A/G genotypes of the TNF promoter -308A/G polymorphism, which leads to an increase in the transcriptional activity of the TNF gene, may confer susceptibility to SLE, especially in a European-derived population.¹⁷²

TNF clearly has immunosuppressive functions in systemic autoimmune disease such as SLE, as shown by an increasing number of experimental and clinical observations.¹⁹ Yet, we are only beginning to understand the underlying role of TNF in immune regulation and apoptosis in autoimmunity. On the other hand, the powerful proinflammatory effects of TNF leading to local tissue destruction also are operative in SLE. Interventional studies in lupus-prone MRL/lpr and C3H.SW mice showed that TNF blockade is beneficial in experimental SLE.¹⁷³⁻¹⁷⁵ Of note, even in (NZB × NZW)F1 mice, in which early TNF administration delays autoimmune disease and the development of lupus nephritis, low dosages of TNF administered in later disease accelerated renal damage.¹⁶⁰ Moreover, a limited number of uncontrolled clinical data support the proinflammatory role of TNF in human SLE and lupus nephritis, and suggest a therapeutic efficacy of anti-TNF treatment. Infliximab, a chimeric monoclonal antibody to TNF, significantly reduced proteinuria in 2 patients with lupus nephritis treated for a short period of time.^{160,168} In a subsequent open-label safety trial of infliximab in 6 patients with mild to moderate SLE, who had refractory lupus nephritis or lupus arthritis, inflammatory organ disease improved rapidly in all treated patients.¹⁷⁶ Two thirds of the patients experienced a transient increase in anti-DNA autoantibodies, but this did not lead to lupus flares.¹⁷⁶ Given these encouraging results, further

prospective studies in larger populations under controlled conditions are warranted, with careful monitoring of potential side effects as a result of possible interference with the immune-modulatory functions of TNF.

Interestingly, the proinflammatory and immune-regulatory functions of TNF in SLE may segregate at the level of the 2 TNF receptors. Zhou et al⁵⁶ described a greatly accelerated lymphadenopathy and autoimmune disease in B6/lpr mice that were deficient for TNFR1. In line with these data are our own results showing an increased renal T-cell infiltration in TNFR1-deficient mice with immune-complex GN.^{56,112} A study in transgenic mice overexpressing the human TNFR2 provided evidence for a proinflammatory role for TNFR2 because these mice spontaneously developed a severe systemic inflammatory syndrome.⁵⁷ In view of these and our own data obtained in the nephrotoxic serum nephritis model, one could speculate on an important role of locally expressed TNFR2 in mediating tissue injury in SLE, including lupus nephritis. Of note, a recent meta-analysis on the functional 196M/R polymorphism of TNFR2 suggested that the M/R and R/R genotypes were associated with SLE susceptibility, especially in the Asian population.¹⁷⁷

ANCA-Associated Vasculitis

Renal expression of TNF has been shown in human biopsy specimens of ANCA-associated vasculitis.¹⁰⁹ A pathogenic contribution of TNF to ANCA-associated GN was shown in several rodent models. For example, pauci-immune necrotizing GN induced in mice by transfer of anti-myeloperoxidase (MPO) antibodies was accelerated by the administration of LPS. LPS transiently induced circulating TNF, and administration of anti-TNF antibodies attenuated the severity of glomerular injury after LPS injections in this model.¹⁷⁸ These data suggest that TNF may mediate relapses induced by intercurrent infections in patients with systemic vasculitis. In a different model of ANCA-associated vasculitis induced by the transfer of anti-proteinase 3 antibody-containing serum, intradermal injection of TNF triggered a more robust local inflammation compared to animals receiving non-immune serum.¹⁷⁹ This indicates that locally

produced TNF may exacerbate the pathogenic effects of ANCA. When Wistar Kyoto rats were immunized with MPO, the animals developed circulating anti-MPO antibodies and a pauci-immune crescentic nephritis, characteristics of ANCA-associated microscopic polyangiitis.¹⁸⁰ The administration of a blocking anti-TNF antibody, starting at a time point when glomerulonephritis already was established, reduced albuminuria, glomerular macrophage infiltration, and crescent formation in this model. In addition, it was shown that TNF blockade decreased leukocyte transmigration induced by anti-MPO antibodies *in vivo*, as shown by intravital microscopy.¹⁸¹ These studies indicate the importance of TNF in leukocyte recruitment and renal injury in ANCA-associated vasculitis.

Despite the correlation of increased TNF levels and function with ANCA-associated pathology, inhibition of TNF in clinical trials of ANCA-associated vasculitis has yielded inconsistent results in human beings. Three uncontrolled studies with the TNF-blocking antibody infliximab found beneficial effects of TNF blockade in inducing remission of acute disease and in treating refractory disease.¹⁸²⁻¹⁸⁴ In contrast, in the Wegener's Granulomatosis Etanercept Trial study of etanercept, no benefit in inducing and maintaining remission in Wegener's granulomatosis could be shown.³¹

Interestingly, a discrepant therapeutic efficacy of infliximab and etanercept also has been reported in Crohn's disease. Although treatment with infliximab clearly is effective, beneficial effects of etanercept could not be shown.¹⁸⁵ The reason for these differences is not well understood. However, both reagents differ in some properties that influence their mode of action. For example, infliximab leads to cell lysis more efficiently than the soluble IgG-TNFR2 fusion protein etanercept when it interacts with membrane-bound TNF, for example, in macrophages. In contrast, because TNF receptors not only bind TNF but also lymphotoxin- α , etanercept neutralizes both ligands, which may lead to as yet unanticipated effects.¹³ The negative results of the Wegener's Granulomatosis Etanercept Trial do not rule out a potential therapeutic effect of etanercept in Wegener's disease. Because etanercept treatment or placebo

was given in addition to a standard treatment with glucocorticoids plus cyclophosphamide or methotrexate, a disease-suppressing effect of etanercept may have been obscured by the concomitant immunosuppression known to itself down-modulate increased TNF levels. An important finding of this trial was a significantly increased incidence of solid tumors in the etanercept-treated patients, who all received additional cyclophosphamide therapy.^{31,186} Clearly, these data indicate that TNF blockade with etanercept in addition to standard immunosuppressive regimens has no additional therapeutic efficacy in Wegener's granulomatosis, but is associated with an increased risk of malignancy when combined with cyclophosphamide. On the other hand, in view of the encouraging experimental studies and uncontrolled data in human beings with neutralizing TNF antibodies, controlled studies with infliximab in ANCA-associated vasculitis are needed. Given the data discussed earlier it is intriguing to speculate that locally expressed TNFR2 may play an important role in mediating TNF-dependent endothelial cell injury and local tissue destruction in vasculitis, a possibility that needs further investigation.

THE TNF SYSTEM IN RENAL TRANSPLANTATION

In rat models of acute and chronic rejection, expression of renal TNF mRNA is up-regulated in rejecting allografts.^{187,188} Interestingly, cytomegalovirus infection increased renal TNF expression in a rat model of chronic rejection, with TNF protein localizing to the vascular wall and tubules. This was associated with vessel wall thickening, vascular luminal narrowing, and the destruction of tubules, suggesting the possibility that enhanced graft vasculopathy in cytomegalovirus-infected grafts is mediated by TNF.¹⁸⁹ Expression of TNF mRNA and protein also is increased significantly in human kidney graft biopsy specimens with acute rejection.^{190,191} In addition, up-regulation of TNF has been reported in human chronic allograft nephropathy.¹⁹¹⁻¹⁹⁴ Early studies found significantly increased plasma levels of TNF in 65% of renal allograft rejection episodes, with increased urinary TNF being detected in association with

acute rejection in 49% of the cases.¹⁹⁵ In human transplants with acute cellular rejection TNF was localized to infiltrating interstitial leukocytes and adjacent tubular epithelial cells. TNFR1 was abundantly detected in infiltrating leukocytes, whereas TNFR2 expression was induced in epithelial cells of the distal convoluted tubule.⁶⁷ These data could indicate that leukocyte-expressed TNFR1, and tubular epithelial cell-expressed TNFR2, may contribute to rejection episodes in allografts. The latter would be in line with the functional role of TNFR2 in other nephropathies that are associated with primary tubular damage (ie, cisplatin nephrotoxicity or obstructive nephropathy).^{120,132} Tubulitis is a hallmark of renal allograft rejection, characterized by local infiltration of donor T cells. Interestingly, the synthesis of soluble TNFR1 and TNFR2 in tissue cultures of human renal allograft biopsy specimens was associated significantly with acute rejection episodes.¹⁹⁶ Both TNF receptors also have been implicated in graft arterial disease in murine cardiac allografts.¹⁹⁷ Whether TNFR1 or TNFR2 are involved in chronic allograft nephropathy has not been investigated.

In a nonhuman primate model of renal allograft rejection TNF blockade with a soluble TNF receptor, administered as the only immunosuppressive agent or in combination with subtherapeutic doses of cyclosporine, successfully prolonged renal allograft survival.¹⁹⁸ Single-center studies evaluating the role of TNF inhibitors in human kidney transplantation have been initiated but results are not yet available.¹⁹⁹ Functional relevance of TNF in human renal allograft rejection is suggested by studies that link genetic polymorphisms in the TNF gene of the recipients to the development of acute rejections. In particular, the TNF high-producer genotypes A/A and A/G of the TNF promoter -308A/G polymorphism are associated with an increased risk of acute rejection episodes, especially recurrent acute rejections and steroid-resistant rejections.²⁰⁰⁻²⁰⁸ However, this association was not found in all populations studied.²⁰⁹ In 1 report, no differences in the overall acute rejection frequency between the genotypes were seen, but recipients with the A/A and A/G genotypes and rejection episodes had significantly worse

graft survival compared with the G/G recipients with rejection episodes.²¹⁰ Importantly, all of these studies investigated TNF genotypes in recipients, not in kidney donors. Thus, an increased systemic TNF production or local secretion by recipient-derived renal cells, such as infiltrating leukocytes or endothelial cells, apparently contributes to acute allograft rejection. The role of intrinsic donor renal cell-derived TNF remains to be established, although its quantitative contribution might be small compared with recipient-derived TNF. Interestingly, no significant differences in distribution of TNF genotypes between patients with stable graft function and chronic allograft nephropathy were found.^{194,211}

TNF AND TNF-RECEPTOR BLOCKADE IN HUMAN NEPHROPATHIES: THERAPEUTIC IMPLICATIONS

Treatment options for both acute and chronic renal diseases are limited. Blockade of the renin-angiotensin system and nonspecific immunosuppression with steroids and cytotoxics are the mainstay of therapy, the latter being associated with severe side effects.²⁹ Thus, new, more efficient, and more specific therapeutics are needed urgently. With the advancements of modern molecular biology, genomics, and proteomics, our understanding of the pathogenesis of renal disease has advanced considerably, as have the techniques applied for identification and validation of potential drug targets and lead compounds. Biologic therapeutics have been developed that modulate humoral and cellular mediator systems of the innate and adaptive immune response. Today an increasing number of these agents is used in clinical practice in a variety of diseases, including autoimmune disease, cancer, and transplantation. Among the newly developed biologicals, TNF inhibitors are now used in routine clinical practice, being highly beneficial in patients with chronic autoimmune disease such as rheumatoid arthritis and Crohn's disease. In this review we discussed experimental and clinical evidence for the TNF system being centrally involved in the pathogenesis of immune and nonimmune renal injury. Thus, TNF blockade may constitute an efficacious therapeutic option in many human renal diseases.

However, large prospective trials that prove the utility of TNF targeting therapies in human nephropathies are missing. One reason for this is the numerous possible roles of TNF in mediating not only inflammatory responses in the kidney, but also in controlling the systemic immune response by virtue of its immune-regulatory functions. This pleiotropism of functions has become evident especially in patients with anti-TNF therapy who developed autoantibodies, lupus-like syndromes, and even GN. Moreover, there is increasing experimental evidence for an immune-suppressive function of TNF. These findings have raised concerns about using TNF-blocking therapies, especially in the setting of systemic autoimmune diseases such as SLE and vasculitis.¹⁶⁸ The dual functions of TNF in autoimmunity are difficult to define because of different TNF-dependent effects in different experimental models and under different settings. Blocking TNF, especially in autoimmune-prone chronic inflammatory diseases, therefore may lead to unpredictable outcomes.¹⁹ To establish a role of TNF blockade in the treatment of such disease, carefully designed prospective trials with detailed monitoring of potential side effects are needed.

Importantly, recent experimental evidence has indicated heterogeneity in the use of TNF receptors for immunosuppression versus inflammatory tissue damage. As summarized in this review, TNFR2 expressed in the kidney plays an essential role in mediating local renal injury in models of acute and chronic renal disease, including cisplatin nephrotoxicity and immune-complex glomerulonephritis.^{112,120} An important exception from this may be endotoxin-mediated kidney damage, which apparently is mediated through locally expressed TNFR1.¹¹³ In contrast, immune-suppressive effects of TNF predominantly may require signaling through TNFR1, or in some cases involve both TNF receptors.^{19,49} A specific blockade of TNFR2 that reduces inflammatory tissue damage in the kidney without compromising TNFR1-mediated immunosuppressive effects, may therefore be advantageous to anti-TNF treatments, especially in chronic renal diseases and systemic autoimmunity. Moreover, given

that TNFR1, but not TNFR2, plays a crucial role in lymphoid organogenesis³⁷ and the priming of sensitized T cells,^{19,49} which potentially may be important in homeostatic immune surveillance, a TNFR2-specific blockade may not compromise the antitumor response of the immune system.

In conclusion, there is increasing experimental evidence that TNF, by delivering signals of cellular differentiation, cytokine production, proliferation, or apoptosis through its 2 membrane receptors, not only orchestrates acute responses to infection and immunologic injury, but acts as an immunosuppressive factor required for re-establishing physiologic homeostasis and immune regulation.¹⁹ The proinflammatory, immune-activating, or immunosuppressive effects of TNF in a given pathophysiologic condition (ie, type and phase of disease) likely is dictated by the level, timing, and duration of TNF activity.¹⁹ Moreover, variable activation of the 2 TNF receptors and their associated intracellular signaling pathways appear to be important determinants of the cellular response to TNF. Thus, the development of specific inhibitors that pharmacologically dissociate the immunosuppressive from the tissue-damaging effects of TNF is required. To establish a role for TNF-receptor-specific therapies in renal diseases, more experimental studies in relevant models are needed. Results from such studies and the availability of specific TNF-receptor inhibitors will provide a rationale for clinical trials that ultimately could test the therapeutic potential of TNFR2-specific inhibition of inflammatory TNF effects in glomerular and interstitial injury associated with immune and nonimmune renal diseases.

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