Tumor Necrosis Factor Receptors: Biology and Therapeutic Potential in Kidney Diseases

Marijn M. Speeckaert^{a, b} Reinhart Speeckaert^b Margo Laute^a
Raymond Vanholder^a Joris R. Delanghe^b

Departments of ^aNephrology and ^bClinical Chemistry, Ghent University Hospital, Ghent, Belgium

Key Words

Renal pathology • Tumor necrosis factor- α • Tumor necrosis factor receptor

Abstract

The major evolutionary advance represented in the human immune system is a mechanism of antigen-directed immunity in which tumor necrosis factor (TNF)- α and TNF receptors (TNFRs) play essential roles. Binding of TNF- α to the 55-kDa type I TNFR (TNFR1, TNFRSF1A, CD120a, p55) or the 75-kDa type II TNFR (TNFR2, TNFRSF1B, CD120b, p75) activates signaling pathways controlling inflammatory, immune and stress responses, as well as host defense and apoptosis. Multiple studies have investigated the role of TNFRs in the development of early and late renal failure (diabetic nephropathy, nephroangiosclerosis, acute kidney transplant rejection, renal cell carcinoma, glomerulonephritis, sepsis and obstructive renal injury). This article reviews the general characteristics, the analytical aspects and the biology of TNFRs in this domain. In addition, the potential therapeutic application of specific TNFR blockers is discussed.

Copyright © 2012 S. Karger AG, Basel

Introduction

Tumor necrosis factor- α (TNF- α) is a functional 26-kDa homotrimer transmembrane protein, which is cleaved by a disintegrin and metalloprotease protein-17 (ADAM-17) and released into the circulation as a functional 17-kDa soluble form [1]. In plasma, TNF- α appears as free or is bound to circulating TNF receptor 1 (TNFR1, TNFRSF1A, CD120a, p55) and TNF receptor 2 (TNFR2, TNFRSF1B, CD120b, p75) (collectively referred to as markers of the TNF pathway), which originate from separate gene products [2]. TNFR1 can be detected in almost all cell types [3], while TNFR2 is only located in oligodendrocytes [4], astrocytes [5], T cells [6], myocytes [7], thymocytes [8], endothelial cells [9] and in human mesenchymal stem cells [10]. In healthy subjects, TNF- α and TNFR2 are usually not present in the kidneys, whereas TNFR1 can be found in normal glomerular endothelium, where it is primarily localized within the Golgi apparatus [11]. After stimulation (e.g. with lipopolysaccharide, interleukin (IL)-1 α) and during inflammation, TNF- α and its receptors are expressed in glomerular (endothelial, mesangial and epithelial) and tubular cells [12]. Based on those findings, we will focus in this review on the biology of TNFRs and the therapeutic potential of specific TNFR blockers in kidney diseases.

General Characteristics

TNFR1 and TNFR2 belong to the TNF- α receptor superfamily, a group of type I single transmembrane glycoproteins. Their extracellular regions are characterized by four repeated cysteine-rich domains (CRDs) (CRD1 or the pre-ligand binding assembly domain (PLAD), CRD2, CRD3 and CRD4) and the intracellular domains of the two TNFRs are unrelated. The obligatory threefold symmetry of the receptor defines the essential signaling stoichiometry and structure [13]. In contrast to TNFR1, TNFR2 has no death domain on its intracellular region, suggesting activation of different downstream transduction pathways. TNFR1 and TNFR2 have a molecular weight of respectively 55 and 75 kDa and are encoded by the human TNFR genes, which are located on the short arm of chromosome 12 (12p13) [14] and chromosome 1 (1p36) [15]. Restriction mapping, DNA blot analysis and sequence analysis have shown that the TNFR1 gene is composed of 10 exons covering protein coding and 3' untranslated regions and spanning 13 kb [14]. In a similar way, the TNFR2 gene comprises 10 exons (ranging from 34 base pairs to 2.5 kb pairs) and 9 introns (343 base pairs to 19 kb pairs) [15]. Polymorphisms of the TNFR genes have been described and associated with the susceptibility for development of different diseases, e.g. diabetes type 1 [16] and sepsis [17].

The cytoplasmic domains of TNFRs are modest in length and function as docking sites for signaling molecules (fig. 1): (a) TNF-receptor-associated factors (TRAFs), which mediate the downstream activation of transcription factors such as nuclear factor- κ B (NF- κ B) [18], c-Jun NH₂-terminal kinase and kinases of the mitogen-activated protein kinase kinase kinase family and (b) 'death domain' molecules (a family of cysteine proteases called caspases) [3, 19]. The exact roles of the receptors are not yet completely understood and may differ depending on the organ type. While TNFR1 modulates the immune response (IL-6 synthesis) and apoptosis (apoptotic signaling kinase 1 and NF- κ B of mesangial cells), TNFR2 is one of the proinflammatory mediators in glomerulonephritis [20, 21]. Activation of TNFR1 requires the coordinate expression of TNFR2. Besides formation of heterocomplexes with TNFR1, TNFR2 may also potentiate responses mediated by TNFR1 by increasing the local concentration of surface-associated TNF- α available to TNFR1 [22].

Cleavage of TNF- α and TNFRs from the cell surface by a disintegrin and metalloproteinase-17 results in soluble forms [23]. However the predominant form of soluble TNFR1 (sTNFR1) in human serum is a full-length 55-

kDa protein (released in exosome-like vesicles) and not the 28–34 kDa ectodomain of TNFR1 [24]. It is not well known whether the same mechanisms are applicable to TNFR2 release and how this process is regulated. The receptors have different affinities for the soluble and the membrane-bound forms of TNF- α : TNFR1 binds both forms equally well, whereas TNFR2 has a higher affinity for the membrane-bound form [23]. Recent findings point to the importance of soluble circulating levels of TNFRs and not to the cellular signal transduction through these receptors. In 1992, Aderka et al. [25] nicely described the variation in serum levels of sTNFRs among healthy individuals. Their data showed that individual differences of sTNFRs are stable over time and may contribute to differences in the severity of the harmful effects of TNF- α in disease states. The regulation mechanisms of sTNFR have not yet been clarified. Some studies have suggested that TNF- α is the main regulator, as this cytokine induces shedding of the TNFRs [26]. However, this theory has not been confirmed in patients with diabetes type 1 or type 2. In two recent studies, renal function loss was strongly associated with elevated concentrations of circulating TNFR1 and TNFR2, but not with the other TNF pathway markers (free or total TNF- α) [27, 28]. So there is a need to investigate further the factors that influence the concentration of those soluble TNFRs.

Although the exact physiological role of sTNFRs remains unknown, it has been suggested that the circulating forms of TNFRs may function as decoys for TNF- α or their concentrations may reflect long-term exposure to this proinflammatory cytokine [27]. At low concentrations, sTNFRs enhance the actions of TNF- α , but at higher sTNFR concentrations the effects of TNF- α are abrogated [25]. Also in urine, increased TNFR1 excretion reflects increased TNFR shedding from the cell membranes and may be interpreted as a marker of increased TNF- α expression in activated renal cells [29]. Another possibility is that the shedding of receptors represents a mechanism for desensitizing the cells that shed the receptors from the effects of TNF- α [30]. Recent evidence suggests that sTNFRs have immunomodulatory functions, as illustrated by the sTNFR1-induced apoptosis which depends on autocrine transforming growth factor- β_1 signaling through the mitogen-activated protein kinase p38- α [31]. Besides serving as a TNF-binding protein, circulating TNFRs might have additional functions as serum levels of TNFRs are 100–500 times higher than serum levels of TNF- α . For that reason, it should be interesting to investigate further the specific biology of the cleaved, intact and urinary form of TNFRs.

Analytical Methods

Nowadays, the gold standard for quantitative detection of human sTNFR in tissue lysate, cell culture supernate, serum, plasma or other body fluids is the standard sandwich enzyme-linked immunosorbent assay (ELISA) technology. The assay is characterized by a high sensitivity (>20 pg/ml) and specificity, a fast performance, an easy handling, a low background signal and a reliable calibration. No interference by other serum and urine proteins and no cross-reactivity with any other cytokine have been observed. The principle of this method is based on the use of two monoclonal antibodies, directed against two different epitopes of sTNFR1 or sTNFR2. One antibody recognizes an epitope involved in TNF-binding, preferentially enabling the determination of biologically active sTNFR. During the first incubation step, a complex is formed between the sTNFR, the biotin-labeled antibody and the peroxidase-conjugated detection antibody. Via the biotin-labeled antibody, the complex binds to the streptavidin-coated surface of the microtiter plate. After a washing step, a photometrical determination of the concentration of sTNFRs is performed by the interaction of horseradish peroxidase bound in complex with tetramethylbenzidine as substrate. Standards of defined concentrations are run in each assay, allowing the construction of a calibration curve. The sTNFR concentration of unknown samples is then calculated from this calibration curve. Assay results for the receptors are identical in serum and plasma. The sensitivity of the assay is characterized by a mean minimum detectable dose of 0.77 pg/ml. Although recombinant human, mouse, rat and porcine TNF- α did not show any significant cross-reactivity with sTNFR1 in this immunoassay, these factors show a low level of interference [32, 33]. TNFR ELISAs may not only reflect the biologically active part of sTNFR, but can also include the possibly inactive amount of sTNFR. TNFR1 and TNFR2 ligand binding assays may solve this problem as this technology uses TNF- α as the complementary ligand of sTNFR1 and sTNFR2, allowing the determination of the specific biological active part of sTNFR in undiluted sera/plasma and urine. The system uses biotin-labeled TNF- α and a monoclonal antibody from mouse directed against an epitope not involved in receptor binding, which allows the exclusive determination of functionally active sTNFR in this assay. TNF- α does not give false-negative results when used up to a concentration of 5 ng/ml for TNFR1 or 40 ng/ml for TNFR2 [34].

Immunohistochemical analysis of cells or tissue sections stained with specific anti-human monoclonal anti-TNFR antibodies is widely used to understand the distribution and localization of TNFRs in different parts of a biological tissue [11, 35–38]. For instance, the glomerular presence of TNFR1 in proliferative lupus nephritis in association with subepithelial immune deposits may be of pathogenetic significance and may have diagnostic value [39]. Besides, this method is particularly useful to investigate the role of TNFR1 and TNFR2 in cellular events such as proliferation or cell death [36]. Visualizing the antibody-antigen interaction can be accomplished by immunoperoxidase staining (an antibody is conjugated to peroxidase that can catalyze a color-producing reaction) [38] or immunofluorescence (the antibody is tagged to a fluorescein) [40]. In comparison with such a semi-quantitative method, flow cytometry analysis has the advantage to evaluate quantitatively the receptor expression and to gain novel insights into the TNF-mediated pathophysiology of kidney dis-

eases using monoclonal TNFR antibodies [21, 41–43]. The utility of spectral variants of green fluorescent protein in flow cytometric fluorescence resonance energy transfer analysis of TNFRs has been demonstrated. This method allows the quantitative determination of non-covalent molecular associations at Ångström level in living cells and is applicable for quantitative analyses on a cell-by-cell basis for a large number of cells [44]. Finally, the functional characteristics of TNFRs can be further investigated by simple immunochemical techniques like immunoprecipitation, Western blotting [45] or block-and-activation method [46].

Clinical Aspects of TNFR1 and TNFR2 in Renal Pathology (table 1, fig. 1)

Diabetes

The specific involvement of the TNFR-mediated pathway rather than a general inflammation in the development of early and late renal function loss in both types of diabetes has been recently suggested [27, 28]. Although the underlying mechanisms are not yet fully understood, type 2 diabetes is associated with activation of the innate immune system and chronic low-grade inflammation (increased concentrations of TNF- α , IL-6 and C-reactive protein) [47]. Since the first publication of TNF- α in the pathogenesis of diabetic nephropathy by Hasegawa et al. [48], multiple studies have focused on the relationship between circulating markers of the TNF pathway and the risk of abnormal urinary albumin excretion, impaired renal function and cardiovascular death. In a Japanese study of type 2 diabetic patients without overt proteinuria, higher levels of sTNFR1 and sTNFR2 were associated with a greater decline in estimated glomerular filtration rate (eGFR). In comparison with the group of patients with a high level of only one receptor or a low level of both receptors, patients with a high level of both sTNFR had a fourfold higher risk for an eGFR decline of $\geq 25\%$ [49]. In another study with almost all Caucasian patients, analysis of plasma markers of the TNF pathway showed that the 12-year risk of end-stage renal disease (ESRD) in type 2 diabetes was strongly associated with elevated concentrations of circulating TNFR1 and TNFR2, but not with free or total TNF- α [28]. Moreover a single measurement of the plasma TNFR concentration (TNFR1 $>$ TNFR2) was a predictor of ESRD in patients with type 2 diabetes, even after adjustment for clinical covariates such as urinary albumin excretion. The associations of these receptors with mortality unrelated to ESRD were moderate. The questions how and which TNFR is more causally related to the risk of ESRD have not yet been solved. There is a hypothesis that elevated

Table 1. Diagnostic value of TNFRs in kidney diseases

Pathology	Comment	Reference
Diabetes	Circulating TNFRs predict stage 3 CKD in type 1 diabetes and ESRD in type 2 diabetes	27, 28
Renal vascular disease	Kidney function is the most important predictor of TNFR concentrations in plasma	50
Kidney transplantation	An increased local shedding of TNFR2 is seen during an acute renal rejection. In comparison with IL-6/sIL-6R and sVCAM-1, the potential of urinary TNFR1 is considered inferior in predicting an early acute transplant rejection	55, 57
Renal cell carcinoma	TNFR2 expression is related to the malignant grade/tumor stage	37
Glomerulonephritis	ANCA-associated systemic vasculitis: increased expression of TNFRs at active sites	60
	Inflammatory proliferative kidney diseases: increased expression of TNFR1 in glomeruli and urine	29, 35, 62
	Immune complex glomerulonephritis: TNFR1 is not essential for the development	21
	Hepatitis C virus-associated glomerulonephritis: increased expression of TNFR2	64
Sepsis and burns	Essential role of TNFR1 in the signaling TNF- α pathway	65, 67
Obstructive renal injury	A much larger increase of TNFR2 protein is observed in comparison with TNFR1 protein	68

CKD = Chronic kidney disease; IL-6 = interleukin 6; sIL-6R = soluble IL-6 receptor; sVCAM-1 = soluble vascular cell adhesion molecule-1.

concentrations of TNFRs contribute directly to renal injury and progressive renal function decline. Besides this consideration, the interactions between TNFRs and the effects of metabolic control, systemic inflammation or endothelial injury on the risk of ESRD should be further investigated.

Prospective studies in patients with diabetes type 1 have demonstrated that early GFR loss is observed even before the onset of proteinuria [33]. In those patients, a strong association has been found between elevated serum concentrations of TNFR1 and TNFR2 and early renal function loss (progression to CKD3 or higher), independent of circulating free or total TNF- α and relevant clinical covariates. In contrast to diabetes type 2, the strongest association was observed with TNFR2. A systemic rather than a local kidney source of TNFR is contributing to the increased risk for early renal function loss [27]. However in contrast to TNF- α , which increases the albumin permeability, a thorough investigation of TNFR in animal models with diabetic nephropathy has not yet been carried out.

Renal Vascular Disease

Although not directly associated with the severity of lesions in coronary arteries, increased plasma levels of TNF- α , sTNFR1 and sTNFR2 have been reported in patients with coronary artery disease as a reflection of the

impairment of cardiac and renal function. In a multivariate analysis, kidney function was the most important predictor of plasma concentrations of the TNFRs, which might be associated with decreased renal clearance of these cytokines. This association might be partly explained by comorbidities [50]. As previously described, elevated levels of sTNFR1 and sTNFR2 are strongly associated with renal failure in diabetes [27]. Besides a positive correlation between TNFR and the metabolic syndrome [51], COPD and asthma have been reported. The elevated sTNFR1 level is a marker for the proinflammatory state in subjects with atherogenic dyslipoproteinemia. Those receptors contribute to dyslipidemia of insulin resistance and this association is not related to obesity [52]. In contrast to sTNFR2, elevated sTNFR1 levels correlate with worse subjective, echocardiographic and biochemical (brain natriuretic peptide) parameters of cardiac performance [50].

A critical renal protective role for TNFR1 is postulated since deletion of this receptor is associated with higher systolic pressure and urinary albumin excretion, as well as an altered GFR and fractional sodium excretion (FENa%) in response to infusion of angiotensin II. Selective activation or deletion of TNFR1 contributes to a mechanism that may lower or raise blood pressure. Recent data have demonstrated that the activation of TNFR1, not the type 2, is mainly involved in mediating the acute

Fig. 1. TNFR1- and TNFR2-mediated signaling pathways and the involvement of those receptors in human kidney diseases.

renal vasoconstriction and natriuretic actions of TNF- α [53]. Alternatively, TNFR2 has been shown to exert positive inotropic effects in cardiac myocytes via the ERK/MSK1/cPLA2 pathway, which enhances calcium handling and cell fractional shortening [54]. Moreover, TNFR2 may play a role in the increased susceptibility to albuminuria [53].

Kidney Transplantation

A number of cytokines (e.g. IL-6 and TNF- α) and soluble vascular cell adhesion molecules (sVCAM-1) promote B- and T-cell activation, the key reaction leading to acute and chronic renal allograft rejection [55]. During an acute cellular rejection, the influx of lymphocytes in the renal interstitium is accompanied by the detection of epithelial cells expressing upregulated TNF- α converting enzyme (TACE), which are surrounded by a higher level

of TACE-activating molecules (histamine, protein kinase C activator, reactive oxygen species and IL-1), stimulating the local shedding of TNFR2. This finding has been confirmed in an in vitro model, showing an upregulation of TACE in tubular epithelial cells during an acute renal rejection, which colocalized with TNFR2, but not with TNF- α or TNFR1. The primary function of TACE is to produce more sTNFR2 to antagonize the proinflammatory effect of TNF- α in the local environment [56]. TNFR2 protein is synthesized by epithelial cells in situ and is mainly located on the cytoplasmic membrane in an acute cellular rejecting kidney [11]. Besides the upregulation of TNFR2 in tubular epithelial cells, human renal transplant biopsies and rat allografts with acute rejection have demonstrated an increased number of TNFR2-positive podocytes. Although the functional role of TNFR2 in podocytes has to be further clarified, the significantly

elevated urinary TNFR2 protein concentration in rats with acute rejection could be an indirect sign of damage to the basement membrane, besides being an indicator for an ongoing inflammatory reaction. Furthermore, TNFR2 seems to mediate preferentially early effects of TNF- α in acute allograft rejection as a significantly lower TNFR2 expression has been observed in biopsies with chronic allograft lesions [57].

Multiple studies have investigated the potential value of individual cytokine profiles to predict acute rejection episodes. Elevated urinary concentrations of IL-6, sIL-6R, TNFR1 and sVCAM-1 clearly indicate an early acute transplant rejection. However, in comparison with IL-6/sIL-6R and sVCAM-1, the potential of urinary TNFR1 is considered as inferior [55].

Renal Cell Carcinoma

Clear cell renal cell carcinoma is characterized by inactivating mutations of the von Hippel-Lindau tumor suppressor gene, which induces an increased transcription of hypoxia-inducible genes, such as vascular endothelial growth factor (VEGF) and stimulates the translation of TNF mRNA. Fundamental research on clear cell renal cell carcinoma organ culture showed that TNF- α functions as an autocrine growth factor. Released by tubular epithelial cell malignancies, TNF- α may selectively activate TNFR2 responses, which results in activation of epithelial and endothelial tyrosine kinase (Etk) and apparent transactivation of VEGFR-2 by phosphorylation at Tyr(P)-1054-1059. This pathway promotes entry into the cell cycle and activation of NF- κ B. In contrast to TNFR1, the TNFR2 expression in such tumors is correlated with the malignant grade, which is in line with the significantly higher sTNFR2 plasma levels in patients with advanced stages of renal cell carcinoma [58]. Besides its action via Etk-VEGFR2 cross-talk, TNF- α can initiate apoptosis signaling pathways after TNFR1 ligation. This results in a TNF receptor-associated factor-2 (TRAF2)-dependent formation of a death-inducing signaling complex or a TRAF2-dependent activation of apoptosis signal-regulating kinase 1 (ASK1). TNFR2-induced NF- κ B activation stimulates the transcription of antiapoptotic proteins, including cFLICE (procaspase-8) inhibitory protein (c-FLIP), cellular inhibitors of apoptosis (c-IAPs), A20 and mitogen-activated protein kinase-specific phosphatases [37].

Glomerulonephritis

Glomerulonephritis is characterized by a variety of human immune-mediated disorders, including IgA ne-

phropathy, anti-glomerular basement membrane disease, systemic vasculitis and systemic lupus erythematosus. As the kidney is frequently affected in inflammatory and autoimmune diseases, the contrasting proinflammatory and immunosuppressive roles of TNF- α have been studied in the inflammatory cascade leading to renal injury. Besides the experimental animal models of lupus nephritis, several studies have investigated the important role of TNF- α and its receptors in patients with glomerulonephritis [21, 35, 39, 59–64]. An immunological injury is followed by an upregulation of TNF- α in the glomeruli, activating tubular epithelial cells and increasing the production of cytokines. In anti-neutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis, both TNF- α and the TNFRs are hyperexpressed at sites of vasculitis. TNF- α priming is necessary before ANCA can induce neutrophil activation, endothelial and vascular damage [61].

In inflammatory proliferative kidney diseases, such as human lupus nephritis (WHO class III/IV), an increased expression of TNFR1 in the glomeruli and a stimulated shedding of TNFR1 from the membranes of glomerular cells has been demonstrated [62]. This is reflected in elevated, but not statistically significant urinary TNFR1 concentrations in patients with different forms of primary glomerulonephritis (focal segmental glomerulosclerosis > IgA nephropathy > mesangial proliferative glomerulonephritis). In those cases, the kidneys are probably the only source for the production of urinary TNFR1. In comparison with nephrotic patients, significantly lower urinary TNFR1 concentrations are reported in the non-nephrotic group. This finding may be explained by the additional production of TNFR1 by infiltrating lymphocytes and macrophages in the interstitial tissue of patients with a more advanced glomerular barrier function impairment. Multiple regression analysis has shown a significant negative association of urinary TNFR1 with eGFR values at presentation, which suggests that this marker indicates the activation of the TNF- α pathway in the kidneys and may be considered as an additional risk factor for progressive GFR deterioration [35]. Besides the described apoptosis of human kidney cells after exposure to sTNFRs (sTNFR1 > sTNFR2), the underlying mechanisms of this association should be further investigated [39].

However, other authors have questioned the described role of TNFR1 and have reported the presence of TNFR2 in various glomerular diseases [59]. In a model of immune complex glomerulonephritis dependent on complement, $\alpha\beta$ and $\gamma\delta$ T cells and induced by anti-glomer-

ular basement membrane antibody, it was demonstrated that the TNFR1 function is not essential for the development of glomerulonephritis, although it accelerates the systemic immune response towards the glomerular immune complexes. Due to an impaired adaptive immune response, TNFR1-deficient mice exhibited only a delay in the development of glomerulonephritis, but were not protected from disease. The modulation of the glomerular complement activation/deposition may be crucial for the development and progression of disease, which is fulfilled by the TNFR2 expression on intrinsic and glomerular endothelial cells of nephritic kidneys. This finding was confirmed by the nearly complete protection of TNFR2-deficient mice in glomerulonephritis [21]. TNFR2 is required for the effector phase of nephrotoxic nephritis. Bruggeman et al. [63] found a de novo expression of TNFR2 on podocytes prior to hyperplastic injury in crescentic glomerulonephritis of mice with nephrotoxic nephritis and in collapsing glomerulopathy of Tg26^{HIV/ni} mice, *kd/kd* mice and humans. They showed that neutralization of TNFR2 on podocytes with blocking antibodies abrogates NF- κ B activation and the induction of cyclin D1 by TNF- α in proliferative podocytopathies. TNFR2 is also present on infiltrating renal T cells, intrinsic renal cells and glomerular endothelial cells during glomerulonephritis, but is absent on renal macrophages. Endothelial cell-expressed TNFR2 has also been demonstrated in post-capillary venules of the cortical interstitium. Leukocyte-expressed TNFR2 only partly contributes to renal injury [21].

In hepatitis C virus-associated glomerulonephritis, interferon- γ -inducible protein (IP-10) and TNF- α are upregulated, which is specifically mediated by the viral receptor Toll-like receptor-3 expressed on mesangial cells. In the proinflammatory environment, stimulation of viral receptors leads to a ligand-increased expression of TNFR2, which is the anchor for TNF- α to stimulate IP-10 induction [64].

Sepsis

Gut-derived bacteria or endotoxin translocation might play a role in the development of sepsis and multiple organ damage in critically ill patients. The underlying pathophysiological mechanisms are regulated by (pro)inflammatory cytokines and mediators (e.g. TNF- α) [65]. Fang et al. [66] investigated the mRNA expression patterns of TNF- α and TNFR1 in some vital organs of male Wistar rats after thermal injury. In contrast to the transient increased TNF- α mRNA expression in the liver, a sustained elevation of TNF- α gene expression was ob-

served in the kidneys (peak at 48 h post-burn with a ten-fold increased expression) and lungs. The TNFR1 mRNA expression in liver, lung, kidney and intestine was decreased throughout the observation period with the lowest levels at 24–48 h post-burn (26.1–46.7% of the control values). The ratio of TNF- α to renal TNFR1 mRNA reached its maximum between 24 and 48 h. A highly negative correlation between levels of renal TNFR1 and serum creatinine was reported, suggesting that the imbalance of TNF- α to TNFR1 might be involved in the development of acute kidney failure following burns. The essential role of TNFR1 in signaling TNF- α toxicity was further illustrated by experiments with TNFR1^{-/-} mice as those animals were protected from bolus shock reactions induced by lipopolysaccharide/D-GalN and *Staphylococcus aureus* superantigen/D-GalN [67].

Obstructive Renal Injury

Upper urinary tract obstruction is an important cause of renal failure and is associated with significant collecting system dilatation, apoptotic renal tubular cell death and tubulointerstitial fibrosis. In an experimental rat model with unilateral ureteral obstruction (UUO), proliferation and apoptosis of renal tubular and interstitial cells have been associated with an overexpression of TNF- α and TNFRs [68]. TNFR knockout mice displayed around 40% less tubulointerstitial fibrosis than wild-type mice [69]. The TNFR2-mediated TNF- α signaling pathway plays probably a more important role in the pathophysiology of UUO kidney lesions, as a much larger increase in TNFR2 protein is observed in comparison with TNFR1 protein [68]. The combination of an increased TNFR1 mRNA expression and a low protein concentration suggests the involvement of an ubiquitin-dependent degradation of TNFR1 in UUO kidneys [69]. A biphasic course of TRADD mRNA expression is reported with an initial increase, followed by degradation and ubiquitination of TRADD in the kidneys at day 1 after UUO [68]. However in a mice model, an increased transcription of apoptosis-related molecules (Fas ligand, Fas-associated death domain, TRADD and several caspases) associated with apoptotic cell death was found after 1–4 days and was maximal after 7–15 days of renal obstruction. After 1 month, tubular cell apoptosis reverted to control level, but interstitial apoptosis was probably still going on, which might be explained by a different molecular control system of the two cellular compartments through the entire course of the development of obstructive renal injury [70, 71]. The non-concordant results could be explained by the difference in animal species studied and/

or the antibodies used to detect TRADD [68]. Inhibition of TNF- α by the administration of sTNFR2 (etanercept) or pegylated sTNFR ameliorated obstructive renal injury [68, 71–73].

Therapeutic Use of TNFR Blockers in the Field of Nephrology

As illustrated above, the TNF signaling pathway is involved in the pathophysiology of several kidney diseases. Anti-TNF therapy interferes with the pathogenic processes at multiple levels, inhibiting inflammatory cell recruitment, inducing cell death in inflammatory cells and lowering the cytokine production [74]. Besides the increased risk of infection, a slightly increased risk for malignancy and an aggravation of congestive cardiac failure, anti-TNF- α treatment may lead in a subset of patients to a paradoxical induction of autoimmunity, systemic lupus erythematosus, type 1 diabetes, vasculitis, multiple sclerosis and psoriasis [75]. Due to its different effect on B cells, T cells, dendritic cells and the process of apoptosis, TNF- α plays an important role in the production of autoantibodies, which could explain the production of antinuclear antibodies (anti-DNA, anti-dsDNA) and anti-cardiolipin antibodies, which has been observed during anti-TNF treatment [76].

In contrast to potent inhibitors of TNF-dependent responses, regardless of whether the biological response is initiated via TNFR1 or TNFR2 signaling (e.g. etanercept, which is a fusion protein composed of the extracellular part of two TNFR2 molecules coupled to the Fc region of an IgG1 molecule), next-generation therapeutics should focus more specifically on the individual TNFRs, which regulate the balance between the proinflammatory and immunosuppressive functions of TNF- α through distinct intracellular signals. Some studies have suggested benefits of anti-TNF- α treatments in renal inflammation. Targeted TNF- α blockade has the potential to improve the safety of induction regimens by permitting reduced glucocorticoid exposure and may also improve the degree of renal recovery in ANCA-associated systemic vasculitis [61]. However, randomized controlled trials are still lacking, which could be explained by the small and heterogeneous group of study patients. As illustrated in this work, the quest for the most suitable target (TNFR1 or TNFR2) is still going on. Autoimmune disease models in mice have illustrated that TNFR2 might be the preferred target as its expression is induced during renal inflammation and TNFR2 deficiency in mice confers sig-

nificant protection from renal tissue. This selective approach should result in a preservation of the immunosuppressive effects of TNF- α such as apoptosis of autoreactive T cells and in a blocking of the TNF- α dependent proinflammatory functions that lead to glomerular dysfunction [63]. However in a recent paper, Wang et al. [77] provided evidence that targeting PLAD of TNFR1 by PLAD.Fc ameliorates autoimmune diabetes. PLAD.Fc-mediated Th17 suppression is fulfilled through blockage of TNFR1 signaling on antigen presenting cells and T cells. Also in renal vascular disease patients, selective targeting of individual TNFRs can be an entry point to improve cardiovascular and renal function based on the underlying mechanisms of higher pulse pressure, especially in those with systolic hypertension [38]. The identification of key signaling events downstream specifically of TNFR2 may reveal additional therapeutic targets for modulating TNFR2 function [23].

Conclusion

Acting as key communication systems between cells of the immune system, TNF- α and its receptors (TNFR1 and TNFR2) play a crucial role in the pathogenesis of renal diseases. The interaction between TNF- α and TNFR1 mediates several cellular processes, including cell survival, inflammation, apoptosis and necrosis. Besides, the TNF- α -TNFR2 signaling pathway is also involved in proliferation, gene activation and apoptosis [78]. Several studies have suggested the existence of a functional cross-talk between TNFR1 and TNFR2, which is of great biological relevance [79, 80]. Due to the existence of a complex regulation network after the activation of the receptor, the final cellular response depends on the cellular context and the microenvironment conditions [3]. This means that the TNF superfamily members are active targets for drug development. It has become clear from sophisticated mouse models that a new approach based on specific inhibition or deletion of sTNF or TNFRs could be a much more appropriate strategy than blocking all TNF signaling, which is what current anti-TNF therapies do. Overall, we believe that further research is still necessary to clarify the prognostic and therapeutic role of TNFRs in clinical nephrology.

Disclosure Statement

The authors have no conflicts of interest.

References

- Lorenzen I, Trad A, Grötzinger J: Multimerisation of A disintegrin and metalloprotease protein-17 (ADAM-17) is mediated by its EGF-like domain. *Biochem Biophys Res Commun* 2011;415:330–336.
- Richter C, Messerschmidt S, Holeiter G, et al: The TNF receptor stalk regions define responsiveness to soluble versus membrane bound ligand. *Mol Cell Biol* 2012;32:2515–2529.
- Cabal-Hierro L, Lazo PS: Signal transduction by tumor necrosis factor receptors. *Cell Signal* 2012;24:1297–1305.
- Inukai T, Uchida K, Nakajima H, et al: Tumor necrosis factor- α and its receptors contribute to apoptosis of oligodendrocytes in the spinal cord of spinal hyperostotic mouse (twy/twy) sustaining chronic mechanical compression. *Spine (Phila Pa 1976)* 2009;34:2448–2457.
- Yan M, Xia C, Cheng C, et al: The role of TNF- α and its receptors in the production of Src-suppressed C kinase substrate by rat primary type-2 astrocytes. *Brain Res* 2007;1184:28–37.
- Chen X, Oppenheim JJ: Contrasting effects of TNF and anti-TNF on the activation of effector T cells and regulatory T cells in autoimmunity. *FEBS Lett* 2011;585:3611–3618.
- Defer N, Azroyan A, Pecker F, Pavoine C: TNFR1 and TNFR2 signaling interplay in cardiac myocytes. *J Biol Chem* 2007;282:35564–35573.
- Grell M, Becke FM, Wajant H, Männel DN, Scheurich P: TNF receptor type 2 mediates thymocyte proliferation independently of TNF receptor type 1. *Eur J Immunol* 1998;28:257–263.
- Ding B, Kirkiles-Smith NC, Pober JS: FOXO3a regulates oxygen-responsive expression of tumor necrosis factor receptor 2 in human dermal microvascular endothelial cells. *J Biol Chem* 2009;284:19331–19339.
- Kelly ML, Wang M, Crisostomo PR, et al: TNF receptor 2, not TNF receptor 1, enhances mesenchymal stem cell-mediated cardiac protection following acute ischemia. *Shock* 2010;33:602–607.
- Al-Lamki RS, Wang J, Skepper JN, Thiru S, Pober JS, Bradley JR: Expression of tumor necrosis factor receptors in normal kidney and rejecting renal transplants. *Lab Invest* 2001;81:1503–1515.
- Baud L, Ardaillou R: Tumor necrosis factor in renal injury. *Miner Electrolyte Metab* 1995;21:336–341.
- Locksley RM, Killeen N, Lenardo MJ: The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 2001;104:487–501.
- Fuchs P, Strehl S, Dworzak M, Himmler A, Ambros PF: Structure of the human TNF receptor 1 (p60) gene (TNFR1) and localization to chromosome 12p13. *Genomics* 1992;13:219–224.
- Beltinger CP, White PS, Maris JM, et al: Physical mapping and genomic structure of the human TNFR2 gene. *Genomics* 1996;35:94–100.
- Nishimura M, Obayashi H, Mizuta I, et al: TNF, TNF receptor type 1, and allograft inflammatory factor-1 gene polymorphisms in Japanese patients with type 1 diabetes. *Hum Immunol* 2003;64:302–309.
- Gordon AC, Lagan AL, Aganna E, et al: TNF and TNFR polymorphisms in severe sepsis and septic shock: a prospective multicentre study. *Genes Immun* 2004;5:631–640.
- Rangan G, Wang Y, Harris D: NF- κ B signaling in chronic kidney disease. *Front Biosci* 2009;14:3496–3522.
- Fujita K, Srinivasula SM: Ubiquitination and TNFR1 signaling. *Results Probl Cell Differ* 2009;49:87–114.
- Zhu LJ, Yang X, Li XY, et al: Suppression of tumor necrosis factor receptor associated factor-2 attenuates the proinflammatory and proliferative effect of aggregated IgG on rat renal mesangial cells. *Cytokine* 2010;49:201–208.
- Vielhauer V, Stavrakis G, Mayadas TN: Renal cell-expressed TNF receptor 2, not receptor 1, is essential for the development of glomerulonephritis. *J Clin Invest* 2005;115:1199–1209.
- Tartaglia LA, Pennica D, Goeddel DV: Ligand passing: the 75-kDa tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor. *J Biol Chem* 1993;268:18542–18548.
- Ernandez T, Mayadas TN: Immunoregulatory role of TNF- α in inflammatory kidney diseases. *Kidney Int* 2009;76:262–276.
- Hawari FI, Rouhani FN, Cui X, et al: Release of full-length 55-kDa TNF receptor 1 in exosome-like vesicles: a mechanism for generation of soluble cytokine receptors. *Proc Natl Acad Sci USA* 2004;101:1297–1302.
- Aderka D, Engelmann H, Maor Y, Brakebusch C, Wallach D: Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. *J Exp Med* 1992;175:323–329.
- Lantz M, Malik S, Slevin ML, Olsson I: Infusion of tumor necrosis factor (TNF) causes an increase in circulating TNF-binding protein in humans. *Cytokine* 1990;2:402–406.
- Gohda T, Niewczas MA, Ficociello LH, et al: Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. *J Am Soc Nephrol* 2012;23:516–524.
- Niewczas MA, Gohda T, Skupien J, et al: Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. *J Am Soc Nephrol* 2012;23:507–515.
- Idasiak-Piechocka I, Oko A, Pawliczak E, Kaczmarek E, Czekalski S: Urinary excretion of soluble tumour necrosis factor receptor-1 as a marker of increased risk of progressive kidney function deterioration in patients with primary chronic glomerulonephritis. *Nephrol Dial Transplant* 2010;25:3948–3956.
- Porteu F, Nathan C: Shedding of tumor necrosis factor receptors by activated human neutrophils. *J Exp Med* 1990;172:599–607.
- Waetzig GH, Rosenstiel P, Arlt A, et al: Soluble tumor necrosis factor (TNF) receptor-1 induces apoptosis via reverse TNF signaling and autocrine transforming growth factor- β . *FASEB J* 2005;19:91–93.
- Bouma MG, Buurman WA: Assay of soluble tumor necrosis factor receptors. *Methods Mol Med* 2000;36:91–100.
- Niewczas MA, Ficociello LH, Johnson AC, et al: Serum concentrations of markers of TNF- α and Fas-mediated pathways and renal function in nonproteinuric patients with type 1 diabetes. *Clin J Am Soc Nephrol* 2009;4:62–70.
- Rauert H, Stühmer T, Bargou R, Wajant H, Siegmund D: TNFR1 and TNFR2 regulate the extrinsic apoptotic pathway in myeloma cells by multiple mechanisms. *Cell Death Dis* 2011;2:e194.
- Al-Lamki RS, Wang J, Vandenabeele P, et al: TNFR1- and TNFR2-mediated signaling pathways in human kidney are cell type-specific and differentially contribute to renal injury. *FASEB J* 2005;19:1637–1645.
- Bachmeier BE, Nerlich AG, Weiler C, Paesold G, Jochum M, Boos N: Analysis of tissue distribution of TNF- α , TNF- α receptors, and the activating TNF- α -converting enzyme suggests activation of the TNF- α system in the aging intervertebral disc. *Ann NY Acad Sci* 2007;1096:44–54.
- Al-Lamki RS, Sadler TJ, Wang J, et al: Tumor necrosis factor receptor expression and signaling in renal cell carcinoma. *Am J Pathol* 2010;177:943–954.
- Castillo A, Islam MT, Prieto MC, Majid DS: Tumor necrosis factor- α receptor type 1, not the type 2, mediates its acute responses in the kidney. *Am J Physiol Renal Physiol* 2012;302:F1650–F1657.
- Aten J, Roos A, Claessen N, Schilder-Tol EJ, Ten BI, Weening JJ: Strong and selective glomerular localization of CD134 ligand and TNF receptor-1 in proliferative lupus nephritis. *J Am Soc Nephrol* 2000;11:1426–1438.
- Gaeta ML, Johnson DR, Kluger MS, Pober JS: The death domain of tumor necrosis factor receptor 1 is necessary but not sufficient for Golgi retention of the receptor and mediates receptor desensitization. *Lab Invest* 2000;80:1185–1194.
- Wittebole X, Coyle SM, Kumar A, Goshima M, Lowry SF, Calvano SE: Expression of tumour necrosis factor receptor and Toll-like receptor 2 and 4 on peripheral blood leucocytes of human volunteers after endotoxin challenge: a comparison of flow cytometric light scatter and immunofluorescence gating. *Clin Exp Immunol* 2005;141:99–106.

- 42 Takasugi K, Yamamura M, Iwahashi M, et al: Induction of tumour necrosis factor receptor-expressing macrophages by interleukin-10 and macrophage colony-stimulating factor in rheumatoid arthritis. *Arthritis Res Ther* 2006;8:R126.
- 43 Zola H, Flego L, Weedon H: Expression of membrane receptor for tumour necrosis factor on human blood lymphocytes. *Immunol Cell Biol* 1993;71:281–288.
- 44 Chan FK, Siegel RM, Zacharias D, et al: Fluorescence resonance energy transfer analysis of cell surface receptor interactions and signaling using spectral variants of the green fluorescent protein. *Cytometry* 2001;44:361–368.
- 45 Blackwell K, Zhang L, Thomas GS, Sun S, Nakano H, Habelhah H: TRAF2 phosphorylation modulates tumor necrosis factor- α -induced gene expression and cell resistance to apoptosis. *Mol Cell Biol* 2009;29:303–314.
- 46 Zhou M, Zhang Y, Ardans JA, Wahl LM: Interferon- γ differentially regulates monocyte matrix metalloproteinase-1 and -9 through tumor necrosis factor- α and caspase-8. *J Biol Chem* 2003;278:45406–45413.
- 47 Donath MY, Shoelson SE: Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol* 2011;11:98–107.
- 48 Hasegawa G, Nakano K, Sawada M, et al: Possible role of tumor necrosis factor and interleukin-1 in the development of diabetic nephropathy. *Kidney Int* 1991;40:1007–1012.
- 49 Miyazawa I, Araki S, Obata T, et al: Association between serum soluble TNF- α receptors and renal dysfunction in type 2 diabetic patients without proteinuria. *Diabetes Res Clin Pract* 2011;92:174–180.
- 50 Safranow K, Dziedzicko V, Rzeuski R, et al: Plasma concentrations of TNF- α and its soluble receptors sTNFR1 and sTNFR2 in patients with coronary artery disease. *Tissue Antigens* 2009;74:386–392.
- 51 Kowalska I, Straczkowski M, Nikolajuk A, et al: Insulin resistance, serum adiponectin, and proinflammatory markers in young subjects with the metabolic syndrome. *Metabolism* 2008;57:1539–1544.
- 52 Rosenson RS, Tangney CC, Levine DM, Parker TS, Gordon BR: Elevated soluble tumor necrosis factor receptor levels in non-obese adults with the atherogenic dyslipoproteinemia. *Atherosclerosis* 2004;177:77–81.
- 53 Chen CC, Pedraza PL, Hao S, Stier CT, Ferreri NR: TNFR1-deficient mice display altered blood pressure and renal responses to ANG-II infusion. *Am J Physiol Renal Physiol* 2010;299:F1141–F1150.
- 54 Defer N, Azroyan A, Pecker F, Pavoine C: TNFR1 and TNFR2 signaling interplay in cardiac myocytes. *J Biol Chem* 2007;282:35564–35573.
- 55 Reinhold SW, Straub RH, Krüger B, et al: Elevated urinary sVCAM-1, IL-6, sIL-6R and TNFR1 concentrations indicate acute kidney transplant rejection in the first two weeks after transplantation. *Cytokine* 2012;57:379–388.
- 56 Wang J, Li Z, Al-Lamki R, et al: The role of tumor necrosis factor- α converting enzyme in renal transplant rejection. *Am J Nephrol* 2010;32:362–368.
- 57 Hoffmann U, Bergler T, Rihm M, et al: Up-regulation of TNF receptor type 2 in human and experimental renal allograft rejection. *Am J Transplant* 2009;9:675–686.
- 58 Qi H, Ohh M: The von Hippel-Lindau tumor suppressor protein sensitizes renal cell carcinoma cells to tumor necrosis factor-induced cytotoxicity by suppressing the nuclear factor- κ B-dependent antiapoptotic pathway. *Cancer Res* 2003;63:7076–7080.
- 59 Rosenkranz AR, Knight S, Sethi S, Alexander SI, Cotran RS, Mayadas TN: Regulatory interactions of $\alpha\beta$ and $\gamma\delta$ T cells in glomerulonephritis. *Kidney Int* 2000;58:1055–1066.
- 60 Hasegawa M, Nishii C, Ohashi A, et al: Expression of tumor necrosis factor receptors on granulocytes in patients with myeloperoxidase anti-neutrophil cytoplasmic autoantibody-associated vasculitis. *Nephron Clin Pract* 2009;113:c222–233.
- 61 Wu T, Xie C, Wang HW, et al: Elevated urinary VCAM-1, P-selectin, soluble TNF receptor-1, and CXC chemokine ligand 16 in multiple murine lupus strains and human lupus nephritis. *J Immunol* 2007;179:7166–7175.
- 62 Zhu L, Yang X, Ji Y, et al: Up-regulated renal expression of TNF- α signalling adapter proteins in lupus glomerulonephritis. *Lupus* 2009;18:116–127.
- 63 Bruggeman LA, Drawz PE, Kahoud N, Lin K, Barisoni L, Nelson PJ: TNFR2 interposes the proliferative and NF- κ B-mediated inflammatory response by podocytes to TNF- α . *Lab Invest* 2011;91:413–425.
- 64 Merkle M, Ribeiro A, Wörnle M: TLR3-dependent regulation of cytokines in human mesangial cells: a novel role for IP-10 and TNF- α in hepatitis C-associated glomerulonephritis. *Am J Physiol Renal Physiol* 2011;301:F57–F69.
- 65 Spies M, Chappell VL, Dasu MR, Herndon DN, Thompson JC, Wolf SE: Role of TNF- α in gut mucosal changes after severe burn. *Am J Physiol Gastrointest Liver Physiol* 2002;283:G703–G708.
- 66 Fang WH, Yao YM, Shi ZG, et al: The mRNA expression patterns of tumor necrosis factor- α and TNFR-I in some vital organs after thermal injury. *World J Gastroenterol* 2003;283:G703–G708.
- 67 Pfeffer K, Matsuyama T, Kündig TM, et al: Mice deficient for the 55-kD tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *L. monocytogenes* infection. *Cell* 1993;73:457–467.
- 68 Masaki T, Yamamoto T, Suzuki S, et al: Decrease in tumor necrosis factor- α receptor-associated death domain results from ubiquitin-dependent degradation in obstructive renal injury in rats. *Am J Pathol* 2009;175:74–83.
- 69 Guo G, Morrissey J, McCracken R, Tolley T, Klahr S: Role of TNFR1 and TNFR2 receptors in tubulointerstitial fibrosis of obstructive nephropathy. *Am J Physiol* 1999;277:F766–F772.
- 70 Choi YJ, Baranowska-Daca E, Nguyen V, et al: Mechanism of chronic obstructive uropathy: increased expression of apoptosis-promoting molecules. *Kidney Int* 2000;58:1481–1491.
- 71 Misseri R, Meldrum DR, Dinarello CA, et al: TNF- α mediates obstruction-induced renal tubular cell apoptosis and proapoptotic signaling. *Am J Physiol Renal Physiol* 2005;288:F406–F411.
- 72 Meldrum KK, Metcalfe P, Leslie JA, Misseri R, Hile KL, Meldrum DR: TNF- α neutralization decreases nuclear factor- κ B activation and apoptosis during renal obstruction. *J Surg Res* 2006;131:182–188.
- 73 Meldrum KK, Misseri R, Metcalfe P, Dinarello CA, Hile KL, Meldrum DR: TNF- α neutralization ameliorates obstruction-induced renal fibrosis. *Am J Physiol Regul Integr Comp Physiol* 2007;292:1456–1464.
- 74 Van Hauwermeiren F, Vandenbroucke RE, Libert C: Treatment of TNF-mediated diseases by selective inhibition of soluble TNF or TNFR1. *Cytokine Growth Factor Rev* 2011;22:311–319.
- 75 Faustman D, Davis M: TNF receptor-2 pathway: drug target for autoimmune diseases. *Nat Rev Drug Discov* 2010;9:482–493.
- 76 Aringer M, Smolen JS: The role of tumor necrosis factor- α in systemic lupus erythematosus. *Arthritis Res Ther* 2008;10:202.
- 77 Wang GB, Li CR, Yang J, Wen PQ, Jia SL: A regulatory polymorphism in promoter region of TNFR1 gene is associated with Kawasaki disease in Chinese individuals. *Hum Immunol* 2011;72:451–457.
- 78 Keystone EC, Ware CF: Tumor necrosis factor and anti-tumor necrosis factor therapies. *J Rheumatol Suppl* 2010;85:27–39.
- 79 Fotin-Mleczek M, Henkler F, Samel D, et al: Apoptotic crosstalk of TNF receptors: TNFR2 induces depletion of TRAF2 and IAP proteins and accelerates TNF-R1-dependent activation of caspase-8. *J Cell Sci* 2002;115:2757–2770.
- 80 Rodríguez M, Cabal-Hierro L, Carcedo MT, et al: NF- κ B signal triggering and termination by tumor necrosis factor receptor-2. *J Biol Chem* 2011;286:22814–22824.