AUS DEM LEHRSTUHL FÜR IMMUNOLOGIE
PROFESSOR DR. DANIELA N. MÄNNEL
DER FAKULTÄT FÜR MEDIZIN
DER UNIVERSITÄT REGENSBURG

THE ROLE OF TNFR2 IN EXPERIMENTALLY INDUCED GLOMERULONEPHRITIS

Inaugural – Dissertation
zur Erlangung des Doktorgrades
der Medizin

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Zusammenfassung

TNF ist ein wichtiges Zytokin und spielt eine entscheidende Rolle in der Entstehung von entzündlichen Gewebsschädigungen, die durch immunologische Prozesse vermittelt werden. TNF entfaltet seine Wirkung über zwei verschiedene Signalwege, unter Verwendung von TNFR1 oder TNFR2.
Im Jahre 2005 fanden Vielhauer et al. heraus, dass TNFR2 eine wichtige Rolle in der Entstehung von Glomerulonephritis spielt, welche eine der wichtigsten Ursachen für die Entwicklung eines Nierenversagens ist und mit Proteinurie sowie einer gestörten Nierenfunktion einhergeht. Vielhauer stellte fest, dass TNFR2-/- Mäuse vor der Entstehung einer Glomerulonephritis geschützt waren. Ausgehend von dieser Hypothese wollten wir dies näher untersuchen und versuchten daher zunächst, Vielhauers Erkenntnisse in unserem Versuchsaufbau der experimentell induzierten Glomerulonephritis zu reproduzieren. Als Parameter zur Beurteilung der Glomerulonephritis dienten die Messung der Proteinkonzentration im Urin und typische histologische Veränderungen der Niere.
Ausgehend von Vielhauers Erkenntnis, dass das Vorhandensein von TNFR2 eine entscheidende Rolle für die Entwicklung einer Glomerulonephritis spielt, untersuchten wir außerdem einen möglichen Zusammenhang zwischen der Entstehung einer Glomerulonephritis und der Entwicklung der Konzentration an TNFR2 im Urin. Hierbei fanden wir heraus, dass es keine Korrelation zwischen der Schwere der Glomerulonephritis und der Menge an löslichem TNFR2 im Urin der Mäuse gab.
Im Gegensatz zu Vielhauer konnten wir jedoch nicht zeigen, dass die TNFR2-/-Mäuse in unserem Versuch gegen Glomerulonephritis geschützt waren, und nahmen an, dass dies möglicherweise auf eine sogenannte TNF-Toleranz zurückzuführen sei, die die TNFR2-/-Mäuse in unserem Versuch aufgrund einer vorherigen Exposition gegenüber höheren Konzentrationen an TNF entwickelt haben könnten.
Darüber hinaus untersuchten wir die Entstehung einer Glomerulonephritis bei Mäusen, die transgen für den humanen TNFR2 (hTNFR2) waren, über den mäusliches TNF ebenfalls wirken kann. Während der Arbeit mit den transgenen Mäusen war es uns möglich, eine einfache und gut zu reproduzierende Methode zu entwickeln, diese transgenen Mäuse zu identifizieren, indem wir die Konzentration an löslichem hTNFR2 im Urin dieser Mäuse bestimmten.
Entgegen unserer Erwartungen jedoch zeigten die transgenen Mäuse weder Anzeichen einer vermehrten Entwicklung einer Glomerulonephritis noch einer verstärkten Entzündungsreaktion. Eine mögliche Erklärung hierfür könnte die Tatsache liefern, dass die transgenen Mäuse einer krankheitsunabhängigen, kontinuierlichen Überexpression des hTNFR2 ausgesetzt waren und nicht einer mit der Schwere der Krankheit korrelierenden

Konzentration an mäuslichem TNFR2, die entscheidenden Einfluss auf die Entwicklung der Glomerulonephritis hat.
Zusammenfassend führten unsere Ergebnisse uns zu der Annahme, dass das Herbeiführen einer Glomerulonephritis entsprechend unseres Versuchsaufbaus keinen entscheidenden Einfluss auf die Konzentration an TNFR2 hat und zu keiner verstärkten Signalwirkung von TNF über TNFR2 führt. Entgegen Vielhauers Annahme bietet daher das Antagonisieren von TNFR2, wie es derzeit in der Therapie chronisch entzündlicher Darmerkrankungen oder auch der rheumatoiden Arthritis eingesetzt wird, möglicherweise keine entscheidende Verbesserung in der Therapie der Glomerulonephritis.

Abstract

TNF is an important cytokine and acts as mediator of inflammatory tissue damage which is caused by immunologically mediated processes. TNF provides its effects via two signalling pathways using its two receptors, TNFR1 and TNFR2.

In 2005 Vielhauer et al. revealed that TNFR2 plays an important role in the development of glomerulonephritis, which is one of the most important causes for renal failure and leads to proteinuria and renal dysfunction. Vielhauer found that TNFR2-/- mice were protected from the development of glomerulonephritis. Based on this hypothesis, we intended to further investigate and, therefore, tried to reproduce it in our experimental setup of glomerulonephritis induction. As parameters for the development of glomerulonephritis we observed proteinuria and typical histological changes in the renal structure. We also tested whether we could find a correlation between the development of glomerulonephritis and the concentration of TNFR2 in urine. We found out that amounts of soluble mTNFR2 in urine showed no correlation to the severity of disease.

However, we were not able to reproduce Vielhauer's findings, since TNFR2-/- mice in our setup were not protected against glomerulonephritis. We assumed that his findings could be explained by the presence of a so-called TNF-tolerance that has developed in the organism of TNFR2-/- mice because of former exposure to higher levels of TNF.

Furthermore, we intended to study the development of experimental glomerulonephritis in mice that were transgenic for human TNFR2 (hTNFR2), which is able to interact with mouse TNF in a functional way. Working with these transgenic mice, we were able to establish an easy and reproducible way to identify mice that were transgenic for the hTNFR2 by detecting soluble hTNFR2 in urine of these mice.

Contrary to our expectations, mice transgenic for hTNFR2 showed no signs of increased pathology and no enhanced inflammatory response to the induction of glomerulonephritis. One possible explanation may be provided by the fact that the mice we used in our experimental setup were exposed to constitutive overexpression of hTNFR2 instead of disease-correlating levels of mouse TNFR2 which may have an important impact on the development of glomerulonephritis. According to these findings, we assumed that inducing glomerulonephritis in mice according to our experimental protocol has no striking impact on the concentration of signalling TNFR2. Contrary to Vielhauer's assumptions, antagonizing TNFR2 might not provide such special improvement in treatment of glomerulonephritis as TNF blockade does in the current clinical treatment of other chronic inflammatory diseases such as inflammatory bowel diseases or rheumatoid arthritis.

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Abbreviations

AIDS	acquired immunodeficiency syndrome
ATPase	adenosin triphosphatase
BSA	bovine serum albumine
cDNA	copy desoxyribonucleic acid
Con-A	concanavalin A
d	day
DISC	death inducing signaling complex
DNA	desoxyribonucleic acid
ELISA	enzyme-linked immunoabsorbent assay
ERK	extracellular signal related kinase
FADD	Fas-associated protein with death domain
g	gram
GBM	glomerular basement membrane
GFR	glomerular filtration rate
h	hour
H.E.	hematoxylin and eosin
hicp75TNFR2	intracellular human tumor necrosis factor receptor type 2
HRP	horseradish peroxidase
hTNFR2	human tumor necrosis factor receptor type 2
hTNFR2tg	transgenic for human tumor necrosis factor receptor type 2
i.p.	intraperitoneal
IgG	immunglobulin G
IKK	IκB proteinkinase
JNK	c-Jun-N-terminal kinase
kDa	kilodaltons
LPS	lipopolysaccharide
MAPK	mitogen activated protein kinase
mg	milligram
min.	minute
mL	milliliter
mTNFR2	mouse tumor necrosis factor type 2
NaCl	sodium chloride
NF-κB	nuclear factor-kappa B

ng	nanogram
nm	nanometer
No.	Number
OD	optical density
PAS	periodic acid-schiff
PBS	phosphate buffered saline
pg	picogram
pI3K	phosphoinositide 3-kinase
RIP	receptor interacting protein
sec.	second
sTNF	soluble tumor necrosis factor
TACE	tumor necrosis factor alpha converting enzyme
TIM	tumor necrosis factor receptor associated factor interacting motif
TNF	tumor necrosis factor
TNFR1	tumor necrosis factor receptor type 1
TNFR2	tumor necrosis factor receptor type 2
TNFR2-/-	tumor necrosis factor receptor type 2-deficient
TRADD	tumor necrosis factor receptor associated death domain
TRAF	tumor necrosis factor receptor associated factor
µg	microgram
µL	microliter
℃	degree celsius

1 Introduction

1.1 Glomerulonephritis

The term glomerulonephritis involves a number of different renal diseases which are accompanied by glomerular inflammation and cellular proliferation (Chadban and Atkins, 2005).
The different forms of glomerulonephritis can be classified into primary and secondary forms. Primary forms of glomerulonephritis evolve from intrinsic kidney causes which are mainly autoimmune processes or which develop due to unknown reasons. Secondary forms of glomerulonephritis describe the renal involvement in different systemic disorders such as systemic lupus erythemathosus or systemic vasculitis (Chadban and Atkins, 2005; Herold, 2009).
The different forms of glomerulonephritis can also be characterized by histological criteria. Depending on the histological findings non-proliferative forms of glomerulonephritis can be separated from proliferative forms which show signs of hypercellularity. Minimal-change glomerulonephritis, focal segmental glomerulosclerosis and membranous glomerulonephritis are regarded as non-proliferative forms which clinically lead to symptoms of the nephrotic syndrome, whereas post-infectious glomerulonephritis, membrano-proliferative glomerulonephritis and rapidly progressive glomerulonephritis belong to the proliferative forms showing clinical signs of the nephritic syndrome. Proliferative forms are likely to progress to end-stage renal failure more often than non-proliferative forms (Stahl et al., 2004, Herold, 2009).

1.1.1 Renal Physiology

The renal perfusion requires about 20% of the cardiac output (Deetjen and Speckmann, 1999). This reflects the importance of the different renal functions and metabolic activities. One of the most important functions is the elimination of substances that are obligatory excreted by urine such as urea, creatinine, and uric acid. Moreover, the kidneys are involved both in the regulation of the electrolyte and water balance and in the regulation of the acid-base metabolism. In addition, important hormones are produced in the kidneys for example erythropoietin, which induces the red cell production, or other certain enzymes which regulate the blood pressure such as renin. (Huppelsberg and Walter, 2003)

The kidney can be separated into cortex and medulla. The renal functional structures are the nephrons which are composed of a filtering component, the renal corpuscle, and an absorbing and secreting component, the renal tubule.
Blood reaches the renal corpuscle through the vas afferens and leaves it after filtration through the vas efferens. Together they form the vascular pole.
The renal corpuscle, which serves the filtration of the primary urine, consists of the glomerulus and the Bowman's capsule. The glomerulus is composed of a tuft of small capillaries with a fenestrated endothelium (Lüllmann-Rauch, 2003). The endothelium is covered by the glomerular basement membrane which is lined by podocytes. Podocytes form the visceral layer of the Bowman's capsule and have several foot processes which surround the capillaries and form filtration slits (Pavenstädt et al., 2003; Rodewald and Karnovsky, 1974). The parietal layer of the Bowman's capsule consists of squamous epithelial cells (Lüllmann-Rauch, 2003).
The cell layers described above form the renal filtration system which, on the one hand, is permeable for water, ions, and other small molecules but, on the other hand, prevents larger molecules, especially proteins such as the important albumin, from penetrating the glomerular barrier, thereby retaining them in the circulation (Haraldsson and Sörensson, 2004).
The so-called filtrated primary urine is collected between the visceral and the parietal layer of the Bowman's capsule and leaves the renal corpuscle at the urinary pole into the proximal convoluted tubule (Lüllmann-Rauch, 2003). This primary urine not only contains the substances that are obligatorily excreted by urine but also molecules such as glucose, amino acids, and electrolytes. These molecules mostly have to be reabsorbed. During the passage through the proximal tubule nearly all of the amino acids and glucose and about two-thirds of the filtrated NaCl are resorbed from the lumen (Silbernagl, 1991). The required gradient is maintained by the Na^+/K^+-ATPase. The primary urine then reaches the loop of Henle where a concentration gradient is built up and more NaCl is resorbed into the interstitium. The following distal convoluted tubule and collecting tubule are responsible for the final concentration and composition of the primary urine. The antidiuretic hormone (ADH) is responsible for the concentration of the urine. The concentrated urine reaches the renal pelvis, the bladder and is eliminated via the ureter (Huppelsberg and Walter, 2003, Schmidt et al., 2004).
Physiological urine contains only small proteins which make up for about 150mg/d (Herold, 2009, Ehrich et al., 1984).
Renal function can be measured by quantifying the amount of liquid that is filtrated by the nephrons in a certain time. This volume is called glomerular filtration rate (GFR) and indicates the functional status of the kidney.

Creatinine serves as a tracer substance for glomerular filtration due to its characteristics. Creatinine is an endogenous product of the muscle metabolism and is obligatorily excreted by urine. Moreover, creatinine is freely filtered in the nephrons and is hardly not secreted or reabsorbed in the kidney. These characteristics lead to the following formula to calculate GFR:

$$GFR = \frac{\text{Creatinine Concentration in Urine}}{\text{Creatinine Concentration in Serum}} * \text{Urine flow (mL/min)}$$

This formula only indicates a limited renal function if 60% or more of the nephrons have lost there function and is therefore only appropriate to identify severe renal damage (Peronne et al., 1992; Silbernagel and Despopoulos, 2003).

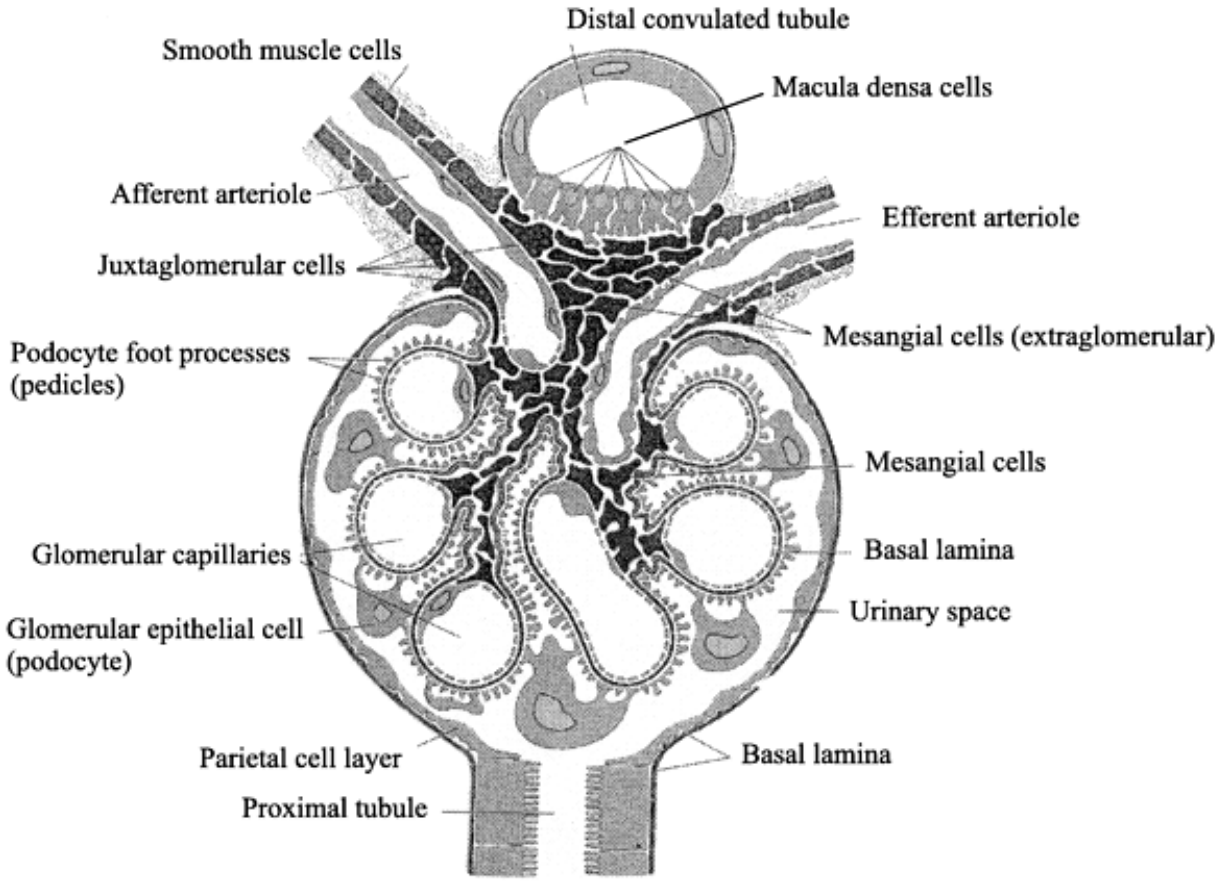


Figure 1: Schematical anatomy of the renal corpuscle. (Homepage of the University of Oulu, Finland)

1.1.2 Pathophysiology of Glomerulonephritis

Glomerular damage is caused by immune mediated processes. Two different pathogenetic mechanisms can be distinguished. One causes glomerular damage because of the deposition of immune complexes on the surface of the glomerular basement membrane (GBM) (Dixon et al., 1961), while the other one is caused by the binding of nephrotoxic antibodies which are specifically directed against components of the GBM (Lerner et al., 1967).
The immune-complex deposition leads to activation of the complement system which results in the damage of the podocytes (Abbate M et al., 2008). This causes a loss of integrity of the slit membrane which is formed by podocytes and their foot processes (Somlo and Mundel, 2000). The dysfunction of the glomerular filter allows the passage of larger molecules such as proteins from the circulation to urine and consequently leads to proteinuria (Adler et al., 1984; Chadban and Atkins, 2005). Besides the glomerular damage the deposition of immune complexes also leads to an overproduction of certain components of the GBM such as collagen type IV and laminin. This overproduction is histologically described as “crescent formation” and results in extracapillary hypercellularity and in the broadening of the GBM (Andres et al., 1966; Bach et al., 1997, D’Agati et al., 2005). There are a lot of different antigens which form the immune complexes with IgG antibodies: double-strand DNA in connection with systemic disorders such as lupus erythemathosus (Brentjens et al., 1975, McCluskey 1982), bacterial or viral components due to infections (Mohammed et al., 1977), and specific antigens in combination with tumor diseases (Couser et al., 1974; Keur et al., 1989).

The other form of the development of glomerular damage is called “anti-GBM-disease”. This term describes a rare autoimmune disorder in which autoantibodies are produced against antigenic targets of the GBM (Lerner et al., 1967), such as collagen type IV (Hudson et al., 1993). Owing to an antigen affinity between the glomerular and alveolar basement membrane, most patients suffering from this form of rapidly progressive glomerulonephritis are also likely to develop alveolar hemorrhage. This combination of symptoms is described as the Goodpasture Syndrome (Butkowski et al., 1985). Glomerular damage leads to progressing renal failure with proteinuria, hematuria, and an increase of serum creatinine (Wilson and Dixon, 1973).
There are also characteristic histological findings like segmental necrosis, crescent formation, the destruction of the GBM, and tubulointerstitial damage (Kluth and Rees, 1999; Chadban and Atkins, 2005).

In order to find out something about the immunologic pathways which are responsible for the glomerular damage and increased glomerular permeability in connection with glomerulonephritis, anti-GBM glomerulonephritis is often induced in experimental models from various species as “passive anti-GBM nephritis” or “nephrotoxic nephritis”. “Passive anti-GBM nephritis” is induced by the intravenous injection of nephrotoxic antibodies directed against GBM. These antibodies can be maintained by immunizing another species with an extract of renal tissue of the laboratory animal. Therefore, they are later able to bind to the GBM of the laboratory animal. To accelerate and specify this binding the laboratory animal is pre-immunised with IgG of the other species. This, in turn, should induce the specific production of antibodies against these IgGs. In the following, the application of the heterologous anti-GBM-antibodies leads to the formation of immune complexes with the produced antibodies of the laboratory animal. The immune complexes are able to activate both the complement system and inflammatory cells such as granulocytes and monocytes which mediate the formation of glomerular crescents as well as leading to a loss of glomerular function (Dixon et al., 1961; Wilson and Dixon, 1973).

1.1.3 Clinical Symptoms of Glomerulonephritis

Depending on the type of glomerulonephritis the symptoms can either be assigned to the nephritic or the nephrotic syndrome.
The nephritic syndrome in the human organism is characterized by a glomerular hematuria and distorted erythrocytes in urine, while proteinuria is less distinctive (< 3g/d). Most patients develop arterial hypertension and generalised edema due to the increased oncotic pressure. A progressive and rapid loss of renal function is also associated with the nephritic syndrome. It can be detected on the basis of increased serum levels of creatinine, which is normally eliminated by the kidney and serves as a parameter for the renal filtration function (Chadban and Atkins, 2005).
The nephrotic syndrome in the human organism is characterized by a distinctive proteinuria (> 3,5g/d up to 20g/d) and a resulting hypoproteinemia with a decrease of serum albumin. Patients also suffer from generalized edema and develop a hyperlipidemia with increased cholesterol and triglyceride levels (Mason and Pusey, 1994; Orth and Ritz, 1998; Chadban and Atkins, 2005; Dendorfer and Mann, 2006).
Both the nephritic and the nephrotic syndrome may differ a lot in their distinctness and can also often merge into one another (Herold 2009).

1.2 Tumor Necrosis Factor

Tumor necrosis factor (TNF, TNFSF2 = tumor necrosis factor superfamily member 2, formally also known as cachectin), a member of the TNF/TNF-R superfamily, is a cytokine which plays an important role in many different functions of the immune system such as the regulation of the activity of immune cells, the immune response to infections, local or systemic inflammation, and the organogenesis and homeostasis of the secondary lymphoid organs (Beutler and Cerami, 1989; Aggarwal 2003; Hehlgans and Pfeffer, 2005).

1.2.1 Structure of TNF

TNF is produced as a 26-kDa 233-amino acid-long type II transmembrane protein. It exists as a stable homotrimer in its membrane-bound form (Kriegler et al., 1988; Tang et al., 1996) but is also biologically active in a soluble form (sTNF). sTNF is a trimer, too, and is released by proteolytic cleavage of the membrane–integrated form. This cleavage reaction is catalyzed by TACE, the TNF-α Converting Enzyme, a membrane-bound metalloprotease and disintegrin (Black et al., 1997, Solomon et al., 1999).

1.2.2 Discovery of TNF

Experimental researches and observations over the last 100 years brought up the theory of an immune response to tumor diseases. In 1968 a cytokine produced by lymphocytes was discovered and was named lymphotoxin (Kolb and Granger, 1968). In 1975 another cytokine produced by macrophages was identified and characterized. It was found to initiate necrosis of cells in an animal model of fibrosarcoma. Following these findings this cytokine was named tumor necrosis factor (Carswell et al., 1975).
As further researches showed a close functional similarity of lymphotoxin and tumor necrosis factor, they were grouped in a large cytokine family, the TNF/TNF-R superfamily.
In 1985 Bruce Beutler revealed that the hormone cachectin, which until then was known to modulate the metabolism of both neoplastic and non-neoplastic cells, was the same protein as the detected TNF (Beutler et al., 1975). These investigations also disclosed the crucial role of TNF in important immune responses such as sepsis (Beutler et al., 1985).

1.2.3 TNF-Receptors and Signaling Pathways

TNF is able to bind to two different types of transmembrane type I receptors which are responsible for the different effects of TNF (Hohmann et al., 1989).
TNFR1 (p55) is a 55 kDa, 425-amino acid-long glycoprotein. It is constitutively expressed on nearly all types of cells and tissues and is mainly responsible for the known effects of TNF.
TNFR2 (p75) is a 75 kDa, 452-amino acid-long glycoprotein which is mainly expressed on activated cells belonging to the immune system but also on other cells, for example epithelial or renal intrinsic cells.
The membrane-bound form of the TNF homotrimer is capable of activating both TNFR1 and TNFR2 whereas the soluble TNF trimer mainly induces responses mediated by TNFR1 (Grell et al., 1995).
Similar to the two forms of TNF both receptors can also be cleaved from the membrane and can exist as soluble receptors. This process is often described as “shedding” of the TNF receptor. The soluble receptors are capable of binding TNF and, therefore, antagonizing or inhibiting the effects of TNF (Nophar et al., 1990; Engelmann et al., 1990; Wallach et al., 1991).

TNFR1 contains an intracellular death domain and, hence, is capable of inducing apoptosis (Tartaglia et al., 1993). When the TNF homotrimer binds to TNFR1 the receptor is able to ligate TRADD (TNF-receptor associated protein via death domain). This ligation provides the further binding of FADD (Fas-associated protein with death domain) and, therefore, the recruitment of Caspase 8. Together FADD and Caspase 8 form the death inducing signaling complex (DISC). If sufficient levels of Caspase 8 are reached the autoproteolytic activation is initiated and the resulting effector caspases induce apoptosis (Schulze-Osthoff et al., 1995).

Whereas the death signaling effects of TNF are rather marginal compared to those of the other receptors containing an intracellular death domain such as Fas, the induction of specific transcription of inflammatory genes is the main effect mediated by the two TNF-receptors. This induction is mediated by both TNFR1 and TNFR2.
As described in the death signaling pathway TNFR1 is able to ligate TRADD when the TNF homotrimer is bound. In addition to TRADD the death domain is capable of binding the kinase RIP (Receptor interacting protein) and recruiting TRAF2 (TNF-receptor associated factor). These ligations provide the activation of IKK (IκB proteinkinase) and therefore the release of NF-κB, an important transcription factor which is responsible for the transcription of various genes. Among these genes are those responsible for the regulation and development of the immune system, cell proliferation, cell differentiation, and inflammatory

processes. Additionally, NF-κB plays a role in the prevention of apoptosis and, therefore, antagonizes the effects of the death signaling pathway of TNFR1 (Chinnaiyan et al., 1996; Legler et al., 2003; Dempsey et al., 2003).

The release of NF-κB can also be mediated by TNFR2. TNFR2 contains an intracellular TIM (TRAF-interacting motif) domain which is capable of binding TRAF2 when the TNF trimer is bound to the receptor. As in the TNFR1 mediated pathway this ligation leads to the release of NF-κB and the effects on transcription described above. Besides the NF-κB pathway other signal transduction pathways are activated via TNFR2 which are important for the regulation of immune response, inflammatory processes and apoptosis, such as JNK (c-Jun N-terminal kinase), p38 MAPK (mitogen activated protein kinase), ERK (extracellular signal related kinase), pI3K (phosphoinositide 3-kinase) and AKT (also known as protein kinase B) (Darnay et al., 1998; Lee et al., 2000, Dempsey et al., 2003).

As mentioned above most effects of TNF are mediated by TNFR1 but in a few cases the interaction of TNFR1 with TNFR2, by the formation of hetero complexes, is essential to acquire the required activation of TNFR1 (Pinckard et al., 1997).
Studies also revealed that TNFR2 is able to intensify the effects mediated by TNFR1 (Wajant et al., 2003), amongst others because it leads to an increased concentration of bound TNF trimers which are consequently available for TNFR1 (Tartaglia et al., 1993).

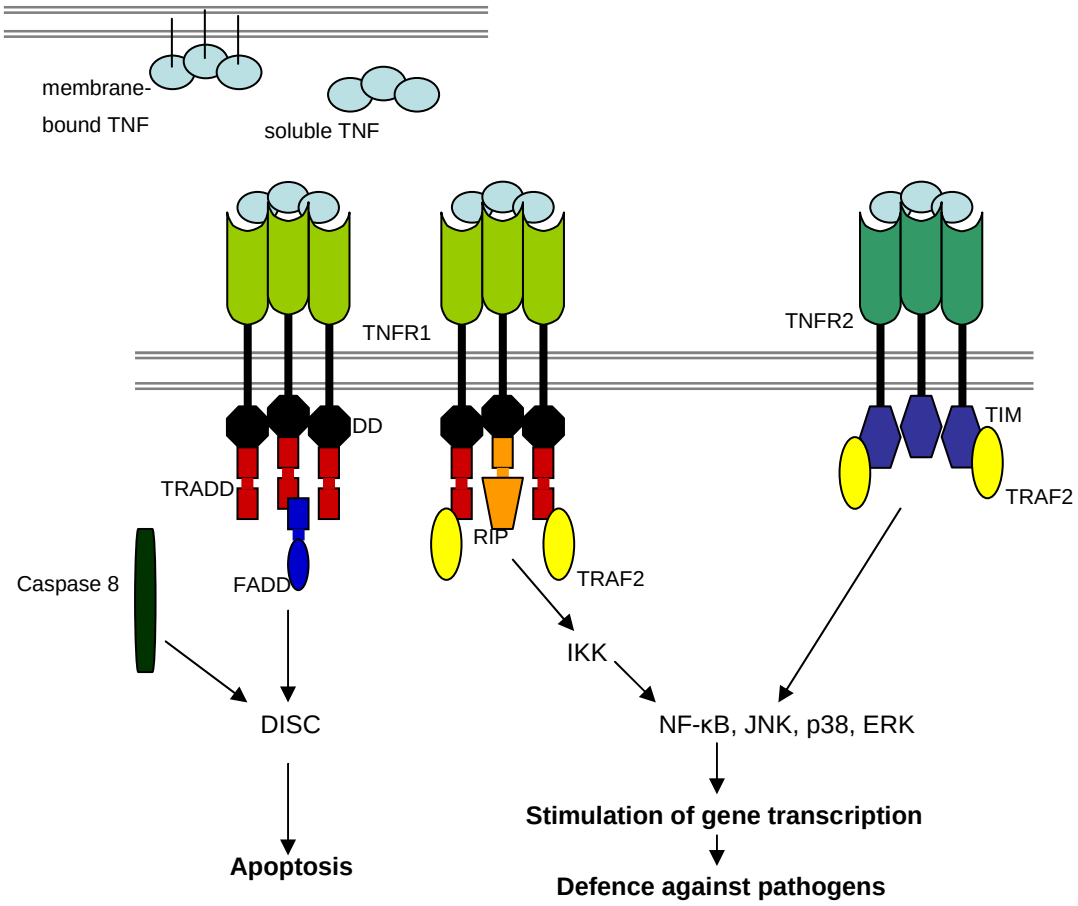


Figure 2: Overview of the signaling pathways of TNF, TNFR1 and TNFR2.

DD=death domain, TRADD= TNF-receptor associated protein via death domain, FADD= Fas-associated protein with death domain, RIP= receptor interacting protein, TRAF2= TNF-receptor associated factor 2, TIM= TRAF-interacting motif, IKK= IκB kinase, p38=p38 mitogen activated protein kinase, ERK=extracellular signal related kinase.

1.2.4 Functions of TNF

TNF is mainly produced and released by activated macrophages but other cells were also found to secrete TNF, among them neutrophils, mast cells, endothelial cells and others depending on the respective tissue which is affected by a immune response taking place (Chensue et al., 1988; Baud et al., 1989; Neale et al., 1995).

One of the most common stimuli for the production of large amounts of TNF is the exposure to endotoxins like lipopolysaccharides (LPS). But other bacterial substances and interleukins also induce the release (Morrison and Ryan, 1987; Baud et al., 1989).

In addition to the induction of necrosis of certain types of tumor cells, TNF plays a crucial role in various processes concerning the immune response to bacteria, viruses and parasites, the regulation of the inflammatory response, and several autoimmune diseases.
In the regulation of the immune response TNF acts as an early inflammatory mediator and enables the host to defend itself against pathogens. This is accomplished by the recruitment of T-cells and macrophages which lead to the production of other proinflammatory cytokines. Inflammatory cells such as neutrophils are also recruited and their proliferation is induced by TNF (Van der Poll et al., 1992). This results in the release of chemokines and cell adhesion molecules which are responsible for the local inflammatory response (Roach et al., 2002; Aggarwal, 2003).
Besides its own function as an early inflammatory mediator TNF is also able to induce the production of acute-phase proteins in the liver which affect coagulation, vascular permeability, and the complement system such as C-reactive protein and complement factors (Perlmutter et al., 1986; Meijer et al., 1993).
Contrary to the described proinflammatory effects there are also immunosuppressive impacts of TNF such as the induction of apoptosis of activated T-cells and neutrophils (Zheng et al., 1995; Elzey et al., 2001). Furthermore, studies revealed that TNF promotes the regression of autoimmune reactivity (Cope, 1998).

Studies in the past also discovered that besides its host defensive effects TNF is a mediator in various pathologies and autoimmune diseases and that its release can have systemic endotoxic effects (Tracey et al., 1986; Kollias et al., 1999).
A dysregulation of the production of TNF resulting in a prolonged overproduction may lead to inflammatory disorders, for example the induction of sepsis and the development of a septic shock syndrome as a response to bacteria. In this case high levels of TNF are brought into connection with various symptoms that characterize sepsis such as fever, falling blood pressure, systemic edema, and the progressing multi-organ failure (Tracey et al., 1986; Kilbourn et al., 1990).
TNF and its overproduction are also involved in other pathologies, for example AIDS and the accompanying cachexia, transplant rejection (Dörge et al., 1994), and various autoimmune disorders such as rheumatoid arthritis and inflammatory bowel diseases (Kollias et al., 1999).

1.2.5 Anti-TNF-Therapies

Dysregulation in the production of TNF and resulting high levels of TNF are not only responsible for immune responses such as sepsis but also for various autoimmune disorders

caused by the proinflammatory effects of TNF. These findings led to the development of a new way to treat patients who suffer from chronic inflammatory diseases. It provides the opportunity to antagonize TNF by the application of monoclonal antibodies which bind to TNF or the application of recombinant soluble human TNFR2. These monoclonal antibodies are available as chimeric mouse-human antibodies (Infliximab, distributed as Remicade®), recombinant human antibodies (Adalimumab, distributed as Humira®) and humanized antibodies (Certolizumab, distributed as Cimzia®). Furthermore, a fusion protein consisting of the soluble form of the human TNFR2 and the Fc portion of human IgG1 (Etanercept, distributed as Enbrel®) has been developed.

Anti-TNF-antibodies are indicated for the treatment of chronic inflammatory diseases such as inflammatory bowel diseases (van Dullemen et al., 1995; Suenaert et al., 2002), rheumatoid arthritis (Feldmann and Maini, 2001), ankylosing spondylitis, and psoriasis (Mease et al., 2000; Reimold, 2003; Antoni et al., 2005). Anti-TNF antibodies are administered if the basis therapy containing glucocorticoids and Methotrexat provide no sufficient amelioration of the symptoms.

Several clinical studies confirmed the efficiency of anti-TNF antibody therapies. But apart from the high costs of these therapies patients have to cope with the risk of adverse side effects which may turn out as unpredictable due to the contrasting effects of TNF as described above (Kollias and Kontoyiannis, 2003; Reimold, 2003). Among these adverse side effects are the risk of the reactivation of arrested tuberculosis and other infections, the formation of auto-antibodies resulting in the development of lupus-like syndromes.

In the treatment of multiple sclerosis anti-TNF-antibody therapy even deteriorated symptoms and led to an exacerbation of the disease (Sicotte and Voskuhl, 2001).

Among others, these findings have led to the consideration that developing therapy opportunities aiming at the TNF-receptors instead of TNF itself may provide an alternative, improved option (Kollias and Kontoyiannis, 2002).

1.3 Role of TNF in Renal Diseases

Several studies in the past few years revealed that TNF plays a decisive role in renal injuries and diseases (Bertani et al., 1989; Tomosugi et al., 1989; Vielhauer and Mayadas, 2007). Renal mesangial cells, glomerular and tubular epithelial cells were found to produce high amounts of TNF, but only when exposed to cell damage or stimulation (Tipping et al., 1991; Timoshanko et al., 2003). Increased levels of TNF were discovered in several experimentally

induced renal injuries such as endotoxin-induced renal failure (Ramesh et al., 2007), obstruction-induced renal tubular cell apoptosis, cisplatin-induced (Ramesh and Reeves, 2003) and ischema-induced renal injury (Donnahoo et al., 1999). Other animal models which focused on the damage of glomerular structures revealed that animals which were exposed to experimentally-induced nephrotoxic nephritis developed glomerular injuries due to high systemic TNF levels (Le Hir et al., 1998).
These various models showed that TNF is an important mediator of different processes which lead to renal injury and failure such as apoptosis of renal cells, resulting in renal dysfunction, the induced production of other members of the inflammatory response, and the recruitment of proinflammatorily active cells.

These findings were confirmed by the fact that treatment with TNF inhibitors and the soluble TNF-receptor led to the improvement of renal functions and alleviation of renal damage (Hruby et al., 1991; Lan et al., 1997).
In addition, TNF-deficient mice were found to develop less distinctive injuries, suffered less from nephrotic symptoms, and showed fewer histological alterations such as crescent formation and inflammatory infiltrates (Le Hir et al., 1998).

Clinical researches also revealed increased production of TNF in patients who developed rejection reactions after kidney transplantation as one example for a special immunological situation (Kutukculer et al., 1995). Histological analysis of renal biopsies showed that both renal parenchymal cells and mononuclear leukocytes were responsible for high levels of TNF resulting in an excessive inflammatory and immune response (Morel et al., 1993; Noel et al., 2000, Al-Lamki et al., 2001).

1.3.1 Role of TNF and TNF-Receptors in Glomerulonephritis

As described above, the induction of “nephrotoxic nephritis” or “passive anti-GBM nephritis” in animal models provides the opportunity to get to know the pathogenetic mechanisms that lead to the development of glomerulonephritis. It was shown that TNF plays an important role in this development but the signaling pathways that are responsible weren’t completely understood.

In 2005 Vielhauer and colleagues (Vielhauer et al., 2005) focused on the specific role of the two TNF-receptors TNFR1 and TNFR2 in the pathogenesis of glomerulonephritis.

They induced accelerated nephrotoxic nephritis in mice and examined the role of the two TNF-receptors by looking at different parameters characterizing glomerulonephritis such as the development of proteinuria, glomerular lesions, activation of the complement system, T-cell and macrophage infiltration, and complement deposition.
They found out that TNFR2-deficient mice developed a less severe and a later onset of proteinuria than TNFR1-deficient or wild type mice. Furthermore, they were not affected by other symptoms related to the nephrotic syndrome. Neither glomerular lesions nor infiltration of inflammatory cells such as T-cells and macrophages were observed in the histological analyses of the kidneys of TNFR2-deficient mice. In addition TNFR2-deficient mice showed lower activation of the complement system and lower amounts of glomerular complement deposition.
Vielhauer also examined the renal expression of TNFR2 both in mice suffering from nephrotoxic nephritis and mice that were not exposed to any treatment. Kidney stainings revealed that the receptor was expressed on intrinsic renal cells such as glomerular endothelial cells in glomerulonephritis-affected mice but could not be found on the renal cells of the untreated mice.
In order to find out whether this renal TNFR2 expression was responsible for the progression of glomerulonephritis, Vielhauer compared bone marrow-chimeric mice. He showed that TNFR2 on intrinsic renal cells and not on bone marrow-derived cells such as T cells or macrophages is required for the development of the disease.

On the basis of these findings he put forward his hypothesis that TNFR2 plays a central role in the inflammatory processes in glomerulonephritis and that, therefore, TNFR2-deficient mice are protected from glomerulonephritis.

1.4 Aim of the Study

On the basis of the hypothesis proposed by Vielhauer (Vielhauer et al., 2005) we intended to investigate whether the protection of the TNFR2-deficient mice against the development of experimental glomerulonephritis could be reproduced in our experimental setup of glomerulonephritis induction. As appropriate parameters for the development of glomerulonephritis we intended to examine whether TNFR2-deficient mice developed less severe proteinuria and less severe glomerular damage than C57Bl/6 control animals. Furthermore, we intended to study the development of experimental glomerulonephritis in mice that were transgenic for the human TNFR2 (hTNFR2). Apparently, hTNFR2 is able to

interact with mouse TNF in a functional way (Bäumel et al., 2008). Due to these findings, transgenic mouse lines were generated as described in 2.2.7. We backcrossed the transgenic mice with C57Bl/6 mice and phenotyped them according to 2.1.4. These transgenic mice expressed both mouse and human TNFR2 (Figure 20; Figure 22). hTNFR2 was expressed constitutively in every cell and expression was not subjected to any regulatory elements. Soluble hTNFR2 could be detected in urine (Figure 30).

Several studies in the past few years revealed that mice transgenic for hTNFR2 were more sentizised to the toxic effects of LPS and TNF than their nontransgenic littermates. They developed more severe inflammatory symptoms and increased histological pathologies could be observed in diseases such as severe inflammatory syndrome, experimental hepatitis induced by Con-A (Concanavalin A) as well as in intestinal inflammatory diseases (Küsters et al., 1997; Douni and Kollias, 1998; Holtmann et al., 2002).
Transgenic mice used by Douni and Kollias (Douni and Kollias, 1998) differed from those we used in our experiments. In their animals transgenic constructs containing the hTNFR2 gene also contained regulatory elements. These elements controlled the expression of hTNFR2 depending on influences and impacts to which mice were exposed.
Other studies revealed that mice transgenic for an hTNFR2 isoform, which is mainly expressed intracellularly and, therefore, termed hicp75TNFR were also found to be more susceptible for TNF-dependent inflammation than nontransgenic littermates. The effect induced in mice transgenic for hicp75TNFR, although, was less striking than the one induced in mice transgenic for hTNFR2 (Bäumel et al., 2008; and unpublished results).

As demonstrated by Vielhauer the development of experimental glomerulonephritis also seems to be dependent on TNFR2. According to these findings and the effects described above of hTNFR2 we intended to investigate whether the constitutive overexpression of hTNFR2 on intrinsic renal cells in our animals is also capable of inducing enhanced susceptibility to the induction of experimental glomerulonephritis. Therefore, we intended to induce experimental glomerulonephritis in both mice transgenic for hTNFR2 and there nontransgenic littermates and compare their reaction regarding the development of proteinuria and glomerular damage.

2 Materials and Methods

2.1 Methods

2.1.1 Induction of Glomerulonephritis

First the mice were immunised by injecting 200 µL of a 1:1 emulsion of 0,2 mg rabbit IgG and complete Freund's Adjuvans subcutaneously. This should result in the production of antibodies against rabbit IgG.
6 days after the immunisation 250 µL of rabbit "anti-GBM-serum" were injected into the retro-bulbar blood vessel of each mouse. For this purpose the mice were sedated with Ketanest-Xylazin by injecting 100 µL per 10g body weight intraperitoneally (i.p.). The rabbit "anti-GBM-serum" was produced by Drs. Männel, Echtenacher, and Witzgall by immunising Chinchilla rabbits with a solution containing an extract of homogenized glomeruli isolated from mouse kidneys for several times. The blood was taken and serum was produced. The specificity of the binding of the serum to the murine glomerular basement membrane was tested on paraffin sections of mouse kidneys. The specific binding could be detected up to a dilution of 1:10,000. In all our experiments serum of rabbit No. 194 was used. In experiment 3.1, 3.2, 3.3, 3.4, and 3.5 serum was used which was taken at August 17, 2006 after the 5th immunisation of the rabbit. In experiment 3.7 serum was used which was taken at August 22, 2006 as well after the 5th immunisation of the rabbit.

Experimental setup:

Days from induction of glomerulonephritis:

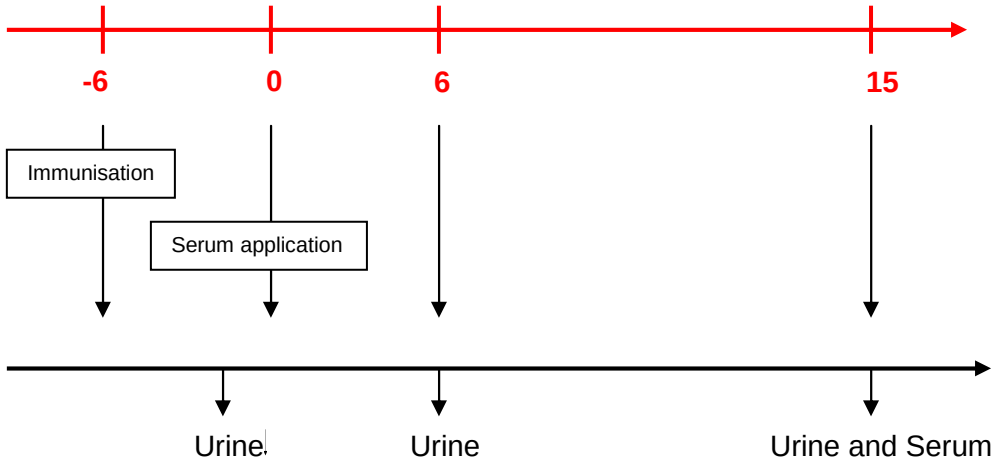


Figure 3: Experimental setup of the induction of glomerulonephritis.

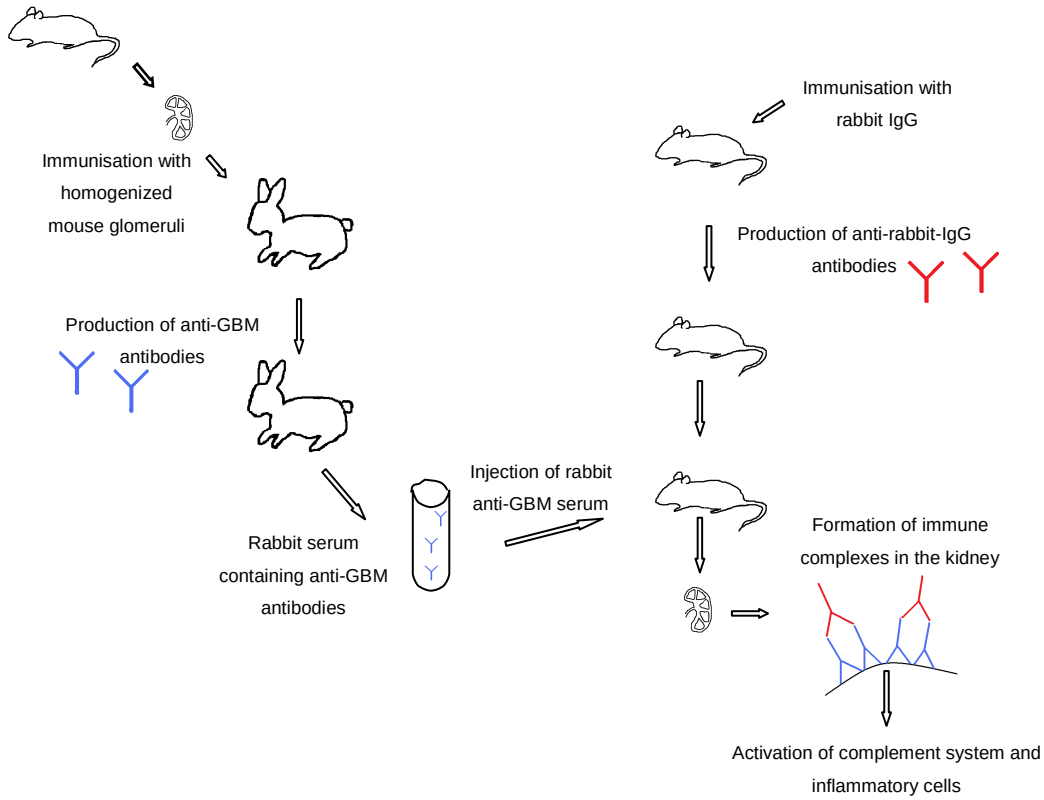


Figure 4: Schematical overview of the induction of glomerulonephritis with rabbit anti-GBM serum.

2.1.2 Measurement of Proteinuria

In order to quantify the extent of the glomerulonephritis the protein concentration in the murine urine had to be measured before and after immunisation and throughout the progression of the disease. Therefore urine was collected from all mice once a day and stored at -20℃ until the measurement. The protein concentration was measured according to the method of Bradford, which involved the addition of an acidic dye solution to a solution containing proteins. The binding of the acid dye to the protein resulted in a shift of the absorbance maximum from 465 nm to 595 nm. A standard row was created for each measurement, using an Albumin Standard from the BCA Protein Assay Kit in a log2-dilution serial from 25 µg/mL up to 1000 µg/mL in PBS. Urines were diluted in PBS in order to reach a linear measurement range and used in two technical replicates. As dye reagent Dye Reagent Concentrate from the Bio-Rad Protein Assay was diluted 1:5 with deionised water. 5µL of diluted urine or standard were pipetted into microtiter plate wells and 200 µL of dye reagent were added to each well and dispensed with the pipette. The plate was incubated at

room temperature for 5 minutes. Depending on the protein concentration of the urine sample different color changes of the dye reagent were observed and the absorbance was measured at 595nm in the photometer. The protein concentrations in the urine samples were then calculated by using the standard row in a linear way.

2.1.3 ELISAs for Measuring the Soluble Mouse TNFRII, Human TNFRII and Mouse TNF in Urine and Serum

All ELISAs were carried out in accordance with the general ELISA protocol given in the corresponding product data sheet of R&D Systems (Homepage of R&D Systems, see References).

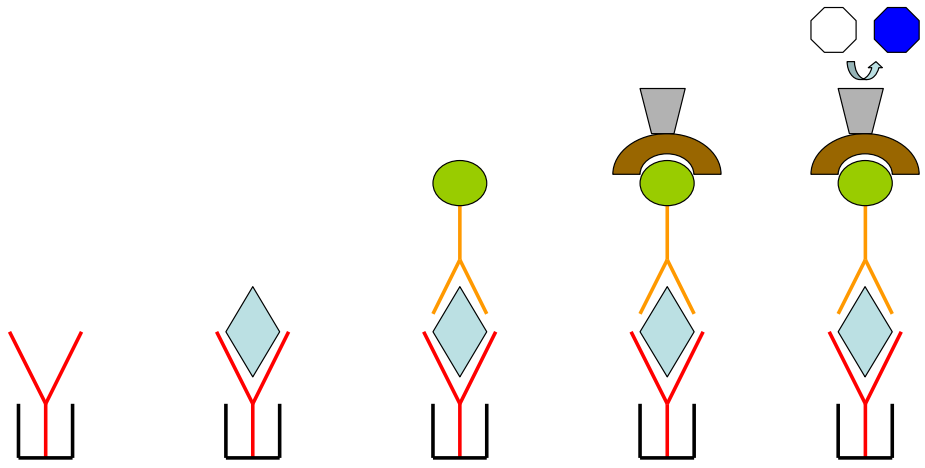
Plate preparation:

1. The Capture Antibody was diluted to the working concentration in PBS and a 96-well assay plate was covered with 100 µL per well of the diluted Capture Antibody. The plate was covered and kept overnight at room temperature in the dark. (1, Figure 5)
2. Each well was washed by filling it with 400 µL of Wash Buffer using a manifold dispenser and Wash Buffer was removed by emptying the plate on paper towels. The wash process was repeated another three times.
3. The plate was blocked with 300 µL of Reagent Diluent to each well in order to saturate free binding opportunities for proteins. The plate was then incubated at room temperature for at least 1 hour.
4. The washing was repeated as described in step 2.

Assay procedure:

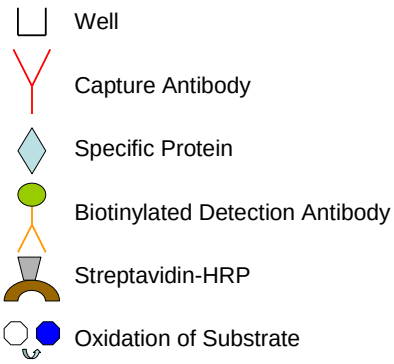
1. Samples of urine or serum and the standard were diluted in Reagent Diluent to appropriate concentrations in order to reach a linear measurement range. The standard was diluted in a 2-fold serial dilution starting with a highest standard of 500 pg/mL. 100 µL of the dilutions were added to each well and the plate was incubated overnight at room temperature in the dark. (2, Figure 5)
2. The washing was repeated as described in step 2.

3. The biotinylated Detection Antibody was diluted in Reagent Diluent and 100 µL were added to each well in order to bind to the protein which should be detected. The plate was incubated for another 2 hours at room temperature. (3, Figure 5)
4. The washing was repeated as described in step 2.
5. Streptavidin-HRP was diluted 1:200 in Reagent Diluent and 100 µL were added to each well in order to bind to the biotin attached to the Detection Antibody. The plate was incubated for 20 minutes in the dark. (4, Figure 5)
6. The washing was repeated as described in step 2.
7. 100 µL of the Substrate Solution, containing an 1:1 mixture of H_2O_2 and Tetramethylbenzidine were added to each well and the plate was incubated for 20 min. at room temperature in the dark. Streptavidin-HRP catalyzed the oxidation of Tetramethylbenzidine with H_2O_2 which resulted in a colour change to blue. (5, Figure 5)
8. 50 µL of 2 N H_2SO_4 were added as Stop Solution to each well in order to stop the reaction which leads to a yellow colouration.
9. The optical density was then immediately measured at 450nm.



(1) (2) (3) (4) (5)

Figure 5: Schematic overview of the sandwich-ELISA method: (1)Plate is coated with the capture antibody. (2) Sample is added and antigen binds. (3) Biotinylated detection antibody binds to the antigen. (4) Streptavidin-HRP binds to the biotin. (5) Substrate is added and product can be detected.



Result calculation:

In all measurements technical replicates were used. A standard curve was created by plotting the logarithm of the different concentrations of the standard versus the logarithm of the corresponding mean optical density and then drawing the regression line. The standard curve was created newly for each plate.
The sample concentrations were calculated by using the standard curve. The results were shown in a non-logarithmic form.

Sensitivity of the test system:

The lower detection limit in the respective ELISA setup is 31,25pg/mL.

2.1.4 Phenotyping of Mice Transgenic for hTNFR2

Urine of the hTNFR2 mice was taken from the naïve mice at two consecutive days and the concentration of hTNFR2 was measured using the human sTNFRII ELISA Assay Kit according to the manual. Measurements revealed that the urines of transgenic naïve mice contained up to 15 ng/mL of hTNFR2. As negative controls served the urines of wild type mice. The results of the ELISA were compared to the results of a Southern Blot done by another working group. Results were consistent in both types of genotyping. Therefore, in the following the human sTNFRII ELISA was used for genotyping as a very simple, accurate and efficient method.

2.1.5 Measurement of Creatinine in Serum and Urine

Creatinine levels were measured in urine and serum in order to quantify the extend of glomerular damage of mice in the anti-GBM glomerulonephritis experiment.
The assay was carried out in accordance with the QuantiChrom^{TM} Creatinine Assay Kit protocol given in the corresponding product data sheet of BioAssay Systems (Homepage of Bio Assay Systems, see Reference).

Serum assay:

1. Standard was diluted in deionised water to a concentration of 2 mg/100mL. 30 µL of both standard and undiluted serum were added in technical replicates to the wells of a 96-well assay plate.
2. Working Reagent was prepared by mixing 100 µL Reagent A and 100 µL Reagent B per well. 200 µL Working Reagent were quickly added to each well using a multi-channel pipette.
3. The optical density was measured at 510nm after 1 min (OD1) and after 5 min (OD5).

Urine assay:

1. Standard was used in the stock concentration of 50mg/100mL. 5 µL of both standard and undiluted urine were added in technical replicates to the wells of a 96-well assay plate.
2. Working Reagent was prepared by mixing 50 µL Reagent A and 50 µL Reagent B with 100µL tap water. 200 µL Working Reagent were quickly added to each well using a multi-channel pipette.
3. The optical density was measured at 510nm after 1 min (OD1) and after 5 min (OD5).

Calculation:

The concentration of creatinine in the respective serum or urine samples was calculated referring to the following formula:

$$\text{Creatinine [mg/100mL]} = (OD5_{Sample} - OD1_{Sample})/(OD5_{Standard} - OD1_{Stanard}) * [\text{Standard}]$$

Sensitivity of the test system:

The lower detection limit in the respective creatinine assay setup is 2mg/100mL.

2.1.6 Fixation and Staining of Mouse Kidney Tissue

On day 15 after the induction of glomerulonephritis, blood was taken by retro-bulbar bleeding of all mice, they were killed by cervical dislocation and both kidneys were removed.

Cryo-fixation and sectioning:

1. The right kidney of each mouse was put into mounting medium at room temperature, snap frozen in liquid nitrogen immediately and then stored at -80℃.
2. The mounted and frozen kidneys were sectioned with a thickness of 5-10 µm, put on a slide and were then fixed and dehydrated with cold acetone for 10 min at -20℃. After that they had to be dried for 10 min at room temperature.
3. The whole cryosection had to be encircled with a hydrophobic emulsion using Dako Pen and then dried again at room temperature for 15 min.
4. The slides were stored at -20℃ until staining.

Paraffin fixation and sectioning:

1. The left kidney of each mouse was put into buffered formalin solution and fixed for 3 months at +8℃.
2. The kidneys were rinsed with tap water for 2-6 h and then dehydrated in ascending alcohol series (20% Isopropanol, 40% Isopropanol, 60% Isopropanol, 80% Isopropanol, 90% Isopropanol, twice 100% Isopropanol for 1 h each, 100% Isopropanol for 16 h).
3. Then they were put three times into Xylol for one hour before they were paraffinated in Paraffin at 55-65℃ for 4 h, 16h and again 4 h. The n they were embedded in hot paraffin and sectioned with a thickness of 5-10 µm. The sections were applied to slides and ready for staining.

H.E. Staining:

The H.E. staining method was used to gain an overview of the tissue structure.

1. The slides with paraffin sections were placed in a slide holder and first thermally deparaffinized for 35 min at 72℃ in the heating ca binet and then twice chemically deparaffinized in Xylol for 10 min.

2. The slides were then dehydrated and the Xylol was washed out by putting them twice into 99% Ethanol for 1 min, twice into 96% Ethanol for 1 min and once into 70% Ethanol. After that they were well rinsed with deionised water.
3. The slides were then put into Haematoxylin for 3 min, rinsed with deionised water, and put into tap water for 5 min to allow the staining to develop.
4. The slides were fast dipped into 2% HCl-Ethanol for about 10 times, twice rinsed in tap water for one minute, and finally rinsed with deionised water for 2 min. Excessive water had to be removed by blotting the slide holder against paper towels.
5. The slides were then put into aqueous Eosin for a maximum of 10 sec and then shortly rinsed in tap water.
6. After that the slides were shortly dipped into 70% Ethanol, then twice put into 96% Ethanol for 5 sec, twice into 99% Ethanol for 1 min, and twice into Xylol for 3 min.
7. The slides were then cover slipped using Entellan™ and dried overnight.
8. As a result the nuclei should be stained blue or violet, cytoplasm, collagen, and erythrocytes should be stained purple.
9. The slides were analyzed using the Leitz Diaplan microscope and pictures were taken using the Color View™ camera.

Periodic Acid-Schiff Staining:

The PAS staining method was used to demonstrate the carbohydrates in the tissue and therefore to visualize glomerular structures and lesions.

1. The slides with paraffin sections were placed in a slide holder and first thermally deparaffinized for 35 min at 72℃ in the heating ca binet and then twice chemically deparaffinized in Xylol for 10 min.
2. The slides were then dehydrated and the Xylol was washed out by putting them twice into 99% Ethanol for 1 min, twice into 96% Ethanol for 1 min, and once into 70% Ethanol. After that they were well rinsed with deionised water.
3. The slides were oxidized in Periodic Acid Solution for 5 min and then rinsed with deionised water.

4. After that they were put into Schiff's Reagent for 2 min and rinsed with tap water.
5. The slides were then counterstained in Haematoxylin for 2 min and again rinsed with lukewarm tap water.
6. After that the slides were dipped twice into 70% Ethanol for 10 sec and then twice put into 96% Ethanol for another 10 sec, twice into 100% Propanol for 10 sec, and twice into Xylol for 2 min.
7. The slides were then cover slipped using Entellan™ and dried overnight.
8. As a result glycogen and basement membranes should be stained purple, the nuclei blue, proteins and cytoplasm should be stained yellow.
9. The slides were analyzed using the Leitz Diaplan and pictures were taken using the Color View™ camera.

Staining of mTNFR2:

1. The stored slides with cryo sections were rehydrated in Wash Buffer for 10 min.
2. Then they were blocked with Block Buffer for 30 min at room temperature.
3. After that the slides had to be washed three times with Wash Buffer for 10 min.
4. During the washing the antibody dilution was prepared in Staining Buffer by diluting the anti-mTNFR2 AF 647 antibody 1:100. The slides were then stained for 30 min at room temperature.
5. The washing was repeated as described in step 3.
6. The slides were then covered with mounting medium and analyzed at the Zeiss Axio.

2.1.7 Statistics

Statistics were done by using a T-test. Significance was determined for values of $p<0,05$.

2.2 Materials

2.2.1 Chemicals

Acetone, Lot. No. 0688950 (Acros, New Jersey, USA)
BD OptEIA™ Substrate Reagent A&B, Cat. No. 51-2607 and 51-2606, Lot. No. 85847 and 91028 (Becton Dickinson, Heidelberg)
Bio RAD™ Protein Assay, Cat. No. 500-0006, Lot. No. 106819 (Bio RAD, München)
Bovine Serum Albumin (BSA), Cat. No. P06-1391050, Lot. No. 108 (PAN Biotech GmbH, Aidenbach)
Complete Freund's Adjuvans, Lot. No. 014K8927 9007-81-2 (Sigma Aldrich, Deisenhofen)
Crystal Mount™ Aqueous Mounting Medium (for staining) Lot. No. 025K1195 (Sigma Aldrich, Deisenhofen)
Dako Pen™, Lot. No. 00040914 (Dako, Glostrup, DK)
Entellan™, Lot. No. 1007961.0500 (Merck, Darmstadt)
Eosin-G solution, 0.5% aqueous, Lot. No. X8832 (Roth, Karlsruhe)
Mayers Hämalaun, Lot. No. 109249250 (Merck, Darmstadt)
Mounting Medium Killik (for cryosections), Lot. No. 200830 (Bio Optica, Milano, I)
Periodic Acid Solution, Lot. No. 395-1 (Sigma Aldrich, Deisenhofen)
Schiff Reagent, Lot. No. 395-2 (Sigma Aldrich, Deisenhofen)
Streptavidin HRP, Part. No. 890803, Lot. No. AEM5407092 (R&D Systems, Wiesbaden)
Tween™ 20, Lot. No. 1337618 32807046 (Fluka, Buchs, CH)
Xylol, Lot. No. 9713.3 (Roth, Karlsruhe)

2.2.2 Consumables

Assay plates, used for ELISAS, 96-well BD Falcon™™, REF 353912, Lot.No. 012835 (Becton Dickinson, Heidelberg)
BD Falcon Microtest Tissue Culture plates, 96-well, REF 353075 (Becton Dickinson, Heidelberg)
Cover slips (Marienfeld, Lauda-Königshofen)
Embedding caskets for paraffin fixation, Lot. No. 053761(Kabe, Nürnbrecht-Eisenroth)

Eppendorf tubes, 1.5 mL and 2 mL (Eppendorf Hamburg)
Microscope Slides (Engelbrecht, Edermünden)
Needles (Becton Dickinson, Heidelberg)
Pipettes (Sarstedt, Nürnbrecht)
Specimen Molds for cryo fixation: Cryomold Standard, Lot. No. 2-0114557 (DiaTec, Hallstadt)
Syringes, 1 ml, 2 mL and 5 mL (Becton Dickinson, Heidelberg)
Tips, steril (Sarstedt, Nürnbrecht)

2.2.3 Instruments

Camera for microscopy: Color View™, Soft Imaging Systems (Olympus, Münster)
Centrifuge: Centrifuge 5418 (Eppendorf, Hamburg)
Microscopes: Leitz Diaplan™ (Leitz, Wetzlar)
Zeiss Axio™ (Zeiss, Göttingen)
Photometer: Bio Photometer (Eppendorf, Hamburg)
Reader for the ELISA plates: Molecular Devices Emax™ (MWG Biotech, Ebersberg)
Scales: Mettler™ PJ 400 (Mettler-Toledo, Giessen)
Sartorius™ CP 224S (Sartorius, Göttingen)

2.2.4 Buffer Solutions

Buffered Formalin Solution, pH 7.4, containing: 9.07 g KH_2PO_4
11.86 g Na_2HPO_4 soluted in 860 ml of deionised water
140 ml Formalin (37% stock solution)
PBS, pH 7.3, containing: 137 mM NaCl
1.5 mM $KH_2 PO_4$
6.5 mM Na_2HPO_4
2.7 MM KCl
Reagent Diluent: 1% BSA in PBS
Wash Buffer: 0.05% Tween 20 in PBS

2.2.5 Assay Kits

BCA Protein Assay Kit™, Prod. No. 23225, Lot. No. IH114297B (Thermo Scientific, Schwerte)
QuantiChrom™ Creatinine Assay Kit, Cat. No. DICT-500 (BioAssay Systems, Hayward, USA)

ELISAs:

Mouse sTNF RII/ TNFRSF1B, Cat. No. DY426 (R&D Systems, Wiesbaden)
Mouse TNF-α/ TNFSF1A, Cat. No. DY410 (R&D Systems, Wiesbaden)
Human sTNF RII/ TNFRSF1B, Cat. No. DY726 (R&D Systems, Wiesbaden)

2.2.6 Antibodies

Chrom Pure Rabbit IgG, whole molecule, Code: 011-000-003, Lot. No. 79330 (Jackson Immuno Research, Suffolk, UK)
Anti-mTNFR2 antibody: Hamster Anti Mouse CD120b: ALEXA 647, MCA2351A647, Batch No. 0105 (Serotec, Düsseldorf)

2.2.7 Animals

The animals were kept according to the keeping regulations. They were exposed to a 12-hour day and night rhythm and achieved a standard diet and tap water.

C57Bl/6, 8-10 weeks old (Charles River, Sulzfeld)
C57Bl/6, 7 months old (Charles River, Sulzfeld)
hp75tg (huTNFR2tg), 8-10 weeks old

(To generate hp75TNFRtg mice, full length hp75TNFR-cDNA was cloned into *Hind*III and *Xho*I sites of an expression vector driven by the human ubiquitin C promotor. The sequence of the cloned hp75TNFR construct was confirmed by automated sequencing. The transgenic hp75TNFR fragment was released by using *Nde*I/*Kpn*I digestion of the

vector and microinjected into pronuclei of fertilized oocytes prepared from FvB mice using standard protocols. The offspring were screened for transgene integration by DNA extraction from tail biopsies. The purified DNA was analyzed by Southern blot analysis using a α-P^{32}.dCTP-labeled hp75TNFR-cDNA fragment as probe. As positive control, a part of the transgenic hp75TNFR fragment was removed by *Eco*RI digestion of the vector (on the basis of Bäumel et al., 2008). Confirmed hp75tg founder mice were backcrossed to the F2 and F3 generation with C57Bl/6 mice and genotypizised both by Southern Blots and ELISAs, according to 2.1.4 Phenotyping of Mice Transgenic for hTNFR2. Nontransgenic littermates served as control animals.)

C57Bl/6, 8 weeks old (Charles River, Sulzfeld)

P75-/-, 8-10 weeks old (Breed of the Department of Immunology)

P75-/-, 11-12 months old (Breed of the Department of Immunology)

CD45.1, 10 weeks old (Breed of the Department of Immunology)

2.2.8 Others

Ketanest-Xylazin, containing 0.9 mL Ketamin 5%, Lot. No. 72201-10 (WDT, Garbsen)
0.48 mL Xylazin 2%, Lot. No. 0056 (Serumwerk, Bernburg)
4.62 mL PBS, sterile

3 Results

3.1 Glomerulonephritis Induction in TNFR2-/- Mice and Control Animals – Part 1

3.1.1 Evaluation of the Induction of Glomerulonephritis

In order to test our protocol of immunization and induction of glomerulonephritis, we compared 10 C57Bl/6 animals in which glomerulonephritis was induced according to 2.1.1 with 10 C57Bl/6 animals which were not immunized and merely received rabbit anti-GBM serum. Mice which were not immunized and merely received rabbit anti-GBM serum on day 0 developed no proteinuria, whereas mice which received both rabbit-IgG on day -6 and rabbit anti-GBM serum on day 0 developed proteinuria up to day 15, when the experiment was finished (data not shown). These findings exclude the possibility that mere injection of rabbit anti-GBM serum could be capable of inducing severe glomerulonephritis and point out the importance of immunization with rabbit-IgG in order to induce the development of glomerulonephritis.

Glomerulonephritis was induced in 7 TNFR2-/- mice and 7 control animals according to 2.1.1. As control animals served C57Bl/6 mice. TNFR2-/- mice were 11 to 18 weeks old, control animals were 15 weeks old when they were taken into the experiment. Urine was collected once to twice a day. However, we were not able to get urine from each mouse once or twice a day. The mice were killed on day 15, blood was taken, serum produced and the kidneys were removed and fixated according to 2.1.6.
One control mouse died on day 14 after induction of glomerulonephritis.

3.1.1 Proteinuria

Proteinuria was measured according to 2.1.2. Urines were collected and stored at -20℃ for two or three days until the measurement was done.
On day 14 proteinuria was found to be significantly higher in control animals than in TNFR2-/- animals. On the other days no significant differences could be seen between the two mouse lines.

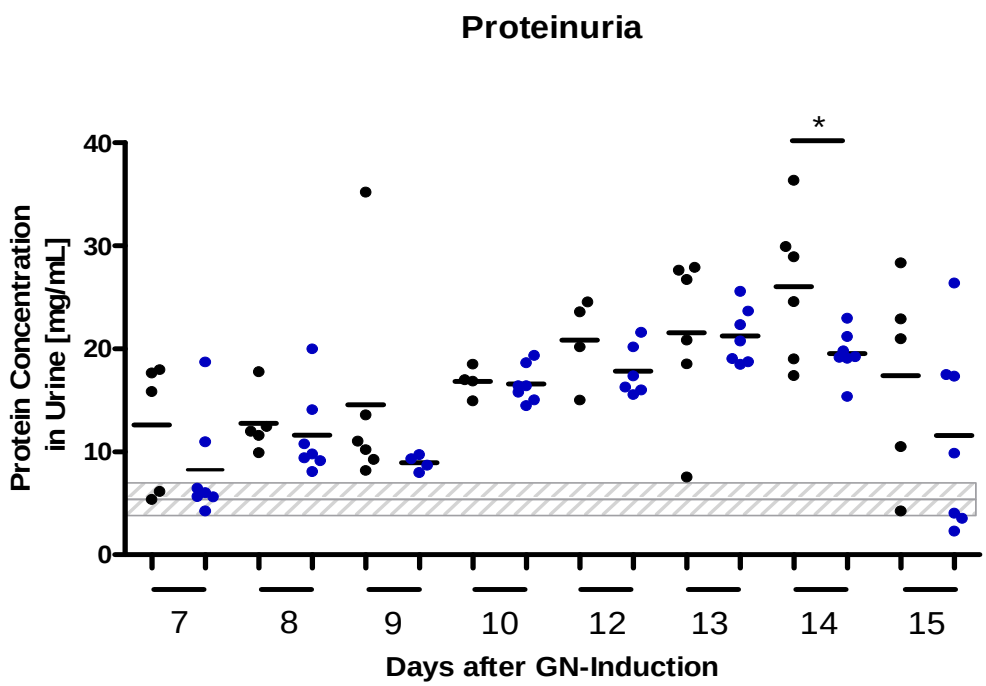


Figure 6: Development of proteinuria in TNFR2-/- animals and control animals during glomerulonephritis. Black dots represent control animals (C57Bl/6), blue dots represent TNFR2-/- mice. Dots represent single animals and means are shown. Grey shaded box shows mean and standard deviation of the normal range of proteinuria of all animals before the induction of glomerulonephritis (5,89±1,02mg/mL). If urine was taken twice a day the average protein concentration is shown. Values and means are shown from day 7 to 15 since no development of proteinuria could be seen until day 7.

3.1.2 Creatinine Concentration

Creatinine was measured in urine according to 2.1.5.
On day 15 creatinine concentrations turned out to be significantly higher in control animals than in TNFR2-/- animals.

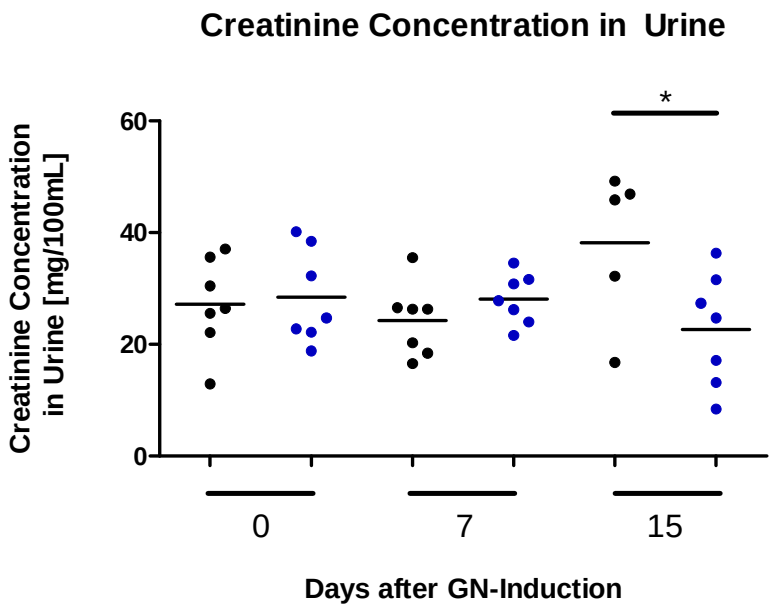


Figure 7: Creatinine concentration in urine of TNFR2-/- animals and control animals during glomerulonephritis. Black dots represent control animals (C57Bl/6), blue dots represent TNFR2-/- mice. Dots represent single animals and means are shown.

3.1.3 Relation of Proteinuria to Creatinine

The measured proteinuria and creatinine concentrations in urine were put in relation to each other on two selected points of time.
No significant differences could be seen between control animals and TNFR2-/- animals at any point of time.

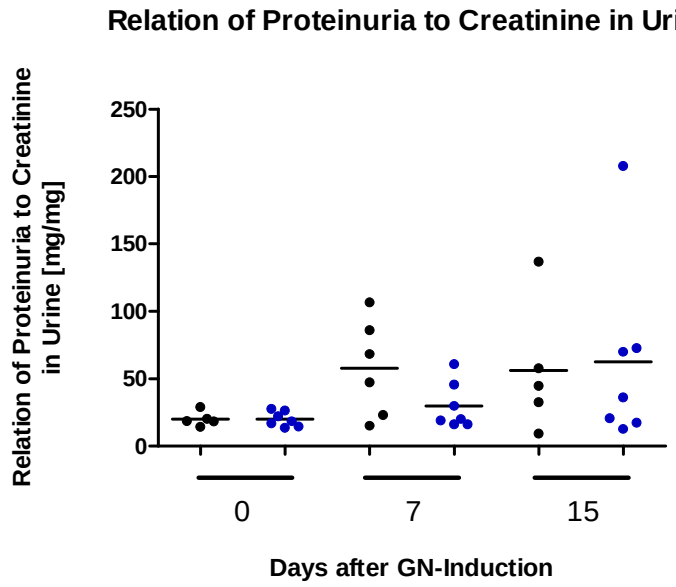


Figure 8: Relation of proteinuria to creatinine concentration in urine of TNFR2-/- animals and control animals during glomerulonephritis. Black dots represent control animals (C57Bl/6), blue dots represent TNFR2-/- mice. Dots represent single animals and means are shown. Urine on day 0 was taken before the induction of glomerulonephritis.

3.1.4 Histological Findings

On day 15 after the induction of glomerulonephritis mice were killed and their kidneys were removed. Kidneys were fixed and stained according to 2.1.6.
Kidneys of two representative mice are shown, one which developed merely light proteinuria and one which suffered from severe proteinuria.

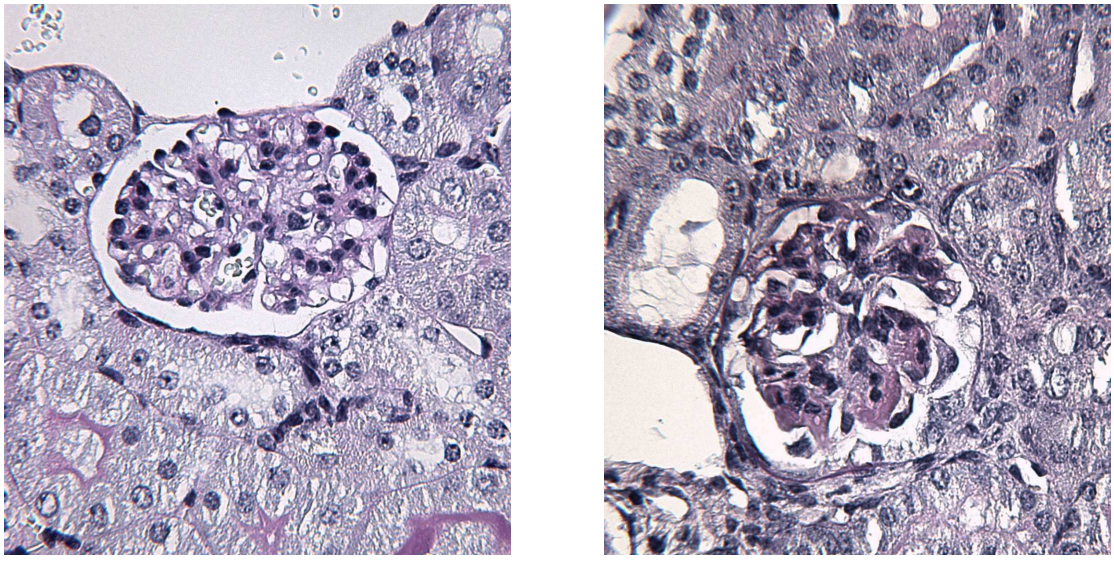


Figure 9

Figure 9: Comparison of two glomeruli after the induction of glomerulonephritis.
On the left: PAS-stained mouse kidney. After the induction of glomerulonephritis the respective mouse merely developed light proteinuria in a range between 5mg/mL and 12mg/mL. There are no lesions visible by light microscopy. Normal cellularity and no clustering of cells can be seen. The glomerular capillaries show open lumens and the Bowman's capsule can be seen as a very narrow structure surrounding the glomerulum.
On the right: PAS-stained mouse kidney. After the induction of glomerulonephritis the respective mouse developed severe proteinuria up to a concentration of 27mg/mL on day 15. There are signs of mesangial hypercellularity and the formation of crescents can be seen. The constitution of glomerular capillaries is partially broken up and the lumina can not be identified. These findings suggest severe glomerular damage in the course of crescentic or mesangoproliferative glomerulonephritis and correlate with the severe proteinuria.

3.2 Glomerulonephritis Induction in TNFR2-/- Mice and Control Animals – Part 2

Glomerulonephritis was induced in 8 TNFR2-/- mice and 7 control animals according to 2.1.1 using the respective rabbit anti-GBM serum (see 2.1.1). As control animals served C57Bl/6 mice. TNFR2-/- mice were 11 to 12 months old, control animals were 7 months old when they were taken into the experiment. On day -18 before the induction of glomerulonephritis, all animals were moved from a keeping facility with a very high standard of hygiene to a facility with a lower standard.
Urine was collected once a day. The mice were killed on day 13. One TNFR2-/- mice and one control animal died immediately after the injection of the rabbit anti-GBM serum. Two TNFR2-/- mice and 5 control animals died during glomerulonephritis.

3.2.1 Proteinuria

Proteinuria was measured according to 2.1.2. Urines were collected and stored at -20℃ for the time of the experiment until the measurement was done on day 13 after the induction of glomerulonephritis.
On day 2 to 4 after the induction of glomerulonephritis control animals showed an increase in protein levels in urine, which, on day 3, were found to be significantly higher than in TNFR2-/-

mice. During this time, TNFR2-/- showed no changes in protein concentrations in urine at all, and only slight increases and decreases on day 5 to 13. So did control animals on day 5 to 13.

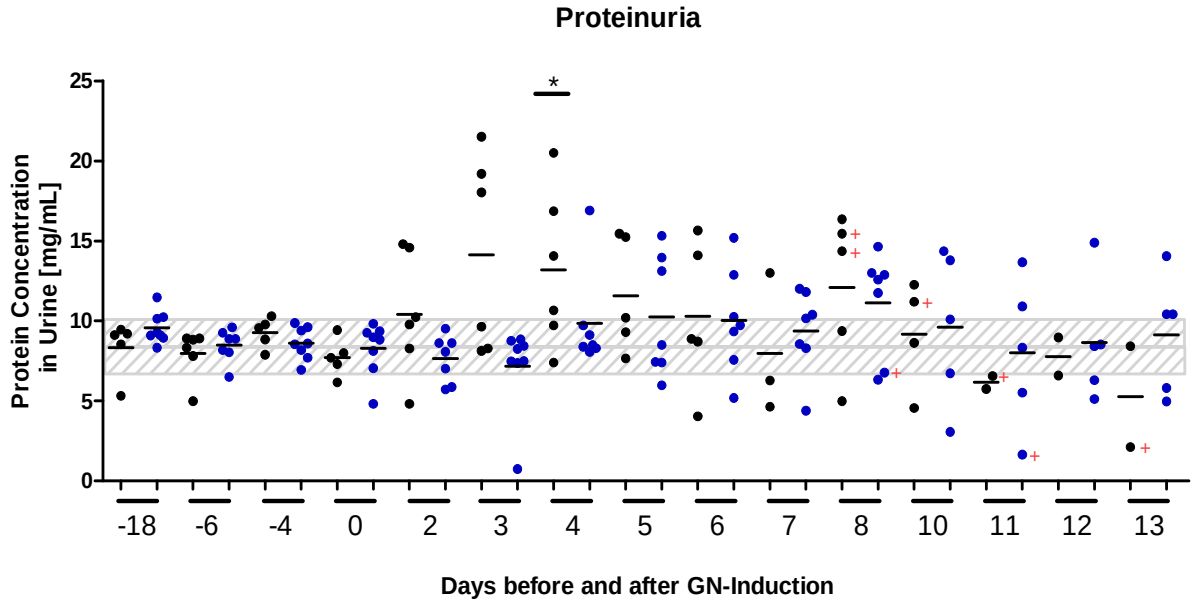


Figure 10: Development of proteinuria in TNFR2-/- animals and control animals during glomerulonephritis. Black dots represent control animals (C57Bl/6), blue dots represent TNFR2-/- mice. Dots represent single animals and means are shown. Grey shaded box shows mean and standard deviation of the normal range of proteinuria of all animals before the induction of glomerulonephritis (8,66±1,37mg/mL). Red crosses mark final values of animals which died afterwards.
Values and means are shown starting on day -18, when animals were taken into the experiment.

3.2.2 Survival

TNFR2-/- mice showed a better survival throughout the development of glomerulonephritis compared to control animals with 2 TNFR2-/- mice dying on day 10 and day 11 and 5 control animals dying on day 10, day 11 and day 13 after the induction of glomerulonephritis.

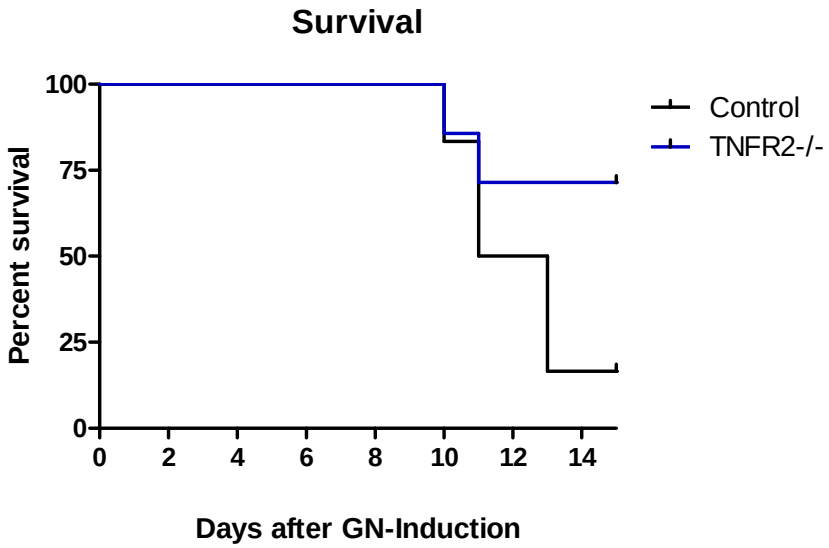


Figure 11: Survival of TNFR2-/- mice and control animals during glomerulonephritis. Black line represents control animals, blue line represents TNFR2-/- mice.

3.2 Glomerulonephritis Induction in hTNFR2tg Mice and Control Animals – Part 1

Glomerulonephritis was induced in 13 hTNFR2tg mice and 9 control animals according to 2.1.1. As control animals served littermates of the hTNFR2tg mice. All mice were 12 weeks old when they were taken into the experiment. Urine was collected once to twice a day. However, we were not able to get urine from each mouse once or twice a day. The mice were killed on day 14, blood was taken, serum produced and the kidneys were removed and fixated according to 2.1.6.

3.2.1 Proteinuria

Proteinuria was measured according to 2.1.2. Urines were collected and stored at -20℃ for two or three days until the measurement was done.
No significant difference in proteinuria could be seen between control animals and hTNFR2tg animals at any point of time.

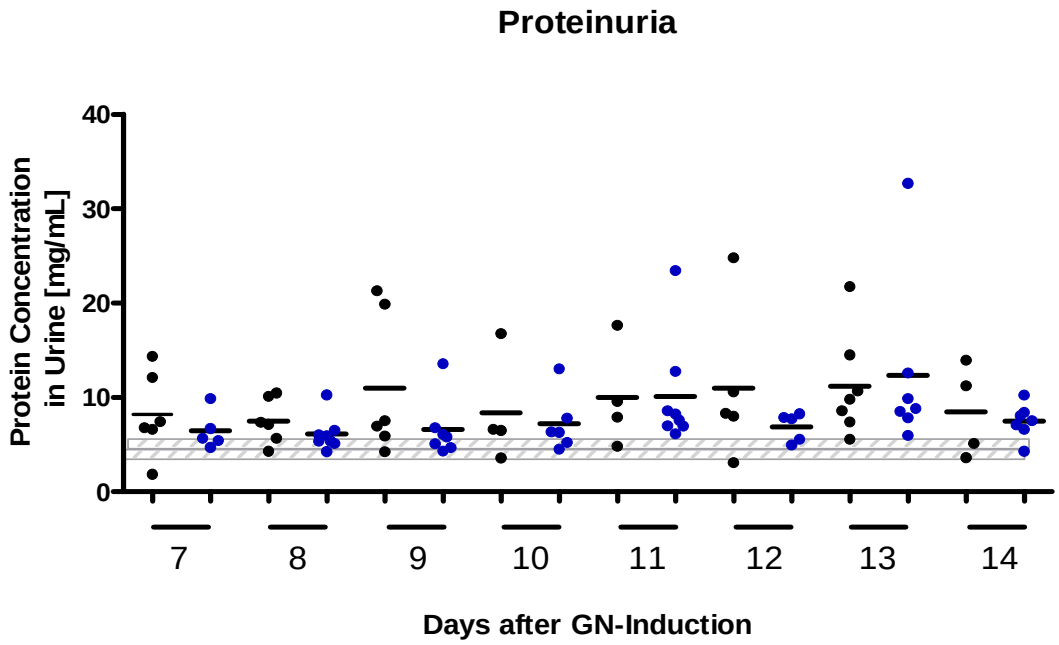


Figure 12: Development of proteinuria in hTNFR2tg animals and control animals during glomerulonephritis. Black dots represent control animals (littermates), blue dots represent mice transgenic for hTNFR2. Dots represent single animals and means are shown. Grey shaded box shows mean and standard deviation of the normal range of proteinuria of all animals before the induction of glomerulonephritis (4,68±0,94mg/mL). If urine was taken twice a day the average protein concentration is shown. Values and means are shown from day 7 to 14 since no development of proteinuria could be seen until day 7.

3.2.2 Creatinine Concentration

Creatinine was measured in urine and serum according to 2.1.5.
On day 8 and 14 after the induction of glomerulonephritis creatinine concentrations in urine and serum showed no significant differences between control animals and hTNFR2tg animals.

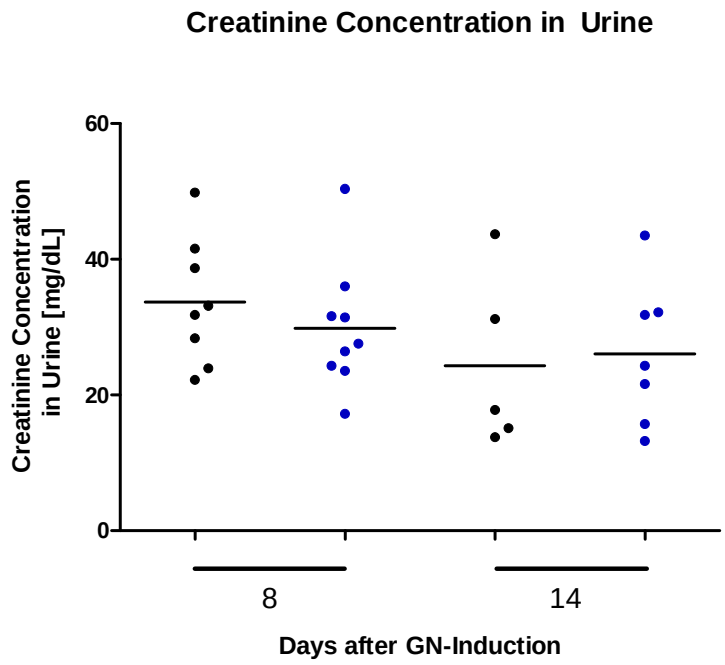


Figure 13: Creatinine concentration in urine of hTNFR2tg animals and control animals during glomerulonephritis. Black dots represent control animals (littermates), blue dots represent mice transgenic for hTNFR2. Dots represent single animals and means are shown.

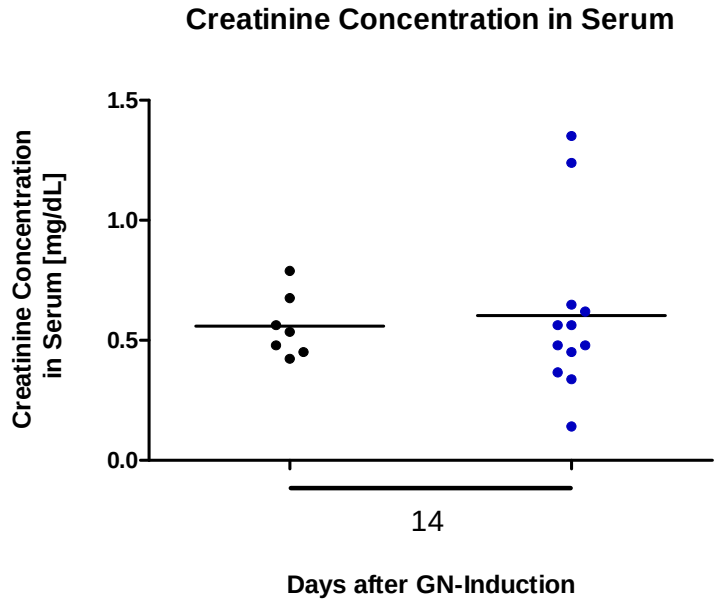


Figure 14: Creatinine concentration in serum of hTNFR2tg animals and control animals during glomerulonephritis. Black dots represent control animals (littermates), blue

dots represent mice transgenic for hTNFR2. Dots represent single animals and means are shown. Serum was merely available on day 14 due to the experimental setup.

3.2.3 Relation of Proteinuria to Creatinine

The measured proteinuria and creatinine concentrations in urine were put in relation to each other on two selected points of time.
Relations of proteinuria to creatinine showed an increase from day 8 to day 14 after the induction of glomerulonephritis, but no significant difference between control animals and hTNFR2tg animals could be seen.

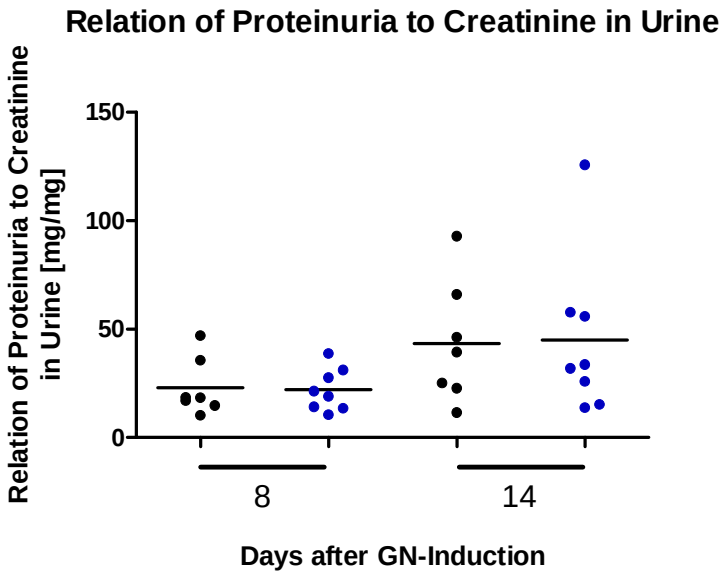


Figure 15: Relation of proteinuria to creatinine concentration in urine in hTNFR2tg animals and control animals during glomerulonephritis. Black dots represent control animals (littermates), blue dots represent mice transgenic for hTNFR2. Dots represent single animals and means are shown.

3.2.4 Histological Findings

On day 14 after the induction of glomerulonephritis mice were killed and their kidneys were removed. Kidneys were fixed and stained according to 2.1.6.
Kidneys of two representative mice are shown, one which developed merely light proteinuria and one which suffered from severe proteinuria.

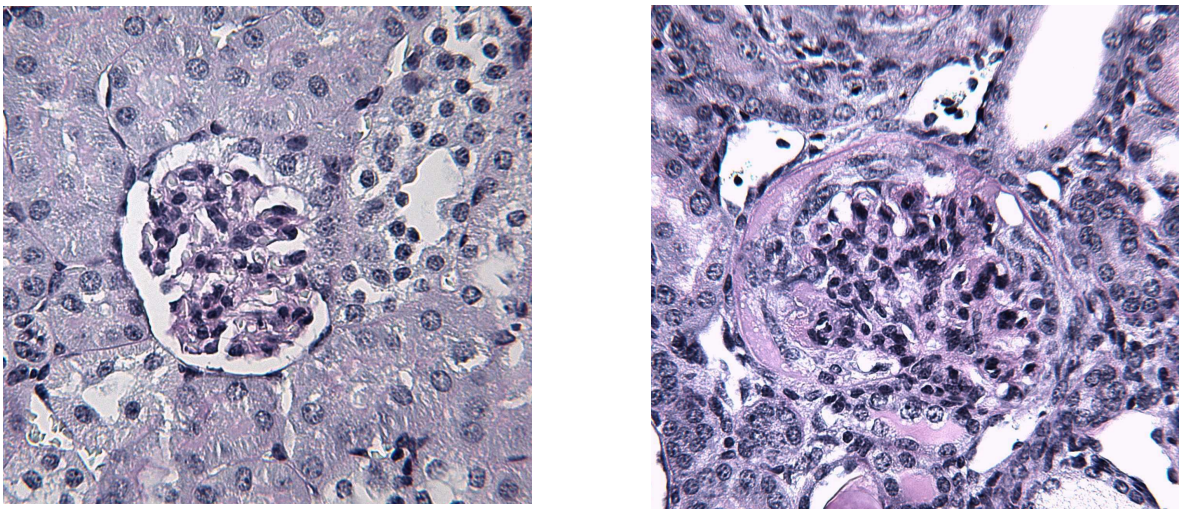


Figure 16

Figure 16: Comparison of two glomeruli after the induction of glomerulonephritis.
On the left: PAS-stained mouse kidney. After the induction of glomerulonephritis the respective mouse developed no proteinuria at all. Protein concentrations stayed in the normal range during the whole observation period. There are no lesions visible by light microscopy. Normal cellularity and normal constitution of glomerular capillaries can be seen. Bowman's capsule can be seen as a very narrow structure surrounding the glomerulum.
On the right: PAS-stained mouse kidney. After the induction of glomerulonephritis the respective mouse developed massive proteinuria with concentrations up to 48mg/mL. A large crescent-shaped zone can be seen enclosing the glomerular capillaries due to massive extracapillary hypercellularity. Capillaries show a clustering of cells. Bowman's capsule shows signs of hypercellularity and is broadened. These findings suggest a severe glomerular damage in the course of crescentic or mesangoproliferative glomerulonephritis and correlate with the massive proteinuria.

3.3 Glomerulonephritis Induction in hTNFR2tg Mice and Control Animals – Part 2

The experiment as described in 3.2 was repeated with younger mice. Glomerulonephritis was induced in 12 hTNFR2tg mice and 12 control animals according to 2.1.1. As control animals served littermates of the hTNFR2tg mice. All mice were 8 weeks old when they were taken into the experiment. Urine was collected once to twice a day. However, we were not able to get urine from each mouse once or twice a day. The mice were killed on day 15, blood was taken, serum produced and the kidneys were removed and fixed according to 2.1.6.
One of the littermates died on day 9 after induction of glomerulonephritis, one of the hTNFR2tg mice on day 11. Two littermates and one hTNFR2tg mice were killed on day 12 after they developed ascites.

3.3.1 Proteinuria

Proteinuria was measured according to 2.1.2. Urines were collected and stored at -20℃ for two or three days until the measurement was done.
No significant difference in proteinuria could be seen between control animals and hTNFR2tg animals at any point of time.

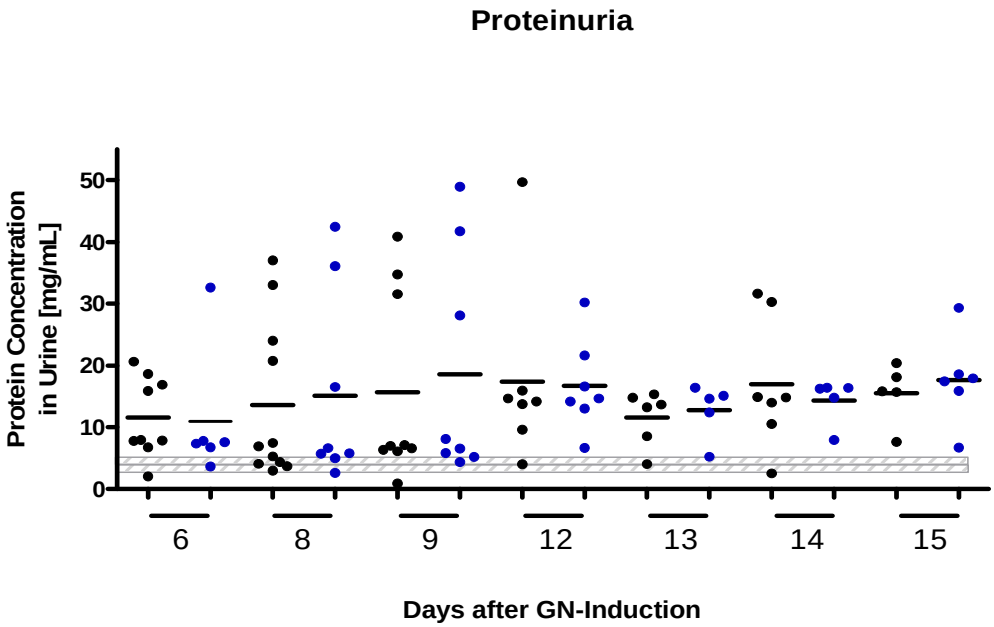


Figure 17: Development of proteinuria in hTNFR2tg animals and control animals during glomerulonephritis. Black dots represent control animals (littermates), blue dots represent mice transgenic for hTNFR2. Dots represent single animals and means are shown. Grey shaded box shows mean and standard deviation of the normal range of proteinuria of all animals before the induction of glomerulonephritis (4,18±0,79mg/mL). If urine was taken twice a day the average protein concentration is shown. Values and means are shown from day 6 to 15 since no development of proteinuria could be seen until day 6.

3.3.2 Creatinine Concentration

Creatinine was measured in urine according to 2.1.5.
Neither on day 6 nor on day 15 after the induction of glomerulonephritis a significant difference could be seen between control animals and hTNFR2tg animals.

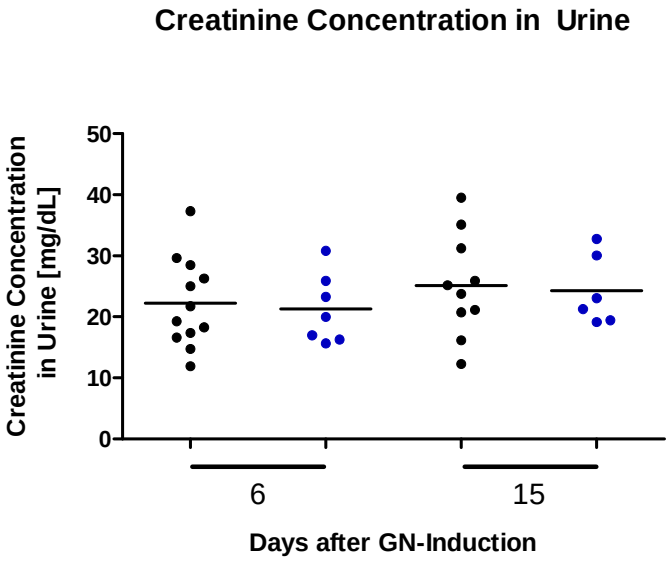


Figure 18: Creatinine concentration in urine of hTNFR2tg animals and control animals during glomerulonephritis. Black dots represent control animals (littermates), blue dots represent mice transgenic for hTNFR2. Dots represent single animals and means are shown.

3.3.3 Relation of Proteinuria to Creatinine

The measured proteinuria and creatinine concentrations in urine were put in relation to each other on two selected points of time.
No significant difference in the relation of proteinuria to creatinine between control animals and hTNFR2tg animals could be seen at any point of time.

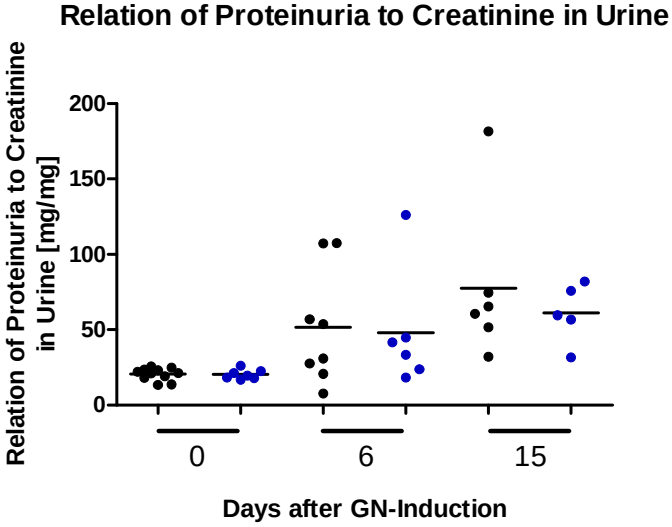


Figure 19: Relation of proteinuria to creatinine concentration in urine of hTNFR2tg animals and control animals during glomerulonephritis. Black dots represent control animals (littermates), blue dots represent mice transgenic for hTNFR2. Dots represent single animals and means are shown. Urine on day 0 was taken before the induction of glomerulonephritis.

3.3.4 mTNFR2 Concentration

In order to see whether secretion of mTNFR2 is changing during glomerulonephritis the concentration of mTNFR2 was measured in urine and serum during glomerulonephritis according to 2.1.3.
No significant difference in mTNFR2 concentrations both in urine and serum could be seen between hTNFR2tg animals and control animals after the induction of glomerulonephritis. Concentrations of mTNFR2 turned out to be higher in urine than in serum.

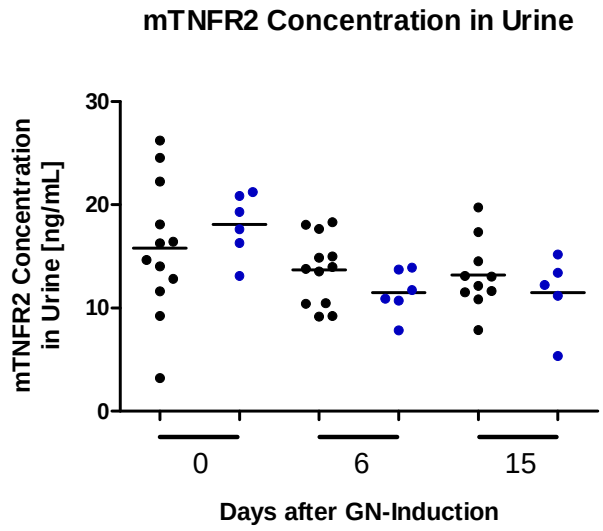


Figure 20: mTNFR2 concentration in urine of hTNFR2tg animals and control animals during glomerulonephritis. Black dots represent control animals (littermates), blue dots represent mice transgenic for hTNFR2. Dots represent single animals and means are shown. Urine on day 0 was taken before the induction of glomerulonephritis.

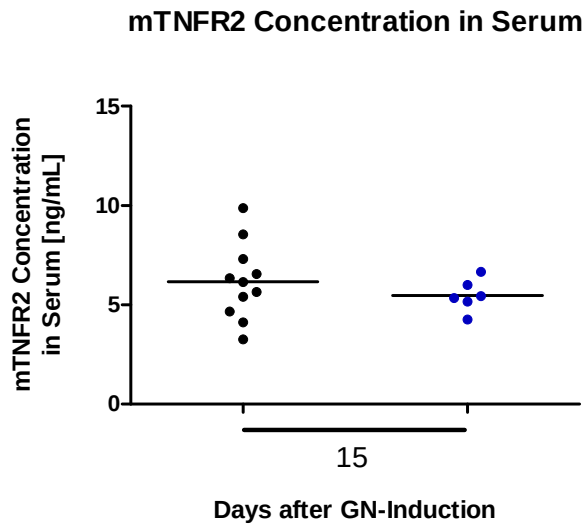


Figure 21: mTNFR2 concentration in serum of hTNFR2tg animals and control animals during glomerulonephritis. Black dots represent control animals (littermates), blue dots represent mice transgenic for hTNFR2. Dots represent single animals and means are shown. Serum was only available on day 15 due to the experimental setup.

3.3.5 hTNFR2 Concentration

In order to see whether secretion of hTNFR2 is changing during glomerulonephritis the concentration of hTNFR2 was measured in urine and serum of hTNFR2tg mice during glomerulonephritis according 2.1.3.

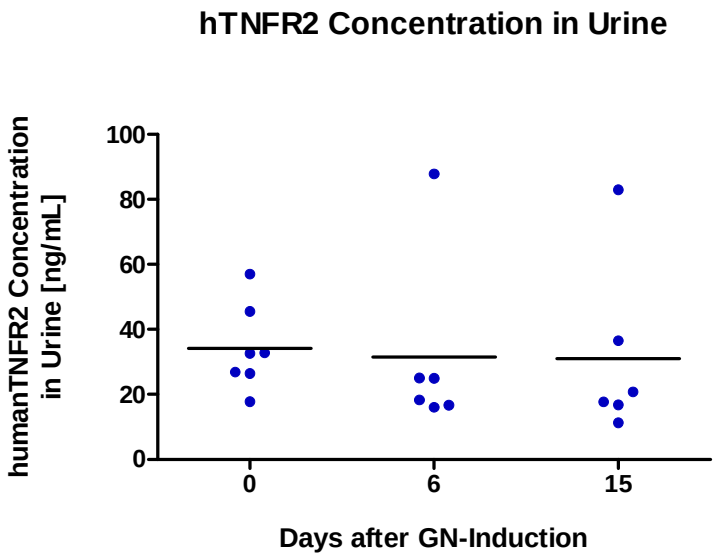


Figure 22: hTNFR2 concentration in urine of hTNFR2tg animals during glomerulonephritis. Blue dots represent mice transgenic for hTNFR2. Dots represent single animals and means are shown. Urine on day 0 was taken before the induction of glomerulonephritis.

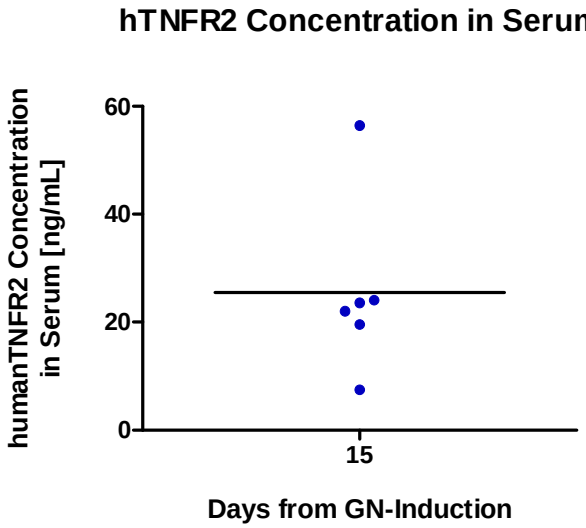


Figure 23: hTNFR2 concentration in serum of hTNFR2tg animals during glomerulonephritis. Blue dots represent mice transgenic for hTNFR2. Dots represent single animals and mean is shown. Serum was only available on day 15 due to the experimental setup.

3.3.6 Histological Findings

On day 15 after the induction of glomerulonephritis mice were killed and their kidneys were removed. Kidneys were fixed and stained according to 2.1.6.
Kidneys of two representative mice are shown, left kidney was taken from a mouse which developed merely light proteinuria and right kidney was taken from a mouse which suffered from severe proteinuria.

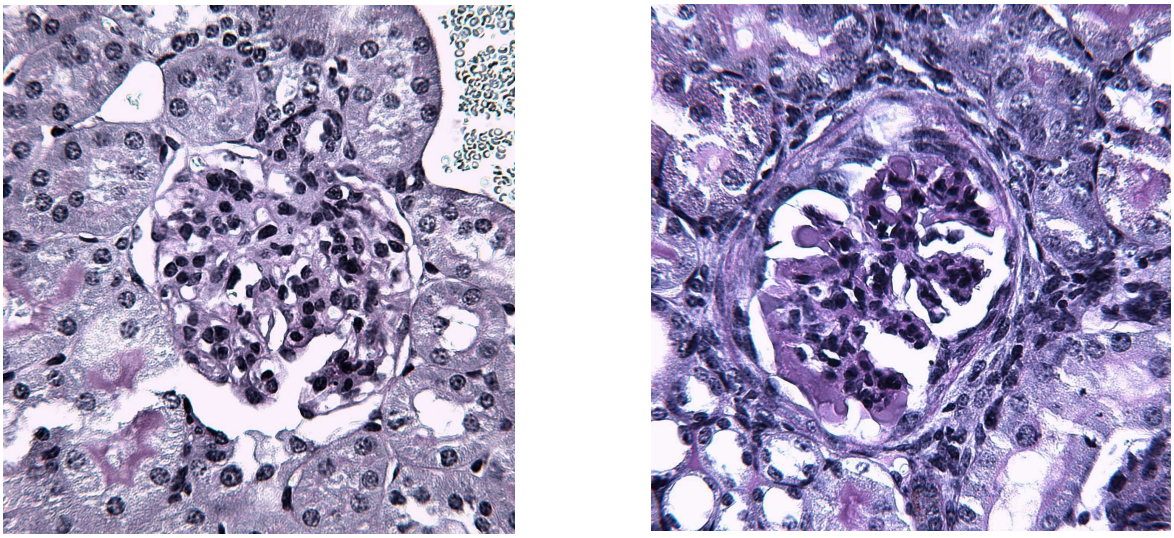


Figure 24

Figure 24: Comparison of two glomeruli after the induction of glomerulonephritis.
On the left: PAS-stained mouse kidney. After the induction of glomerulonephritis the respective mouse developed no proteinuria at all. Protein concentrations stayed in the normal range during the whole observation period. There are no lesions visible by light microscopy. Glomerular capillaries show normal lumina and no signs of hypercellularity can be seen. Bowman's capsule can be seen as a narrow structure surrounding the glomerulum.
On the right: PAS-stained mouse kidney. After the induction of glomerulonephritis the respective mouse developed massive proteinuria with concentrations up to 49mg/mL. The formation of crescents can be seen as a sign of massive mesangial hypercellularity. The constitution of glomerular capillaries is mainly broken up and no capillary lumens can be identified. These findings suggest a severe glomerular damage and correlate with the massive proteinuria.

3.4 mTNFR2 Concentration in Urine With and Without Induction of Glomerulonephritis

In order to determine whether mice already secrete mTNFR2 with the urine after the immunisation with rabbit-IgG or merely after the induction of glomerulonephritis, we performed two experiments. In one experiment 9 CD45.1 mice were used which were 10 weeks old when they were taken into the experiment. The mice were immunised and glomerulonephritis was induced according to 2.1.1. The other experiment used 6 CD45.1 mice which were 7 weeks old when they were taken into the experiment. These mice were only immunised on day 0 according to 2.1.1 but got no further treatment. Mice were killed on day 14 or 15 respectively.

Concentration of mTNFR2 in urine was measured according to 2.1.3.
In the first experiment concentrations of mTNFR2 increased significantly until day 12 with the exception of day -3 and day -1.
In the second experiment concentrations of mTNFR2 increased significantly on day 1, day 2, day 7, day 9, and day 12. Furthermore, a significant increase could be seen from day 1 to day 2 and from day 6 to day 7.

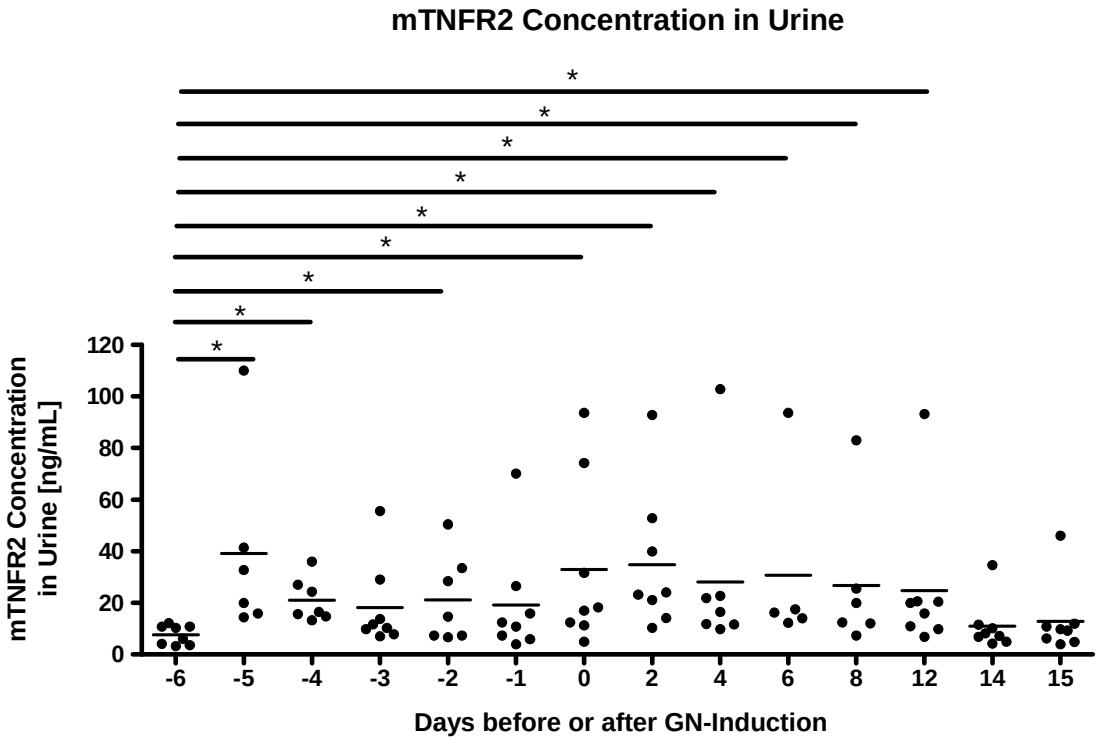


Figure 25: mTNFR2 concentration in urine of CD45.1 mice during glomerulonephritis. Mice were immunised on day -6, urine on day -6 was taken before the immunisation. Values on day -6 represent base levels without any treatment of the mice. Glomerulonephritis was induced on day 0. Dots represent single animals and means are shown.

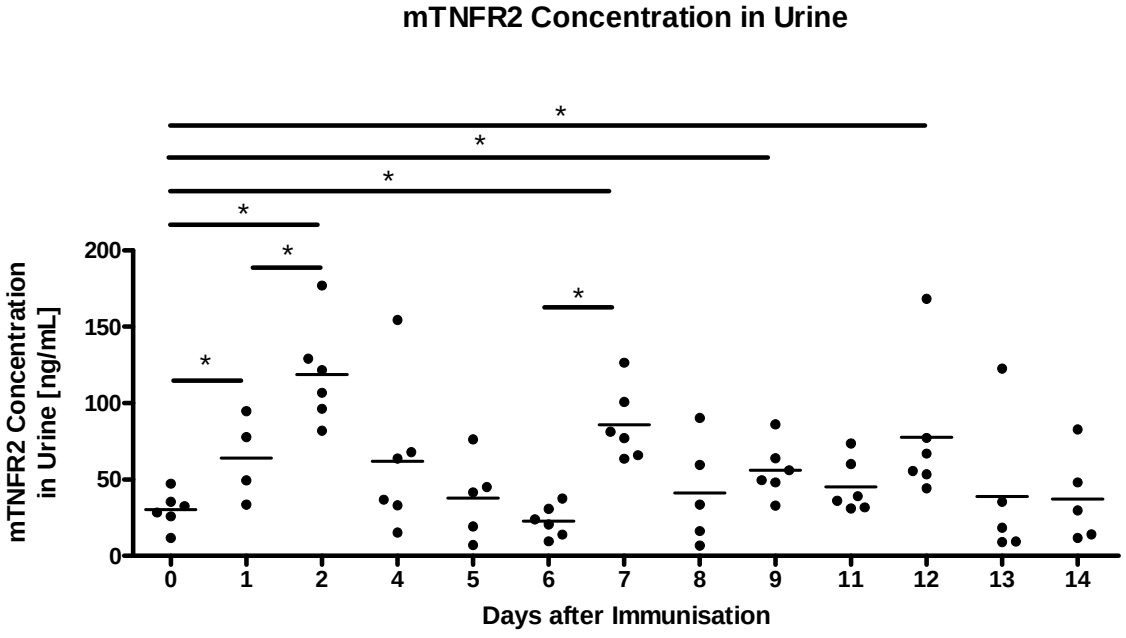


Figure 26: mTNFR2 concentration in urine of CD45.1 mice after immunisation. Mice were immunised on day 0, urine on day 0 was taken before the immunisation. Dots represent single animals and means are shown.

3.5 Titration of Anti-GBM-Serum in Induction of Glomerulonephritis

In order to test whether the amount of anti-GBM serums affects the severity of glomerulonephritis mice were challenged with different dosages of the anti-GBM serum. Glomerulonephritis was induced in 9 CD45.1 mice according to 2.1.1, but the mice received different amounts of anti-GBM-serum: 3 mice received 250µL of rabbit anti-GBM-serum, 3 mice received 200µL of rabbit anti-GBM-serum and 50µL PBS, 3 mice received 150µL of rabbit anti-GBM-serum and 100µL PBS. All mice were 10 weeks old when they were taken into the experiment. Urine was collected once a day and proteinuria was measured according to 2.1.2. The mice were killed on day 34.
Mice which had received the highest amount of anti-GBM serum developed proteinuria earlier than the other 2 groups. Mice challenged with 200µL of anti-GBM serum developed severe proteinuria after day 25 while mice challenged with the lowest amount of anti-GBM serum only showed a transient proteinuria on day 14 and 16.

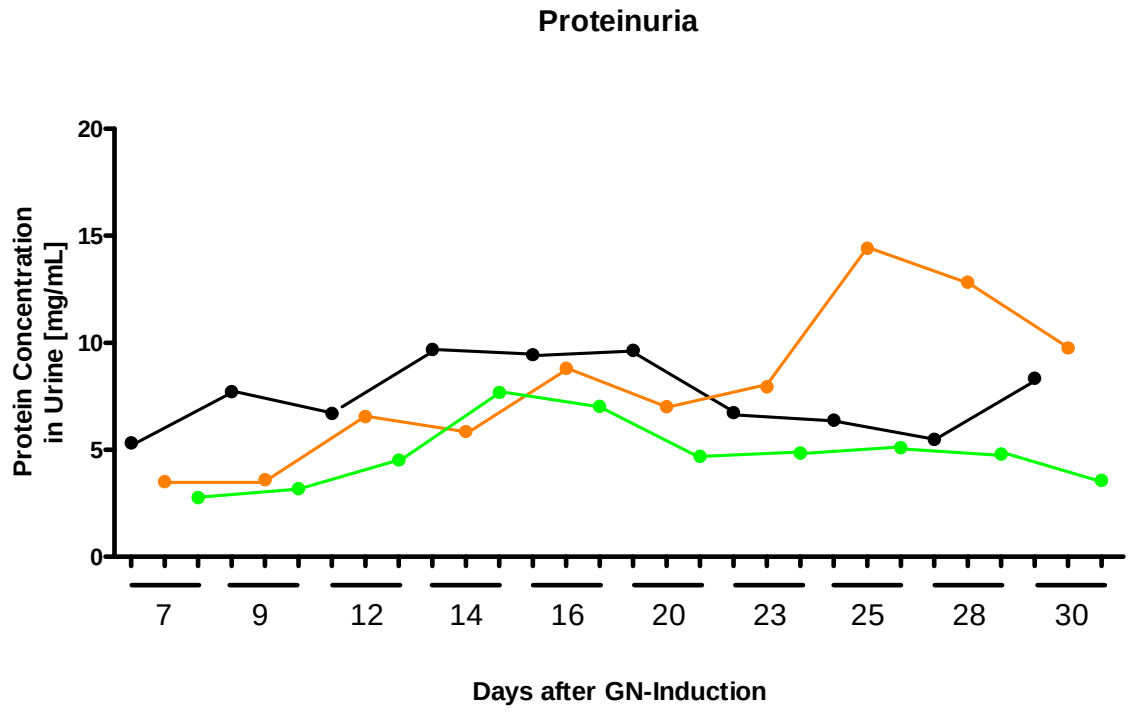


Figure 27: Development of proteinuria in CD45.1 mice during glomerulonephritis. Mice received different amounts of rabbit anti-GBM-serum: black dots represent mice which received 250µL, orange dots represent mice which received 200µL, green dots represent mice which received 150µL. Dots represent means of 3 animals in each case. Values are shown from day 7 to 30 since no development of proteinuria could be seen until day 7.

3.6 Induction of Glomerulonephritis after pretreatment with E. coli LPS and humanTNF

Glomerulonephritis was induced in 30 C57Bl/6 mice according to 2.1.1. Deviant from the standard operating procedure of immunization and glomerulonephritis induction described in 2.1.1, mice were pretreated before the injection of rabbit anti-GBM serum on day 0, in order to investigate the influence of precursory activation of the immune system on the development of glomerulonephritis. Pretreatment was accomplished on day -1 according to the following: 10 animals received 20µg E. coli LPS in 200µL PBS, 10 animals received 20µg humanTNF 200µL PBS and 10 animals merely received 200µL PBS and, therefore, served as control group.
Animals were 6 weeks old when they were taken into the experiment. Urine was collected once a day, whereas it was not possible to get urine from every mouse on every day. The mice were killed on day 15.
4 animals of the group which received E. coli LPS died during glomerulonephritis.

3.6.1 Proteinuria

Proteinuria was measured according to 2.1.2. Urines were collected and stored at -20℃ for the time of the experiment until the measurement was done after the experiment was finished. Animals which received E. coli LPS developed high amounts of proteinuria during the development of glomerulonephritis, with an immediate increase on day 1. Mice which received humanTNF also showed increased levels of proteinuria up from day 1 but levels were lower compared to those of the LPS group. Control animals developed merely slight proteinuria throughout the experiment.

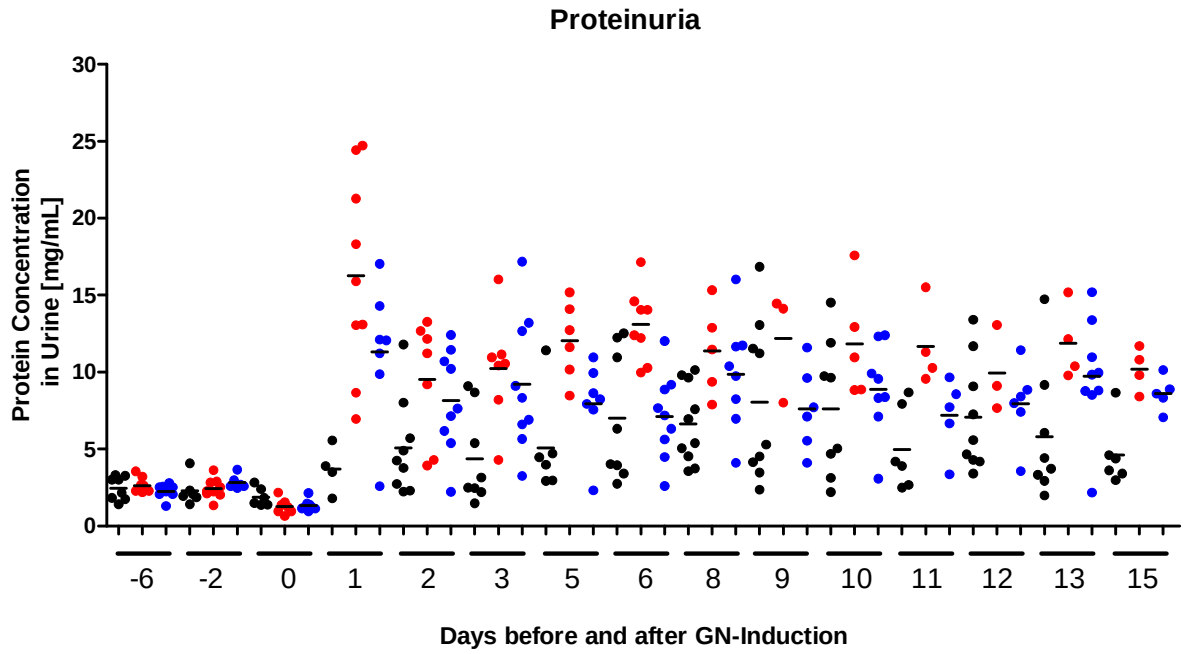


Figure 28: Development of proteinuria in mice pretreated with PBS, E. coli LPS and humanTNF during glomerulonephritis. Black dots represent control animals merely treated with PBS, red dots represent mice treated with E. coli LPS, blue dots represent mice treated with humanTNF. Values and means are shown.

3.6.2 Survival

All mice treated with humanTNF and mice merely treated with PBS survived glomerulonephritis, whereas 2 animals treated with E. coli LPS died on day 7 and 2 on day 9 after the induction of glomerulonephritis.

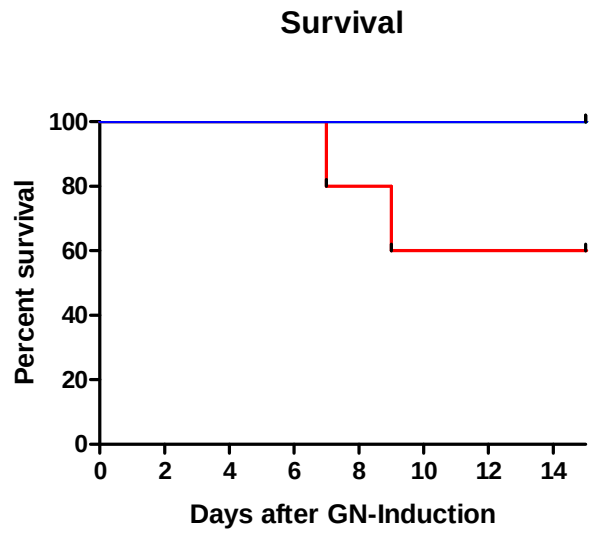


Figure 29: Survival of mice pretreated with PBS, E. coli LPS and humanTNF during glomerulonephritis. Blue line represents mice treated with humanTNF, the line representing animals treated with PBS is identical to the blue line and can, therefore, not be seen in this figure. Red line represents mice treated with E. coli LPS.

3.7 Phenotyping of hTNFR2tg Mice

hTNFR2tg mice and there littermates had to be genotyped before taking them into the experiments. A total of 94 mice were genotyped according to 2.1.4. All mice were 6 weeks old when they were genotyped.
The difference between the two groups was highly significant with no exception of mouse wrongly genotyped.

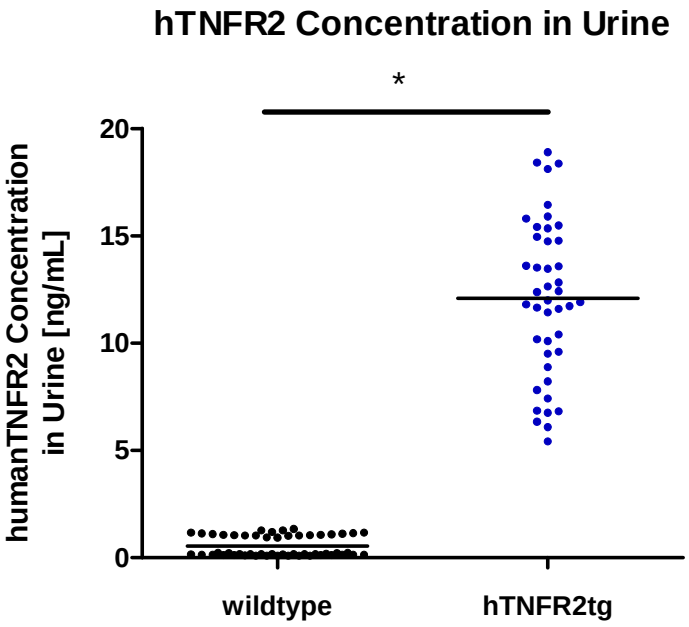


Figure 30: hTNFR2 concentration in urine of hTNFR2tg mice and littermates. Dots represent single animals (43 hTNFR2tg mice, 51 littermates) and means are shown.

4 Discussion

4.1 Development of Glomerulonephritis in TNFR2-deficient Mice

On the basis of the studies carried out and the hypothesis put forward by Vielhauer (Vielhauer et al., 2005) that TNFR2-deficient mice are protected against the development of glomerulonephritis we tried to reproduce their results. Therefore, we induced experimental glomerulonephritis in both TNFR2-deficient mice and control animals. As parameters which served as a measure for the development and severity of glomerulonephritis we determined the protein concentration in urine on the days after the induction of glomerulonephritis with rabbit anti-GBM serum.
Our measurements revealed that changes in protein concentration in urine could be seen in all animals from day 6 or 7 after the injection of anti-GBM serum and proteinuria increased up to day 14 both in TNFR2-deficient mice and control animals. A significant difference between the two mouse lines could only be observed on day 14 with significantly lower proteinuria in TNFR2-deficient mice compared to control animals. Protein concentrations in control animals ranged between 10mg/mL and about 36mg/mL during glomerulonephritis, protein concentrations in TNFR2-deficient mice varied between 10mg/mL and 25mg/mL (Figure 6). Alterations in creatinine concentration in urine could only be slightly observed during glomerulonephritis. A significantly lower concentration of creatinine in urine of TNFR2-deficient mice could be seen on day 15 after the induction of glomerulonephritis compared to control animals. These findings may be due to low sensitivity of creatinine measurement in just beginning renal failure since changes in creatinine concentration do not appear until more than 50 percent of the functional glomeruli are damaged (Figure 7). Histological findings in PAS-stained kidney sections were also observed. Glomerular damages, hypercellularity in mesangial cells and the development of crescents as a sign of a proliferating GBM could be seen in several sections. Some kidneys even showed signs of acute renal failure with destroyed renal structures. The extent of histological changes correlated reasonably well with the measured amount of proteinuria in the respective animals with high-grade damages in severely affected animals and normal renal patterns in mice with normal protein concentrations (Figure 9).

Contrary to Vielhauer's findings of total protection against glomerulonephritis, TNFR-2 deficient mice in our experiment developed proteinuria at the same point of time as control animals and differed merely in the range of protein concentrations. They were not protected

against glomerular damage either since histological changes could be observed both in TNFR2-deficient mice and control animals.
The protection Vielhauer observed in his TNFR2-deficient mice could be explained by the mechanism of TNF-induced apoptosis and the role TNFR2 plays in this signalling pathway. Wajant and Pfizenmaier (Wajant et al., 2003) described the mechanism of TNFR2-induced apoptosis a few years ago. According to their findings, stimulation of TNFR2 results in an increased production and release of membrane-bound TNF, which is capable to activate TNFR1 and therefore TNFR1-induced apoptosis via signalling pathways described above. Besides this increased TNF-expression, activated TNFR2 is able to inhibit the TNFR1-induced expression of anti-apoptotic genes, which subsequently leads to an enhanced cell death. These findings may suggest that mice lacking TNFR2 and, thus, the TNF inhibitor soluble TNFR2 as well as TNFR2 signalling, are protected against TNF-induced apoptosis to a certain amount and therefore, amongst others against glomerular damage leading to the development of proteinuria.

One possible way of explaining the susceptibility to glomerulonephritis we observed in our TNFR2-deficient mice may be the difference in levels of TNF to which mice were exposed during the experiment and before. These levels may be possibly different between our mice and the ones Vielhauer examined due to different ways of keeping the animals and, therefore, different exposures to pathogens challenging the murine immune system, for example LPS. LPS is usually known to induce the production of proinflammatory cytokines amongst others TNF and, therefore, plays a leading role in the immune response and the development of sepsis. Freudenberg and Galanos (Freudenberg and Galanos, 1988) discovered that a regular administration of a non-lethal dose of LPS leads to the development of a certain tolerance and an attenuated immune response to further exposure to toxic amounts of LPS. This dampened immune response is mainly contributed to the decreased production of proinflammatory mediators such as TNF. Further studies revealed a relationship and cross tolerance between LPS and TNF according to which animals constantly exposed to non-lethal doses of TNF or LPS subsequently are refractory to usually toxic doses of both TNF and LPS (Fraker et al., 1988). Due to the lack of TNFR2 and its TNF-antagonizing effects described above, TNFR2-deficient mice may constantly be exposed to higher levels of endogenous TNF produced because of external immunological challenges. This may lead to the development of tolerance towards proinflammatory effects of TNF and, therefore, a lower susceptibility to inflammatory diseases such as experimentally induced glomerulonephritis. Mice not exposed to high levels of TNF due to immunological non-challenging keeping conditions (as in our experiment) may not develop a comparable tolerance to TNF. This missing tolerance to TNF and the not existing cross tolerance to LPS

may, therefore, explain the susceptibility to the development of glomerulonephritis with the leading symptoms of proteinuria and glomerular damages in our TNFR2-deficient mice.

A too strong induction of glomerulonephritis might overcome the deleterious effect of TNFR2 seen by Vielhauer's group.
To eliminate the possibility of too highly dosed injections of rabbit anti-GBM serum, we investigated the effect of different doses of serum on the development of glomerulonephritis. Dosages below 250µL of anti-GBM serum, however, did not induce proteinuria before day 12.
We came to the conclusion that the amount of 250µL rabbit anti-GBM serum, which we used in all our experiments, was most likely adequate to induce glomerulonephritis (Figure 27).

However, because of the method of injecting the serum into the retro-bulbar blood vessels it was difficult to determine the actually injected amount of serum in each individual mouse. On account of this, the lacking development of glomerulonephritis in some animals may be owed to low serum injection.

4.2 Development of Glomerulonephritis in hTNFR2-transgenic Mice

As mentioned above various studies in the past few years revealed an increased susceptibility to inflammatory diseases in mice which were transgenic for the human TNFR2 and, therefore, expressed human TNFR2 constitutively. According to these findings, we wanted to investigate whether hTNFR2-transgenic mice were more likely than non-transgenic animals to develop increased proteinuria and severe glomerular damage after the experimental induction of glomerulonephritis. We induced glomerulonephritis in two independent experiments both consisting of hTNFR2-transgenic mice and their non-transgenic littermates which served as control animals.

Our measurements revealed that first increases in protein concentration in urine could be seen on day 6 after the induction of glomerulonephritis and that no difference in the starting point of time between transgenic mice and littermates could be observed. Highest values of proteinuria up to about 32mg/mL in the first experiment and up to about 49mg/mL in the second experiment were reached on day 12 and occurred in both transgenic mice and littermates. Some animals, both transgenic and non-transgenic, of each group did not

develop any proteinuria at all after the injection of rabbit anti-GBM serum (Figure 12, Figure 17). Creatinine concentrations in urine in all animals were not significantly lower during glomerulonephritis (Figure 13, Figure 18). Histological findings in PAS-stained sections correlated with the measured proteinuria and showed severe glomerular damage and signs of acute renal failure in single animals whereas those who did not develop proteinuria showed no signs of glomerular changes
(Figure 16, Figure 24). Additionally, no changes in concentrations of soluble hTNFR2 in urine could be observed during glomerulonephritis in transgenic mice which could have been expected because of the constitutive expression of soluble hTNFR2 (Figure 22). Soluble mTNFR2 concentrations in urine merely decreased slightly and without any significant difference between transgenic animals and littermates (Figure 20).
Contrary to our expectations based on previous studies our measurements revealed that mice transgenic for human TNFR2 were not more susceptible to experimental glomerulonephritis compared to their non-transgenic littermates and showed no signs of increased inflammatory response to the injected anti-GBM serum.

Previous studies with mice transgenic for hTNFR2 revealed an increase in pathology and inflammatory symptoms due to the overexpression of hTNFR2 as well in experimental hepatitis, as in severe inflammatory syndrome and inflammatory bowel diseases. The overexpression of hTNFR2, which was held responsible for these effects could both be demonstrated by increased levels of shedded soluble receptor and elevated expression of cell surface receptor (Douni and Kollias, 1998; Küsters et al., 2002; Holtmann et al., 2002).

According to our data, these findings could not be transferred to the development of experimental glomerulonephritis as an example of inflammatory renal disease in hTNFR2 transgenic mice. One possible explanation for this difference may be provided by the difference in the gene expression of hTNFR2 in our animals compared to those used by Douni and Kollias as described above. Douni and Kollias revealed that mice being exposed to Con-A induced hepatitis or merely to a challenge with LPS showed upregulated expression of hTNFR2 in their sera compared to transgenic mice which were not exposed to any treatment. Sera levels of soluble human TNFR2 turned out to be correlating with the severity of pathology in the treated mice. Therefore, Douni and Kollias reasoned that induced and not constitutive expression of hTNFR2 is responsible for the increased pathologic effects in their experimental setup. In contrast to this regulated expression, our mice transgenic for hTNRF2 expressed hTNFR2 constitutively and were, therefore, not able to enhance the expression when treated with rabbit anti-GBM serum. This lacking ability of upregulation may be causal for the equal susceptibility in transgenic mice and their littermates.

Another possible explanation for our discovery may be provided by findings which revealed different amounts of expression of mTNFR2 and human TNFR2 on renal cells. For example, recent studies by Hoffmann (Hoffmann et al., 2009) investigated the expression and localization of TNFR2 on renal cells during renal allograft rejection in rats. They revealed high expression of TNFR2 on intrinsic renal cells such as podocytes and tubular epithelial cells in control animals which were not exposed to any treatment. There was an even higher expression in animals which suffered from acute transplant rejection and elevated levels of TNFR2 could be observed in their urine.
These findings describing the constitutive expression of TNFR2 on intrinsic renal cells which could be detected without the presence of any inflammatory progress or disease might provide a possibility to explain the lacking impact of the expression of hTNFR2 on the development of inflammatory renal diseases such as glomerulonephritis. Interestingly, we also found higher levels of mTNFR2 in urine than in serum.
Additional constitutive and non-regulated expression of hTNFR2 might not be capable to induce further increase of inflammatory symptoms on top of the symptoms already caused by elevated expression of mTNFR2. This seems very likely especially in light of the fact that spontaneous release of mTNFR2 in urine was found to about 17ng/mL while hTNFR2 in urine of transgenic mice was about 23ng/mL. The relatively high amount of secreted mTNFR2 might be completely sufficient to prevent the above mentioned development of tolerance and, therefore, no additional effect of the hTNFR2 is to be expected.

This hypothesis could be supported by the fact that concentrations of hTNFR2 did not change significantly during the development of glomerulonephritis and, furthermore, did not differ from those in untreated animals in our experiments (Figure 22, Figure 30).

We failed to analyse the expression and localization of mTNFR2 on sections of both kidneys from mice suffering from glomerulonephritis and normal mice kidneys since we were not able to stain mTNFR2 specifically with anti-mTNFR2 AF 647 antibody according to 2.1.6 Fixation and Staining of Mouse Kidney.
Therefore, we are not able to make a statement concerning the localization and extent of expression of mTNFR2 in kidneys affected by glomerulonephritis.

Another study group at our department examined the effects of the presence of hTNFR2 in Con A-induced hepatitis. Along the lines of our experimental design they compared mice transgenic for hTNFR2 with their non-transgenic littermates and could not observe any difference in the reaction to experimental hepatitis between transgenic and non-transgenic

mice. Mice showed no changes in concentrations of relevant liver enzymes and no histological differences could be seen (unpublished).
These findings might present another experimental setup in which overexpression of hTNFR2, additionally to the expression of mTNFR2, did neither increase inflammatory symptoms nor organ failure.

4.3 Phenotyping of hTNFR2-transgenic Mice

Since the mice transgenic for hTNFR2 we used in our experiments were crossed back to C57Bl/6 mice up to the F2 and F3 generation we had to genotype them clearly and distinguish them from their littermates before they could be taken into the experiment.

A first genotyping was carried out by Southern Blots, for which a small part of the tail had to be cut off from the mice. In order to develop a non-invasive and more definite way of typing we examined whether soluble hTNFR2 could be detected in the urine of mice transgenic for hTNFR2.
According to 2.1.4 we collected urine from all animals which had to be typed and measured soluble hTNFR2. Concentrations turned out to range between 5ng/mL and 20ng/mL in about half of the animals, whereas the other half showed concentrations below the detection limit which was determined at 1.25ng/mL because of the experimental setup (Figure 30). Therefore, we claimed mice with concentrations of hTNFR2 below detection limit as non-transgenic littermates and mice with concentration above detection limit as mice transgenic for hTNFR2.

To verify our hypothesis we compared our findings with those detected by Southern Blots. Results consisted in 7 of 9 cases and animals which could not be definitely genotyped by Southern Blot showed unambiguous concentrations of hTNFR2 in their urine.
Hence, the constitutive overexpression and, therefore, constitutive elimination of soluble hTNFR2 in urine of mice transgenic for hTNFR2 provides the opportunity of definite identification of transgenic animals.

Summing up these findings, we were able to establish a non-invasive, easily accomplished and very effective way to phenotype mice transgenic for hTNFR2. Moreover, the method turned out be highly sensitive and reproducible.

4.4 Detection of Soluble TNFR2 in Urine

Several studies on the TNF receptors over the past few years discovered the existence of both cell surface and soluble TNF receptors. These soluble forms are shedded from the membrane and are capable of binding TNF thus antagonizing its effects. In 1990 Engelmann first described the detection and purification of soluble forms of TNF receptors from human urine (Engelmann et al., 1990). Further studies revealed a correlation between the concentration of soluble TNF receptors in serum and urine and the extent of activity of certain diseases such as chronic inflammatory bowel diseases. Especially the measurement of soluble TNF receptors in urine turned out to be an appropriate and above all non-invasive method of assessing disease activity and therapeutic outcome in patients suffering from chronic inflammatory bowel diseases (Hadziselimovic et al., 1995). In particular, levels of TNFR2 were found to be increased in infectious or chronic inflammatory diseases and turned out to be correlating with the severity of disease (Diez-Ruiz et al., 1995). These correlations and further studies on the kinetics of receptor shedding lead to the assumption that cleavage of cell surface TNFR2 may be a highly controlled mechanism both in the presence (Aderka et al., 1998) and absence of TNF (Douni and Kollias, 1998).

In order to assess the concentration of soluble mTNFR2 in urine during the development of glomerulonephritis, we measured soluble mTNFR2 on day 0 before the induction and on day 6 and 15 after the induction of experimental glomerulonephritis. Both hTNFR2-trangenic mice and littermates showed a decreased level of soluble TNFR2 in urine during glomerulonephritis declining from about 17ng/mL before induction and about 10ng/mL on day 6 and 15 (Figure 20).

To distinguish whether immunisation with rabbit IgG or induction of glomerulonephritis with rabbit anti-GBM serum leads to the described effect on concentrations of soluble mTNFR2 we compared mice in two experiments. Animals in the first experiment were exposed both to immunisation with rabbit IgG and, 6 days later, to the induction of glomerulonephritis while animals in the second experiment only received immunisation with rabbit IgG.
Mice in the first experiment showed a base level of about 8±4ng/mL of soluble mTNFR2 in urine without any preceded treatment. After immunisation concentrations of soluble mTNFR2 went up to a mean of 39±36ng/mL on day 1 and were, in the following, significantly increased on nearly all points of time up to day 12. They nearly gained back their base level by day 6 after immunisation. After the induction of glomerulonephritis on day 7 an increase of soluble mTNFR2 in urine up to a mean of 35±27ng/mL could be seen on the second day after the induction of glomerulonephritis. Single animals developed concentrations up to 92ng/mL. Up

to day 15 after the induction of glomerulonephritis a decrease of soluble TNFR2 concentrations to the base level of 10±9ng/mL could be observed in most animals (Figure 25).
Mice in the second experiment turned out to have higher base levels of soluble mTNFR2 in urine of 30±12ng/mL. Immunisation with rabbit IgG led to a significantly increased concentration of soluble mTNFR2 up 64±28ng/mL on day 1 and 119±33ng/mL on day 2 after immunisation. Concentrations went back to the base level of 23±10ng/mL by day 6 but showed a significant second increase of concentrations up to 86±24ng/mL on day 7 after immunisation without having the mice exposed to any further treatment. Apart from a slight increase on day 12 up to 78±46ng/mL, concentrations of soluble mTNFR2 decreased to a level of about 37±29ng/mL by day 14 (Figure 26).

Summing up, both experiments showed the same development in the elimination of soluble mTNFR2 in urine with a first significant increase on day 1 and 2 after immunisation and a second one on day 7, regardless of whether immunisation was followed by the injection of anti-GBM serum on day 6 or not.
Interestingly, it turned out that all animals in both experiments showed relatively high amounts of soluble mTNFR2 in their urine ranging between 5ng/mL and 50ng/mL without being exposed to any treatment at all (Figure 25, Figure 26). This supports the idea that continuously secreted TNFR2 is available to dampen inflammatory TNF effects.

These findings suggest that treatment with rabbit anti-GBM serum had no impact on the concentration of soluble mTNFR2 in the urine. Our measurements lead to the conclusion that merely immunisation with rabbit IgG, probably triggering a systemic immune response, is responsible for the described kinetics in the shedding an elimination of TNFR2 in urine. Hence, the actual development of glomerulonephritis can not be implicated with varying concentrations of TNFR2.

4.5 Conclusion

First of all, we were not able to reproduce the findings described by Vielhauer (Vielhauer et al., 2005). TNFR2-deficient mice in our experimental setup did not turn out to be protected against the development of experimental glomerulonephritis. We, therefore, assume that the existence of protection may be dependent on the presence of a so-called TNF-tolerance that

has developed in the organism because of accidental exposure to TNF in preceded diseases or because of certain keeping conditions. To verify this hypothesis further experiments comparing TNFR2-deficient mice and control animals in different environments would provide more information.

Contrary to our expectations, mice transgenic for hTNFR2 showed no signs of increased pathology and no enhanced inflammatory response to the induction of glomerulonephritis. As mentioned above, one possible explanation may be provided by the differences in the way the transgenic fragment was integrated into the murine DNA. We assume that regulated expression and, therefore, disease-correlating levels of hTNFR2 instead of constitutive overexpression may have an important impact on the development of glomerulonephritis. Generating mice transgenic for hTNFR2 by integrating regulatory elements may offer a future possibility to examine the impact of additional regulated expression of hTNFR2 during experimental glomerulonephritis and to investigate whether enhanced susceptibility to glomerulonephritis can be observed.
In addition, we found out that amounts of soluble mTNFR2 in urine showed no correlation to the severity of disease during glomerulonephritis in contrast to former studies (Hoffmann et al., 2009). Furthermore, elimination of soluble mTNFR2 in urine turned out to be merely dependent on immunisation and independent of the following treatment with rabbit anti-GBM serum to induce glomerulonephritis.
According to these findings, we assume that inducing glomerulonephritis in mice according to our experimental protocol has no striking impact on the concentration and, therefore possibly, on the elimination of TNFR2. Contrary to Vielhauer's assumptions, antagonizing TNFR2 might not provide such special opportunities in the treatment of glomerulonephritis as it does in the treatment of other chronic inflammatory diseases such as inflammatory bowel diseases or rheumatoid arthritis as mentioned above.

5 References

Abbate M, Zoja C, Corna D, Rottoli D, Zanchi C, Azzollini N, Tomasoni S, Berlingeri S, Noris M, Morigi M, Remuzzi G (2008). “Complement-mediated dysfunction of glomerular filtration barrier accelerates progressive renal injury.” J Am Soc Nephrol. Jun;19(6):1158-67.

Aderka D, Sorkine P, Abu-Abid S, Lev D, Setton A, Cope AP, Wallach D, Klausner J (1998). “Shedding kinetics of soluble tumor necrosis factor (TNF) receptors after systemic TNF leaking during isolated limb perfusion. Relevance to the pathophysiology of septic shock.” J Clin Invest. Feb 1;101(3):650-9.

Adler S, Baker PJ, Pritzl P, Couser WG (1984). “Detection of terminal complement components in experimental immune glomerular injury.” Kidney Int. Dec;26(6):830-7.

Aggarwal BB (2003). “Signalling pathways of the TNF superfamily: a double-edged sword.“ Nat Rev Immunol. Sep;3(9):745-56. Review.

Al-Lamki RS, Wang J, Skepper JN, Thiru S, Pober JS, Bradley JR (2001). “Expression of tumor necrosis factor receptors in normal kidney and rejecting renal transplants.” Lab Invest. Nov;81(11):1503-15.

Andres GA, Accinni L, Hsu KC, Zabriskie JB, Seegal BC (1966). “Electron microscopic studies of human glomerulonephritis with ferritin-conjugated antibody. Localization of antigen-antibody complexes in glomerular structures of patients with acute glomerulonephritis.” J Exp Med. Feb 1;123(2):399-412.

Antoni C, Krueger GG, de Vlam K, Birbara C, Beutler A, Guzzo C, Zhou B, Dooley LT, Kavanaugh A; IMPACT 2 Trial Investigators (2005). “Infliximab improves signs and symptoms of psoriatic arthritis: results of the IMPACT 2 trial.” Ann Rheum Dis. Aug;64(8):1150-7. Epub 2005 Jan 27.

Bach D, Klein BC, Grabensee B (1997). “Long-term clinical course and histopathologic scoring in acute crescentic glomerulonephritis.” Ren Fail 19: 85-98.

Baud L, Ardaillou R (1994). “Tumor necrosis factor alpha in glomerular injury.” Kidney Int 45 (Suppl 45): 32-36.

Baud L, Oudinet JP, Bens M, Noe L, Peraldi MN, Rondeau E, Etienne J, Ardaillou R (1989). “Production of tumor necrosis factor by rat mesangial cells in response to bacterial lipopolysaccharide.” Kidney Int. May; 35(5):1111-8.

Bäumel M, Lechner A, Hehlgans T, Männel DN (2008). “Enhanced susceptibility to Con A-induced liver injury in mice transgenic for the intracellular isoform of human TNF receptor type 2.” J Leukoc Biol. Jul;84(1):162-9. Epub 2008 Apr 24.

Bertani T, Abbate M, Zoja C, Corna D, Perico N, Ghezzi P, Remuzzi G (1989). “Tumor necrosis factor induces glomerular damage in the rabbit.” Am J Pathol. Feb;134(2):419-30.

Beutler B, Cerami A (1989). “The biology of cachectin/TNF--a primary mediator of the host response.” Annu Rev Immunol. ;7:625-55.

Beutler B, Greenwald D, Hulmes JD, Chang M, Pan YC, Mathison J, Ulevitch R, Cerami A (1985). “Identity of tumour necrosis factor and the macrophage-secreted factor cachectin.” Nature. Aug 8-14;316(6028):552-4.

Beutler B, Milsark IW, Cerami AC (1985). “Passive immunisation against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin.” Science. Aug 30;229(4716):869-71.

Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP (1997). “A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells.” Nature. Feb 20;385(6618):729-33.

Brentjens JR, Sepulveda M, Baliah T, Bentzel C, Erlanger BF, Elwood C, Montes M, Hsu KC, Andres GA (1975). “Interstitial immune complex nephritis in patients with systemic lupus erythematosus.” Kidney Int. May;7(5):342-50.

Butkowski RJ, Wieslander J, Wisdom BJ, Barr JF, Noelken ME, Hudson BG (1985). “Properties of the globular domain of type IV collagen and its relationship to the Goodpasture antigen.” J Biol Chem. Mar 25;260(6):3739-47.

Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975). “An endotoxin-induced serum factor that causes necrosis of tumors.” Proc Natl Acad Sci U S A. Sep;72(9):3666-70.

Chadban SJ, Atkins RC (2005). “Glomerulonephritis.” Lancet May 21-27;365(9473):1797-806.

Chensue SW, Remick DG, Shmyr-Forsch C, Beals TF, Kunkel SL (1988). “Immunohistochemical demonstration of cytoplasmic and membrane-associated tumor necrosis factor in murine macrophages.” Am J Pathol. Dec;133(3):564-72.

Chinnaiyan AM, Tepper CG, Seldin MF, O'Rourke K, Kischkel FC, Hellbardt S, Krammer PH, Peter ME, Dixit VM (1996). “FADD/MORT1 is a common mediator of CD95 (Fas/APO-1) and tumor necrosis factor receptor-induced apoptosis.” J Biol Chem. Mar 1;271(9):4961-5.

Cohen AH, Border WA, Shankel E, Glassock RJ (1981). “Crescentic glomerulonephritis: immune vs. nonimmune mechanisms”. Am J Nephrol 1(2): 78-83.

Cope AP (1998). “Regulation of autoimmunity by proinflammatory cytokines.” Curr Opin Immunol. Dec;10(6):669-76.

Couser WG, Wagonfeld JB, Spargo BH, Lewis EJ (1974). “Glomerular deposition of tumor antigen in membranous nephropathy associated with colonic carcinoma.” Am J Med. Dec;57(6):962-70.

D’Agati VD, Jennette JC, Silva FG (2005). “Atlas of Nontumor Pathology. Non-Neoplastic Kidney Diseases.“ American Registry of Pathology, Washington, DC. 189-198.

Daniels BS, Hauser EB, Deen WM, Hostetter TH (1992). “Glomerular basement membrane: in vitro studies of water and protein permeability”. Am J Physiol. Jun; 262(6 Pt 2):F919-26.

Darnay BG, Haridas V, Ni J, Moore PA, Aggarwal BB (1998). “Characterization of the intracellular domain of receptor activator of NF-kappaB (RANK). Interaction with tumor necrosis factor receptor-associated factors and activation of NF-kappab and c-Jun N-terminal kinase.” J Biol Chem. Aug 7;273(32):20551-5.

Deetjen P, Speckmann EJ (1999). „Niere“. Physiologie. München, Urban & Fischer: 371-407.

Dempsey PW, Doyle SE, He JQ, Cheng G (2003). “The signaling adaptors and pathways activated by TNF superfamily.” Cytokine Growth Factor Rev. Jun-Aug;14(3-4):193-209.

Dendorfer U, Mann J (2006). „Glomerulonephritis.“ Dtsch Med Wochenschr. Jan 20;131(3):93-100; quiz 101-4.

Diez-Ruiz A, Tilz GP, Zangerle R, Baier-Bitterlich G, Wachter H, Fuchs D (1995). “Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis.” Eur J Haematol. Jan;54(1):1-8. Review.

Dixon FJ, Feldman JD, Vasquez JJ (1961). “Experimental glomerulonephritis. The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis.” J Exp Med. May 1;113:899-920.
Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, Meldrum DR (1999). “Early kidney TNF-alpha expression mediates neutrophil infiltration and injury after renal ischemia-reperfusion.“ Am J Physiol. Sep;277(3 Pt 2):R922-9.

Dörge SE, Roux-Lombard P, Dayer JM, Koch KM, Frei U, Lonnemann G (1994). “Plasma levels of tumor necrosis factor (TNF) and soluble TNF receptors in kidney transplant recipients.” Transplantation. Nov 15;58(9):1000-8.

Douni E, Kollias G (1998). “A critical role of the p75 tumor necrosis factor receptor (p75TNF-R) in organ inflammation independent of TNF, lymphotoxin alpha, or the p55TNF-R.“ J Exp Med. Oct 5;188(7):1343-52.

Ehrich JH, Foellmer HG, Krull F, Winnecken HJ, Wurster U (1984). “Significance, sources of error and limits of protein diagnosis in urine. II. Normal values and classification of pathologic proteinurias.Monatsschr Kinderheilkd. Mar;132(3): 144-50.

Elzey BD, Griffith TS, Herndon JM, Barreiro R, Tschopp J, Ferguson TA (2001). “Regulation of Fas ligand-induced apoptosis by TNF.” J Immunol. Sep 15;167(6):3049-56.

Engelmann H, Novick D, Wallach D (1990). “Two tumor necrosis factor-binding proteins purified from human urine. Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors.” J Biol Chem. Jan 25;265(3):1531-6.

Feldmann M, Maini RN (2001). “Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned?” Annu Rev Immunol.;19:163-96.

Fraker DL, Stovroff MC, Merino MJ, Norton JA (1988). “Tolerance to tumor necrosis factor in rats and the relationship to endotoxin tolerance and toxicity.” J Exp Med. Jul 1;168(1):95-105.

Freudenberg MA, Galanos C (1988). “Induction of tolerance to lipopolysaccharide (LPS)-D-galactosamine lethality by pretreatment with LPS is mediated by macrophages.” Infect Immun. May;56(5):1352-7.

Grell M, Douni E, Wajant H, Löhden M, Clauss M, Maxeiner B, Georgopoulos S, Lesslauer W, Kollias G, Pfizenmaier K, Scheurich P (1995). „The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor.” Cell. Dec 1;83(5):793-802.

Hadziselimovic F, Emmons LR, Gallati H (1995). “Soluble tumour necrosis factor receptors p55 and p75 in the urine monitor disease activity and the efficacy of treatment of inflammatory bowel disease.” Gut. Aug;37(2):260-3.

Haraldsson B, Sörensson J (2004) “Why Do We Not All Have Proteinuria? An Update of Our Current Understanding of the Glomerular Barrier”. News Physiol Sci 19: 7-10.

Hehlgans T, Pfeffer K (2005). “The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games.” Immunology. May;115(1):

Herold G (2009), “Nephrologie”. Innere Medizin. Köln: 566-615.

Hohmann HP, Remy R, Brockhaus M, van Loon AP (1989). “Two different cell types have different major receptors for human tumor necrosis factor (TNF alpha).” J Biol Chem. Sep 5;264(25):14927-34.

Holtmann MH, Douni E, Schütz M, Zeller G, Mudter J, Lehr HA, Gerspach J, Scheurich P, Galle PR, Kollias G, Neurath MF (2002). “Tumor necrosis factor-receptor 2 is up-regulated on lamina propria T cells in Crohn's disease and promotes experimental colitis in vivo.” Eur J Immunol. Nov;32(11):3142-51.

Hruby ZW, Shirota K, Jothy S, Lowry RP (1991). “Antiserum against tumor necrosis factor-alpha and a protease inhibitor reduce immune glomerular injury.” Kidney Int. Jul;40(1):43-51.

Hudson BG, Reeders ST, Tryggvason K (1993). “Type IV collagen: structure, gene organization, and role in human diseases. Molecular basis of Goodpasture and Alport syndromes and diffuse leiomyomatosis.” J Biol Chem. Dec 15;268(35):26033-6.

Huppelsberger J, Walter K (2003). “Wasser- und Elektrolythaushalt, Nierenfunktion.“ Kurzlehrbuch Physiologie. Stuttgart, Thieme: 178-201.

Keur I, Krediet RT, Arisz L (1989). “Glomerulopathy as a paraneoplastic phenomenon.” Neth J Med. Jun;34(5-6):270-84.

Kilbourn RG, Gross SS, Jubran A, Adams J, Griffith OW, Levi R, Lodato RF (1990). “NG-methyl-L-arginine inhibits tumor necrosis factor-induced hypotension: implications for the involvement of nitric oxide.” Proc Natl Acad Sci U S A. May;87(9):3629-32.

Kluth DC, Rees AJ (1999). “Anti-glomerular basement membrane disease.” J Am Soc Nephrol. Nov;10(11):2446-53.

Kolb WP, Granger GA (1968). “Lymphocyte in vitro cytotoxicity: characterization of human lymphotoxin.” Proc Natl Acad Sci U S A. Dec;61(4):1250-5.

Kollias G, Douni E, Kassiotis G, Kontoyiannis D (1999). “The function of tumour necrosis factor and receptors in models of multi-organ inflammation, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease.” Ann Rheum Dis. Nov;58 Suppl 1:I32-9.

Kollias G, Kontoyiannis D (2002). “Role of TNF/TNFR in autoimmunity: specific TNF receptor blockade may be advantageous to anti-TNF treatments.” Cytokine Growth Factor Rev. Aug-Oct;13(4-5):315-21.

Kriegler M, Perez C, DeFay K, Albert I, Lu SD (1988). “A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF.” Cell. Apr 8;53(1):45-53.

Küsters S, Tiegs G, Alexopoulou L, Pasparakis M, Douni E, Künstle G, Bluethmann H, Wendel A, Pfizenmaier K, Kollias G, Grell M (1997). “In vivo evidence for a functional role of both tumor necrosis factor (TNF) receptors and transmembrane TNF in experimental hepatitis.“ Eur J Immunol. Nov;27(11):2870-5.

Kutukculer N, Shenton BK, Clark K, Rigg KM, Forsythe JL, Kirby JA, Proud G, Taylor RM (1995). “Renal allograft rejection: the temporal relationship and predictive value of plasma TNF (alpha and beta), IFN-gamma and soluble ICAM-1.” Transpl Int. ;8(1):45-50.

Lan HY, Yang N, Metz C, Mu W, Song Q, Nikolic-Paterson DJ, Bacher M, Bucala R, Atkins RC (1997). “TNF-alpha up-regulates renal MIF expression in rat crescentic glomerulonephritis.” Mol Med. Feb;3(2):136-44.

Le Hir M, Haas C, Marino M, Ryffel B (1998). “Prevention of crescentic glomerulonephritis induced by anti-glomerular membrane antibody in tumor necrosis factor-deficient mice.” Lab Invest. 1998 Dec;78(12):1625-31.

Lee ZH, Kwack K, Kim KK, Lee SH, Kim HH (2000). “Activation of c-Jun N-terminal kinase and activator protein 1 by receptor activator of nuclear factor kappaB.” Mol Pharmacol. Dec;58(6):1536-45.

Legler DF, Micheau O, Doucey MA, Tschopp J, Bron C (2003). “Recruitment of TNF receptor 1 to lipid rafts is essential for TNFalpha-mediated NF-kappaB activation.” Immunity. May;18(5):655-64.

Lerner RA, Glassock RJ, Dixon FJ (1967). “The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis.” J Exp Med. Dec 1;126(6):989-1004.

Lüllmann-Rauch R (2003). “Niere“. Histologie. Stuttgart, Thieme: 376-95.

Mason PD, Pusey CD (1994). “Glomerulonephritis: diagnosis and treatment.” BMJ. Dec 10;309(6968):1557-63.

McCluskey RT (1982). “Evidence for an immune complex disorder in systemic lupus erythematosus (SLE).” Am J Kidney Dis. Jul;2(1 Suppl 1):119-25.

McDevitt H, Munson S, Ettinger R, Wu A (2002). “Multiple roles for tumor necrosis factor-alpha and lymphotoxin alpha/beta in immunity and autoimmunity”. Arthritis Res. 4 Suppl 3:S141-52. Epub 2002 May 9.

Mease PJ, Goffe BS, Metz J, VanderStoep A, Finck B, Burge DJ (2000). “Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial.” Lancet. Jul 29;356(9227):385-90.

Meijer C, Huysen V, Smeenk RT, Swaak AJ (1993). “Profiles of cytokines (TNF alpha and IL-6) and acute phase proteins (CRP and alpha 1AG) related to the disease course in patients with systemic lupus erythematosus.” Lupus. Dec;2(6):359-65.

Mohammed I, Ansell BM, Holborow EJ, Bryceson AD (1977). “Circulating immune complexes in subacute infective endocarditis and post-streptococcal glomerulonephritis.” J Clin Pathol. Apr;30(4):308-11.

Morel D, Normand E, Lemoine C, Merlio JP, Velours J, Astier T, Bonnet F, Aparicio M, Potaux L, Bloch B (1993). “Tumor necrosis factor alpha in human kidney transplant rejection-analysis by in situ hybridization.” Transplantation. Apr;55(4):773-7.

Morrison DC, Ryan JL (1987). “Endotoxins and disease mechanisms.” Annu Rev Med.;38:417-32.

Neale TJ, Rüger BM, Macaulay H, Dunbar PR, Hasan Q, Bourke A, Murray-McIntosh RP, Kitching AR (1995). “Tumor necrosis factor-alpha is expressed by glomerular visceral epithelial cells in human membranous nephropathy.” Am J Pathol. Jun; 146(6):1444-54.

Noel C, Copin MC, Hazzan M, Labalette M, Susen S, Lelievre G, Dessaint JP (2000). “Immunomodulatory effect of pentoxifylline during human allograft rejection: involvement of tumor necrosis factor-alpha and adhesion molecules.” Transplantation. Mar 27;69(6):1102-7.

Nophar Y, Kemper O, Brakebusch C, Englemann H, Zwang R, Aderka D, Holtmann H, Wallach D (1990). “Soluble forms of tumor necrosis factor receptors (TNF-Rs). The cDNA for the type I TNF-R, cloned using amino acid sequence data of its soluble form, encodes both the cell surface and a soluble form of the receptor.” EMBO J. Oct;9(10):3269-78.

Orth SR, Ritz E (1998). “Nephrotic syndrome“. Internist (Berlin). Dec;39(12):1246-52.

Pavenstädt H, Kriz W, Kretzler M (2003). “Cell biology of the glomerular podocyte”. Physiol Rev. Jan;83(1): 253-307.

Perlmutter DH, Dinarello CA, Punsal PI, Colten HR (1986). “Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression.” J Clin Invest. Nov;78(5):1349-54.

Perrone RD, Madias NE, Levey AS (1992). “Serum creatinine as an index of renal function: new insights into old concepts.” Clin Chem. Oct;38(10):1933-53.

Pinckard JK, Sheehan KC, Schreiber RD (1997). “Ligand-induced formation of p55 and p75 tumor necrosis factor receptor heterocomplexes on intact cells.” J Biol Chem. Apr 18;272(16):10784-9.

Ramesh G, Kimball SR, Jefferson LS, Reeves WB (2007). “Endotoxin and cisplatin synergistically stimulate TNF-alpha production by renal epithelial cells.” Am J Physiol Renal Physiol. Feb;292(2):F812-9. Epub 2006 Oct 10.

Ramesh G, Reeves WB (2003). “TNFR2-mediated apoptosis and necrosis in cisplatin-induced acute renal failure.” Am J Physiol Renal Physiol. Oct;285(4):F610-8. Epub 2003 Jul 15.

Reimold AM (2003). “New indications for treatment of chronic inflammation by TNF-alpha blockade.” Am J Med Sci. Feb;325(2):75-92.

Roach DR, Bean AG, Demangel C, France MP, Briscoe H, Britton WJ (2002). “TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection.” J Immunol. May 1;168(9):4620-7.

Rodewald R, Karnovsky MJ (1974). “Porous substructure of the glomerular slit diaphragm in the rat and mouse.” J Cell Biol. Feb;60(2):423-33.

Schmidt R, Lang F, Heckmann M, Kurtz A (2004). “Niere“. Physiologie des Menschen mit Pathophysiologie. Heidelberg, Springer: 668-99.

Schulze-Osthoff K, Ferrari D, Los M, Wesselborg S, Peter ME (1998). “Apoptosis signaling by death receptors.” Eur J Biochem. Jun 15;254(3):439-59.

Sicotte NL, Voskuhl RR (2001). “Onset of multiple sclerosis associated with anti-TNF therapy.” Neurology. Nov 27;57(10):1885-8.

Silbernagel S, Despopoulos A (2003). “Niere.“ Taschenatlas der Physiologie. Stuttgart, Thieme: 148-186.

Silbernagl S (1991). “Niere, Salz- und Wasserhaushalt.“ Taschenatlas der Physiologie. Stuttgart, Thieme.

Silbernagl S, Klinke R (2001). “Die Funktion der Nieren.“ Lehrbuch der Physiologie. Stuttgart, Thieme: 287-336.

Solomon KA, Pesti N, Wu G, Newton RC (1999). “Cutting edge: a dominant negative form of TNF-alpha converting enzyme inhibits proTNF and TNFRII secretion.” J Immunol. Oct 15;163(8):4105-8.

Somlo S, Mundel P (2000). “Getting a foothold in nephrotic syndrome.” Nat Genet. Apr;24(4):333-5.

Stahl RAK, Schlöndorff D, Braun N, Boesken W, Kühn K, Risler T. “Leitlinien-basierte Empfehlungen für die Praxis: G4 Glomeruläre Nierenkrankheiten“. Stand Dezember 2004.

Suenaert P, Bulteel V, Lemmens L, Noman M, Geypens B, Van Assche G, Geboes K, Ceuppens JL, Rutgeerts P (2002). “Anti-tumor necrosis factor treatment restores the gut barrier in Crohn's disease.” Am J Gastroenterol. Aug;97(8):2000-4.

Tang P, Hung M-C, Klostergaard J(1996). “Human pro-tumor necrosis factor is a homotrimer.” Biochemistry. Jun 25;35(25):8216-25.

Tartaglia LA, Ayres TM, Wong GH, Goeddel DV (1993). “A novel domain within the 55 kd TNF receptor signals cell death.” Cell. Sep 10;74(5):845-53.

Tartaglia LA, Pennica D, Goeddel DV (1993). “Ligand passing: the 75-kDa tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor.” J Biol Chem. Sep 5;268(25):18542-8.

Tartaglia LA, Rothe M, Hu YF, Goeddel DV (1993). “Tumor necrosis factor's cytotoxic activity is signaled by the p55 TNF receptor.” Cell. Apr 23;73(2):213-6.

Timoshanko JR, Sedgwick JD, Holdsworth SR, Tipping PG (2003). “Intrinsic renal cells are the major source of tumor necrosis factor contributing to renal injury in murine crescentic glomerulonephritis.” J Am Soc Nephrol. Jul;14(7):1785-93.

Tipping PG, Leong TW, Holdsworth SR (1991). ”Tumor necrosis factor production by glomerular macrophages in anti-glomerular basement membrane glomerulonephritis in rabbits.” Lab Invest. Sep;65(3):272-9.

Tomosugi NI, Cashman SJ, Hay H, Pusey CD, Evans DJ, Shaw A, Rees AJ (1989). “Modulation of antibody-mediated glomerular injury in vivo by bacterial lipopolysaccharide, tumor necrosis factor, and IL-1.” J Immunol. May 1;142(9):3083-90.

Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey TJ 3rd, Zentella A, Albert JD, et al (1986). “Shock and tissue injury induced by recombinant human cachectin.” Science Oct 24;234(4775):470-4.

van der Poll T, van Deventer SJ, Hack CE, Wolbink GJ, Aarden LA, Büller HR, ten Cate JW (1992). “Effects on leukocytes after injection of tumor necrosis factor into healthy humans.” Blood. Feb 1;79(3):693-8.

van Dullemen HM, van Deventer SJ, Hommes DW, Bijl HA, Jansen J, Tytgat GN, Woody J (1995). “Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2).” Gastroenterology. Jul;109(1):129-35.

Vielhauer V, Mayadas TN (2007). “Functions of TNF and its receptors in renal disease: distinct roles in inflammatory tissue injury and immune regulation.” Semin Nephrol. May;27(3):286-308.

Vielhauer V, Stavrakis G, Mayadas TN (2005). “Renal cell-expressed TNF receptor 2, not receptor 1, is essential for the development of glomerulonephritis.” J Clin Invest. May;115(5):1199-209. Epub 2005 Apr 1.

Wajant H, Pfizenmaier K, Scheurich P (2003). “Tumor necrosis factor signaling.” Cell Death Differ. Jan;10(1):45-65.

Wallach D, Engelmann H, Nophar Y, Aderka D, Kemper O, Hornik V, Holtmann H, Brakebusch C (1991). “Soluble and cell surface receptors for tumor necrosis factor.” Agents Actions Suppl. ;35:51-7. Review.

Wilson CB, Dixon FJ (1973). “Anti-glomerular basement membrane antibody-induced glomerulonephritis.” Kidney Int. Feb;3(2):74-89.

Zheng L, Fisher G, Miller RE, Peschon J, Lynch DH, Lenardo MJ (1995). “Induction of apoptosis in mature T cells by tumour necrosis factor.” Nature. Sep 28;377(6547):348-51.

Product data sheet of mouse sTNF RII/ TNFRSF1B DuoSet ELISA, September 2006, R&D Systems (2010-01-13) http://www.rndsystems.com/pdf/dy426.pdf

Product data sheet of mouse sTNF RII/ TNFRSF1B DuoSet ELISA, October 2008, R&D Systems (2010-01-13) http://www.rndsystems.com/pdf/dy410.pdf

Product data sheet of human sTNF RII/ TNFRSF1B DuoSet ELISA, November 2008, R&D Systems (2010-01-13) http://www.rndsystems.com/pdf/DY726.pdf

Product data sheet of QuantiChrom Creatinine Assay Kit (DICT-500), 2005, Bio Assay Systems (2010-01-21) http://www.bioassaysys.com/Search.asp?Key=blood

Homepage of the University of Oulu, Finland (2010-01-19) http://herkules.oulu.fi/isbn9514264290/html/x782.html

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Eva Pfeifer, Johannes Polz, Sybille Grieβl, Sven Mostböck, Thomas Hehlgans, Daniela N. Männel (2012). **“Mechanisms of immune complex-mediated experimental glomerulonephritis: possible role of the balance between endogenous TNF and soluble TNF receptor type 2.”** European Cytokine Network. Volume 23, Number 1, 15-20.

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