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It is remarkable to be alive and it is astonishing to be healthy. Our environment contains an impressive number of life-threatening viruses, bacteria, fungi, parasites, and other pathogens that may cause disease.\(^1\) In addition, our body hosts a great number of processes that can cause life-threatening diseases, if homeostasis is disturbed.\(^2\text{-}^5\) Fortunately, throughout evolutionary history, the human body has developed an equally remarkable series of barriers and defenses to protect itself from these perils: the immune system.

Two immune systems
The immune system is a network of molecules, cells, and tissues that protects the body by detecting pathogens and responding to eliminate them.\(^6\) It is traditionally divided into two distinct, but interconnected systems: the innate immune system and the adaptive immune system.\(^6\)

The innate immune system is a primitive system that is already present at birth – hence the name – in healthy individuals. It has two main goals: to quickly detect the presence of pathogens and to recruit effector mechanisms that eliminate them.\(^7\) When a pathogen succeeds in breaching the anatomic barriers of the body, the innate immune system is the first line of defense. It is activated within seconds of the encounter with a pathogen. Enzymes and peptides break down the pathogen’s cell walls and cell membranes, and a cascade of highly orchestrated proteins cooperate swiftly in recruiting effector mechanisms that eliminate the pathogen. Thus, the innate immune system is a non-specific, fast system that is effective against all sorts of pathogens at an early stage.\(^6\)

In contrast, the adaptive immune system is a more specific, but a much slower immune system that is required once the host’s innate immune system is compromised, evaded, or overpowered.\(^6\text{-}^7\) In several days to weeks after the encounter with a pathogen, the adaptive immune system strikes with full force using a tailored immune response to the particular pathogen.\(^6\text{-}^7\) The adaptive immune system is organized around two types of cells, T- and B-lymphocytes, that adapt to the threat by proliferating, dividing and differentiating into highly specialized cells, such as cytotoxic T cells and antibody-producing B cells.\(^6\text{-}^8\) After the battle is won, some of these cells become long-lived memory cells that
enable the immune system to respond faster and more vigorously whenever a particular pathogen is encountered again.\textsuperscript{6}

A powerful effector mechanism of the innate immune system, and the linking pin between the innate and the adaptive immune systems, is the complement system.
GENERAL INTRODUCTION

Overview of the complement system

Complement evolution
The complement system in vertebrates is estimated to be at least 600 million years old, pre-dating the existence of our human predecessors by approximately 597 million years. Although genes encoding complement proteins are not present in all animals, even primitive species such as arthropods, corals, and sea urchins have a functioning complement system. Over millions of years, the complement system has evolved into an efficient and highly versatile system that can respond within seconds according to a hard-wired, ‘standard protocol’. This response is possible because of the architectural backbone of the complement system: a proteolytic cascade. In such a cascade, many proteins are zymogens, inactive precursors of enzymes that are activated by proteolytic cleavage. The complement zymogens are widely distributed throughout the body without any adverse effect. When the zymogen is activated locally, it forms an active complement enzyme that cleaves its substrate – another complement zymogen – thereby activating the next zymogen in the cascade, and so on. This enzyme-triggered cascade ensures that activation of a small number of complement proteins at the start of the pathway results in a rapidly amplified complement response.

Complement discovery
A series of experiments in the late 19th century led to the first recognition of the complement system, as was reviewed previously. In 1874, Traube and Gscheideln showed that microorganisms injected into the circulation were killed quickly and that blood remained sterile. In 1884, Grohmann demonstrated that microorganisms could be killed by cell-free serum in vitro, indicating that a component in the serum was bactericidal. In 1888, Nuttall discovered that this bactericidal activity could be destroyed by heating blood serum above 55°C. One year later, Buchner demonstrated that the bactericidal activity in serum was caused by a heat-labile substance in serum which he named ‘Alexin’ (derived from the Greek word ‘αλεξειν’, or ‘alexein’, which means to defend). But the discovery of the complement system is generally credited to Bordet
for performing the critical experiments needed to identify the complement system. In 1895, he inactivated the bactericidal activity of serum from an immunized animal by heating the serum. Next, he added fresh serum from a non-immunized healthy control to this inactivated serum, which restored the bactericidal activity in the serum. Thus, he concluded that bactericidal activity in serum was caused by two different factors. The first was a heat-stable factor that was only present in immunized serum and which he termed ‘sensitizer’ (now known as antibodies). The second was a heat-labile, lytic factor, that was present in normal serum and which he thought to be Alexin. In 1899, Ehrlich was responsible for replacing the term ‘Alexin’ with ‘complement’. He hypothesized that immune cells had receptors on their surface that could recognize antigens and that after immunization with the antigen, these receptors were shed from the cells as ‘amboceptors’ (now known as antibodies). He introduced the term ‘das Komplement’ to emphasize that antibodies have a site for recognizing the antigen and a different site that attaches to the heat-labile serum factor which ‘complemented’ or aided the bactericidal effect of the antibodies. Today, the term ‘complement’ is widely accepted even though it is now known that the functions of complement proteins exceed merely ‘complementing’ the antibody-mediated response.

**Complement system: main functions and proteins**

More than a century after its discovery, a wide variety of functions have been attributed to the complement system, which by far exceed the effector arm of the innate immune system. As was reviewed previously, these functions include: recognizing and clearing foreign pathogens and antigens, stimulating phagocytosis of opsonized targets, promoting humoral immune responses, modulating cellular immune responses, clearing self-antigens derived from apoptotic processes, facilitating immune complex transport, promoting the auto-inflammatory response to injured self-tissue following recognition of neo-epitopes by natural antibodies, shaping the composition of the natural antibody repertoire, regulating the growth of inflammatory tumors, and enhancing tissue regeneration. Furthermore, several complement proteins are able to elicit responses from different cell types and different tissues that are not directly related to host-defense but rather bridge immunity and developmental biology.
The complement system has a large number of soluble and membrane-bound proteins that are found in the circulation and in tissues. In general, the complement proteins have one of three functions in the complement system. The first function is activating the complement cascade. Proteins with this function are often present as zymogens and serve as proteolytic enzymes, enzyme cofactors, or enzyme substrates. The second function is regulating the complement cascade. Complement regulatory proteins typically inhibit enzymes and inactivate peptides, to ensure that the extent of complement activation is proportional to the required amount and duration. The third function is serving as a cellular receptor for complement proteins and their fragments. Some proteins overlap these functional categories and several proteins have additional functions in other physiological systems.

**Complement nomenclature**

The nomenclature of the complement proteins is not the most amiable aspect of the complement system. It follows the chronological order of discovery, which has led to inconsistencies that may form an important stumbling block in the understanding of the complement system. Thus, before discussing the complement cascade in more detail, some comments are given on the nomenclature of the complement system. These comments are based on the recent recommendations made by the International Complement Society and the European Complement Network to harmonize the complement nomenclature while making minimal changes to long-established conventions. Nevertheless, for several proteins consensus has not been reached and some inconsistencies remain.

The processes that initiate complement activation are traditionally divided into three pathways: the classical pathway, the lectin pathway and the alternative pathway (Figure 1). These three pathways converge at a final common pathway: the terminal complement pathway. The classical complement pathway was the first to be discovered, and the first eleven proteins that constitute this pathway are designated by the capital letter C, followed by a number. Unfortunately, these proteins were numbered in the order of discovery instead of the sequence of reactions which is: C1, C4, C2, C3, C5, C6, C7, C8, and C9. Furthermore, C1 is a complex of three distinct proteins, termed C1q, C1r, and C1s; the letters q, r, and s designate their elution order on ion exchange.
Proteolytic cleavage fragments of the native complement protein are designated by adding a lower-case letter, in which the smaller fragment is designated a, and the larger fragment is designated b (e.g. C4a and C4b). C2a is the exception to this rule; C2a originally indicated the activated C2 fragment in the C3 and C5 convertases of the classical pathway, but was found to be larger than C2b. Consensus has not yet been reached, thus in some literature C2b is used to describe the smaller, inactive C2 fragment, whereas C2b is used in other literature, including this thesis, to describe the larger active C2 fragment. Inactive proteins or inactive protein fragments are designated by the lowercase letter i (e.g. iC3b).

The alternative pathway was the second pathway to be discovered and was named ‘alternative’ for being an alternative to the already established ‘classical pathway’. Newly discovered proteins in the alternative pathway were first designated by a ‘factor’ and a capital letter (e.g. factor H), and are now also designated by their abbreviation (e.g. FH is factor H). Properdin is the exception to this rule, and debate exists on whether to rename this protein to factor P or FP. Just as in the classical pathway, the cleavage products of the proteins in the alternative pathway are designated, by adding a lower case letter (e.g. Ba and Bb). Spontaneous hydrolysis of C3 is an important part of the alternative pathway, and hydrolyzed C3 is designated by adding H₂O in parenthesis (e.g. C3(H₂O)).

The lectin pathway was the most recently discovered pathway and was originally termed so for being organized around the protein mannose-binding lectin. Proteins that were discovered in the lectin pathway are designated by trivial names or their abbreviations (e.g. ficolins; MBL, for mannose-binding lectin; MASP, for MBL-associated serine protease).

The terminal complement pathway has protein complexes consisting of multiple proteins. Each complex is designated by a hyphen between the first and last protein of the complex (e.g. C5b-9 is the membrane attack complex which consists of the proteins C5b, C6, C7, C8, and C9). The soluble variant of C5b-9 is designated by the lowercase letter s (i.e. sC5b-9).

Some final remarks: convertases are designated by the active fragments from which they are composed (e.g. C3bBb is the C3 convertase of the alternative pathway, which consists of the protein fragments C3b and Bb). Receptors are usually designated by the capital letter R; four complement receptors are...
designated by CR, followed by the numbers 1-4 (e.g. CR1), the remaining receptors are designated by the protein or protein fragment to which they bind, followed by the capital letter R (e.g. C1qR). Membrane-bound proteins also have been assigned a CD number (e.g. DAF is also known as CD55). Proteins with trivial names may have other trivial names (e.g. Ficolin-1 was formerly known as M-Ficolin).

**Complement activation**

Although the proteins involved in the complement system have many functions and are structurally diverse, in general, complement activation is characterized by striking operational simplicity. Complement activation can be divided into four main phases (Figure 1): 1) initiation of complement activation, 2) C3 convertase activation and amplification, 3) C5 convertase activation and 4) the assembly of the membrane attack complex (C5b-9) in the terminal pathway.

*Phase 1: Initiation of the three main complement pathways*

The processes that initiate complement activation are traditionally divided into three pathways: the classical, the lectin, and the alternative pathway. The classical complement pathway is typically activated by immune complexes, but this pathway can also be activated in an antibody-independent manner, for instance by C reactive protein (CRP), viruses and Gram-negative bacteria. Classical pathway activators are recognized by C1q, a protein with six pattern-recognition globular heads (Figure 2A). The binding of initiating activators to C1q induces a conformational change in C1q which activates C1r, which in turn activates C1s. Activated C1s then cleaves C4 into C4a and C4b. C4b attaches to the activator surface and binds to C2. C2 is then cleaved by C1s into C2a and C2b. In the presence of magnesium ions, C2a binds to C4b, which was already bound on the activator surface, thereby creating the C4b2a complex that serves as the classical pathway C3 convertase.

The lectin pathway is initiated when e.g. carbohydrates on microbial surfaces are recognized via mannose-binding lectin (MBL), ficolins 1–3, and collectin 11 (CL-11) (Figure 2B). Other processes can also activate the lectin pathway, such as the direct recognition of self-proteins, the binding of MBL to autoantibodies containing agalactosyl (G0) carbohydrates, or the binding of MBL to pathogenic natural IgM antibodies. After recognition, activation
of the lectin pathway proceeds through the activities of MBL-associated serine proteases (MASP) that cleave and activate C4 and C2, into a C3 convertase, quite like the cleavage of C4 and C2 in the classical pathway.\(^{23}\)

The alternative pathway does not require specific activation and is spontaneously and constantly activated under physiological circumstances in a process called ‘tick over’ (Figure 2C).\(^{30}\) The alternative pathway starts with the hydrolysis of C3 into C3(H\(_2\)O). C3(H\(_2\)O) can bind to factor B which, in the presence of magnesium ions, can be cleaved by factor D into two fragments: Ba and Bb.\(^{27}\) The Bb fragment remains bound to C3(H\(_2\)O), thereby forming the initiation C3 convertase, C3(H\(_2\)O)Bb. Under physiological circumstances, this initiation C3 convertase has a short half-life and is continuously inhibited by

![Figure 1. Simplified flowchart of complement activation](image-url)
regulatory proteins. However, this spontaneous activation is readily amplified if C3b binds to cellular surfaces such as that of virally infected cells, bacteria, parasites, and fungi. Following the binding of properdin to a C3bBb complex, a stabilized C3 convertase C3bBbP is formed and the half-life is extended from 1.5 minutes to approximately 8-15 minutes. In addition to activation by spontaneous hydrolysis, the alternative pathway can be activated via repeating polysaccharides, endotoxin, IgA-containing immune complexes, C3 nephritic factor, and immunoglobulin light chains.

In conclusion, there are three main pathways of complement activation with different pattern-recognition proteins that recognize the surface structures of the different activators. Following activation of a pathway, a cascade of highly orchestrated protein-protein interactions and proteolytic cleavages can lead to the generation of a C3 convertase.

Phase 2: C3 convertase and amplification
Depending on the nature of the initiating activator, different pathways are activated and different proteins are used to form a C3 convertase. All C3-convertases however, cleave C3 into its two active fragments: C3a and C3b. C3a is an anaphylatoxin that recruits and activates the effector cells of the innate immune system. C3b amplifies the cascade and coats microbial or apoptotic surfaces, thereby opsonizing or “marking” the attached target as distinct molecules for phagocytosis. On the surface membranes of intact self-cells, regulators prevent further complement activation, whereas on the surfaces of microbes or modified self-cells, activation proceeds.

Phase 3: C5 convertase
If complement activation proceeds, an additional C3b molecule binds to the C3 convertase, which transforms the protein complex into a C5 convertase (Figure 2D). Thus the C5 convertase for the alternative pathway is C3bBbC3b and the C5 convertase for the classical and lectin pathways is C4bC2bC3b. Complement can also be activated by additional pathways that act independently of C3 and may bypass the C3 convertase, such as thrombin acting on the C5 convertase. The C5 convertases cleave C5 into C5a and C5b. C5a is a powerful anaphylatoxin and chemotactic factor with many biological functions. C5b is necessary for the assembly of the membrane attack complex (C5b–9).
Figure 2. Schematic view of complement activation
Phase 4: Formation of the membrane attack complex
The formation of the membrane attack complex begins with the binding of C5b to C6, and the sequential binding to C7, C8, and several molecules of C9 (Figure 2D). During these reactions, hydrophobic domains become exposed on the surface. The assembly and conformational changes generate C5b-9, a lipophilic, membrane-inserting, and pore-forming complex, that can lead to cell lysis.

Complement regulation
A powerful system needs powerful regulation to protect the hosts’ own tissues from harmful effects. Complement regulatory proteins ensure that the extent of complement activation is proportional to the required amount and duration of complement activation. It is therefore not surprising that the number of regulators that inhibit complement activation is far greater than the number of regulators that stimulate complement activation. Complement regulators are categorized into three groups: soluble regulators, membrane-bound regulators that are attached to the surface of host cells, and complement clearance receptors. Currently known regulators of complement activation include factor H, factor I, C1-inhibitor (C1-INH), C4b binding protein (C4BP), Vitronectin, Clusterin, Membrane Cofactor Protein (MCP; CD46), Decay-Accelerating Factor (DAF; CD55), Complement Receptor 1 (CR1), Thrombomodulin, and CD59. Interestingly, several regulators have additional activities beyond complement-mediated host defense, such as mediating cell adhesion, interacting with the extracellular matrix, or linking the complement cascade with other important physiological systems, such as the coagulation cascade.
Complement activation in disease
The importance of the complement system is best illustrated when it is defective. A clinically relevant concept in the pathogenesis of complement-mediated diseases is a disturbance in the balance between insufficient and excessive complement activation. Insufficient complement activation results in diseases such as severe infections and lupus-like disease. These diseases reflect defective physiologic functions of the complement system, such as protecting the host from infection, clearing immune-complexes, and removing debris. On the other hand, excessive complement activation that is caused by inadequate regulation, excessive stimulation, or both, can result in rare, but life-threatening systemic diseases such as atypical hemolytic uremic syndrome and paroxysmal nocturnal hemoglobinuria. These diseases reflect tissue damage that occurs when the powerful mechanisms that are focused on recognition and clearance of foreign pathogens and antigens, are directed to self-tissues. Thus, while complement activation is essential for protection and repair, it can also cause detrimental inflammation and cell injury. Much like the concept of Yin and Yang, it is the balance of these seemingly opposite forces within the same system that determines health or disease. Although the complement system can affect many organs, the kidney is frequently affected and is, therefore, the main focus of this thesis.
The kidney

According to the theory of evolution, life began in the seas. The cells of the first primitive organisms had an interior milieu that was similar to the salt water that surrounded them. For the transition to life on land, these primitive organisms required kidneys.37

Gross anatomy

The kidneys are paired organs that lie in the retroperitoneal space of the abdominal cavity and extend from the twelfth thoracic vertebra to the third lumbar vertebra (Figure 3A).38 To produce urine, they filter more than 180 L of fluid from the blood plasma, every day. Urine is conducted through the ureters to the urinary bladder and exits the body via the urethra. The shape of the kidney is oval with a convex border and a concave border. This shape is common in nature and is so characteristic that several languages have a single word to describe objects with this shape (e.g. “reniform” in English and Dutch, “reniformia” in Italian, and “reniforme” in French and Spanish), derived from the Latin words for kidneys “renes” and shape “forma”. The size of a normal kidney (approximately 11 x 6 x 3 cm) is comparable to a human fist, and the mass of a normal kidney (approximately 125-170 grams in adult males, and 115-155 grams in adult females) is comparable to a small apple.39 A fibrous capsule covers the surface of the kidney. The concave medial border of the kidney contains the renal hilum, a vertical fissure that serves as a portal for structures that enter or exit the kidney, such as the renal artery, vein, nerve, and ureter.

Gross examination of a cross-sectioned kidney shows two distinct regions: the cortex and the medulla, collectively termed the renal parenchyma (Figure 3B).40 The cortex is the brown-reddish outer layer between the medulla and the fibrous cap. It contains most of the initial blood-filtering structures; more than 90% of the blood that passes the kidney flows through the cortex. The medulla is the lighter-colored middle part of the kidney, which only receives approximately 10% of the blood that passes through the kidney. The medulla contains two distinct regions: renal pyramids and renal columns. The renal pyramids resemble inverted pyramids and help transport urine towards the ureters. The apex of the pyramid is called the papilla, and each papilla has perforated openings of the collecting ducts through which urine is excreted.
Figure 3. The kidney, from gross to ultrastructural examination
The papilla empties urine into a minor calyx; minor calyces empty urine into a major calyx, and major calyces empty urine into the renal pelvis, the funnel-shaped beginning of the ureter. Projections of cortical tissue lie between the renal pyramids in the medulla; these projections are called renal columns.

**The nephron**

Over the course of evolution, the human kidneys developed into a pair of organs that serves crucial functions: filtering toxins and metabolic products from the blood and excreting them through the urine, maintaining homeostasis by regulating the body’s extracellular fluid status, electrolyte balance, and acid-base balance, contributing to the metabolism of glucose, and serving as an endocrine organ by producing hormones that are involved in erythropoiesis, calcium metabolism, and the regulation of blood pressure and blood flow. The nephron is the smallest functional unit of the kidney that converts blood to urine (Figure 3C). Each kidney has approximately one million nephrons, which can be classified based on their location into superficial cortical nephrons, mid-cortical nephrons, and juxtamedullary nephrons. The nephron consists of the glomerulus (also known as the renal corpuscle), the tubules, and the collecting ducts.

The glomerulus is the initial blood-filtering component of the nephron and consists of a small ball-shaped cluster of capillaries, known as the glomerular tuft, and a surrounding capsule, known as Bowman’s capsule (Figure 3D). Blood that enters the glomerular capillaries is forced to pass the glomerular filtration barrier in a process called ultrafiltration. The determinants of glomerular ultrafiltration are hydraulic and colloid osmotic pressure differences, and the permeability of the glomerular filtration barrier. The glomerular filtration barrier consists of three components that blood must pass (Figure 3E): the fenestrated glomerular endothelium and its glycocalyx, the glomerular basement membrane with three distinct layers (lamina rara interna, lamina densa, and lamina rara externa), and the slit diaphragm created by the visceral epithelial cells (podocytes) and their foot processes. The glomerular filtration barrier can prevent the passage of particles according to their molecular size, electrical charge, and stereotypical configuration: large and negatively charged molecules have more difficulty in passing the glomerular filtration barrier than small particles with an electroneutral or positive charge. As a result, blood cells and...
large proteins, such as antibodies and albumin, remain in the blood, whereas small waste products are filtered out of the blood to form the ultrafiltrate. In addition to the endothelial cells and the podocytes, the glomerulus contains two other main resident cell types: parietal epithelial cells and mesangial cells. Parietal epithelial cells are simple squamous cells that form Bowman’s capsule. They are continuous with the visceral epithelial cells. Mesangial cells are positioned between the capillary loops and have direct contact with the fenestrated endothelium. This position enables the mesangium to stabilize the glomerular endothelium, alter the intra-glomerular capillary flow and ultrafiltration surface area, and clear filtration residue through phagocytosis by mesangial cells or macrophages located within the mesangium. The space between the parietal and the visceral layers of Bowman’s capsule is termed Bowman’s space. Bowman’s space receives the ultrafiltrate and drains into the renal tubules at the urinary pole of the glomerulus. Tubuloglomerular feedback is regulated through the juxtaglomerular apparatus, which consists of the macula densa, juxtaglomerular cells, and extraglomerular mesangial cells. After ultrafiltration, the remaining blood proceeds through the efferent arteriole, whereas the ultrafiltrate flows from Bowman’s space into the tubules.

The tubules modify the glomerular ultrafiltrate and can be subdivided into the proximal tubules, the loop of Henle, and the distal tubules (Figure 3C). The proximal tubule is lined with epithelial cells containing microvilli and mitochondria. In the proximal tubules most of the water, electrolytes, and other nutrients in the ultrafiltrate are reabsorbed from the lumen into the peritubular capillaries. Reabsorption ensures that important substances that pass the glomerular filtration barrier, such as glucose and amino acids, are not lost by urinary excretion. The proximal tubule is also important for active solute secretion and hormone production. Substances that are secreted include hydrogen ions (for pH homeostasis), potassium ions (for salt homeostasis), ethanol, toxins, drugs, and other “foreign” substances. Following the proximal tubules, the ultrafiltrate flows into the loop of Henle, which encompasses the thin descending limb, the thin ascending limb, and the thick ascending limb. The main function of the loop of Henle is to create an osmolality gradient within the renal medullary interstitium, enabling the downstream nephron segments to concentrate urine. The loop of Henle is also responsible for maintaining calcium, magnesium, bicarbonate, and ammonium homeostasis. Following the
loop of Henle, the ultrafiltrate flows into the distal tubules, which include the distal convoluted tubules and the connecting tubules.\textsuperscript{50} The distal tubules are responsible for sodium, potassium, calcium, and magnesium homeostasis, and are regulated by hormones.\textsuperscript{50}

The distal tubules of several nephrons drain into a collecting duct (Figure 3C).\textsuperscript{42} Collecting ducts run through the osmotic gradient in the medulla and reabsorb water under the influence of hormones. Collecting ducts merge into larger collecting ducts, which are also known as the ducts of Bellini.\textsuperscript{40} The end result is concentrated urine, which leaves the collecting ducts via the renal papilla, renal pelvis, and ureter, respectively.

**The renal microvasculature**

The kidneys are highly vascularized organs and receive approximately 20-25\% of the cardiac output, which constitutes approximately 1 L of blood per minute.\textsuperscript{51} Considering that the volume of each kidney is only approximately 200 mL,\textsuperscript{52} each kidney is perfused with more than 2.5 times the total volume, every minute. This high renal blood flow is required to ensure that sufficient plasma is delivered to the glomeruli for filtration.

Each kidney receives blood from the renal artery, a direct branch of the abdominal aorta.\textsuperscript{40} After entering the hilum of the kidney, the renal artery branches into interlobar arteries, followed by arcuate and interlobular arteries, which in turn give rise to the afferent arterioles that feed the glomerular capillaries (Figure 2B and C).\textsuperscript{51} Most capillary beds in the body convey blood between arterioles and venules, enabling the exchange of gases, nutrients, and other substances with the surrounding tissue. In contrast, the glomerular capillaries are sandwiched between two arterioles: the afferent and efferent arteriole. The main function of the afferent and efferent arterioles is to regulate the glomerular blood flow and the glomerular filtration rate.\textsuperscript{51} Both arterioles can constrict or dilate separately, thereby influencing the hydrostatic pressure gradient in the glomerular capillaries. Blood flows out of the glomerular capillaries and into the efferent arteriole, which drains into the peritubular capillaries in cortical nephrons, or the vasa recta in the juxtamedullary nephrons.\textsuperscript{51} The peritubular capillaries and the vasa recta are both important for the delivery of oxygen and nutrients to the surrounding cells.\textsuperscript{51} Additionally, the peritubular capillaries are essential for the tubular modification of urine in the cortex, whereas the
vasa recta are particularly important for the maintenance of the medullary concentration gradient. The peritubular capillaries and the vasa recta progress into venules, followed by a series of veins that parallel the arterial system, and drain into the inferior vena cava.

The endothelium that lines the interior surface of the renal microvasculature plays an important role in health and disease. Under physiological circumstances, endothelial cells ensure appropriately regulated blood flow and express an anti-inflammatory phenotype, inhibiting platelet aggregation, coagulation, and inflammation which includes resistance to complement activation. In a physiologic response to a disruption of homeostasis, such as in injury or infection, the endothelial cells become activated and cause the opposite: they promote vasoconstriction, platelet aggregation, coagulation, and inflammation, which may help resolve the disorder. The kidney has a remarkably heterogeneous population of endothelial cells, each with structural and functional features, as was reviewed previously. The glomerular endothelial cells are an essential component of the glomerular filtration barrier. These cells are highly specialized and have a unique morphology: they are characterized by a flattened shape and contain many fenestrae with a diameter of approximately 60 nm that lack a classic diaphragm. Moreover, they are covered by the glycocalyx, a layer of negatively-charged macromolecules that contribute to permselectivity and repel blood cells from the vascular wall. There is cross-talk between glomerular endothelial cells, mesangial cells, and podocytes to maintain the function and morphology of the glomerular filtration barrier in relation to the local microenvironment. As a result, the glomerular microvasculature may be affected in glomerular diseases by direct injury to the glomerular endothelial cells, but also by injury or disturbance of the glomerular microenvironment. The endothelium of peritubular capillaries also contains fenestrations but these fenestrae have a thin diaphragm that modulates the filtration property. Because these post-glomerular capillary beds do not have a collateral circulation, events in the glomerular capillary bed may have downstream influences. For example, inflammatory mediators released in the glomerular capillaries can activate the endothelium of the peritubular capillaries. The afferent and efferent arterioles are phenotypically distinct. The proximal section of the afferent arteriole has fenestrated endothelial cells that face the extraglomerular mesangial cells, whereas the distal section of the
afferent arteriole, along with the efferent arteriole, does not contain fenestrae. The afferent arteriole and the proximal section of the efferent arteriole have a mono-layer of smooth muscle cells, the distal section of the efferent arterioles, along with the peritubular capillaries, is covered by pericytes. These properties help in regulating blood flow and filtration.

While the larger arteries are important for delivering blood from the systemic circulation into the kidney, the renal microvasculature is important because it supplies the renal cells with oxygen and nutrients, and maintains renal function by providing an adequate glomerular filtration rate, modulating the urinary composition, and sustaining the medullary concentration gradient. Consequentially, microvascular endothelial injury and dysfunction are central in the pathogenesis of various kidney diseases.
Complement in renal microangiopathies

This thesis comprises studies on complement proteins in the kidneys from patients with various renal microangiopathies. Renal microangiopathies are diseases of the renal microvasculature. The term ‘microangiopathy’ is derived from the Ancient Greek words ‘μικρόν’ (micron) which means small, ‘αγγείο’ (angeios) which means blood vessel, and ‘πάθος’ (pathos) which means suffering or disease. Renal microangiopathy refers to a status of injury or dysfunction of the renal microvascular endothelium. This may occur in the context of common diseases such as diabetes mellitus, hypertension, and preeclampsia, or in rare diseases such as atypical hemolytic uremic syndrome, primary glomerulonephritides, systemic vasculitides with renal manifestations, and rejection of the kidney allograft. The renal endothelium can be injured directly by a variety of factors (such as toxins, hyperglycemia, complement proteins, autoantibodies, alloantibodies, immune cells, and cytokines) or by defects in protective mechanisms (such as complement dysregulation or an imbalance between pro-angiogenic and anti-angiogenic factors). The microvasculature of the kidney is particularly vulnerable to complement-mediated injury, which is reflected by the broad range of renal diseases that have been linked to abnormal complement activation and the predominant renal manifestations of systemic diseases caused by a dysfunctional complement system. The reason for this susceptibility is not fully elucidated, but it has been suggested that it results from the kidney’s unique microvascular bed, which is subjected to high levels of shear stress and a wide range of substances in the bloodstream that can activate the complement system, such as immune complexes, pathogens, toxins, and cytokines. Several diseases relevant to this thesis will be discussed.

Glomerulonephritides

Glomerulonephritides are a relatively heterogeneous group of rare diseases characterized by glomerular inflammation, either as part of a primary renal disease (such as IgA nephropathy) or as a manifestation of a systemic disease (such as anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, or systemic lupus erythematosus). They often affect young people, are mostly incurable, and can lead to chronic kidney disease with progression to end-stage renal disease and the need for dialysis or kidney transplantation.
In several glomerulonephritides, complement has been well studied as an important mediator of renal injury, causing activation of granulocytes and platelets, chemotaxis of leucocytes via the anaphylatoxins C3a and C5a, and direct cytotoxicity via the assembly of C5b-9. As was reviewed previously, glomerular pathology in glomerulonephritis often results from immune complexes that activate the classical pathway, although recent studies show that the lectin and alternative pathways are also involved in the pathogenesis of several glomerulonephritides. The consequences of complement activation in glomerulonephritis depend on various factors. For example, the human immunoglobulin isotypes differ in their ability to cause classical pathway activation: IgM, IgG1, and IgG3 are strong complement activators, whereas IgG2 is a relatively weak complement activator; IgG4, IgA, IgD, and IgE are incapable of activating the classical pathway. Moreover, complement activation can take place in different glomerular compartments, following passive entrapment of pre-formed immune complexes, or in situ immune complex formation; antibodies may bind to antigens that are either intrinsic constituents of glomerular structures or to soluble antigens that are taken up by the mesangium or glomerular capillary wall. As a result, different cell types may be affected, causing different histopathological lesions and immune staining patterns (Figure 4). For example, subendothelial deposits are accessible to circulating cells, such as neutrophils and platelets, and may cause endothelial cell injury, hemostasis, coagulation, and exudative lesions. Mesangial deposits can activate and injure mesangial cells, causing them to proliferate and produce growth factors, cytokines, and extracellular matrix. Linear immune complex deposits indicate the binding of antibodies to autoantigens in the glomerular basement membrane. Subepithelial deposits can cause complement-mediated podocyte injury without an extensive inflammatory reaction; subepithelial immune complexes are separated from the circulation by the glomerular basement membrane, and the ultrafiltration flow carries mediators towards the urine, rather than toward the circulation. A pauci-immune staining pattern indicates an absence of immune complex deposition.
Thrombotic microangiopathies
Thrombotic microangiopathy (TMA) may result from various disorders that are characterized by extensive endothelial cell injury (Table 1), but it is historically connected to two disorders: thrombotic thrombocytopenic purpura (TTP), and hemolytic uremic syndrome (HUS). An improved understanding of the pathogenesis of HUS and TTP has changed the way in which these historically defined terms are now used.\textsuperscript{65} Therefore, a short summary of the first descriptions of TTP and HUS and how these terms developed in medical history is given below.

\textit{The first description of TTP}

The first clinical and pathological description of TMA is generally attributed to Moschcowitz who presented a case history of TMA to the New York Pathological Society in New York, on the 7th of February 1924.\textsuperscript{66} He gave a detailed account of a 16-year old girl who presented with acute fever, petechiae, pallor, and anemia, followed rapidly by paralysis, coma, and death. Her urine showed marked traces of albumin with hyaline and granular casts, but renal failure was absent. At autopsy, hyaline thrombi were observed in the arterioles and capillaries of the heart, liver, spleen, and kidney. It took more than 10 years before Baehr, Klemperer, and Schifrin published the clinical and morphological findings of four cases with ‘Moschcowitz syndrome’ in 1936.\textsuperscript{67} They suggested
that the microthrombi were composed of platelets and that the associated thrombocytopenia was caused by the excessive consumption of these platelets. In 1947, Singer et al. introduced the term ‘thrombotic thrombocytopenic purpura’ and in 1952, Symmers introduced the term ‘thrombotic microangiopathy’, brief for ‘thrombotic microangiopathic haemolytic anaemia’. Symmers considered ‘TMA’ a better diagnostic term to describe the syndrome than Moschcowitz’s syndrome, TTP, and other contemporary terms, because it was not eponymous, remained appropriate for patients without thrombocytopenia or purpura, and described the most striking histological lesions.

*The first description of HUS*

The first publications suggestive of HUS include a case-report on a soldier who died in 1918 following gastroenteritis, and the descriptions of severe cases during an epidemic of gastroenteritis due to *Escherichia coli* in 1955. In 1955, von Gasser et al. introduced the term “Hämolytisch-urämische Syndrome” in their description of five children with bilateral necrosis of the renal cortex, and four characteristic clinical features: acquired hemolytic anemia, acute renal failure, thrombocytopenia, and cerebral symptoms. There were several reasons why HUS was considered distinct from TTP. First, the condition primarily affected children, whereas TTP was considered a disease of the adults. In addition, the children presented with acute renal failure and had histopathological lesions of bilateral renal cortical necrosis, which were not considered typical of TTP at the time. Interestingly, von Gasser et al. suggested the existence of multiple syndromes, instead of one hemolytic uremic syndrome. The German word “Syndrome” translates to the plural form ‘syndromes’, in contrast to the singular “Syndrom”, which translates to ‘syndrome’. This distinction seems to have gone lost in translation in the subsequent papers but appears to be accurate in retrospect, as the reported patients may have had various syndromes, such as TTP and pneumococcal-associated HUS. The lesions of HUS were subsequently defined by Habib et al. who found that cortical necrosis was not a requirement for HUS and that the lesions observed in kidneys from patients with TTP and HUS were similar. Habib et al. used the term ‘TMA’, with permission from Symmers, and TMA became used to denote a morphological manifestation in addition to a clinical syndrome. From the initial description of HUS, the diagnostic triad of thrombocytopenia, microangiopathic hemolytic anemia, and acute renal injury remained, and are still in use today.
Summary of diseases, disorders, and syndromes that may manifest with systemic or local TMA. Recent publications listed multiple causes and differential diagnoses for TMA. Some of these TMAs are entities with a specific pathophysiology. Other TMAs are described in association with clinical conditions that often cause endothelial cell injury or dysfunction, but the pathophysiological mechanisms that drive these processes are poorly understood. For many of these TMAs, it is unknown if TMA is a separate entity, or related to a specific cause that can only be found if it is meticulously looked for. Defects in the complement system may contribute to various of these TMAs.

Table 1. Differential diagnosis of TMA

<table>
<thead>
<tr>
<th>TTP: caused by a functional deficiency of ADAMTS-13</th>
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<tbody>
<tr>
<td>Genetic abnormalities in ADAMTS-13 (Upshaw Schulman Syndrome)</td>
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<tr>
<td>Acquired autoantibodies against ADAMTS-13</td>
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</tbody>
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<tr>
<th>Infectious-TMA</th>
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<tbody>
<tr>
<td>STEC-HUS caused by Shiga-toxin or Shiga-like toxins such as in E. Coli or Shigella dysenteriae type-1</td>
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<tr>
<td>Pneumococcal-HUS</td>
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<tr>
<td>Human immunodeficiency virus (HIV)-associated TMA</td>
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<tr>
<td>Influenza-TMA</td>
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<td>TMA following other infections</td>
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<tr>
<th>Atypical HUS</th>
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<tr>
<td>Complement-mediated TMA</td>
</tr>
<tr>
<td>Genetic abnormalities in complement regulatory proteins or complement effector proteins</td>
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<tr>
<td>Acquired autoantibodies against complement regulatory proteins</td>
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<tr>
<td>Broad term for all other causes than TTP or STEC-HUS</td>
</tr>
</tbody>
</table>

| DGKE-TMA: caused by mutations in diacylglycerol kinase epsilon (DGKE) |

| CblC deficiency associated TMA: caused by a functional deficiency in cobalamin C (CblC) |

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<tr>
<th>Secondary TMA: TMA secondary to non-infectious clinical conditions</th>
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<tr>
<td>Solid organ transplantation</td>
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<td>Hematopoietic stem cell transplantation</td>
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<td>Post-radiation</td>
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<td>Post-surgery</td>
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<tr>
<td>Drug-induced</td>
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<tr>
<td>Chemotherapy (mitomycin, cisplatin, bleomycin, and gemcitabine)</td>
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<tr>
<td>Immunosuppressive drugs (cyclosporine, tacrolimus, OKT3, IFN, and quinidine)</td>
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<tr>
<td>Antiplatelet agents (ticlopidine and clopidogrel)</td>
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<tr>
<td>Anti-VEGF therapy (bevacizumab, sunitinib)</td>
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<td>Pregnancy</td>
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<tr>
<td>Preeclampsia/HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets)</td>
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<td>Other TMA following pregnancy</td>
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<td>Malignancy</td>
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<td>Pancreatitis</td>
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<td>Severe and malignant hypertension</td>
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<td>Glomerular diseases</td>
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<td>IgA nephropathy</td>
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<td>Membranous nephropathy</td>
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<td>C3 glomerulopathy/ Membranoproliferative glomerulonephritis (MPGN)</td>
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<tr>
<td>Focal segmental glomerulosclerosis (FSGS)</td>
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<tr>
<td>Diabetic nephropathy</td>
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<tr>
<td>Systemic autoimmune diseases</td>
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<tr>
<td>Systemic lupus erythematosus</td>
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<tr>
<td>Antiphospholipid syndrome</td>
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<tr>
<td>Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis</td>
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<tr>
<td>Systemic sclerosis</td>
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Advancements in the understanding of HUS and TTP

In 1966, Amorsi and Ultmann published a case-series of 16 patients with TTP and reviewed the literature of 255 previously described cases. They concluded that TTP had a dramatically high mortality rate of 90% in the era before effective therapy. Moreover, they established the diagnostic criteria for TTP consisting of the ‘pentad’ of hemolytic anemia, thrombocytopenia, renal abnormalities, neurological abnormalities, and fever. Given the overlap with the diagnostic triad of criteria for HUS, the classification of patients with TMA became based on clinical features: TTP for patients with predominantly neurological manifestations, and HUS for patients with predominantly renal manifestations. However, this distinction was complicated by the significant overlap in clinical features: both TTP and HUS could have severe neurological, renal, or multi-organ involvement, both could present in children and adults, and there were case-reports of patients with recurrent episodes that had alternating phenotypes of HUS and TTP, as well as case-reports of family members in which some individuals had HUS and others had TTP. 

For more than 40 years since the von Gasser publication, controversy remained as to whether HUS and TTP were separate disease entities or different clinical expressions of the same disease. As the clinical and histopathological findings of both syndromes were often indistinguishable and the underlying pathophysiology was largely unknown, in 1987, Remuzzi introduced the term ‘HUS/TTP’ to describe patients with thrombocytopenia, microangiopathic hemolytic anemia, and renal injury. Others suggested that the term TMA could be used to encompass the spectrum of TTP and HUS, or that TMA could be appropriate for cases that could not unequivocally be considered as having either TTP or HUS.

Subsequent discoveries were useful for untangling the HUS/TTP knot. In the field of TTP, major discoveries include the description of familial TTP in 1975 and the association between TTP and large von Willebrand factor (vWF) multimers leading to platelet agglutination. Plasma-exchange therapy was found to be effective for patients with TTP, reducing mortality from 90% to approximately 25%. The efficacy of plasma-exchange therapy for patients meant that more inclusive diagnostic criteria were required to allow rapid initiation of treatment: in the clinical trial that demonstrated the efficacy of plasma-exchange therapy in patients with TTP, only microangiopathic hemolytic anemia and thrombocytopenia, without an apparent alternative cause, were required for the
diagnosis of TTP. In 1997, two siblings with TTP were described who were deficient in a protease that cleaves vWF. In 2001, this protease was identified as ADAMTS-13, short for *A Disintegrin-like And Metalloprotease with ThromboSpondin type 1 motif, member 13*, and mutations in ADAMTS-13 were discovered in families with TTP. These studies supported the hypothesis that patients with TTP are deficient in ADAMTS-13, preventing adequate cleavage of vWF and leading to ultra large vWF multimers and increased platelet aggregation.

In the field of HUS, the distinction between the most common form (termed typical HUS), and other variants (termed atypical HUS) became relevant. After the introduction of HUS, diarrhea became recognized as a prodromal feature, and this diarrhea-associated HUS (D+ HUS or typical HUS) was associated with epidemics of *Escherichia coli* (E. coli) infection. Following the discovery of verocytotoxigenic E. coli (VTEC) strains, also known as enterohemorrhagic E. coli (EHEC), Karmali et al. showed in 1983 that D+HUS could be caused by the Shiga-like verotoxins produced by these strains. Subsequently, it was recognized that most cases of D+ HUS were caused by infection with strains of Shiga toxin-producing E. Coli, and the term STEC-HUS became common.

In contrast, it was observed that a minority of HUS patients had an atypical, diarrhea-negative course, for whom the term ‘D- HUS’ or ‘atypical HUS’ was used. Atypical HUS was often described in families and patients with atypical HUS were found to have evidence of complement activation as determined by serological testing and deposition of complement proteins in renal biopsies. Subsequently, loss-of-function mutations were found in genes encoding important regulators of the complement system, such as factor H, factor I, and MCP; gain-of-function mutations were found in genes encoding effector proteins factor B, and C3; and auto-antibodies were discovered that lead to a functional deficiency of factor H. In 2013, the efficacy of terminal complement inhibitor eculizumab was demonstrated in patients with atypical HUS. The criteria for atypical HUS in the clinical trials of eculizumab were designed to exclude patients with TTP and patients with STEC-HUS: evidence of hemolysis (e.g., lactate dehydrogenase level at or above the upper limit of the normal range, haptoglobin level below the lower limit of the normal range, or the presence of schistocytes) and impaired renal function (creatinine level at or above the upper limit of the normal range), without plasma ADAMTS13 activity below 5% or STEC; identification of
complement gene mutations or factor H autoantibodies was not required.\textsuperscript{136} At least half of the patients with atypical HUS are now known to have an inherited or acquired abnormality in the complement system, causing endothelial injury.\textsuperscript{137} The availability and efficacy of complement-inhibiting therapy in patients with atypical HUS, including those with and without proven mutations in complement regulatory genes, has led to a significant reduction in morbidity and mortality in these patients.\textsuperscript{138, 139}

\textit{Morphologic TMA}

TMA is also used as a morphologic term for disorders of structure and function with microvascular lesions that reflect endothelial cell injury (such as microthrombi) but lack the microvascular inflammatory cell infiltrate that defines vasculitis (morphologic TMA).\textsuperscript{65, 140, 141} Morphologic TMA is a histopathological diagnosis for a local pattern of lesions and can occur in a wide range of clinical settings.\textsuperscript{140} As was reviewed previously,\textsuperscript{65, 137, 140-142} morphologic TMA in the kidney may manifest with acute and chronic lesions (Table 2). These lesions may result from extrinsic causes, such as Shiga-like toxins and drugs, or from intrinsic causes that lead to endothelial dysfunction and injury (Table 1), but often the underlying cause is unknown.\textsuperscript{143} Typically, a patient with systemic TMA, such as atypical HUS, has morphologic TMA on the renal biopsy. However, morphologic TMA can also be observed in the biopsies of patients who lack thrombocytopenia and/or microangiopathic hemolytic anemia but who presented with other symptoms for which a renal biopsy was performed (local TMA).\textsuperscript{65, 140, 141, 144-147} In these cases, TMA in the renal biopsy may, for example, ‘unmask’ patients with atypical HUS caused by complement dysregulation, or demonstrate TMA in the setting of drug toxicity. Unfortunately, the precise etiology of the underlying disease cannot be distinguished by renal biopsy alone.\textsuperscript{140} When TMA persists, chronic lesions become predominant and there may be a paucity or even absence of microthrombi.\textsuperscript{140} Recently, it was suggested to replace the term ‘\textit{TMA}’ with ‘\textit{microangiopathy with or without thrombosis}’, to account for biopsies with microangiopathic lesions in the absence of microthrombi.\textsuperscript{137} Moreover, microangiopathic lesions consistent with morphologic TMA may co-exist with other kidney diseases, or overlap with lesions seen in other disorders, such as a duplicated glomerular basement membrane in the setting of transplant glomerulopathy, or glomerular endotheliosis in the setting of preeclampsia, complicating histopathological diagnosis.\textsuperscript{140, 146, 148, 149}
Contemporary view on TTP, HUS, and TMA

Currently, the term ‘TTP’ is used to diagnose patients with severe ADAMTS-13 deficiency, although it is recognized that a number of patients meet the clinical criteria for TTP but only have mildly reduced ADAMTS-13 activity levels. Although the term ‘HUS’ is still typically used to describe STEC-HUS, it is also used for all patients with microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury, and can be classified according to primary and secondary causes. Atypical HUS is used by some to describe patients with a primary defect in the complement system (also described as complement-mediated TMA or complement-mediated HUS) and by others to roughly describe all other forms of TMA than TTP or STEC-HUS, including patients with mutations in diacylglycerol kinase epsilon (DGKE), defective cobalamin C metabolism, and TMA secondary to various diseases and therapies that cause endothelial cell injury or dysfunction (Table 1).

As TTP has connotations with beneficial effects of plasma exchange, HUS with an infectious component that is treated with conservative...
therapy, and atypical HUS with mutations in genes affecting complement proteins, for which complement inhibition may be beneficial, some prefer to use the more value-neutral term TMA in all instances. Consequentially, this may lead to miscomprehension as TMA is used as a term for syndromes that are characterized by thrombocytopenia, microangiopathic hemolytic anemia, and evidence of end-organ injury (systemic TMA), for a histopathological pattern of lesions (morphologic TMA) that can also be observed in the absence of thrombocytopenia or microangiopathic hemolytic anemia (local TMA), and as an all-embracing term for disorders that can present with evidence of severe endothelial injury and microvascular thrombosis (the thrombotic microangiopathies; TMAs). As was reviewed recently, case reports and small studies suggest that complement activation is an important contributing factor for many etiologies of TMA, but the pathophysiological mechanisms that lead to microangiopathic lesions are incompletely understood and the exact role of complement in this process remains to be determined.

**Antibody-mediated rejection and transplant glomerulopathy**

Kidney transplantation is the treatment of choice for patients with end-stage renal disease because it has superior outcomes in terms of morbidity, mortality, and quality of life, in comparison to dialysis. As was reviewed previously, the renal microvasculature is the primary target of several acute and chronic immunologic processes directed against the transplanted kidney, causing allograft rejection. Antibody-mediated rejection is a distinct form of allograft rejection in which donor-specific antibodies (DSAs) from the recipient are directed against the antigens of the donor kidney. The DSAs are most commonly directed towards class I or class II human leukocyte antigens (HLAs) that are expressed in the endothelial cells of the renal microvasculature. They cause endothelial injury by activating endothelial cells directly, recruiting inflammatory cells, and activating the complement system. Complement activation can cause endothelial cell injury via various mechanisms that were reviewed previously. For example, the Fc regions of IgM and IgG DSAs can bind to C1q, activating the classical complement pathway and resulting in endothelial injury. In addition, anaphylatoxins C3a and C5a cause inflammation, and C5b–9 can lead to apoptosis, activation, and lysis of endothelial cells, as well as activation of T cells. Moreover, the transplantation process can cause ischemia-reperfusion
injury, which promotes local activation of complement via lectin and alternative pathways. Endothelial cell activation leads to increased expression of adhesion molecules, inflammatory cell recruitment, and a pro-coagulative state, causing further allograft injury. Complement split product C4d forms after C4 cleavage and can attach covalently to endothelial cells and basement membranes. This property makes C4d a stable, target-bound biomarker that can reveal complement activation even though the initiating factors or the subsequent complement proteins have gone into solution. Although several studies have shown that a proportion of patients with antibody-mediated rejection are C4d-negative, the presence of C4d deposition in the peritubular capillaries is both sensitive and specific for antibody-mediated rejection, and is part of the diagnostic criteria for active antibody-mediated rejection.

Over the past three decades, there has been an enormous improvement in renal allograft survival in the first year after transplantation. In contrast, the rate of long-term renal allograft loss has remained almost unchanged. Improving long-term allograft survival is one of the major unmet needs in renal transplantation, but progress is limited by an incomplete understanding of the causes of long-term allograft loss. Chronic active antibody-mediated rejection is an important cause of long-term allograft failure. It is thought to result from undetectable, low-titer DSAs or de-novo antibodies generated after transplantation, which can bind to microvascular endothelial surfaces and activate the complement and coagulation cascades. According to the most recent Banff criteria, chronic active antibody-mediated rejection is diagnosed by identifying evidence of DSAs, interaction of the antibody with vascular endothelium (such as C4d positivity), and morphologic evidence of chronic tissue injury (such as peritubular capillary basement membrane multilayering, arterial intimal fibrosis, and transplant glomerulopathy). Transplant glomerulopathy is a morphologic description of glomerular basement membrane duplication (‘tram tracking’) by light or electron microscopy, that is associated with poor graft survival. The clinical course of patients with transplant glomerulopathy is often insidious, with progressive, unexplained loss of renal function, minor proteinuria, and mild hypertension, although nephrotic range proteinuria has also been documented. In addition to glomerular basement membrane duplication, renal biopsies may show mesangial matrix expansion, mesangial hypercellularity, and glomerulitis. Ultrastructural analysis
shows circumferential multilayering of the glomerular basement membrane, which is frequently accompanied by multilayering of the basement membrane of peritubular capillaries.\textsuperscript{173} Glomerular capillaries also show subendothelial widening with mesangial cell interposition, and podocyte foot process effacement may be seen in patients with proteinuria.\textsuperscript{173}

Transplant glomerulopathy is considered a morphologic manifestation of chronic antibody-mediated rejection; it is associated with DSAs, most notably against HLA class II antigens, prior episodes of antibody-mediated rejection, glomerulitis, and C4d deposition in peritubular capillaries.\textsuperscript{173, 174} However, transplant glomerulopathy is not pathognomonic for chronic antibody-mediated rejection; other etiologies include hepatitis C virus infection, TMA, and T cell-mediated rejection.\textsuperscript{149, 173-175} In addition to C4d staining in peritubular capillaries, transplant glomerulopathy is also associated with C4d deposition in glomerular capillaries.\textsuperscript{176, 177} This glomerular staining pattern is frequently considered a sign of chronic antibody-mediated rejection, but the significance of glomerular C4d deposition in transplant glomerulopathy is unclear, particularly in absence of other signs of antibody-mediated rejection, such as concomitant C4d in peritubular capillaries.

**Preeclampsia**

Preeclampsia is a pregnancy-specific microangiopathy, complicating approximately 5\% of all pregnancies.\textsuperscript{178, 179} It is characterized by hypertension and proteinuria occurring after 20 weeks of gestation.\textsuperscript{179} A more recent definition broadened the diagnostic criteria: preeclampsia can now also be diagnosed in the absence of proteinuria if hypertension occurs after 20 weeks together with any new onset maternal organ dysfunction (including renal insufficiency, impaired liver function, neurologic complications, or hematological complications), or uteroplacental dysfunction as evidenced by fetal growth restriction.\textsuperscript{180} Severe preeclampsia may progress into eclampsia which is characterized by the development of tonic-clonic seizures, or present as HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), a life-threatening variant of preeclampsia.\textsuperscript{180} The current treatment is supportive with the aim of reducing blood pressure levels, preventing the progression of systemic disease, and prolonging the pregnancy in order to maximize fetal development.\textsuperscript{180} There is no cure other than the delivery of the placenta, which causes significant
fetal morbidity and mortality, depending on the gestational age. As a result, preeclampsia remains one of the leading causes of maternal and neonatal mortality in the world.\textsuperscript{179}

The lesions observed in the renal biopsy or autopsy samples of patients with preeclampsia have been reviewed previously.\textsuperscript{146} Light microscopic examination of the kidneys from women with preeclampsia can reveal ‘bloodless glomeruli’, in which swelling of the glomerular endothelial cells (glomerular endotheliosis) causes the occlusion of the glomerular capillaries. Glomerular volume is typically increased, but glomerular hypercellularity or arteriolar involvement is unusual. In severe cases, microthrombi and features of chronic TMA and transplant glomerulopathy can also be observed. In some cases, it is impossible to distinguish preeclampsia from other TMAs.\textsuperscript{146, 152}

The pathogenesis of preeclampsia is incompletely understood but is considered to be multifactorial.\textsuperscript{179, 181} The prevailing hypothesis is that abnormal placentation causes placental hypoxia, triggering the release of placenta-derived circulating factors, which leads to an imbalance between pro-angiogenic factors (such as vascular endothelial growth factor (VEGF) and placental growth factor), and anti-angiogenic factors (such as soluble Fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin).\textsuperscript{57, 178, 179, 181} For example, sFlt-1 can bind to VEGF, leading to a reduced biological availability for VEGF-receptor signaling. This relative deficiency in VEGF can then lead to endothelial dysfunction, endothelial cell injury, and the disruption of the glomerular filtration barrier, reflected by proteinuria and hypertension. Several findings support this hypothesis. For example, serum levels of sFlt-1 are elevated in women with preeclampsia,\textsuperscript{182} and animal models with increased expression of sFlt-1 develop a clinical and morphological phenotype that resembles preeclampsia.\textsuperscript{183, 184} Moreover, patients who receive anti-VEGF drugs for the treatment of malignancies may develop morphologic TMA and a preeclampsia-like phenotype.\textsuperscript{185} Preeclampsia can be distinguished in two stages: placental preeclampsia, caused by placental dysfunction in the first half of pregnancy, and maternal preeclampsia, in which an exaggerated maternal inflammatory response can cause the clinical phenotype of preeclampsia in the second half of pregnancy.\textsuperscript{178, 181} The inflammatory response is amplified in women with other risk factors for endothelial dysfunction such as hypertension, obesity, and diabetes.\textsuperscript{186} Recently, genetic defects in complement regulation were found to predispose to the development of preeclampsia and
our group has shown that patients with preeclampsia have evidence of classical pathway activation in the placenta. The data suggest that abnormal complement activation in the placenta or the kidney could be involved in the pathogenesis of preeclampsia, but it remains to be determined whether this process takes place in the kidney; the molecular mechanisms of complement activation in preeclampsia have not yet been identified.

**Diabetic nephropathy**

Diabetes mellitus is a pandemic disease that affects more than 400 million people worldwide. It is characterized by hyperglycemia, resulting from defective insulin secretion, insufficient insulin action, or both. The two main forms of diabetes are type 1 and type 2, with type 2 diabetes accounting for more than 85% of the total diabetes prevalence. Type 1 diabetes is caused by an autoimmune destruction of the insulin-producing β-cells of the pancreas; type 2 diabetes results from a combination of insulin resistance and a progressive loss of insulin secretion. Prolonged hyperglycemia causes macrovascular and microvascular injury and is associated with complications in various organs, including the eyes, nerves, kidneys, and the heart. Diabetic nephropathy is the renal microvascular complication of diabetes mellitus type 1 and type 2. Currently, it is the leading cause of end-stage renal disease in high-income countries. The clinical diagnosis of diabetic nephropathy is based on persisting microalbuminuria or macroalbuminuria, or an estimated glomerular filtration rate ≤ 60 mL / 1.73 m², along with clinical features, such as diabetes duration and the presence of diabetic retinopathy. Renal biopsy is the gold standard for the diagnosis of diabetic nephropathy, but the majority of diabetic patients with renal involvement are not biopsied because it is an invasive procedure with limited benefits in an otherwise uncomplicated patient. Nevertheless, the renal biopsy can be useful to discern diabetic nephropathy from non-diabetic kidney disease, or a superimposed non-diabetic condition on underlying diabetic nephropathy. Moreover, the renal biopsy can be used to classify lesions by various degrees of severity, guiding therapeutic management and outcome prediction. Diabetic nephropathy is characterized by mesangial matrix expansion, which may be nodular (Kimmelstiel-Wilson nodules) and hyaline arteriolosclerosis in afferent and efferent arterioles by light microscopy, as well as thickening of the glomerular basement membrane by electron microscopy.
Other light microscopical lesions include glomerular hypertrophy, segmental or global glomerulosclerosis, mesangial cell proliferation, mesangiolysis, capillary microaneurysms, hyaline deposits in Bowman’s capsule (capsular drop), interstitial fibrosis, tubular atrophy, and arteriolar intimal sclerosis. By electron microscopy, other ultrastructural lesions include podocyte loss, foot process effacement, glomerular fibrillar extracellular matrix deposition, tubular basement membrane thickening, and subendothelial or transmural hyaline deposits in the small arteries and arterioles.  

Despite the high global burden of disease, 60-70% of patients with diabetes mellitus type 1 or 2 do not develop diabetic nephropathy and only a proportion of the patients with diabetic nephropathy develop advanced stages of glomerular or arteriolar injury. Risk factors for diabetic nephropathy include susceptibility factors (such as age, gender, ethnicity, and genetic predisposition), initiation factors (such as hyperglycemia and acute kidney injury) and progression factors (such as hypertension and dietary intake).  

As was reviewed previously, different molecular processes may cause endothelial dysfunction or injury in diabetic nephropathy, including the accumulation of reactive oxygen species and advanced glycation end products, increased flux through the polyol and hexosamine pathways, and activation of protein kinase C, with downstream effects on various proteins, growth factors, and inflammatory mediators. Two recent reviews highlighted that complement activation may also be involved in the macrovascular and microvascular complications of diabetes mellitus, and in the development of diabetic nephropathy specifically. For example, hyperglycemia can induce the glycation of complement regulatory proteins, leading to a dysfunction of their regulatory capacity and complement-mediated injury. Moreover, diabetes-induced alterations in glycoproteins may stimulate complement activation through the binding of MBL to neo-epitopes. Gene expression analysis of microdissected human renal glomeruli and tubule samples showed that various complement regulators and proteins of the classical pathway were upregulated in patients with diabetic nephropathy. However, complement deposition along the renal microvasculature of patients with diabetic nephropathy has not yet been thoroughly characterized and the involvement of complement activation in the development or progression of diabetic nephropathy is incompletely understood.
Aims and outline of this thesis

This thesis is focused on the clinicopathologic significance of complement deposits along the renal microvasculature of patients with renal microangiopathies. Special attention is paid to C4d, a cleavage product of C4 activation, which remains covalently bound to the surrounding tissue long after the complement-pathway initiating factors have dissociated. C4d is used as a biomarker for complement activation worldwide.168

The specific aims of this thesis are:

• to determine the prevalence, localization, distribution, and staining pattern of complement deposits in kidneys from patients with morphologic TMA, in the setting of various underlying clinical conditions;
• to determine the clinicopathologic significance of microangiopathy with or without thrombosis and complement deposits in the kidneys of patients with IgA nephropathy and IgA vasculitis with nephritis;
• to determine the relationship between glomerular C4d deposits and glomerular basement membrane remodelling in native and transplanted kidneys;
• to determine the clinicopathologic significance of complement activation in glomeruli of patients with preeclampsia; and
• to determine the clinicopathologic significance of complement deposits in kidneys of patients with diabetic nephropathy.
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