Renal heparan sulfate proteoglycans
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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General Introduction
Inflammation in chronic kidney dysfunction

Despite advances in treatment and better knowledge of renal pathophysiology, the prevalence of chronic kidney disease (CKD) continues to increase, with recent data indicating a rise in CKD prevalence of 19.6% in the past decade (1). Patients suffering from CKD can progress to end-stage renal disease (ESRD), requiring renal replacement therapy. Moreover CKD patients have a higher risk of death from cardiovascular disease compared to individuals without CKD (2). CKD has a variety of pathophysiological causes, but most of these causes are incurable at this moment. Therefore treatment of CKD consist predominantly of preventing complications and slowing disease progression, giving rise to the need for new treatment strategies to treat the underlying cause of CKD. On a worldwide basis, diabetic kidney disease is the leading cause of CKD and ESRD, and prevalence of diabetic kidney disease keeps increasing (3,4). Other important causes of CKD are inflammatory diseases of the kidney, especially different forms of glomerulonephritis such as IgA nephropathy, glomerulonephritis related to other (auto-)immune mediated diseases such as systemic lupus erythematosus (SLE) or small-vessel vasculitis or tubulo-interstitial diseases. Also loss of graft function following renal transplantation caused by procedure related damage, medication and immunological processes (rejection) attributes significantly to the incidence of CKD. Importantly, in all these widely different circumstances leading to renal function loss, an inflammatory component with complement activation and/or cellular infiltrates is a major factor in the pathophysiology. In some the inflammatory component is the initiating event, while in most the inflammation is seen as a progressive factor.

The immune system is characterized by a humoral and cellular branch which can both be divided in an innate branch and an acquired branch. Innate immunity is seen as the first line of defense and is largely characterized by the complement system (humoral) and by neutrophils and antigen presenting cells like macrophages and dendritic cells (cellular). Acquired immunity is characterized by antibody producing B-cells (humoral) and antigen-specific T-cells (cellular). In general it can be stated that the innate immunity is the first line of defense and is quick and powerful, however, rather non-specific. The innate immune system also functions as the initiator for the acquired immune system, presenting antigens to T-and B-cells, which subsequently form a specific response to these antigens. This thesis focusses mostly on the initiation of the inflammatory response in several experimental renal diseases. The initial inflammatory stimulus varies among renal diseases, however two processes are critical for the development of an innate immune response i.e. complement activation and leukocyte infiltration.
Complement in renal diseases

There are 3 known complement pathways i.e. the classical (CP), lectin (LP) and alternative pathway (AP) with distinct pathogen/damage recognition processes, but all leading to the cleavage of C3 into C3a and C3b eventually followed by cleavage of C5 and formation of the membrane attack complex (C5b-9). Complement has been identified as an important instigator of the inflammatory response in several renal diseases. The CP has been shown to contribute to renal injury in various renal diseases. These include predominantly antibody mediated diseases like lupus nephritis, anti-GBM glomerulonephritis and membranoproliferative glomerulonephritis (5-7). The LP of complement has been shown to be involved in a number of renal diseases like diabetic nephropathy, ischemia/reperfusion (I/R), transplantation and IgA nephropathy. Although the role of the LP in these diseases has in most cases not been causally established, mice deficient in MBL have been shown to be partially protected from I/R mediated damage, which could be reversed by administering recombinant MBL (8). The same MBL deficient model was used in an experimental model for diabetes and the results showed that MBL deficient mice were partially protected from renal damage due to hyperglycemia (9). A role for the AP of complement has been causally established in I/R injury, as factor B deficient mice were partially protected against functional and morphological renal injury in an experimental renal I/R model. Interestingly factor B deficient mice also showed a reduced influx of neutrophils in the outer medulla after I/R (10). Moreover the AP has been identified as a major player in the initiation of proteinuria mediated tubular epithelial damage. Amongst others, our group has shown that AP factors bind to tubular epithelium and activate the AP, resulting in tubular atrophy (11,12). These findings indicate that complement can be considered a therapeutic target in renal diseases. Complement inhibitors are on the market for some time now, but they have only been implemented as treatment in some rare renal diseases. Already for some years eculizumab, a monoclonal C5 inhibitor, is registered as treatment for atypical hemolytic uremic syndrome and paroxysmal nocturnal hemoglobinuria. Furthermore, some data suggest efficacy of eculizumab treatment in some types of (complement-related) membranoproliferative glomerulonephritis and humoral transplant rejection. However treatment with eculizumab is very expensive and therefore not available to all patients. Attempts to develop other C5 inhibitors have been undertaken with variable results (13). C5 inhibitors inhibit the terminal phase of all pathways of complement. However, as discussed before, specific complement pathways have been linked to specific renal diseases, opening the opportunity for the development of pathway specific complement inhibitors.
Table 1, Complement pathway involvement in renal diseases

<table>
<thead>
<tr>
<th>Classical pathway</th>
<th>Lectin pathway</th>
<th>Alternative pathway</th>
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<tbody>
<tr>
<td>Transplantation (14)</td>
<td>Transplantation (15)</td>
<td>Transplantation (16)</td>
</tr>
<tr>
<td>Lupus nephritis (5)</td>
<td>Ischemia reperfusion (8)</td>
<td>Ischemia reperfusion (16)</td>
</tr>
<tr>
<td>Anti-GBM disease (7)</td>
<td>IgA nephropathy (17)</td>
<td>IgA nephropathy (18)</td>
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<tr>
<td>Diabetic nephropathy (19)</td>
<td>Membranous nephropathy (21)</td>
<td>C3 glomerulopathy (20)</td>
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<tr>
<td>Henoch-Schönlein purpura nephritis (23)</td>
<td>Hemolytic uremic syndrome (22)</td>
<td>Dense deposit disease (24)</td>
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<td></td>
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<td>ANCA-associated vasculitis (25)</td>
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<td></td>
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<td>Membranoproliferative glomerulonephritis (20)</td>
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<td>Henoch-Schönlein purpura nephritis (23)</td>
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Cellular infiltrates in renal disease

The role of leukocyte migration in renal disease has been extensively studied. It has for example been shown that macrophage and T-cell migration from the vasculature to the mesangial and interstitial areas is crucial in the development of diabetic nephropathy (26,27). These same cell types have been shown to infiltrate the renal interstitium in chronic transplant dysfunction (28). In an I/R model, neutrophils and macrophages have been shown to rapidly infiltrate the interstitium, but also CD4+ and CD8+ T-cells and B-cells have been shown to infiltrate under these acute inflammatory conditions (29). These studies clearly demonstrated that leukocyte infiltration is an important mediator of immunological mechanisms in (progressive) renal disease and therefore attempts have been undertaken to target leukocyte infiltration. MCP-1 knockout studies have shown beneficial effects in multiple models including in a model for lupus nephritis. MCP-1 deficient mice showed reduced macrophage and T-cell infiltrates in the kidney, reduced proteinuria and renal damage (30). Moreover, in an experimental rat model for I/R, treatment with a interleukin-1 receptor antagonist resulted in reduced cellular influx and reduced I/R mediated damage (31). In an experimental model of chronic renal allograft dysfunction, inhibition of leukocyte transmigration has shown to be beneficial for allograft survival (32). These studies demonstrate the potential for inflammation based therapies in renal diseases and show that cellular infiltration can be targeted via different routes like inhibiting selectins, inhibiting chemokine release, chemokine receptor antagonists or disruption of the chemokine gradient.
Leukocytes: from the vasculature to the lymphatic system

Inflammation is the influx of leukocytes from the microvasculature into the interstitial space. Whether or not leukocytes will start the process of transmigration is determined by the activation of endothelial cells, which in the kidney can be activated by cytokine producing resident macrophages, tubular epithelial cells and other cell in the interstitium. Activated endothelium starts expressing E- and P-selectin which can interact with glycoprotein ligands on the cellular membrane of leukocytes. These ligands on leukocytes are sialylated fucosylated carbohydrate residues of the Lewis x blood group family attached to membrane proteins (33). Endothelial cells also upregulate ligands for L-selectin, a membrane bound selectin on leukocytes. The interaction between selectins and their ligands induces leukocyte rolling on the endothelium and reduces their velocity. Besides ligands like E- and P-selectin, endothelial cells also start expressing chemokines. These chemokines are presented to high affinity chemokine receptors on leukocytes, resulting in activation and/or expression of integrins (34). Integrins on the surface of leukocytes bind to intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) expressed by the activated endothelium, facilitating the firm adhesion of the leukocyte and starting the transmigration process (35). Once the leukocyte has entered the interstitial space, a chemokine gradients produced by, amongst others, resident macrophages and epithelial cells, guides the leukocyte to the site of inflammation.

Upon entering the inflammatory area, leukocytes mature and have multiple functions. For example macrophages produce chemokines and cytokines to recruit additional leukocytes and activate other cells like fibroblasts and pericytes to become myofibroblasts, which are involved in renal fibrosis. However, macrophages can also phagocytose apoptotic cells, bacteria and debris by a process called phagocytosis. Upon stimulation with pro-inflammatory cytokines such as TNF-α and/or IL-1β, macrophages can change from their phagocytic phenotype to an antigen presenting phenotype (36,37). This phenotypic shift causes a change in the expression of chemokine receptors. One of the receptors newly expressed is CCR7 which can be activated by CCL21 and CCL19, two chemokines expressed by the lymphatic endothelium (38). Migration of antigen presenting cells towards the lymphatic system occurs in the same fashion as migration towards the site of inflammation, via a chemotactic gradient of chemokines, amongst others CCL21 and CCL19 (39). Transmigration through the lymphatic endothelium again occurs via the binding of integrins to ICAM expressed by the lymphatic endothelial cell (40-42). Eventually antigen presenting cells migrate to lymph nodes where they interact with lymphocytes to stimulate the acquired immune response.
Chapter 1

Heparan sulfate proteoglycans in the inflammatory response

Heparan sulfate proteoglycans (HSPGs) are linear carbohydrates composed of repeating disaccharide units (glycosaminoglycans) attached to a protein core and can be found on cell surfaces and in basement membranes. Related to their negative charge, mainly due to carboxyl and sulfate groups within the disaccharide units, HSPGs can bind numerous cytokines, chemokines, growth factors and complement factors. Binding, however, is critically dependent on the special distribution and density of the sulfate groups along the glycosaminoglycan chain. HSPGs have been shown to act as a ligand for L-selectin, immobilize chemokines on the endothelial surface and present them to passing leukocytes and stabilize the chemotactic gradient in the interstitium towards the inflammatory site and the lymphatic system (43,44). Moreover, HSPGs have demonstrated to be of major importance in the chemokine presentation of chemokines to leukocytes migrating toward the lymphatic system (45). Besides that, HSPGs have shown to bind and present growth factors like TGF-β and FGF-2 and play a role in tissue fibrosis besides the inflammatory response (46,47).

It is known that HSPGs bind complement factors and thereby regulate complement activation on certain cells. Our group has shown that both an inhibitor and activator of the AP, factor H and properdin, respectively, can bind HSPGs on the tubular epithelium under proteinuric conditions and that binding of properdin to tubular epithelial HSPGs is important in the process of complement mediated tubular epithelial injury in proteinuria (11,12). Heparin, a highly sulfated glycosaminoglycan similar in backbone to heparan sulfates, has been shown to be able to bind numerous complement factors and is known for its complement inhibiting potential (48,49). These studies all in all demonstrate that HSPGs on the cellular membrane and in the basement membrane function as docking stations for chemokines, L-selectin, but also complement factors. Therefore HSPGs are pivotal in the processes of cellular infiltration and complement activation.

Aim of the thesis

Literature has shown that inflammation plays an important role in various renal diseases, of which many are to date incurable. Therefore inflammation is considered an important target in renal pathologies. Targeting inflammation in renal disease has shown promising results in experimental models and also in some clinical trials. Since inflammation is orchestrated by heparan sulfate proteoglycan expression on endothelial and tubular epithelial cells, the aim of this thesis is to further unravel the role of HSPGs in inflammation, with a focus on leukocyte recruitment and complement activation.
Outline of the thesis

In chapter 2, we reviewed the current state of knowledge regarding the role of glycans in tubulo-interstitial inflammation and pathology. We gave an elaborate introduction on the formation and function of glycans and discuss the role of glycans in renal complement activation, leukocyte recruitment and growth factor responses.

Experimental studies have shown that endothelial HSPGs play a role in leukocyte recruitment and HSPG deficiency leads to increased leukocyte rolling and decreased leukocyte transmigration. Therefore, in chapter 3, we used an endothelium specific Ndst1 knockout mouse in an experimental diabetes model to investigate the role of HSPGs in diabetic nephropathy and whether endothelial HSPGs can be considered a target for therapy in diabetes.

In chapter 4 we investigated the role of two basement membrane proteoglycan/collagen hybrids, namely collagen XV and XVIII, in leukocyte recruitment in a more acute model of inflammation. It was previously shown that L-selectin and MCP-1 binding after an inflammatory stimulus was predominantly located in the subendothelial region and facilitated by HSPGs. Since we could not discriminate between apical endothelial and subendothelial HSPGs in chapter 3, we performed the experiments in chapter 4 to shed light on the leukocyte recruiting function of subendothelial HSPGs.

Since we showed in chapter 3 and 4 that endothelial HSPGs can be a target for treatment in inflammatory renal diseases we performed an experiment, in chapter 5, in which we targeted the interaction between chemokines and HSPGs in an experimental renal transplantation setting. To target the HSPG-chemokine interaction we use heparin and two modified non-anticoagulant heparin derivatives, which we showed have a higher binding affinity for chemokines than native HSPGs.

In chapter 6, we reviewed the current knowledge on the interaction between complement and heparin/heparan sulfates. We extensively discussed the role of complement in renal diseases and the possibility of complement as a target for treatment in renal diseases.

Previous experimental work from our group has shown that properdin can bind to HSPGs on tubular epithelium under proteinuric conditions. However others showed that the binding of properdin to endothelial cells is dependent on initial C3b binding. Therefore we have investigated in chapter 7 whether the binding of properdin to tubular epithelial cells is dependent on initial C3b binding and whether the binding of properdin to tubular epithelial cells is syndecan-1 mediated. Moreover, we identified the molecular interactions of properdin with C3b, HSPG, and its inhibitor SALP20.

Since we have reviewed the possibility of heparin/heparan sulfates as inhibitors of complement in renal disease, we investigated in chapter 8 whether we can identify heparins/heparan sulfates that specifically inhibit preferentially
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one pathway of the complement system. We tested a library of heparin derivatives/heparan sulfates in the WieLISA complement assay. We also determined the inhibitory mechanism of heparin derivatives/heparan sulfates to the LP of complement.

In chapter 9, we summarized the findings of the experiments performed in this thesis, and discussed possible implications of targeting HSPG in renal inflammatory disease, both in leukocyte recruitment and complement activation. We also discussed the future perspectives of our work.
References


Chapter 1


