Multifaceted role of the complement system in health and disease: a focus on properdin
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Role of properdin in complement-mediated kidney diseases

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Abstract

As part of the innate immune system, the complement system is an important mechanism in our first line of defence, but it can also contribute to the onset of various diseases. In renal diseases, the dysregulation of the complement system is often caused by mutations in—and autoantibodies directed against—members of the complement system, and contributes to disease onset and severity. As the only known positive regulator of the complement system, the role of properdin in complement-mediated diseases is largely unknown. In this review, we provide an overview of the detection of properdin in kidney biopsies and urine, serum or plasma samples from patients with complement-mediated renal diseases, such as immune complex-mediated glomerulonephritis and C3 glomerulopathy. Advances towards a better understanding of the role of properdin in (local) complement activation will provide insight into its potential role and offer opportunities to improve diagnosis and therapeutic interventions.

Introduction

The complement system is part of the innate immune system and comprises three pathways: the classical, lectin and alternative pathway. Together, these pathways consist of over 30 soluble and membrane-bound proteins, which are not only important for our innate defence against microorganisms, but also for the opsonization and removal of apoptotic/necrotic cells and immune complexes (reviewed in (1)). Activation of either the classical, lectin or alternative pathway results in the generation of C3 convertases, which cleave C3 into C3a and C3b. The alternative pathway also acts as an amplification loop for the complement cascade. Together with the C3 convertase, C3b is able to form a C5 convertase, which cleaves C5 and thereby generates C5a, a strong anaphylatoxin, and C5b. The latter is involved in the formation of the membrane attack complex (also referred to as C5b-9), causing the lysis of cells (Figure 1). Complement activation is tightly regulated, thereby allowing complement activation on foreign cells and structures, while minimizing the deposition on viable cells. Disturbance of the regulation, for instance, due to mutations or autoantibodies, can result in uncontrolled complement activation, leading to injury, and can contribute to the onset of disease, including various kidney diseases (1). Properdin is the only known positive regulator of the complement system, and has been a subject of debate ever since its discovery. In this review, we will focus on the presence of properdin in kidney biopsies, urine and serum samples of patients with complement-mediated kidney diseases.

Properdin: function and sources

Properdin was discovered in 1954 and is composed of six to seven thrombospondin repeats. By its interaction with C3b and Bb, it stabilizes the C3 convertase and prolongs its half-life by 5–10 times (reviewed by Kemper *et al.* (2)). Via head-to-tail interactions of the monomeric subunits, dimeric, trimeric and tetrameric oligomers of properdin can be formed. It is thought that the higher oligomer states of properdin are more active. Although most complement proteins are produced by the liver, properdin is mainly produced by leucocytes. Neutrophils store properdin in their secondary granules and monocytes and macrophages are able to synthesize and secrete properdin. Concentrations of properdin in serum and plasma are relatively low (4–6 μg/mL) compared with systemic concentrations of most other complement components (2).

Properdin deficiency is an X-linked trait in which males are affected predominantly and that is often associated with meningococcal infections. Three types of deficiencies are known: Type 1 deficiency is characterized by the total absence of properdin. In Type 2 deficiency, properdin levels are reduced (1–10% of the normal levels) and Type 3 is classified by normal serum properdin levels, however, properdin is non-functional.

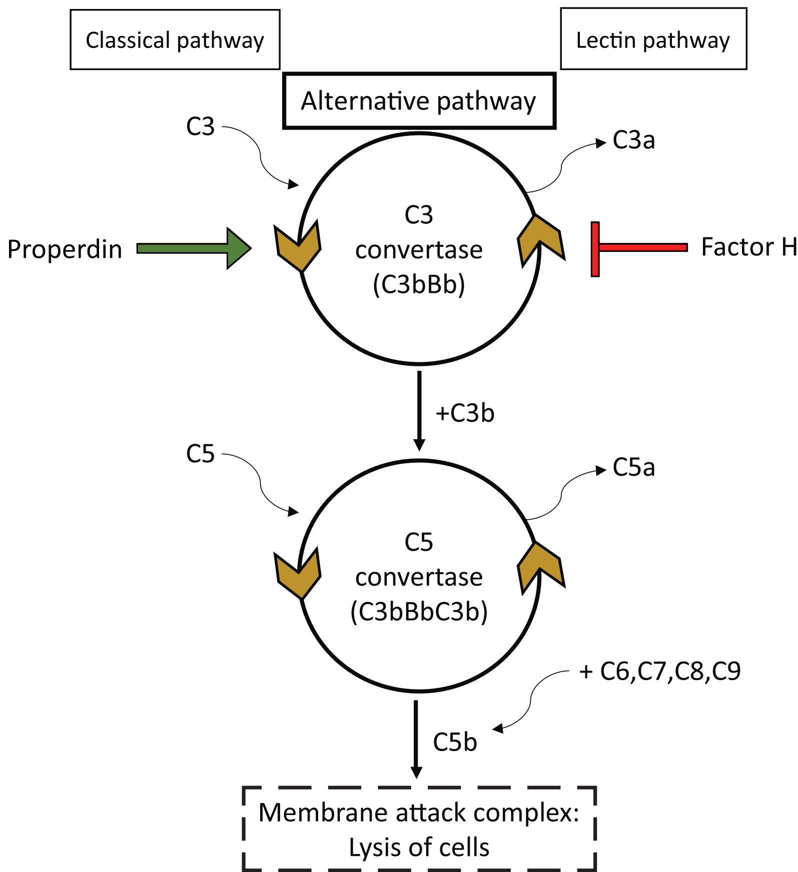

Figure 1: Schematic overview of the complement pathways and complement activation. Classical, lectin or alternative pathway activation results in the formation of C3 convertases. These convertases cleave C3 into C3a (anaphylatoxin) and C3b, involved in the formation of C5 convertases. C5 convertases cleave C5 into C5a, the anaphylatoxin, and C5b, needed for the generation of the membrane attack complex, resulting in lysis of cells. Properdin is able to bind to the C3 convertase, thereby stabilizing the half-life of the convertase. Factor H regulates complement activity by affecting the decay of the convertase.

Next to stabilizing the C3 convertase, several groups have shown a potential role for properdin as a pattern recognition molecule. It has been shown that properdin can bind to different structures independent of C3, including bacterial surfaces, apoptotic, necrotic and renal proximal tubular epithelial cells (3–5). In this configuration, properdin can subsequently bind and concentrate activated C3b, providing a platform for local generation of C3 convertases (2). However, the role of properdin as a pattern recognition molecule remains controversial, since it has been shown by others that properdin was only able to bind in a C3-dependent manner to structures like zymosan, *Escherichia*

coli, human umbilical vein endothelial cells (HUVECs) and *Neisseria meningitidis* (6). More detailed insight of the molecular structure of properdin and the specific interaction with different surfaces will be required to explain these discrepancies.

Complement in renal diseases

The role of the complement system in kidney diseases has been extensively studied. It has been shown that complement dysregulation and activation can contribute to the onset and/or progression of renal diseases, and that products of complement activation are mediators of glomerulonephritis (GN). For the diagnosis of various renal diseases, histological analysis of renal biopsies is of critical importance. In addition, immunofluorescent stainings are performed to detect deposition of complement factors including C1q, C3 and C4. Moreover, C4d deposition has become a determining diagnostic factor for antibody-mediated rejection of kidney allografts (reviewed by Thurman (7)).

Next to the place of complement in pathological analysis, serum measurements of complement haemolytic activity, or circulating levels of C3 and C4, can be helpful in determining whether patients are in disease remission or encounter flares. The measurement of complement in blood is non-invasive and can therefore be performed repetitively, allowing close monitoring in time. However, it should be realized that the levels detected in serum depend on both the production and consumption rate of the complement proteins (7).

In addition, mutations in complement regulators can result in an overactive complement system, such that C3 levels will be lowered. Genetic analyses are performed to determine whether mutations are present and if these are involved in the onset or progression of renal diseases (7). Although properdin has been recognized as an important stabilizer of the alternative pathway C3 convertase, and hence an important indicator of alternative pathway activation, there is relatively limited information on the analysis of properdin. In this present review, we focus on the presence and quantification of properdin in renal biopsies and body fluids.

Properdin: its presence in renal biopsies

Properdin was already detected in kidney biopsies from patients with various renal diseases in the 1970s. In the 1990s and more recently, properdin regained interest, again showing the detection of properdin in kidney biopsies. However, it should be realized that classifications and nomenclature (8), as well as the protocols and reagents used, has changed over time, and most studies relied on frozen sections and often only a semi-quantitative description of the staining profile was provided. With these limitations, we have tried to give an overview of properdin detection in various renal diseases (Table 1).

Table 1: Detection of properdin in kidney biopsies

Disease classification	Properdin positive / total patients	Location of properdin deposition in kidney biopsies	Reference
Immune-complex mediated GN			
Lupus Nephritis - SLE	Membranous, n = 2/2; focal proliferation, n = 1/1 Diffuse proliferation, n = 3/4	Glomerular: granular/globular	(9)
	Various classes, n = 11/17	Glomerular deposition	(10)
IgAN	$C4d^+$, n = 24/27 $C4d^-$, n = 57/69	Mesangial zone/tubular epithelium	(11)
	Group I, n = 9/16 (mild histologic abnormalities)	Glomerular mesangial deposition (distribution similar to IgA)	(12)
	Glomerular MBL/MASP-deposition, n = 6/11	Glomerular deposition	(13)
	N = 71	Glomerular deposition	(14)
	N = 14/15	Glomerular mesangial deposition	(15)
	N = 22/23	Glomerular mesangial deposition	(16)
Anti-GBM	N = 9	Glomerular capillary wall (n = 9/9) Mesangial regions (n = 3/9)	(17)
MPGN type I	N = 6/9	Glomerular deposition	(18)
IgA vasculitis (Henoch-Schönlein purpura)	N = 4/4	Mesangial deposition	(19)
Membranous nephropathy (MN)	Proteinuric renal disease, n = 3	Tubular brush border	(5)
	Idiopathic MN, n = 5	Subepithelial deposits (capillary wall)	(20)

Table 1: (Continued)

Disease classification	Properdin positive / total patients	Location of properdin deposition in kidney biopsies	Reference
C3 Glomerulopathy			
Monoclonal gammopathy and GN	Isolated C3 deposits and gammapathy	Intramembranous, subepithelial and mesangial deposits	(21)
CFHR2-CFHR5Hyb	*N* =1	Mesangial deposition	(22)
MPGN	*N* = 2/2	Glomerular mesangial deposition and in peripheral capillary walls	(23)
MPGN type II	*N* = 3/12	Glomerular deposition	(24)
DDD	*N*= 0/4	No depositions, C3 deposits observed in mesangium	(25)
Diverse			
Pauci-immune GN	MPO-ANCA^{+}, *n* = 7/7	Glomerular capillary wall/mesangial area	(26)
	PR3-AAV^{+}/MPO-AAV^{+}, focal, *n* = 12/43 ; Crescentic, *n* = 12/18; mixed, *n* = 5/14	Glomerular: speckled or granular/mesangial pattern	(27)
Mixed cohort MPGN/SLE/GN etc.	*N* = 21/92	Glomerular deposition	(28)
IRI	*N* = 0/5	No glomerular/cortical depositions	(29)
ESRD	GN, *n* = 15/16; familial and/or congenital, *n* = 17/22; miscellaneous, *n* = 26/32	Glomerular deposition	(30)
Hematuria: C4 deficiency	*N* = 1	Granular mesangial deposits	(31)

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Focusing on complement-related kidney diseases, there are different classifications of GN, depending on the presence or absence of autoantibodies and deposition of complement factors like C3 (reviewed by Salvadori and Rosso (8)).

Immune complex-mediated GN

Immune complex-mediated GN such as in systemic lupus erythematosus (SLE) is characterized by the deposition of immune complexes in the glomeruli. Lupus nephritis is characterized by a full-house immunofluorescence pattern, showing immunoglobulins (IgG, IgA and IgM), combined with C3 deposits (32). In patients diagnosed with SLE, properdin deposits were detected in the glomeruli (9, 10). Furthermore, it was observed that SLE patients with a positive properdin staining had an increased proteinuria (10).

Biopsies taken from patients with IgA nephropathy (IgAN) show focal mesangial proliferation and deposits of IgA, which are mostly associated with co-deposition of C3. In a subgroup of patients, biopsies show glomerular co-deposition of mannose-binding lectin (MBL) and C4d, indicating that complement activation can occur via the lectin pathway (33, 34). In addition, properdin has been detected in the glomerular mesangial area of IgAN patients (12–15). Mesangial C3 deposition was associated with mesangial deposits of properdin and C5b-9, indicating activation of the alternative pathway (11). Glomerular depositions of MBL-Associated Serine Protease (MASP)-1 in kidney biopsies from IgAN patients were associated with C3b/C3c and C5b-9, but there was no association with properdin and various immunoglobulins (13).

In anti-glomerular basement membrane (anti-GBM) GN, linear deposits of IgG are often detected along the GBM in combination with C3, resulting in a focal necrotizing GN with crescents (32). Properdin deposition in anti-GBM GN co-localized with both C5b-9 and C3d (17). Furthermore, properdin was deposited in kidneys of patients with IgA vasculitis (Henoch-Schönlein purpura) in the mesangium, where C3 and IgA were also deposited (19).

Patients diagnosed with idiopathic membranous nephropathy (iMN) are characterized by subepithelial deposits of IgG and complement C3, often in combination with severe proteinuria (32). Since these anti-phospholipase A2 receptor (PLA2R) antibodies are mostly IgG4, a subclass not able to activate complement, the mechanism of complement activation remains unknown (35). One patient with iMN showed an intense staining for C3, C5b-9, factor B and properdin, whereas C1q and C4d were present at low levels. Further analysis revealed MBL deficiency, suggesting that complement was mainly activated via the alternative pathway (20).

Three patients with membranous nephropathy with proteinuria showed strong positive properdin stainings on the tubular brush border of proximal tubular epithelial cells (5). *In vitro* experiments with human tubular epithelial cells confirmed the capacity of properdin to bind these cells, partially through interaction with syndecan-1 (5, 36).

C3 glomerulopathy

C3 glomerulopathy (C3G) is characterized by the dysregulation of the alternative pathway, with the detection of glomerular C3 deposits in the absence of Ig antibodies. C3G can be subdivided into dense deposit disease (DDD) and C3GN. DDD [formerly referred to as membranoproliferative GN (MPGN) Type II] is identified by the presence of electron-dense deposits in the GBM combined with intense C3 deposits located in the mesangium and GBM. In C3GN, C3 is often deposited at mesangial and subendothelial sites (32). Mutations in regulatory proteins or changes in factor B and C3 are responsible for the complement dysregulations. The expression of an aberrant complement factor H-related (CFHR) hybrid protein (CHFR2-CFHR5), originating from genetic alterations, stabilizes the C3 convertase, thereby augmenting the disease (8, 37). Furthermore, in certain DDD patients, autoantibodies directed against C3b and FB can be found, which enhance C3 and C5 cleavage (8). Anti-factor H (FH) antibodies are detected in ~10% of the C3G patients (38). In addition, C3 nephritic factors (NeFs) are found, able to stabilize the convertase in the absence or presence of properdin (8), which will be further addressed below. When properdin was detected in kidney biopsies of C3G patients, it was predominantly detected in the mesangial regions of the glomeruli (21, 23, 24).

In pauci-immune GN, anti-neutrophil cytoplasmic antibodies (ANCAs) against components of the neutrophil are formed, predominantly targeting myeloperoxidase (MPO) and proteinase 3 (PR3). This results in a focal necrotizing and crescentic GN, but limited deposits of immunoglobulins are found in the kidney (32). In a small-scale study utilizing mass spectrometry on kidney biopsies taken from ANCA patients, factor B and properdin were not detected (39). However, in other patients properdin was found deposited in the glomeruli and co-localized with C3d (26). These patients displayed more cellular crescents, more proteinuria and fewer histologically normal glomeruli (27).

In a mixed cohort of patients with various renal diseases, 21 out of 92 patients showed a positive immunofluorescent properdin staining in the glomeruli (28). Moreover, properdin was detectable in the glomeruli of kidney biopsies taken from almost all patients with end-stage renal disease (ESRD) (30). In five patients with early graft failure after transplantation, C3c, C3d and C4d were found to be deposited in the glomerular capillary walls. In contrast, other components (properdin, C1q, C4c, C3a, iC3b, factor

B and immunoglobulins IgG/IgA/IgM) were not detected in the glomeruli or in other cortical structures (29).

Altogether, the detection of complement deposits in kidney biopsies indicates activation of the complement system. Kidney biopsies from patients diagnosed with C3G, pauci-immune GN and IgAN show deposition of C3 and properdin, indicating that complement activation might occur via the alternative pathway.

Properdin detection in serum, plasma or urine

As already briefly addressed, serial measurements of complement factors like C3 and C4 in serum might potentially indicate whether patients are in disease remission or encounter flares (7); however, this remains to be further established. In Table 2, we focus on the detection of properdin in serum, plasma and urine. Over the past years the methods for measuring properdin in serum and plasma shifted from the solid-phase radioimmunoassay/radial immunodiffusion method to the use of enzyme-linked immunosorbent assays. These are more sensitive and less time-consuming, since more samples can be measured simultaneously. The detection of low levels of properdin in otherwise non-deficient patients might correlate with its consumption, implying complement alternative pathway activation (47).

Serum and plasma measurements

In patients with SLE, serum properdin levels were detected within the normal range (9, 42), or were slightly decreased (41). Patients with low C3 levels also had low properdin levels in plasma (40). Patients diagnosed with various types of MPGN had properdin serum levels within the normal range (44), or levels were reduced (18, 45).

In IgAN patients, properdin serum levels were within the normal range (11). However, in another cohort, serum properdin levels were increased (43). In patients with IgA vasculitis (Henoch-Schönlein purpura), no abnormalities in serum properdin levels were detected (19).

A paediatric patient with DDD showed low levels of serum C3 and properdin, combined with elevated levels of sC5b-9 (46). When measuring properdin in both C3GN and DDD patients, levels of properdin in serum were reduced, where C3GN patients had generally lower levels than DDD patients (50). In another C3G cohort, serum properdin levels were low in 17 out of 32 patients (C3NeF-negative patients) and it was shown that these patients had lower levels of C3 and C5, where sC5b-9 was increased (48). Furthermore, the consumption of properdin was negatively correlated with an increase in proteinuria (48).

Table 2: Detection of properdin in serum, plasma en urine

Disease classification	Number of patients	Properdin detection in fluids	Reference
Serum/plasma measurements			
Immune-complex mediated GN			
Lupus nephritis - SLE	*N* = 7	Serum levels within normal range (solid-phase radioimmunoassay)	(9)
	N = 46, of which with low C3: *n* = 29	Low C3: also low properdin levels in plasma (radial immunodiffusion method)	(40)
	N = 6	Serum levels slightly decreased compared to controls (ELISA)	(41)
MPGN and SLE	SLE, *n* = 5; MPGN, *n* = 6; controls, *n* = 9	Plasma levels were comparable between the groups (electroimmunoassay)	(42)
IgAN	IgA $C4d^+$, *n* = 27; IgA $C4d^-$, *n* = 69; controls, *n* = 30	Serum levels in normal range (ELISA)	(11)
	IgAN, *n* =50 ; controls *n* = 50 ; reexamined sera, *n* = 15	Serum levels significantly higher in IgAN patients Serum levels remained higher compared to controls (ELISA)	(43)
MPGN type III	*N* = 3	Serum levels in normal range	(44)
IgA vasculitis (Henoch-Schönlein purpura)	*N* = 3	Serum complement levels: no abnormalities (radial immunodiffusion)	(19)
C3GN			
MPGN Type I/III vs MPGN II	MPGN Type I, *n* = 22; MPGN Type II, *n* = 7 MPGN Type III, *n* = 16; controls, *n* = 163	Serum levels depressed in MPGN Type I and MPGN Type III (radial immunodiffusion exclusion)	(45)
MPGN (no anti-DNA antibodies)	*N* = 42	Low C3: low properdin plasma levels (radial immunodiffusion method)	(40)
MPGN Type I; MPGN Type II	MPGN Type I, *n* = 9; MPGN Type II, n= 9; controls *n* = 45	Serum levels in MPGN Type I/II lower compared to controls. MPGN Type I properdin levels lower compared to Type II (radial immunodiffusion)	(18)
DDD	*N* = 1	Low serum levels	(46)

Table 2: (Continued)

Disease classification	Number of patients	Properdin detection in fluids	Reference
C3GN/DDD	*N* = 13 (DDD, *n* = 3; C3GN, *n* = 10)	Serum levels slightly lowered or within the normal range (ELISA)	(47)
	N =49 (DDD, *n* = 20; C3GN, *n* = 29), of which $C3NeF^+$, *n* = 17; $C3NeF^-$, *n* = 32; controls, *n* = 30	$C3NeF^+$ patients have normal levels; $C3NeF^-$ patients: properdin levels equal or below 17.5 ug/ml (ELISA)	(48)
	$C4NeF^+$ patient: *n* = 5 (C3GN, *n* =4, DDD, *n* =1)	4/5 had reduced properdin levels (ELISA)	(49)
	C3GN, *n* = 17; DDD, *n* = 17; controls, *n* = 100	Serum levels reduced in both DDD and C3GN compared to controls, properdin generally lower in C3GN compared to DDD (ELISA)	(50)
Diverse			
Pauci-immune GN	ANCA-AAV; active disease, *n* = 66; remission, *n* = 54 active + remission, *n* = 20 (total *n* = 100); healthy controls, *n* = 39; LN, *n* = 46	Active disease ANCA-AAV patients: significantly lower properdin plasma levels compared to controls and patients in remission; ANCA-AAV patients in remission: significantly higher properdin levels compared to controls (ELISA)	(51)
aHUS	*N* = 1	Plasma properdin (and factor B) levels were normal (radial immunodiffusion method)	(52)
Urine measurements			
IgAN	IgA $C4d^+$, *n* = 27; IgA $C4d^-$, *n* = 69; controls, *n* = 30	Urine levels significantly higher compared to controls (ELISA)	(11)
	N = 71; controls, *n* = 72	Urinary levels were significantly higher compared to controls (ELISA)	(14)
Various renal diseases	Diabetic nephropathy, *n* = 27; glomerular disease, *n* = 27; other (proteinuria), *n* = 16	Urinary properdin levels detected (*n* =37/70) (ELISA)	(53)
	N = 63 patients; *N* = 48 healthy controls	Urinary levels were significantly higher compared to controls (ELISA)	(54)

Other measurements in C3GN/DDD patients showed that properdin levels were detected within the normal range, or were slightly lowered (47). In C4NeF$^+$ C3G patients, four out of five had reduced properdin levels in serum (49).

In patients with active ANCA-AAV disease, properdin plasma levels were significantly lower, compared with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) patients in disease remission and normal controls. The plasma properdin levels of ANCA-AAV patients in remission were significantly higher compared with the normal controls (51). In addition, the properdin levels measured in the plasma correlated inversely with the detected crescents in renal specimens.

The onset of atypical haemolytic uraemic syndrome (aHUS) is linked to dysregulation of the alternative pathway, also due to genetic abnormalities in genes involved in complement regulation. Mutations in complement regulators FH, factor I and membrane co-factor protein were identified (55). Furthermore, the formation of FH autoantibodies is associated with the presence of genetic alterations in the genes coding for FH-related proteins FHR1 and FHR3. High levels of FH autoantibodies are responsible for a dysfunctional FH, resulting in disease progression. A decline in FH autoantibodies, for example due to plasma exchanges, is associated with disease remission (38). In a patient with HUS, there were no differences in factor B or properdin levels detected in plasma (52).

Urine measurements

Next to measurements in blood samples, detection of biomarkers in urine might be of interest to monitor local injury, especially when dealing with renal diseases. Indeed, complement factors have been detected in urine, including properdin.

The levels of urinary properdin were significantly higher in patients with IgAN compared with control conditions, in which no difference was observed when comparing C4d-positive or negative IgAN patients (11). Urinary levels of properdin, MBL, C4d and C5b-9 were high.

These levels strongly correlated with for example proteinuria, glomerular filtration and the severity of tubulointerstitial fibrotic lesions (11). In another cohort of IgAN patients, the urinary properdin levels were significantly higher compared with urinary levels of healthy controls, which correlated with urinary *N*-acetyl-β-D-glucosaminidase, urinary β_2-microglobulin and urinary protein levels (14).

In a mixed cohort, including patients with glomerular disease and diabetic nephropathy, urinary properdin levels were detected in 37 out of 70. The excretion of properdin was associated with a worse renal function and increased sC5b-9 levels in urine (53). In addition, patients with renal disease had higher urinary properdin, which correlated to the total urinary proteins detected and with tubular damage markers (54).

Convertase stabilizing C3 nephritic factors and properdin

An important biological activity of properdin is the stabilization of the C3 convertase. Although the exact molecular mechanism is not yet resolved, low resolution structural analysis has shed some light on the interaction with the convertase (56). Interestingly, it has been well established that some patients with C3G have autoantibodies with a similar functional capacity, the C3 nephritic factors (C3NeFs) (32). Due to this interaction, FH cannot perform its decay accelerating activity, resulting in stabilization of the C3 convertase. Two distinct types of C3NeFs have been described, depending on the composition of the convertase and location where it can bind to; properdin-independent and properdin-dependent C3NeFs (57). The latter is sometimes also referred to as C5NeF. Both C3NeFs and C5NeFs have been detected in C3G patients (58). C5NeFs are predominantly detected in C3GN (58), where C3NeF-positive patients often had DDD (48).

Properdin in experimental models

The demonstration and role of properdin in various diseases was also addressed *in vivo* in mouse models. In 2008, a mouse deficient for properdin was generated and used in a model of polymicrobial septic peritonitis (59). Due to properdin deficiency, these mice had an impaired survival compared with wild-type mice, suggesting a protective role for properdin in a septic model (59). In a mouse model for arthritis, it was shown that $Cfp^{-/-}$ mice were protected against the development of arthritis (60).

In a model for ischaemia and reperfusion injury (IRI), in which mice were deficient for complement regulators CD59 and decay accelerating factor (DAF), it was shown that properdin deficiency, or blocking its function with an antibody, ameliorated renal injury after IRI (61). In a mouse model of anti-myeloperoxidase vasculitis it was shown that C3-deficient mice were protected; however, properdin-deficient mice were not (62). In a $CFH^{-/-}$ mouse model, it was hypothesized that the additional deficiency of properdin would reduce the observed C3G phenotype. Instead, the opposite was found, as it was shown that the accumulation of C3 in the glomerulus and glomerular inflammation was enhanced in these $CFH^{-/-}FP^{-/-}$ mice (63, 64). Further research pointed to the importance of C5 in the exacerbated C3G $CFH^{-/-}FP^{-/-}$ mouse model, and showed that anti-C5 mAb therapy reduced the lethality (65). At present there are no clear explanations for these

contradictory results on the role of properdin, but one hypothesis is that it might be dependent on either systemic or solid-phase regulatory processes.

Potential role for properdin in immune regulation

The observation that properdin is not produced by the liver, but by several immune cells, already placed properdin as a link between cellular and humoral immune responses (66). In 2002, the local role of C3 was shown in a transplant model where allogeneic kidneys from a $C3^{-/-}$ mouse, transplanted into a wild-type (WT) recipient, were not rejected and showed a better renal function compared with WT kidneys transplanted into a WT recipient, which were quickly rejected (67). The local effector functions of the complement system were assessed by investigating the interaction between dendritic cells and T cells. During this interaction, membrane-bound complement regulator DAF was down regulated, allowing local complement activation, with increased production of the anaphylatoxins C3a and C5a, resulting in enhanced T cell proliferation and activation (68, 69). This implies that other complement factors important for complement activation and regulation could have a role in local antigen-presenting cell (APC)–T cell interactions as well. In this respect, expression of properdin has been demonstrated not only in secondary granules of neutrophils (70), but also in professional APC, including dendritic cells. Under steady state conditions, monocyte-derived dendritic cells express and produce properdin, which can be further regulated by stimulation of the cells (71, 72). In addition, monocytes and macrophage subsets are also able to secrete properdin at basal levels, which can be upregulated upon activation (M.F. van Essen *et al.*, in preparation). Further research is needed to determine the contribution to T cell activation and how this affects the (allo)-immune response.

Conclusions and further directions

In conclusion, properdin is detected in kidney biopsies and in serum/plasma/urinary samples obtained from patients with various complement-mediated renal diseases. Further research is needed to determine whether properdin could be a useful marker in the diagnosis of diseases and for example in determining disease activation or remission. Presence of properdin could indicate involvement of the alternative pathway, important information in the context of the growing list of therapeutic agents able to interfere with different pathways and stages of complement activation (73). This includes the development of therapeutic anti-properdin antibodies, which could potentially ameliorate disease severity. Both Novartis and Novelmed Therapeutics have developed anti-properdin antibodies, which are used in clinical trials for the treatment of age-related macular degeneration, geographic atrophy and paroxysmal nocturnal haemoglobinuria (73, 74). However, in view of the unexpected disease exacerbation in experimental C3G (63, 64), more detailed information on the biological functions

of properdin is required and the effect of properdin inhibition should be carefully evaluated.

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