Complement in Renal Disease as a Potential Contributor to Arterial Hypertension

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Keywords
Kidney · Complement deposition · Hypertension · Renal disease

Abstract

Objective: Complement deposition is prevalent in kidney biopsies of patients with arterial hypertension and hypertensive nephropathy, but an association of hypertension and complement deposition or involvement of complement in the pathogenesis of hypertensive nephropathy has not been shown to date. Methods: In this study, we analyzed complement C1q and C3c deposition in a rat model of overload and hypertension by subtotal nephrectomy (SNX) and in archival human renal biopsies from 217 patients with known hypertension and 91 control patients with no history of hypertension using semiquantitative scoring of C1q and C3c immunohistochemistry and correlation with parameters of renal function. To address whether complement was only passively deposited or actively expressed by renal cells, C1q and C3 mRNA expression were additionally analyzed. Results: Glomerular C1q and C3c complement deposition were significantly higher in kidneys of hypertensive SNX rats and in human renal biopsies. Of note, in patients CKD-stage correlated significantly with the intensity of glomerular C3c staining, but not with that of C1q. Conclusion: Renal complement deposition correlated with experimental hypertension as well as the presence of hypertension in a variety of renal diseases. To answer the question, if and how exactly renal complement is causative for the pathogenesis of arterial hypertension in men, further studies are needed.

Introduction

The complement system and its regulatory proteins are components of the innate immune system consisting of >20 soluble factors, playing an important role in (i) opsonization, (ii) stimulation of different inflammatory...
Renal Complement Deposition in Hypertension

The past 2 decades intensified our knowledge of the complement system and its role in the pathogenesis of different systemic and renal diseases like postinfectious glomerulonephritis (GN), IgA nephropathy (IgAN), lupus nephritis (LN), membranoproliferative GN, and C3 glomerulopathies [1, 5]. An important role of the complement system and its dysfunctions is also known in the pathogenesis of thrombotic microangiopathies, including the atypical hemolytic uremic syndrome [6], IgA vasculitis (former Henoch-Schönlein purpura) [7], and malignant hypertension [8]. While pathomechanisms of thrombotic microangiopathies are already known [6, 7], our knowledge of the significance of the complement system in relation to hypertension is still relatively vague. The majority of patients with CKD present with elevated blood pressure (BP) [9], but the exact pathogenesis of hypertension is unknown. Several studies are available describing an association of plasma C3 and hypertension [10, 11], while another study could not confirm these observations [12].

In the rat DOCA-salt hypertension model, glomerular C3 deposition was increased in DOCA-salt rats and decreased by antihypertensive therapy, that is, spironolactone and triple therapy with hydrochlorothiazide, reserpine, and hydralazine [13]. Recent studies using preclinical models of hypertension suggested a role of complement in the pathogenesis of hypertension [14] mediated by different immune cells like macrophages and regulatory T-cells (Tregs) [15–17] and activation of the renin-angiotensin system leading to phenotypic changes in vascular smooth muscle cells [18]. The detailed role of renal complement deposition in the development of elevated BP is still unclear. Therefore, in this study, we investigated C1q, as a cleavage product of the classical, and C3c as a common marker of all 3 complement activation pathways in renal biopsies from hypertensive and normotensive patients as well as in an established rat model of overload and subsequent hypertension by subtotal nephrectomy (SNX).

Methods

Animal Model

For investigation of renal complement deposition, renal injury, and cardiac changes in hypertensive animals, we used the rat model, which is characterized by glomerular hyperfiltration and overload due to nephron reduction, focal segmental and global glomerulosclerosis, tubulointerstitial scarring and progressive CKD with significantly increased BP. Rats were maintained in a specific pathogen-free facility in a temperature- and light-controlled environment and had ad libitum access to standard chow (sniff Spezialdiäten GmbH, Soest, Germany) and water. The rats were kept on classical aspen wood bedding (sniff Spezialdiäten GmbH, Soest, Germany) in type IV makrolon cages (Tecniplast Deutschland GmbH, Hohenpeißenberg, Germany) with a maximum population of 3. For model induction, 12 male Dark Agouti (DA/HanRj, Janvier, Le Genest-Saint-Ise, France) rats with an age of 12–14 weeks were SNX in a 2 step protocol starting with uninephrectomy followed by weight standardized resection of the upper and lower poles from the remaining kidney 1 week later. For this purpose, the animals were administered Buprenovet (Bayer, Leverkusen, Germany) in a dose of 0.05 mg/kg body weight s.c. before surgery. After anesthesia with isoflurane, the right flank was shaved, disinfected with KODAN tincture forte (Schülke & Mayr GmbH, Norderstedt, Germany), the flank was opened with a 1.5 cm incision and the kidney carefully lifted out and fixed with 2 sterile swabs followed by ligation of the renal blood supply and ureter and removal of the kidney. The abdominal incision was closed with an absorbable suture and a continuous seam as well as the skin closed with single button seams. One week later, the left kidney was lifted out exactly as described for the right kidney. Then the renal artery was clamped with an atraumatic clip and upper and lower pole of the kidney was resected with a scalpel and the cut surface was closed with a collagen sponge (Resorba medical GmbH, Nürnberg, Germany). Finally, the remaining kidney was returned to its original position and the abdominal incision was closed as described above. Postoperative activity, fluid intake, weight, coat texture, and behavior of the animals were assessed but showed no signs of postoperative pain. However, analgesia was maintained at least over 3 days after surgery. Controls received a sham surgery (sham, n = 11). At the end of the experiment, animals were housed for 24 h in metabolic cages to collect urine for measurement of proteinuria using a Bio-Rad Protein Assay (Bio-Rad Laboratories GmbH, Munich, Germany). Adsorption was measured in PBS-diluted urine samples at 595 nm in a microplate reader, and proteinuria was calculated using a standard curve from bovine serum albumin standards ranging from 0.15 to 2 mg (Thermo Scientific, Rockford, IL, USA). After 12 weeks, rats were euthanized after intra-arterial BP measurement for 30 min via insertion of a catheter into A. carotis using AD instruments power lab system (ADInstruments Ltd., Oxford, UK). Finally, the kidneys and hearts were harvested and split into 2 parts, one was snap frozen for RNA analysis and the second part was processed for immunohistochemistry. Blood was collected for detection of serum creatinine using an autoanalyzer (Beckman Instruments, Brea, CA, USA).

Quantitative mRNA Analysis

Total RNA from rat kidneys was isolated using RNasy Mini columns (Qiagen, Hilden, Germany). Reverse transcription reactions and real-time PCR were performed using Power SYBR Green on a 7500 Fast Real-Time PCR system (both Applied Biosystems, Weiterstadt, Germany) according to the manufacturer’s instructions. Real-time PCR data were analyzed using the SDS v1.3 soft-
After washing with 50 mM Tris pH 7.4, bound primary antibodies clonal antibody against human C3c (A0062; DAKO Deutschland). DAKO Deutschland, Hamburg, Germany) and; C3c, a rabbit polyclonal antibody against human C1q (A0126; Roche Diagnostics Deutschland GmbH, Mannheim, Germany). Normalization was conducted against the endogenous 18S rRNA levels applied to the resulting relative fold changes. The following primers were used: 18 s (fw 5′TTGATTAAGTCCCTGCGCCTTTG3′; rev 5′CGATCCGGAGGCTCCTACTA3′); C3 (fw 5′TGGCGGCTGGAGATGAGGAG3′; rev 5′TTACTGGCTGGGAATCTTGTATGG3′) [20]; and ClqB (fw 5′TACAAGACAGGAGGATTTCCATAC3′; rev 5′GACCAGTGACGCTGCTTGG3′) [20].

Immunohistochemistry
For immunohistochemistry kidney biopsies or SNX and sham rat kidney, specimens were fixed in formalin, embedded in paraffin and cut into sections of 1 μm and stained in a Ventana Benchmark machine (Applied Biosystems), and relative expression of target gene mRNA levels was calculated using the comparative delta Ct method [19]. Normalization was conducted against the endogenous 18S rRNA levels applied to the resulting relative fold changes.

TMA, thrombotic microangiopathy; MGN, membranous GN; LN, lupus nephritis; FSGS, focal segmental glomerulosclerosis; IgAN, IgA nephropathy; DN, diabetic NP. *Others: membranoproliferative GN, postinfectious GN, C3 GN, ANCA-associated vasculitis, IgA vasculitis (HSPN), hypertensive nephropathy (NP), TMA, transplant NP, amyloidosis, end-stage renal disease (of unknown origin), Minimal change disease, M. Alport, thin basement disease, acute tubular injury, interstitial nephritis, cholesterol embolism, oxalate NP, light chain cast NP.

### Table 1. Patient characteristics

| No. | Diagnosis | Hypertensive patients, N/n | Percentage of hypertension, % | Sex (m/w), n/n | Age, years (mean ± SD) |
|-----|-----------|---------------------------|-------------------------------|---------------|---------------------|
| **Controls** | | | | | |
| 1 | Zero-biopsy | 46/0 | 0 | 23/23 | 53.5±13.4 |
| **Nephropathies** | | | | | |
| 2 | IgAN | 65/58 | 89.2 | 49/16 | 49.7±17.2 |
| 3 | MGN | 18/11 | 61.1 | 12/6 | 55.8±18.2 |
| 4 | LN | 10/8 | 80.0 | 5/5 | 39.2±15.2 |
| 5 | DN | 28/26 | 92.9 | 14/14 | 60.8±12.8 |
| 6 | FSGS | 17/15 | 88.2 | 10/7 | 52.5±21.1 |
| 7 | Others* | 124/99 | 79.8 | 77/47 | 53.5±18.5 |
| **All** | | 308/217 | 70.5 | 190/118 | 53.5±17.9 |

**Histopathological Evaluation**
Renal biopsy diagnostic was performed by 3 very experienced, specialized nephropathologists during routine work-up with consensus discussion of all ambiguous cases (including M.B.H. and K.A.). Assessment of detailed morphology of human renal biopsies was done by one very experienced scientist (CD) using well-established semiquantitative glomerulosclerosis score, tubular injury score, and vascular injury score (VSI) on PAS-stained paraffin sections as described previously [21]. Interstitial fibrosis/tubular atrophy (IFTA) was graded according to BANFF classification for renal transplant biopsies [22].

**Evaluation of Retrospective Clinical Data**
Clinical parameters that were available at the time-point of renal biopsy were collected. The following parameters were includ-
ed for correlation analysis with C1q- and C3c-scores, complement localization, and injury scores using SPSS software: patient age, presence or absence of hypertension and diabetes, proteinuria, serum creatinine, serum urea, serum cholesterol, serum protein, and serum C3 and C4 complement factors. Unfortunately, information on the duration of renal disease and current medication was not consistently available and could therefore not be systematically analyzed. In renal patients, BP is measured regularly and particularly before performing a kidney biopsy. BP measurements and grading was done according to the KDIGO guidelines for renal patients [23]. Proteinuria was measured by analysis of a 24 h urine collection and significant proteinuria was defined as urinary protein excretion >300 mg/day. In 2/3 of all cases, proteinuria was given as g/24 h urine. Only some patients had proteinuria documented by spot urine measurements, and for very few patients, there was no data on proteinuria available. Since 24 h and spot proteinuria values can be hardly compared, we only included patients with 24 h urine collection for correlation of proteinuria with complement deposition. In order to further standardize our findings, we also subdivided the patients according to different CKD classes using the CKD-EPI formula for calculation of GFR [24] and the CKD classes according to the KDIGO 2012 classification [25].

**Semiquantitative Evaluation of Complement Deposition in Renal Biopsies**

Complement staining by immunohistochemistry in renal biopsies was graded separately in glomeruli and the tubulointerstitium for correlation analysis with C1q- and C3c-scores, complement localization, and injury scores using SPSS software: patient age, presence or absence of hypertension and diabetes, proteinuria, serum creatinine, serum urea, serum cholesterol, serum protein, and serum C3 and C4 complement factors. Unfortunately, information on the duration of renal disease and current medication was not consistently available and could therefore not be systematically analyzed. In renal patients, BP is measured regularly and particularly before performing a kidney biopsy. BP measurements and grading was done according to the KDIGO guidelines for renal patients [23]. Proteinuria was measured by analysis of a 24 h urine collection and significant proteinuria was defined as urinary protein excretion >300 mg/day. In 2/3 of all cases, proteinuria was given as g/24 h urine. Only some patients had proteinuria documented by spot urine measurements, and for very few patients, there was no data on proteinuria available. Since 24 h and spot proteinuria values can be hardly compared, we only included patients with 24 h urine collection for correlation of proteinuria with complement deposition. In order to further standardize our findings, we also subdivided the patients according to different CKD classes using the CKD-EPI formula for calculation of GFR [24] and the CKD classes according to the KDIGO 2012 classification [25].

**Semiquantitative Evaluation of Complement Deposition in Renal Biopsies**

Complement staining by immunohistochemistry in renal biopsies was graded separately in glomeruli and the tubulointerstitial areas.
The semiquantitative scores 0–4 describe the intensity of observable C3c or C1q in kidney biopsies at ×200 magnification. Score 0 indicates no deposits, score 1: weak staining affecting up to 25% of the investigated compartment. Score 2: moderate staining affecting up to 50% or strong staining in <25% of the compartment. Score 3: substantial staining affecting up to 75% of the investigated compartment. Score 4: highest staining intensity affecting >75% of the investigated compartment.

C1q and C3 mRNA in Human Renal Biopsies
Data for C1q and C3 in glomeruli and tubulointerstitium of human renal biopsies with different diseases were evaluated from Nephroseq database using the Ju CKD dataset [26].

Statistical Analyses
After testing for normal distribution of values using Kolmogorov-Smirnov test, data were analyzed using Mann-Whitney test for comparison of 2 groups and Kruskal-Wallis test with Dunn’s Multiple Comparison test as post hoc test for comparison of multiple groups. In all tests, \( p < 0.05 \) was accepted as statistically significant. Spearman’s test was used to test correlation of complement deposition with renal injury scores and clinical data. Statistical analyses were performed using SPSS for Windows software (version 19.0 SPSS, IBM, Munich, Germany) or GraphPad Prism 8 for Windows software (version 8.3, GraphPad Software Inc., San Diego, CA, USA).
Fig. 3. C3c and C1q deposition in renal biopsies. Human renal biopsies were stained for C3c (A, C, E, G) and C1q (B, D, F, H) using immunohistochemistry (brown staining). Representative pictures of glomerular and tubulointerstitial deposition of C3c and C1q show examples of different scores of complement deposition. No staining for C3c in zero-biopsies (A) and C1q in acute tubular necrosis (B). Examples for glomerular C3c deposition in hypertensive/ischemic NP, score 1 (C) and C1q deposition in class II LN, score 2 (D). More prominent C3c deposition in IgAN, score 4 (E) and C1q deposition, score 4, in another case of class II LN (F). Deposition in the tubulointerstitial compartment of C3c in interstitial nephritis, score 4 (G) and C1q deposition, score 4 (H) in a case of postinfectious GN. Scale bars represent 100 µm. LN, lupus nephritis; GN, glomerulonephritis.
Results

Renal Complement C3c and C1q Deposition Is Present in a Hypertensive Rat Model of Stable CKD and Correlates with Renal and Cardiac Changes

Complement deposition was investigated in a hypertensive rat model of CKD, that is, subtotally nephrectomized (SNX) Dark Agouti rats, in which mean arterial BP 12 weeks after model induction was significantly \((p < 0.001)\) increased \((175.7 \pm 21.0 \text{ mm Hg})\) compared to sham-operated controls \((135.8 \pm 2.8 \text{ mm Hg})\) (Fig. 1A). Proteinuria was also significantly higher in SNX compared to sham-operated controls \((78.5 \pm 16.6 \text{ vs. } 8.9 \pm 0.8 \text{ mg/24 h})\). This overload rat model of hypertension and progressive renal insufficiency seemed suitable to examine hypertension-associated changes in renal complement deposition and expression. Glomerular C3c and C1q deposition were absent in normotensive, sham-operated control rats (Fig. 1B, C), and significantly increased in hypertensive SNX rats (Fig. 1D, E, G, H).

In SNX rats, complement factor C3c was not only deposited in renal tissue but also significantly upregulated on mRNA level (Fig. 1F). Renal C1q mRNA expression showed a tendency to higher levels in SNX rats than controls, but this difference did not reach significance (Fig. 1I). Interestingly, in rats both glomerular C3c and C1q deposition highly correlated with mean arterial BP (Fig. 2A, D) and cardiac hypertrophy as assessed by LV weight (Fig. 2B, E). Furthermore, in the SNX model, renal complement deposition is correlated with kidney injury as well as renal function. Glomerular C3c as well as C1q deposition showed a strong correlation with serum creatinine (Fig. 2C, F) and proteinuria (Fig. 2G, H). Correlation of both serum creatinine and proteinuria with complement was not surprising since both parameters showed strong correlation with each other in this rat model (Fig. 2I). Thus, our data in a rat model of hypertensive CKD due to kidney mass reduction and overload suggested an association of renal complement deposition and hypertension. Therefore, we aimed to confirm these findings in a human cohort of hypertensive versus normotensive patients, in whom a renal biopsy was performed for various reasons.

Complement Deposition Is Independent of the Underlying Renal Disease

Here, we used a 2 step approach: first, we included all patients with a reported BP value independent of the underlying kidney disease \((n = 308, \text{ see Table } 1 \text{ for details})\). Of note, 217 of 308 patients \((70.5\%)\) were reported to have hypertension. Second, for further analysis, we focused on subgroups of most prevalent forms of GN, that is, IgAN, MGN, and LN, and 2 nonimmune complex-mediated renal diseases namely DN and focal segmental glomerulosclerosis (FSGS). 46 zero-biopsies from transplant kidney allografts donated from normotensive living donors served as a control group (see Table 1).

To investigate a potential relationship between hypertension and renal complement, deposition of C1q and C3c in the glomerular and tubulointerstitial compartment was analyzed by semiquantitative scoring of immunohistological stainings. The amount and intensity of C1q and C3c staining varied greatly in the investigated biopsies (for details see Fig. 3). In the tubulointerstitial compartment, C3c deposited along the tubular basement membranes and within the interstitial matrix (Fig. 3G), while C1q staining was usually faint and restricted to the interstitium (Fig. 3H).

Glomerular C3c deposition was increased in many renal diseases, including IgAN, MGN, LN, and FSGS, compared to normotensive zero-biopsies (Fig. 4A). Overall, glomerular C1q deposition was fainter than glomerular C3c. It was significantly increased in IgAN, MGN, LN, and DN, but not FSGS compared to zero-biopsy controls (Fig. 4B). Interestingly, complement C3 and C1q were not only deposited in diseased kidneys, but local renal mRNA expression was also increased. Nephroseq data showed that both, C3 and C1q mRNA expression, was significantly upregulated in IgAN, LN, DN, and FSGS compared to the control group, that is, healthy living donors (Fig. 4C, D). C1q mRNA expression was lower than C3 expression. Tubulointerstitial C3c deposition was also increased com-
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Renal C3c and C1q Deposition Are Significantly Increased in Hypertensive Compared to Normotensive Patients

To investigate whether presence of high BP in patients is associated with renal complement deposition, we analyzed biopsies from normotensive and hypertensive patients with various underlying kidney diseases and compared them with complement deposition in zero-biopsies from transplant kidney allografts donated from nonhypertensive donors (zero-biopsies). In IgAN, the most prevalent GN in our region [27], only biopsies from patients with hypertension had significantly higher glomerular (Fig. 5A) and tubulointerstitial C3c (Fig. 5C) as well as glomerular C1q deposition (Fig. 5E) compared to zero-biopsies. When we compared C3c (Fig. 5B, D) and C1q deposition (Fig. 5F) in renal biopsies of the total cohort, both normotensive and hypertensive samples showed significantly increased reactivity when compared to controls. Moreover, in biopsies of hypertensive patients glomerular and tubulointerstitial C3c was significantly higher than normotensive patients (Fig. 5B, D). Since tubular C1q deposition was low in all investigated biopsies, we did not observe any differences between groups neither in IgAN (Fig. 5G) nor in the analysis of all investigated cases (Fig. 5H). Therefore, our data suggest that in particular C3c deposition seems to be relevant in hypertensive renal diseases.

Renal Complement Deposition Is Associated with Kidney Injury and Function

The evaluation of histopathological changes, as assessed by glomerulosclerosis (Fig. 6A), tubulointerstitial injury score (Fig. 6B), and VSI (Fig. 6C), showed significant higher renal injury in biopsies from patients with documented hypertension than normotensive patients. The extent of glomerular C3c deposition correlated positively with glomerulosclerosis (Fig. 6D) and tubulointerstitial C3c with tubulointerstitial injury (Fig. 6E) and VSI (Fig. 6F), indicating that complement deposition is associated with chronic kidney injury. In addition, interstitial fibrosis/tubular atrophy (IF/TA) was significantly higher in renal biopsies of hypertensive patients (Fig. 6G) and correlated well with the extent of tubular C3c deposition (Fig. 6I). Kidney function, as assessed by serum creatinine, was also significantly lower in hypertensive patients (Fig. 6H) and correlated with glomerular C3c deposition (Fig. 6K). No significant difference between normotensive and hypertensive patients was detected with regard to proteinuria (Fig. 6I). However, there was a weak correlation between proteinuria and glomerular C3c (r = 0.238; p = 0.008; Fig. 6L). These results confirmed an association of kidney injury, function, complement deposition, and hypertension.

Finally, we investigated the extent to which complement deposition was dependent on CKD stage. As expected, the mean age of patients increased with higher CKD stage (Fig. 7A). In contrast, the proportion of patients with hypertension was independent on CKD stage (Fig. 7B) confirming our above findings. Glomerular (Fig. 7C) and tubular C3c (Fig. 7D) were significantly lower in CKD stages 1 and 2 than higher CKD stages, whereas no such association was observed for C1q (Fig. 7E, F).

Fig. 5. Renal complement deposition in kidney biopsies from hypertensive and normotensive patients. C3c deposition was analyzed in biopsies from patients with IgAN (A, C) and all investigated biopsies with renal diseases (B, D) separated in patients with normal BP and high BP compared to biopsies from transplant kidney allografts donated from nonhypertensive donors (zero-biopsies) as controls in the glomerular (A, B) and tubulointerstitial (C, D) compartment. Similarly, C1q deposition was also analyzed in biopsies from patients with IgAN (E, G) and all investigated biopsies with kidney diseases (F, H) in the glomerular (E, F) and tubulointerstitial (G, H) compartment. Significant differences are marked by asterisks: *p < 0.05; **p < 0.01 and ***p < 0.001 indicate significant differences. BP, blood pressure.

Fig. 6. Renal complement deposition in human biopsies correlated with renal damage and function. Renal damage as assessed by GSI (A), TSI (B), and VSI (C) was significantly increased in patients with high BP and GSI correlated with glomerular C3c deposition (D), TSI correlated with tubulointerstitial C3c (E), and VSI correlated with tubulointerstitial C3c (F). IF/TA (G) and serum creatinine (H) were increased in hypertensive patients, while proteinuria was similar in both groups (I). Tubular C3c correlated with IF/TA (J) and glomerular C3c with serum creatinine (K) and to a weaker extent glomerular C3c with proteinuria (L). Significant differences are marked by asterisks: **p < 0.01 and ***p < 0.001 indicate significant differences between patients with normal BP and high BP or significant correlations. BP, blood pressure; GSI, glomerulosclerosis; TSI, tubulointerstitial injury; VSI, vascular injury; IF/TA, interstitial fibrosis/tubular atrophy.

(For figure see next page.)
Discussion

Progression of renal disease, especially tubulointerstitial damage, was shown to be mediated, at least in part, by complement activation [28], but the role of complement deposition in hypertension and renal damage is still unclear. In our study, we found increased renal deposition of complement factors, particularly of C3c, which strongly correlated with increased kidney injury and inferior renal function. In addition, very frequently kidney disease is associated with the occurrence of hypertension which itself is linked to increased risk of cardiovascular disease and progression of CKD [9]. Here, we showed higher renal deposition of complement cleavage products C1q, as a marker of the classical activation pathway, and C3c, a component of all 3 activation pathways, in kidneys from hypertensive patients with different renal diseases than normotensive pa-

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**Fig. 7.** Renal C3 deposition in human biopsies, but not hypertension is correlated with CKD stage. Mean age of patients with CKD increased with increasing CKD stage (A), while the percentage of hypertensive patients was not related to CKD stage (B). C3c (C, D) but not C1q (E, F) nicely correlated with different CKD stages. Glomerular C3c (C), tubular C3c (D), glomerular C1q (E), and tubular C1q (F). Significant differences are marked by asterisks: *p < 0.05; **p < 0.01; and ***p < 0.001 indicate significant differences between groups connected by brackets.
tients. There is some evidence that complement plays an important role in the development and worsening of hypertension, but the role of renal complement deposition in this process is unknown. In human renal biopsies and our hypertensive overload rat model, it has become apparent that C3c is more frequently detectable than C1q suggesting that the classical pathway is not exclusively responsible for complement activation in renal disease. Nonetheless, C1q was found to be critically involved in hypertensive arterial remodeling via activation of β-catenin signaling [29]. The question if complement induces hypertension or hypertension activates complement remains to date unclear. In this study, we observed a strong correlation of renal complement deposition, particularly of factor C3c, with the degree of renal injury and function. Most likely hypertension leads to further activation of the complement system, but there are some reports supporting the hypothesis that complement is not only secondary to but also causative for hypertension. In a longitudinal study with a follow-up of 15 years, the incidence of hypertension was significantly higher in patients with elevated plasma complement C3 levels at baseline suggesting a role for plasma C3 in the pathogenesis of hypertension [10]. Additionally, patients with therapy-resistant arterial hypertension had significantly higher C3 plasma levels compared to patients with controlled hypertension [11]. In contrast, in a population-based cohort study elevated plasma C3 was associated with increased incidence of first hospitalization due to CKD but independent of BP [12]. However, the latter study was restricted to the investigation of C3 plasma levels, whilst local expression of complement factors and complement activation were not investigated. C3 cleavage products, that is, C3c, were not only passively deposited but C3 mRNA was also locally expressed in diseased kidneys from hypertensive patients and SNX rats. We did not determine the source of renal C3, however, but there are reports that beside the liver the majority of C3 mRNA is expressed in monocytes and macrophages [30]. In addition fibroblasts, epithelial and endothelial cells have also been reported to be potential extrahepatic C3-sources [31]. Complement activation was also seen in an angiotensin II-induced hypertension model in mice. In spontaneously hypertensive rats, C3 deficiency could abolish salt-sensitive hypertension [18], indicating that C3 is critical involved in hypertension-inducing pathways. However, these effects can also be mediated further downstream in the complement cascade. Using C5a receptor (C5aR) deficient mice Zhang et al. [32] demonstrated that C5aR-signaling plays a pathological role in cardiac inflammation and remodeling. Although C5aR inhibition did not affect the elevated BP induced by angiotensin II, cardiac damage, as assessed by cardiac hypertrophy, inflammation, and perivascular fibrosis could be reduced [33]. In our SNX rat model of hypertension, we confirmed that renal complement deposition correlated well with renal damage but also with cardiac hypertrophy and BP. Complement-induced hypertension was mediated via complement receptors for C3a and C5a. Regulatory Tregs can inhibit activation of effector Tregs and exerts anti-inflammatory effects. In the angiotensin II-induced model of hypertension, Tregs have been shown to be an important modulator of BP and prevention of end-organ damage [15, 34]. Angiotensin II-treatment stimulated expression of C3a and C5a receptor on Tregs leading to a reduced Tregs number in wild-type mice, but not in C3a and C5a receptor double knockout mice [35]. In addition, these double knockout mice developed blunted hypertension and less renal fibrosis and glomerular injury [35]. An association of complement activation and glomerular injury and renal fibrosis, as assessed by glomerulosclerosis and IF/TA, was also confirmed in our biopsy study. Mechanistically, BP increase was induced by C3-mediated activation of the renin-angiotensin system in vascular smooth muscle cells with downstream molecules such as TGF-β/PDGF-A and by changing the phenotype of mesenchymal cells and induction of epithelial-to-mesenchymal transition [31]. Complement signaling was also targeted in other hypertensive diseases. In normal pregnancy, complement activation is present but is excessive in hypertensive disorders of pregnancy and genetic polymorphisms of complement proteins seem to predispose to preeclampsia [36]. Inhibition of complement signaling by treatment with soluble complement receptor 1 resulted in attenuated hypertension in a placental ischemia model of preeclampsia [37].

**Limitations of the Study**

Although we tried to strengthen our findings in human renal biopsies of various renal diseases with and without arterial hypertension by the incorporation of an appropriate animal model to prove an association between complement deposition in kidney tissue and hypertension, we have to acknowledge several limitations and selection biases. In the human study, we face the limitation of a variable cohort with numerous different confounders that cannot be clearly identified or
excluded. In order to address this aspect and to harmonize the study group, we also analyzed the correlation of complement with renal fibrosis (IF/TA) as a surrogate marker of nephron loss and progression of the underlying renal disease as well as with CKD stages where we could show similar findings. Moreover, data on duration of the respective diseases or current medical treatment were not systematically available leaving these aspects as another potential confounder that we have to mention. Since we formally excluded those few patients without clinically well-documented BP, it is likely that those patients might have either no hypertension or controlled hypertension to the point that they might come to regular follow-up which might potentially cause selection bias. In contrast to the animal experiment in our human cohort proteinuria at the time of renal biopsy was not consistently reported for all patients so that we could only include 2/3 of all patients for the respective analyses which may affect some of the results. Of note, proteinuria was not significantly higher in renal patients with and without high BP making a significant bias of proteinuria unlikely.

Conclusion

Taken together, our study underlines a strong association of complement deposition with hypertension but also renal damage in human and experimental renal disease. Although we could not answer the question whether complement deposition is causative of the development of hypertension, the data support an involvement of complement activation in the pathogenesis or progression of hypertension in various kidney diseases, implying a potential role for complement-inhibiting strategies for future management of therapy-refractory cases of hypertension and renal disease. However, additional studies are needed to address the exact role of complement in pathogenesis of hypertensive diseases.

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Statement of Ethics

The study on archived human renal biopsies was conducted according to the principles of the Declaration of Helsinki and approved by the local Ethics Committee of the FAU Erlangen-Nürnberg (reference number 4415). The experimental protocol for the animal studies was approved by the German regional committee for animal care and use, which is equivalent to the US IACUC, and authorized by the governmental department ("Regierung von Mittelfranken" Permit number: 54-2532.1-18/11) prior the animal studies were performed in strict accordance with the German welfare act.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

L.-M.F. conducted all experimental work on human biopsies, analyzed data, and wrote the manuscript; L.A.R. carried out immunohistological staining and data analysis for rat experiments; F.F. performed the statistical analysis; M.B.H. and K.A. made the diagnoses, performed some of the analyses, and wrote the paper; K.B. and C.D. conceived and designed the study, analyzed data, and wrote the manuscript. All the authors had proofread the manuscript.

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