III Complement activation in the kidney during preeclampsia

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Abstract

A growing body of evidence suggests that complement dysregulation plays a role in the pathogenesis of preeclampsia. The kidney is one of the major organs affected in preeclampsia. Because the kidney is highly susceptible to complement deposits, we hypothesized that preeclampsia is associated with renal complement activation. We performed a nationwide search for renal autopsy material in the Netherlands using a computerized database (PALGA). Renal tissue was obtained from 11 women with preeclampsia, 25 pregnant controls, and 14 non-pregnant controls with hypertension. The samples were immunostained for C4d, C1q, MBL, properdin, C3d, C5b-9, IgA, IgG and IgM. Our findings in human samples were validated using a soluble fms-like tyrosine kinase 1 (sFlt-1) mouse model of preeclampsia. Preeclampsia was significantly associated with renal C4d —a stable marker of complement activation— and the classical pathway marker C1q. In addition, the prevalence of IgM was significantly higher in the kidneys of the preeclamptic women. No other complement markers studied differed between the groups. In the preeclampsia mouse model, the kidneys in the sFlt-1–injected mice had significantly more C4 deposits than the control mice. The strong association between preeclampsia and renal C4d, C1q, and IgM levels suggests that the classical complement pathway plays a role in the pathogenesis of renal injury in preeclampsia. Moreover, our finding that sFlt-1–injected mice develop excess C4 deposits indicates that angiogenic dysregulation may play an important role in complement activation within the kidney. We suggest that inhibiting complement activation may be beneficial for preventing the renal manifestations of preeclampsia.

Introduction

Preeclampsia is a severe multisystem pregnancy-related complication; worldwide, preeclampsia causes high maternal and perinatal morbidity and mortality rates.[1] Preeclampsia complicates 2-8% of pregnancies and is characterized by endothelial damage, resulting in maternal hypertension and proteinuria after gestational week 20.[2] The endothelial damage in preeclampsia is believed to arise from a dysregulation of proangiogenic and antiangiogenic factors, particularly the antiangiogenic factor soluble fms-like tyrosine kinase 1 (sFlt-1).[3]

Although the precise pathogenesis of preeclampsia is unknown, a growing body of evidence suggests that complement dysregulation plays a role in the development of preeclampsia.[4] In support of this notion, women with preeclampsia have complement dysregulation in the placenta, as well as elevated circulating levels of complement degradation products.[5, 6] In addition, individuals with mutations in genes that encode complement regulatory proteins are predisposed to developing preeclampsia.[7] Furthermore, treating preeclamptic mice with complement inhibitors can reverse proteinuria and histopathologic lesions.[8] Finally, Eculizumab, a terminal complement inhibitor, has been used successfully to reduce preeclampsia-associated conditions, thereby prolonging pregnancy in a patient with preeclampsia.[9]

In preeclampsia, the kidney is a major target organ that develops severe damage, as demonstrated by renal symptoms that include proteinuria and by abnormal renal histology.[10] These symptoms are believed to reflect endothelial damage due to increased sFlt-1 levels, which prevent vascular endothelial growth factor (VEGF) from maintaining the renal endothelium.[11] It has been shown that damage to the fenestrated glomerular endothelium can activate the complement system.[12-14] Interestingly, a case report showed glomerular complement deposits in a patient with preeclampsia, suggesting that the complement system may indeed play a key role

in the pathogenesis of renal damage in preeclampsia.[15] Furthermore, a recent study reported that patients with severe preeclampsia have a higher prevalence of urinary excretion of the terminal complement complex compared to control subjects.[16]

Because preeclampsia is characterized by complement dysregulation, the kidney plays an important role in preeclampsia, and the kidney is highly susceptible to complement deposits, we hypothesized that complement activation is involved in the renal manifestations of preeclampsia. If correct, this hypothesis would support the notion that inhibiting complement activation might be a viable option for treating the renal manifestations of preeclampsia. To test this hypothesis, we measured the presence of complement components in a unique cohort of renal autopsy tissue samples collected from preeclamptic patients and control patients; in addition, we studied complement components in a sFlt-1–induced mouse model of preeclampsia.

Methods

PATIENT SELECTION AND NATIONWIDE PALGA SEARCH FOR RENAL AUTOPSY TISSUE

To study the role of the complement system in the renal pathology of preeclampsia, we performed a nationwide search for renal autopsy tissues in the Netherlands using the Dutch Pathology Registry (PALGA), a histopathology and cytopathology network and registry that includes all pathology laboratories within the Netherlands.[17] The search parameters were as follows: “autopsy”, “women”, “age between 18 and 45 years”, and “since 1990”. We included all patients who were pregnant and who had a confirmed case of preeclampsia. In addition, two control groups were included in the study. The first control group consisted of pregnant women without a hypertensive disorder either prior to or during their pregnancy; this group was included to investigate the effect of pregnancy alone. The second control group consisted of non-pregnant young women with a medical history of chronic hypertension; this group was included to investigate the effect of hypertension alone. The search yielded paraffin-embedded kidney samples from 11 patients with preeclampsia,[18] 25 pregnant controls, and 14 non-pregnant chronic hypertensive controls. If available, clinical characteristics were obtained from autopsy-reports. The records of the National Maternal Mortality Committee of the Dutch Society of Obstetrics and Gynecology were used to confirm the cause of death of each pregnant case.[19] All tissue samples were coded and treated anonymously in accordance with Dutch national ethics guidelines (Code for Proper Secondary Use of Human Tissue, Dutch Federation of Medical Scientific Societies). This study was approved by the Medical Ethics Committee of the Leiden University Medical Center (P12.107).

sFlt-1 MOUSE MODEL OF PREECLAMPSIA

All animal experiments were performed at the Beth Israel Deaconess Medical Center in accordance with International Animal Care and Use Committee guidelines. We used the sFlt-1–induced mouse model of preeclampsia,[20] which overexpresses the sFlt-1 protein and develops high blood pressure, proteinuria, and endotheliosis. In brief, pregnant female CD1 mice (Charles River, Wilmington, MA) received a tail vein injection of 2×10^9 pfu of either an adenovirus encoding sFlt-1 (Ad-sFlt-1) or an equivalent dose of adenovirus empty vector CMV null (Vector Laboratories, Philadelphia) at gestational day 9.5. This model has been well characterized by a number of groups in both rats and mice and leads to hypertension, proteinuria and glomerular endothelial damage 7-10 days after adenoviral injection of sFlt-1.[3, 20-22] For our studies, mice were euthanized on gestational day 17.5 and one kidney from each mouse was frozen for immunofluorescence, and the other kidney was formalin-fixed and embedded in paraffin. Paraffin-embedded kidney sections were stained with Periodic Acid Schiff (PAS) or silver using standard protocols.

HISTOLOGY, IMMUNOHISTOCHEMISTRY, AND IMMUNOFLUORESCENCE

Sections of human kidney samples were stained with Periodic Acid Schiff (PAS) and silver using a standard protocol. To measure human renal complement activation, various complement system components were stained using immunohistochemistry. We used primary antibodies against the following proteins: C4d (Biomedica Gruppe, 1:50), a split product of C4 that binds covalently to the target tissue and can arise from the classical and mannose-binding lectin (MBL) pathways; C1q (DakoCytomation, 1:800), which reflects activation of the classical complement pathway; MBL (Sigma-Aldrich Biotechnology, 1:500), which reflects activation of the lectin pathway; properdin (kindly provided by the Department of Nephrology, Leiden University Medical Center, 1:200), which reflects activation of the alternative complement pathway; and C3d (Abcam, 1:800) and SC5b-9 (Quidel, 1:150), both of which are formed by activation of any of the three aforementioned pathways. To identify apoptotic cells, the samples were immunostained for caspase-3 (Cell Signaling Technology Inc., 1:300). Immunohistochemistry was performed after the sections were deparaffinized and treated for antigen retrieval. Staining was visualized using the appropriate HRP-labelled secondary antibodies with diaminobenzidine as the chromogen. Finally, the sections were counterstained with hematoxylin.

To examine the presence of immunoglobulin deposits in the human glomeruli, immunofluorescence was performed for IgA, IgG, and IgM separately. First, the sections were treated with protease XXIV (SigmaAldrich) at 37°C for one hour. The sections were then incubated for one hour with FITC-labeled rabbit anti-human IgA (DakoCytomation; 1:20), FITC-labeled goat anti-human IgG (Protos Immuno Research; 1:25), or FITC-labeled rabbit anti-human IgM (DakoCytomation; 1:20). To study classical complement activation in the mouse kidneys, frozen sections were stained for C4d using a rat monoclonal anti-C4 antibody (Cedarlane Laboratories, 1:200), which binds to murine C4, C4b, and C4d. To identify endothelial cells, adjacent sections were stained for CD31 using a rat monoclonal anti-CD31 antibody (BD Pharmingen, 1:100). A FITC-labelled secondary antibody was used to visualize the primary antibodies.

QUANTIFICATION OF IMMUNOHISTOCHEMISTRY AND IMMUNOFLUORESCENCE

The human kidney sections were scored histologically by an experienced renal pathologist who was blinded with respect to the subjects' clinical data. Each stained sample was evaluated and scored by two independent observers. Because the renal pathological manifestations of preeclampsia are present in the glomerulus, we scored the staining of the various markers in the glomerulus only, scoring ≥50 glomeruli per section. The immunostained complement components were scored semi-quantitatively as follows: 0 represents an absence of—or traces of—glomerular staining; 1 represents segmental glomerular staining; and 2 represents global staining of the glomeruli. If positive (i.e., a score of 1 or 2), the kidney sections were further classified as having either focal (10-50% of the glomeruli) or diffuse (>50% of the glomeruli) deposits. Caspase-3 staining was analyzed by counting the number of caspase-3–positive cells in 50 glomeruli and comparing the number of positive cells between the study groups. For immunofluorescence, the slides were analyzed for either the absence or presence of immune deposits in the glomeruli using both a fluorescence microscope (DM5500B, Leica Instruments) and a confocal laser-scanning microscope (LSM 700, Zeiss).

STATISTICAL ANALYSIS

Categorical variables were analyzed using the Chi-square test. Differences in quantitative parameters between groups were analyzed using a one-way ANOVA (for normally distributed data) or the non-parametric Kruskal-Wallis test (for non-normally

distributed data). Correlations between ordinal data and numerical data were calculated using a Spearman's or Pearson's coefficient, respectively. All analyses were performed using the SPSS statistical software package (version 20.0; IBM Corp.). Differences with $p<0.05$ were considered to be statistically significant.

Results

CLINICAL DATA

The clinical characteristics of the human subjects included in the study were previously described, and are shown in chapter 4, page 100.[23] The hypertensive control group was significantly older than the other study groups ($p<0.05$); no other significant differences were observed with respect to the remaining clinical characteristics.

HISTOLOGY

The majority (82%) of the women with preeclampsia had prominent glomerular lesions, including various degrees of endotheliosis, podocyte swelling, and double contours of the glomerular basement membrane (also known as tram tracking). As previously published, in this cohort, endotheliosis was present in 55% of the samples from the women with preeclampsia; in contrast, endotheliosis was less prevalent in the pregnant controls and hypertensive controls (12% and 15%, respectively; $p<0.05$ versus the patients with preeclampsia).[23] Tram tracking and podocyte swelling were present exclusively in the women with preeclampsia (in 36% and 18% of patients, respectively; $p<0.05$ versus the control groups).

IMMUNOHISTOCHEMISTRY

To study complement activation, we stained the kidney sections for several complement components. Figure 1 shows typical examples of immunostained adjacent kidney sections from a patient with preeclampsia ("PE"), a pregnant control ("PC"), and a hypertensive control ("HC"). The glomeruli in all 11 preeclamptic patients were positive for C4d; in contrast, only 15/25 (60%) pregnant controls and 3/14 (21%) non-pregnant hypertensive controls were positive for C4d. The C4d staining in the glomeruli was either segmental or global. Positive C4d staining was strongly associated with preeclampsia ($p<0.0001$). The presence of C4d correlated significantly ($p<0.05$) with endotheliosis and tram tracking. C1q was detected in 9/11 (82%) of the kidney sections obtained from women with preeclampsia; in contrast, C1q was detected in only 6/25 (24%) of the pregnant controls and 2/14 (14%) of the non-pregnant hypertensive controls. Positive C1q staining was significantly associated with preeclampsia ($p<0.01$), and C1q staining and C4d staining were positively correlated ($p<0.0001$). Detailed information regarding the staining patterns of C4d and C1q in the patient and control groups is given in Figure 1.

MBL was present in one preeclamptic patient and one pregnant control; in both samples, the staining pattern was segmental; no significant differences were found between cases and controls with respect to MBL staining. Properdin was not detected in any of kidney samples.

With respect to C3d, the staining pattern was usually segmental. In preeclamptic patients, 5/11 (45%) of the kidney sections were positive for C3d; in contrast, only 2/25 (8%) of the pregnant controls and 2/14 (14%) of the non-pregnant hypertensive controls were positive for C3d subjects. No significant difference was found between the patient and control groups, and no correlation was found between C4d and C3d staining (data not shown). The most abundant C5b-9 staining was detected in sclerotic glomeruli; C5b-9 was present only rarely in functioning glomeruli. However, a significant correlation ($p<0.05$) was found between C5b-9 and C3d staining (Figure S1 shows typical staining patterns of C3d and C5b-9 in the patient and control samples). In all of the aforementioned immunostained sections, all of the positive kidney sections had diffuse deposits of complement components. Finally, no significant

difference was found between the patient and control groups with respect to caspase-3 staining. Specifically, the samples from preeclamptic women had an average of 0.05 caspase-3–positive cells/glomerulus, and the samples from the pregnant controls and hypertensive controls averaged 0.02 and 0.12 caspase-3–positive cells/glomerulus, respectively.

IMMUNOFLUORESCENCE

IgA was not detected in any of the samples. In contrast, IgG deposits were detected at weak levels in a mesangial pattern. No significant difference was found between the three groups with respect to IgG positivity, with 27%, 8%, and 21% of the preeclamptic patients, pregnant controls, and non-pregnant hypertensive controls, respectively, testing positive for IgG. IgM (Figure 2) was detected in 36%, 4%, and 21% of the preeclamptic patients, pregnant controls, and non-pregnant hypertensive controls, respectively ($p<0.05$ between the groups) (Figure 2C). Typical examples of IgM-positive and IgM-negative sections are shown in Figure 2A and 2B, respectively. We also measured the prevalence of IgM staining based on whether the sections were C4d-positive or negative (Figure 2D). We found that 14% of the 21 C4d-negative kidney sections contained IgM deposits; 7% of the 14 kidney sections with segmental C4d staining contained IgM, and 27% of the 15 kidney sections with global C4d staining contained IgM. Although IgM deposits were more prevalent in the kidney sections with global C4d, this correlation was not significant. In contrast, the presence of IgM was correlated significantly with tram tracking ($p<0.001$).

RELATIONSHIP BETWEEN CLINICAL CHARACTERISTICS AND C4D

Among the 36 samples obtained from the preeclamptic patients and pregnant controls, 10 samples were negative for C4d, 11 samples were C4d-positive with segmental staining, and 15 samples were C4d-positive with global staining. Global C4d deposits were significantly correlated with increased gestational age ($p<0.05$), whereas C4d-negative staining was not correlated significantly with gestational age. Neither the level of proteinuria nor peak blood pressure was correlated with the pattern of C4d staining.

sFlt-1 MOUSE MODEL OF PREECLAMPSIA

Next, we used a sFlt-1 mouse model of preeclampsia[3, 20-22] to study whether sFlt-1–induced endothelial damage is associated with complement activation. As reported previously, injecting sFlt-1 into the tail vein caused a preeclampsia-like phenotype, with significantly elevated blood pressure, urinary albumin secretion, and endotheliosis (a characteristic renal lesion in preeclampsia), which was measured using open capillary volume.[3, 20] The percentage of C4-positive kidneys in the sFlt-1–injected mice was significantly higher than in the control mice (Figure 3). The sFlt-1–injected mice had significantly more C4-positive glomeruli ($p<0.05$; Figure 3C). Confocal immunofluorescence microscopy revealed that the C4 deposits were present on the endothelial cells. This finding was confirmed by studying adjacent sections, in which C4 and CD31 co-localized, indicating complement activation in endothelial cells.

Discussion

The mechanisms that underlie the renal pathology in preeclampsia are poorly understood. Here, we report that the kidney sections from all of the preeclamptic women in our study were positive for C4d deposits. In contrast, C4d deposits were significantly less prevalent in two control groups comprised of non-hypertensive pregnant women and non-pregnant women with chronic hypertension. Importantly, the significant correlation between C4d deposits and the classical complement pathway component C1q in glomeruli strongly suggests that the classical complement pathway was activated. The lack of significant correlation between C4d and MBL, and the absence of properdin staining, makes it extremely unlikely that complement activation is attributed to either the lectin or

alternative complement pathway. The hypothesis that angiogenic dysregulation plays an important role in triggering complement activation in the kidney is supported by our finding of excessive numbers of C4 deposits in the glomeruli of sFlt-1-injected mice, an established model of preeclampsia. Taken together, these findings suggest that preeclampsia is associated with activation of the classical complement pathway in the kidney.

We previously described a relationship between preeclampsia and classical complement pathway activation in the placenta.[5] Thus, both the current study and our previous study raise the question of what drives activation of the classical complement pathway in the setting of preeclampsia. In general, complement imbalance can arise from excessive activation and/or inadequate regulation of the complement system. Excessive complement activation can arise from angiogenic dysregulation, which is believed to cause the initial preeclampsia-related renal injury due to increased sFlt-1 levels preventing vascular endothelial growth factor (VEGF) from maintaining the renal endothelium.[3, 11] We hypothesized that the resulting endothelial damage in the kidney might drive excessive complement activation. In our study, the presence of the IgM isotype was significantly associated with preeclampsia. Although glomerular IgM deposits have been observed in a wide range of renal diseases, the role of these deposits has remained elusive, suggesting that these IgM deposits might not always be involved in the pathogenesis of these particular renal diseases. However, the presence of IgM deposits might have other explanations. First, they could reflect the binding of IgM antibodies to damaged endothelium. Natural IgM-antibodies play a major role in the clearance of damaged cells[24, 25], and they can bind to both hypoxic[26] and apoptotic cells[27, 28] through intracellular antigens that become externalized under these conditions. The binding of IgM antibodies to either hypoxic or apoptotic cells triggers the activation of the complement system.[26-28] Taken together, these studies strongly suggest that the initial endothelial damage —mediated via high

sFlt-1 levels in the kidneys of preeclamptic women—could trigger the binding of IgM antibodies, thereby activating the complement system. Our finding of classical complement pathway components in the glomeruli of women with preeclampsia, combined with excess deposits of C4 on glomerular endothelial cells in our sFlt-1–injected mice, highly support this hypothesis.

Secondly, the presence of IgM antibodies and the activation of the classical complement pathway in the kidneys of preeclamptic women could have resulted from auto-antibodies such as angiotensin II type 1 receptor agonistic antibodies (AT_1-AA),[29, 30] anti-phospholipid auto-antibodies,[31] and/or anti-laminin auto-antibodies.[32] In the context of preeclampsia, complement activation could result from these auto-antibodies binding to glomerular structures or by the deposition of circulating antibody–antigen immune complexes and their subsequent entrapment in renal tissue. In our study, although we observed glomerular immunoglobulins both in preeclamptic patients and in some controls, IgM was the only immunoglobulin isotype that was significantly more prevalent in the patients with preeclampsia. If immunoglobulin deposits had resulted from auto-antibodies, we would have expected to find increased IgG deposits in the kidneys of these women. Therefore, our observations suggest that it is unlikely that the glomerular complement deposits in the kidneys of the preeclamptic women were caused by auto-antibodies.

Inadequate regulation of the complement system may also have caused glomerular complement activation. In the kidney, several complement regulatory proteins are expressed at high levels,[33-35] suggesting the importance of renal complement regulation. In our study, we found no correlation between late complement cascade components and preeclampsia, suggesting that the complement cascade does not become activated—at least to an excessive degree—beyond the level of C3. Complement regulatory mechanisms may be responsible for this phenomenon. However, the association between preeclampsia and mutations in genes that encode complement

regulatory proteins suggests that inadequate complement regulation plays a role in preeclampsia.[7] Indeed, a putative mechanism for inadequate regulation is related to complement regulator factor H, which regulates both the alternative and classical complement pathways,[36] and mutations in factor H have been observed in relation to preeclampsia. Importantly, reduced levels of factor H have been related to angiogenic imbalance within the kidney.[37]

Regardless of which mechanism is responsible for renal complement activation in women with preeclampsia, understanding how complement activation contributes to the clinical manifestations of preeclampsia is essential. Given that complement activation is strongly associated with preeclampsia (and its characteristic angiogenic imbalance), inhibiting complement activation may be a promising therapeutic approach for targeting both the placental and renal manifestations of preeclampsia. Importantly, both proteinuria and the typical renal histological changes have been reversed in mouse models of preeclampsia that were treated using complement inhibitors.[8] In one patient with severe preeclampsia, the terminal complement inhibitor Eculizumab has been used successfully to reduce preeclamptic manifestations and to prolong pregnancy.[9]

PERSPECTIVES

Our study is the first to demonstrate extensive activation of the classical complement pathway in the kidneys of women with preeclampsia. The presence of excessive C4 deposits in our sFlt-1–induced preeclampsia mouse model strongly supports the notion that preeclampsia-related renal complement activation is initiated by endothelial damage. In summary, our results suggest that complement activation might contribute to renal injury in preeclampsia. Moreover, our findings provide evidence that inhibiting the complement system might significantly reduce both the renal and placental manifestations of preeclampsia.

NOVELTY AND SIGNIFICANCE

What is new?

- The kidney sections from all of the preeclamptic women in our study were positive for C4d deposits indicating that preeclampsia is associated with activation of the classical complement pathway in glomeruli.
- The hypothesis that angiogenic dysregulation plays an important role in triggering complement activation in the kidney is supported by our finding of excessive numbers of C4 deposits in the glomeruli of sFlt-1– injected mice, an established model of preeclampsia.

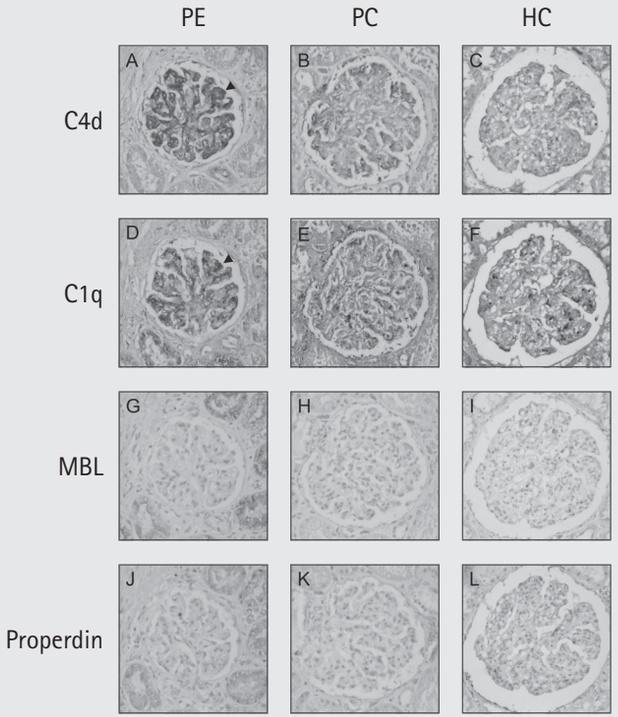
What is relevant?

- Our study suggests that initial endothelial damage —mediated via high sFlt-1 levels in the kidneys of preeclamptic women—could trigger the binding of IgM antibodies, thereby activating the complement system.
- Complement activation might contribute to renal injury in preeclampsia.
- Our findings provide evidence that inhibiting the complement system might significantly reduce the renal manifestations of preeclampsia.

Summary

The strong association between preeclampsia and renal C4d, C1q, and IgM levels suggests that the classical complement pathway plays a role in the pathogenesis of renal injury in preeclampsia. Moreover, our finding that sFlt-1–injected mice develop excess C4 deposits indicates that angiogenic dysregulation may play an important role in complement activation within the kidney. We suggest that inhibiting complement activation may be beneficial for preventing the renal manifestations of preeclampsia.

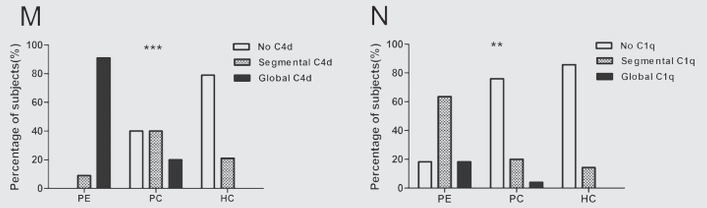
Figure 1



Immunohistochemistry of human kidney sections (full colour version inside cover)

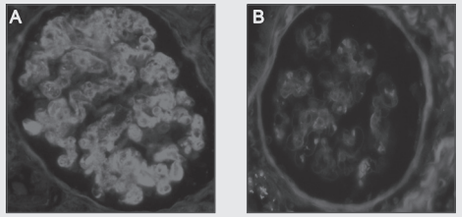
Adjacent sections of glomeruli were immunostained for C4d (A–C), C1q (D–F), mannose-binding lectin (G–I), or properdin (J–L). Each column contains adjacent sections and shows a single glomerulus. The left column shows a glomerulus from a patient with preeclampsia (PE), with global C4d staining. The middle column shows a glomerulus from a pregnant control (PC), with segmental C4d staining. The right column shows a C4d-negative glomerulus from a hypertensive control (HC). C1q staining was present in C4d-positive glomeruli (D) but also in C4d-negative glomeruli. In C4d-positive glomeruli, co-localization of C1q and C4d was observed (A and D). MBL was rarely observed (G-I) and properdin was never observed (J-L). Summary of the prevalence of each C4d (M) and C1q (N) staining pattern in the three groups. Kidneys sections from all preeclamptic patients were positive for C4d, with global staining in the majority of the kidney sections. In contrast, the majority of the pregnant and hypertensive controls showed a segmental or negative C4d staining pattern. Overall comparison revealed that C4d was significantly increased in preeclampsia (p<0.0001).

Figure 1



*Panel N shows the staining patterns for C1q. Overall comparison revealed that C1q was significantly increased in preeclampsia (p<0.01) (N). **p<0.01, ***p<0.0001.*

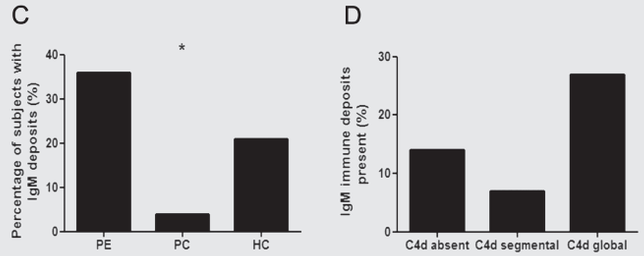
Figure 2AB



Immunofluorescence staining of IgM (full colour version inside cover)

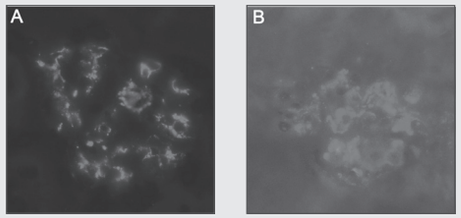
Representative images of an IgM-positive glomerulus (A) and an IgM-negative glomerulus (B). IgM deposits were significantly more prevalent in the kidney sections from the women with preeclampsia compared to the two control groups.

Figure 2CD



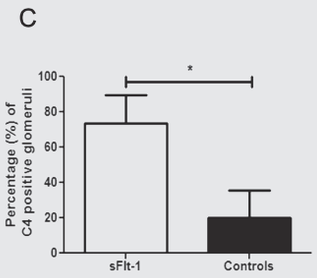
*Distribution of the percentage of IgM-positive sections based on C4d staining pattern (p>0.05) (C). *p<0.05. Distribution of IgM deposits according to C4d staining pattern Complement activation in the kidneys of sFlt-1–injected mice as a model of preeclampsia (D)*

Figure 3AB



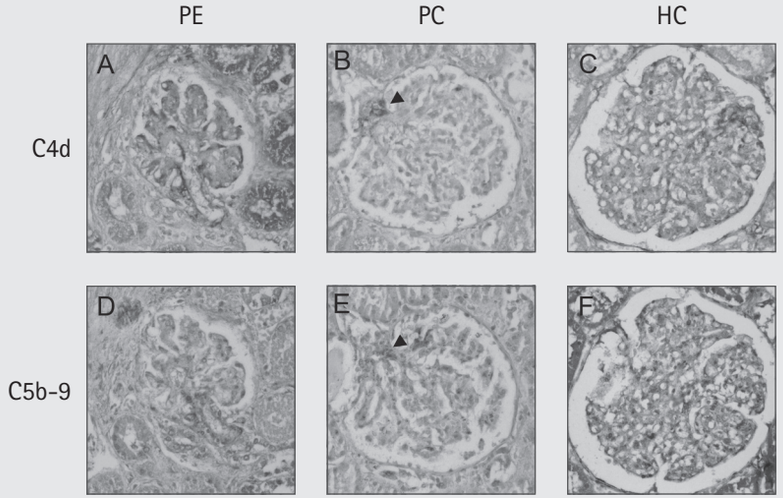
Representative images of C4 deposits in a glomerulus from a sFlt-1–injected (A) and control-injected (B) mouse.

Figure 3C



*Summary of the average (±SD) percentage of C4-positive glomeruli (C) in the kidneys of sFlt-1–injected mice (N=6 mice) and control mice (N=5 mice). *p<0.05.*

Figure S1



Immunohistochemical staining pattern of C3d and C5b-9 in human kidneys

Adjacent sections were immunostained for C3d (A–C) or C5b-9 (D–F). Each column represents an individual glomerulus. The left column shows a glomerulus from a patient with preeclampsia (PE), showing global staining. The middle column shows a glomerulus from a pregnant control (PC), with a segmental staining pattern (arrowhead). The right column shows a glomerulus from a hypertensive control (HC). C3d (A-C) deposits were observed in glomeruli from all study groups whereas C5b-9 (D-F) deposits were infrequently observed. However, C3d does co-localize with C5b-9 (arrowheads).

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