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The lectin pathway does not contribute to glomerular injury in the nephrotoxic nephritis model

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Short Title: Lectin pathway and glomerulonephritis

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Abstract

Aims:

Rapidly progressive crescentic glomerulonephritis occurs in a number systemic and primary glomerular diseases, including anti-glomerular basement membrane disease, anti-neutrophil cytoplasmic antibody vasculitis, and lupus nephritis. Our understanding of pathogenic mechanisms comes from animal models of disease such as the nephrotoxic nephritis model. The lectin pathway of complement activation has been shown to play a key role in several models of inflammation including renal ischaemia reperfusion. However, the lectin pathway is not required for crescentic glomerulonephritis in the anti-myeloperoxidase model of anti-neutrophil cytoplasmic antibody vasculitis. The aim of the current study was to explore the role of the lectin pathway in the nephrotoxic nephritis model, which is another model of crescentic glomerulonephritis.

Methods:

Nephrotoxic nephritis was induced in wild type and mannan-binding lectin-associated serine protease-2 deficient mice. Diseases was assessed by quanitfying glomerular crescents and macrophages, in addition to albuminuria and serum creatinine.

Results:

There was no difference between wild type and MASP-2 deficient mice in any of the histological or biochemical parameters of disease assessed. In addition there was no difference in the humoral immune response to sheep IgG.

Conclusions:

These data show that the lectin pathway of complement activation is not required for the development of crescentic glomerulonephritis in the nephrotoxic nephritis model, reinforcing previous findings in the anti-myeloperoxidase model.

Keywords: glomerulonephritis, complement, inflammation, lectin

Introduction

Rapidly progressive crescentic glomerulonephritis occurs in a number of diseases, including anti-glomerular basement membrane disease, anti-neutrophil cytoplasmic antibody vasculitis, and lupus nephritis. Complement is a system of proteins with activities that include the recruitment of inflammatory cells, cellular activation, cell lysis, antimicrobial defence, clearance of immune complexes, and amplification of the humoral immune response (1). Complement has been implicated in a number of inflammatory and autoimmune pathologies including glomerulonephritis (2). There are three distinct complement activation pathways; the classical, lectin and alternative. All three pathways share many common components but they differ in the nature of initiation. Upon the recognition and subsequent binding to pathogen derived oligosaccharides either by mannose-binding lectin (MBL) or the ficolins (L and H), conformational changes result in the autoactivation of MASP-2 which cleaves C4 in to C4a and C4b. MASP-2 is homologous to the classical pathway C1s and they are closely related. As in the classical pathway, C4b will bind to the surface of the pathogen thus inducing the binding of C2. C2 is subsequently cleaved and activated by MASP-2 to form C2b and C2a. The C4b2a complex forms the lectin pathway C3 convertase.

The identification of complement components on renal biopsy specimens from patients with glomerulonephritis suggests a role in pathogenesis. Further insights into the role of complement come from observations in animal models. In murine models of lupus, there are conflicting data on the role of complement. (3-10). This may be due to effects on both autoimmunity and inflammation. With regards to inflammation there may be proinflammatory effects and protective effects due to clearance of immune complexes or apoptotic cells. The autologous nephrotoxic nephritis model has been used as a model of rapidly progressive, crescentic glomerular nephritis for many years, with disease caused by cellular and humoral immunity a foreign antibody (sheep or rabbit), which acts as a planted glomerular antigen. Published work in this model with mice has suggested a protective role for the classical pathway, with increased disease in both C1q deficient (11) and C3 deficient (12) mice. However other observations have suggested a pathogenic role

for complement in some settings, with increased disease in mice deficient in the complement regulator CD59 (13). Studies in rats and rabbits suggested that complement was not required for the autologous phase (14,15). However, the depletion of complement in these studies with heat aggregated IgG and cobra venom factor respectively was less precise than the use of genetically modified mice. Hence in nephrotoxic nephritis, as in lupus models, there may be a balance between protective and pathogenic effects of complement.

Glomerulonephritis can also be induced in mice by passive transfer of anti-MPO IgG. This results in a focal segmental cresentic glomerulonephritis as seen in patients with anti-MPO antibodies and anti-neutrophil cytoplasmic antibodies. Several observations in this model (reviewed in (16)) support a pathogenic role for complement (17,18). A link between coagulation and crescentic glomerulonephritis is well established (19). Fibrin deposition, initiated physiologically by tissue factor, promotes disease, while activation of fibrinolysis through plasminogen activators activating plasminogen is protective (20,21). There is cross talk between the lectin pathway and coagulation. MASP2 is able to cleave prothrombin, leading to fibrin formation, though less efficiently than factor Xa (22). However, our previous data paradoxically showed evidence of increased prothrombin activation in MASP-2-deficient mice compared with wild-type mice (23). We also showed an increase in crescent formation in MASP-2 deficient mice in this anti-MPO vasculitis model, and wondered if this was due to a tendency to enhanced fibrin generation.

We decided to investigate the role of the lectin pathway in the nephrotoxic nephritis model for two reasons. Firstly, it would establish if the increase in crescent formation seen in the anti-MPO vasculitis model was generally applicable, occurring in other crescentic nephritis models, or if it was specific for vasculitis. If the former was the case, then the nephrotoxic nephritis model would be useful as a tool for further dissecting the mechanism responsible. The anti-MPO vasculitis model requires immunisation of MPO deficient mice to provide the anti-MPO IgG for passive transfer. There are therefore significant advantages in using the nephrotoxic nephritis model, in terms of resources and numbers of animals needed.

Materials and Methods

Mice

Wild type C57BL/6J mice were from Charles River laboratories (Margate, Kent, UK). MASP-2 deficient mice (24) have been described. They had been backcrossed at least 10 generations to C57BL/6J, and were provided by Omeros Corp, (Seattle, WA, USA) and bred at King’s College London. Age and sex matched mice aged 11-12 weeks were used. Animal experiments complied with the Animals (Scientific Procedures) Act 1986. They were approved by the King's College London Animal Welfare Ethical Review Board and by the UK Home Office (project license number PPL 70/7448).

Induction of glomerulonephritis

Mice were immunized subcutaneously with 200 µg sheep IgG, prepared in house with DEAE sepharose, in complete Freund's complete adjuvant (Sigma, Poole, UK). Five days later they were given an intravenous injection of nephrotoxic serum (150µl/20g), prepared by immunizing sheep with a mouse kidney preparation as described as described (25). Blood for serum creatinine was taken at baseline (from the saphenous vein) and at the end of the experiment (terminal anaesthesia). Spot urine samples for assessment of albuminuria were taken before starting the experiment and again at the end.

Assessment of disease

Urine creatinine was measured using a commercial creatinase assay (Diazyme, Dresden, Germany) based on the manufacturer’s instructions, with a standard curve generated for all assays. Serum creatinine was measured using liquid chromatography using tandem mass spectrometry (Evelina Children’s hospital, London, UK). Urine albumin was measured using ELISA (Bethyl laboratories, Montgomery, Tx, USA). For histological assessment, kidneys were fixed in Bouin’s solution with crescent formation and thrombosis assessed on PAS-stained sections. Crescent formation was defined as at least two layers of non-epithelial cells in Bowman’s space.

Thrombosis was scored out of 4 for each glomerulus, with 1-4 denoting up to 25%, 25-50%, 50-75%, and 75-100% thrombosis of the glomerular cross section. A minimum of 50 glomeruli were assessed for crescents and thrombosis. Kidney was also fixed in phosphate-lysine-periodate and transferred to 13% sucrose before freezing. Immunofluorescence staining for CD68 was then performed with clone FA11 (serotec, Oxford, UK). For Mannose-binding lectin (MBL) clone 16A8 (Hycult Biotechnology, Uden, Netherlands) was used. In both cases tissue was fixed with phosphate-lysine-periodate prior to freezing and sectioning. Detection was with DyLight 488 mouse anti-rat IgG (Jacksons Immunoresearch, West Grove, USA). For MBL quantification, glomeruli were manually selected as a region of interest and mean intensity quantified using the software Cell (Olympus, Southend-on-sea, UK). A minimum of 20 glomeruli per sample were included in all cases.

Humoral immune response

The humoral response to sheep IgG was assessed as described (26). Serum was from blood taken under terminal anaesthesia at the end of the experiment. In brief, ELISA plates were coated with an anti-immunoglobulin capture antibody and the detecting antibody was specific for the indicated subclass of mouse IgG. A standard serum from immunised mice was used to generate a standard curve and hence the data are expressed in arbitrary units.

Statistics

GraphPad Prism (GraphPad Software Inc., La Jolla, USA), with Student's t test was used where two groups were compared. Some data were logarithmically transformed before analysis if the variances of the groups were significantly different.

Results

Histological assessment of disease

Disease was induced in 10 wild type and 8 MASP-2 deficient mice. Spot urines were collected from all except 1 wild type mouse on day 6, giving urine albumin creatinine ratio data for 9 wild

type and 8 MASP-2 deficient mice respectively. One wild type and one MASP-2 deficient mouse died on day 7, giving histology and serum creatinine data for 9 wild type and 7 MASP-2 deficient mice respectively. All other mice were killed on day 7 and these data are presented. We assessed both glomerular crescent formation (Fig 1A) and glomerular thrombosis (Fig 1B) in wild type and MASP-2 deficient mice. There was no significant difference in either of these parameters. We also counted glomerular CD68 positive macrophages and again no differences were seen (Fig 1C). We confirmed that the lectin pathway was activated in this model as immunofluorescence staining showed glomerular deposition of MBL. There was no difference between the groups (Fig 1D). Representative PAS-stained sections with immunofluorescence staining for CD68 and MBL are shown in Figure 2.

Biochemical assessment of disease

We assessed function effects on the kidneys by measuring serum creatinine (Fig 3A) and albuminuria (Fig 3B). Baseline creatinines (μmol/L) were 9.00±0.50 and 9.96±0.40, and baseline urine albumin creatinine ratios (mg/mmol) were 2.6±0.12 and 2.9±0.58, in wild type and MASP-2 deficient mice respectively. Data for serum creatinine and urine albumin creatinine ratios are mean ± SEM, with no significant differences at baseline for either. Aligning with the lack of histological changes we found no differences in these biochemical readouts in mice with crescentic glomerulonephritis.

Assessment of the nephritogenic immune response

Using subclass-specific ELISAs we assessed the humoral response to sheep IgG in MASP-2 and wild type mice, at the end of the experiment. There were no differences between groups for any of the subclasses as shown in Fig 4.

Discussion

MASP-2 deficient mice develop robust disease in the autologous nephrotoxic nephritis model. There were no differences in histological or functional parameters when compared with wild type mice. Although the lectin pathway is part of the innate immune system, we thought it important to assess the humoral immune response between groups. Otherwise, a remaining possibility would have been that a difference in downstream inflammation was balanced by an opposing difference in the immune response. Since we found no difference in the immune response, we can conclude that this was not the case, and that MASP-2 had no effect on glomerular inflammation in this model. It is not clear why we found an increase in crescent formation in the anti-MPO vasculitis model but not in the nephrotoxic nephritis model. Although glomerular crescent formation occurs in both models, the pathways leading to this differ. There is far more glomerular thrombosis in the nephrotoxic nephritis model. This may have obscured findings related to cross-talk between the coagulation and complement systems. Further work will be needed to clarify this. The common finding of the previous anti-MPO vasculitis and nephrotoxic nephritis model is the lack of protection from disease in MASP-2 deficient mice. In this sense, the current report is not a new message, shows a consistent lack of protection in different models of crescentic glomerulonephritis.

The lectin pathway is initiated by binding of MBL, ficollins or collectin-11 to carbohydrates on microbes or to abnormal glycosylation patterns on apoptotic, necrotic, malignant of hypoxic cells (27). MASP-2 has been shown to play a role in several other models of inflammation, including renal ischaemia reperfusion (28). Other models are largely those where the primary stimulus for inflammation is ischaemia, for example in the gut, heart (24) and brain (29). This suggests that in these settings the lectin pathway is essential for disease. This does not appear to apply with immune mediated crescentic glomerulonephritis, as shown by the current report and our previous data (23). Despite this finding, we showed evidence that the lectin pathway was activated as MBL was deposited (Figure 2). However, because the study was negative we did not consider it fruitful to try and define the carbohydrate structures responsible for MBL binding in this model.

Although this is a negative study we thought it was important to publish these data for a number of reasons. A bias towards publishing positive data pervades science and this skews the scientific record. Our data are not controversial since there are no previous reports on the role of MASP-2 in the nephrotoxic nephritis model. Therefore, publication of our negative data may prevent others addressing the same question, and potentially wasting resources. This is particularly relevant important when the work involves animal experiments as in the current paper. The balance of protection and pathogenicity shown in previous studies in mice (11-13) is consistent with the lack of effect in the rabbit and rat studies (14,15). The precision afforded by the availability of strains with a genetic deletion of specific complement components means that the studies in mice have been the most informative and are of potential relevance to human disease. However, the limitations in the models and differences between the complement system in mice and humans (30) need to be considered when extrapolating the results to human disease.

Therapeutic reagents based on the lectin pathway are under development and it will be important to define the diseases in which lectin pathway activation contributes to inflammation. Of particular note, the FDA has granted breakthrough designation to OMS721, a monoclonal antibody against MASP-2, for IgA nephropathy. Promising reductions in proteinuria have been seen in a small number of patients (data presented in abstract form). Mesangial proliferation is the major pathology in IgA nephropathy and the pathogenesis is not reflected in the nephrotoxic nephritis model. Consequently our data do not argue against a role for lectin pathway blockade in IgA nephropathy. However, our data do suggest that targeting the lectin pathway in other diseases with crescentic glomerulonephritis may not be beneficial. These includes systemic lupus erythematosus, ANCA vasculitis, and anti-GBM disease.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare

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Figures

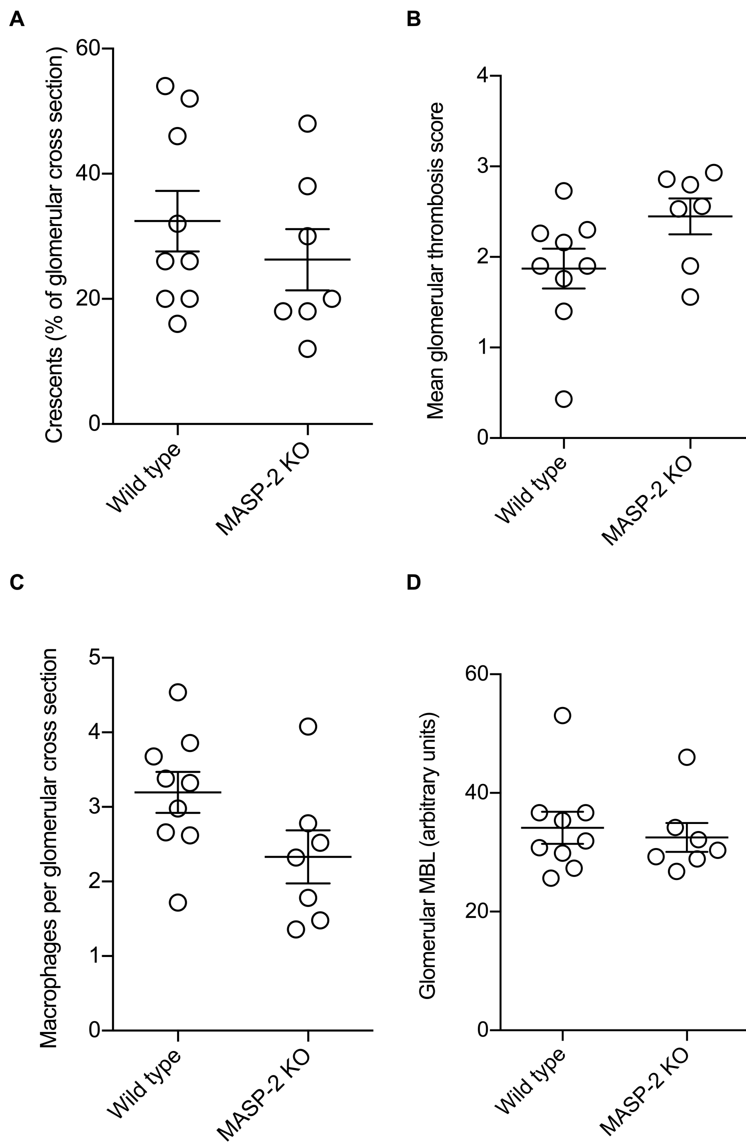

Figure 1. Histological parameters in wild type and MASP-2 deficient mice. Data are for shown for glomerular crescents (A), glomerular thrombosis and (B) glomerular CD68+ macrophages (C) and glomerular mannose-binding lectin (MBL) deposition (D).

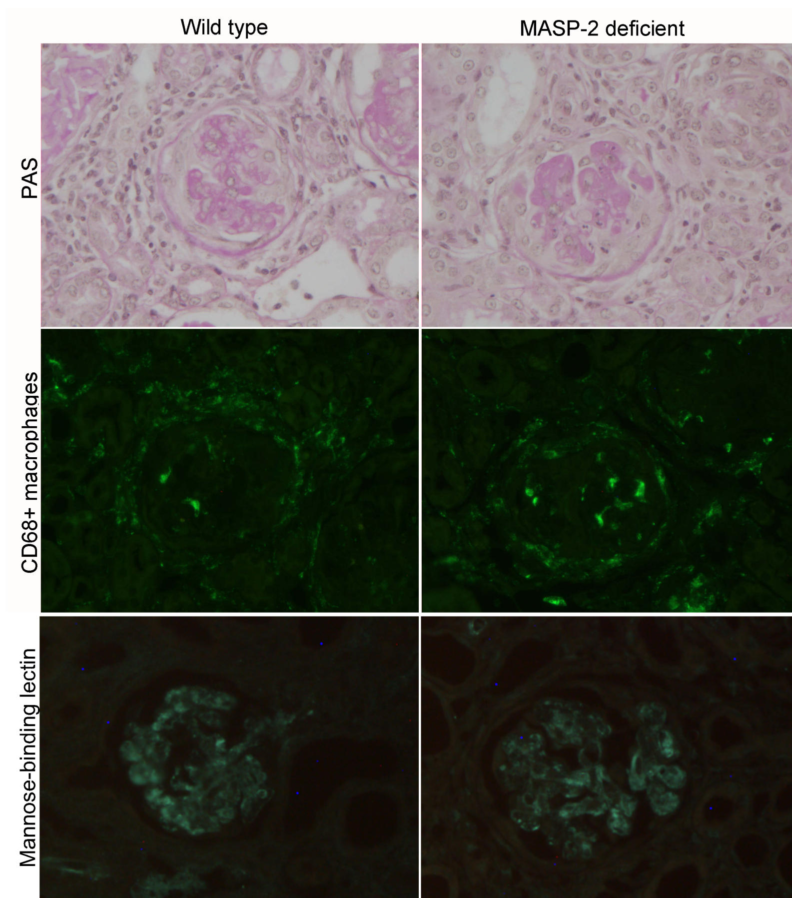

Figure 2. Representative histology for wild type and MASP-2 deficient mice. Periodic acid-Shiff stained sections, with immunofluorescence staining for CD68+ macrophages and mannose-binding lectin is shown.

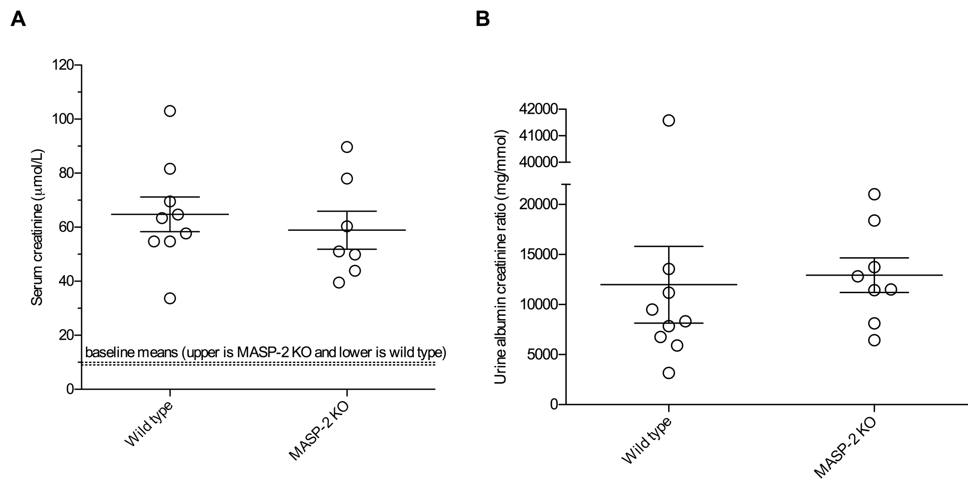

Figure 3. Biochemical disease parameters in wild type and MASP-2 deficient mice. Data are shown for serum creatinine (A), albuminuria (B).

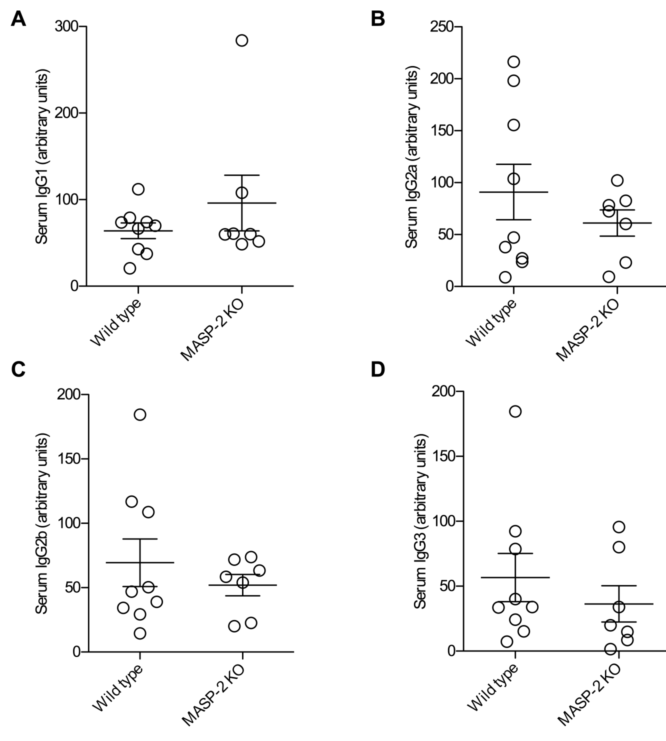

Figure 4. The humoral immune response to sheep IgG in wild type and MASP-2 deficient mice. IgG subclass specific ELISAs were performed using serum taken at the end of the experiment.