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Cross-Reactive Myelin Antibody Induces Renal Disease

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Abstract

Experimental autoimmune encephalomyelitis (EAE) is an autoimmune model for multiple sclerosis (MS). Previously, we reported renal immunoglobulin (Ig) deposition in mice with myelin oligodendrocyte glycoprotein (MOG₉₂₋₁₀₆) induced progressive-EAE and naïve mice injected with MOG₉₂₋₁₀₆ hybridoma cells producing antibody that cross-reacts with various autoantigens including double-stranded DNA. To assess whether MOG₉₂₋₁₀₆ antibodies actually induce kidney changes, the extent of renal Ig deposition and changes in glomerular histology and filtration were investigated. Mice with progressive-EAE exhibited Ig deposition, glomerular hypercellularity and proteinuria indicating kidney dysfunction. MOG₉₂₋₁₀₆ hybridoma cell injected mice also had Ig in the kidneys and proteinuria. Therefore, sensitization with MOG₉₂₋₁₀₆ and transfer of MOG₉₂₋₁₀₆ antibodies can induce both central nervous system and renal pathology. The renal involvement reported in MS is believed to occur as a side effect of nephrotoxic drugs or neurogenic bladder. Our results demonstrate that an autoimmune response against myelin could induce pathologic changes in the kidney and may help explain renal changes reported in patients with progressive MS.

Keywords

Autoantibodies; autoimmunity; CNS-demyelinating autoimmune disease; kidney disease

Introduction

Autoimmune diseases are generally classically divided into two categories, organ-specific and systemic autoimmune diseases. Organ-specific autoimmunity is restricted to a single organ or organ system and includes diseases such as diabetes and polymyositis. In contrast, systemic autoimmunity is not restricted to a single organ or organ system and includes systemic lupus erythematosus (SLE) and systemic sclerosis. Antigenic targets also differ between the two forms of autoimmunity, with the recognized autoantigens being expressed either in a tissue-specific manner or ubiquitously [1]. Although in some disease models or patients the two autoimmune disease types appear to be linked or overlap, whether the two suppress each other, cause one another or contribute to disease progression synergistically remains to be determined.

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Impaired renal function has been reported in patients with primary progressive (PP) and secondary progressive (SP) multiple sclerosis (MS) who had not been treated with nephrotoxic drugs [2]. Immunoglobulin (Ig) and complement (C1q) deposition have also been detected in glomeruli in kidneys of patients with MS [3]. Yet, renal function has generally been considered to be normal and any renal damage observed ascribed to either renal toxicity of drugs or bladder dysfunction induced by damage to the central nervous system (CNS) (neurogenic bladder). Autoantibodies against kidney, liver, heart and brain have been detected in sera of patients with MS [4] and higher levels of natural autoantibodies have been found in cerebrospinal fluid (CSF) compared to both healthy controls and patients with other neurological diseases [5]. In addition, circulating immune complexes and hypocomplementemia have been demonstrated in patients with MS [6,7]. These reports suggest that systemic autoimmunity or immune mediated disease outside the CNS exists in some patients with MS, which has classically been considered to be an organ-specific autoimmune disease.

Support for a link between CNS and systemic autoimmune disease is provided by reports of CNS antibodies, inflammation and/or demyelinating disease similar to MS in systemic autoimmune diseases, such as myasthenia gravis, rheumatoid arthritis, autoimmune thyroid disease and SLE [7-12]. Involvement of organs outside the CNS in the pathogenesis of MS is not clear and systemic autoimmune contributions have not been explored. It also remains to be determined whether there is a transitional form between CNS (organ)-specific versus systemic autoimmune diseases, or whether relapsing-remitting MS (organ-specific) transitions into a more systemic disease resulting in SP-MS.

A.SW mice sensitized with amino acids 92-106 from myelin oligodendrocyte glycoprotein (MOG) developed a PP form of experimental autoimmune encephalomyelitis (EAE), an autoimmune animal model of MS, which is traditionally considered to be organ-specific [13,14]. Surprisingly, mice with PP-EAE had antibody deposition in the glomeruli of their kidneys [13,14]. We found that 93% of A.SW mice with MOG₉₂₋₁₀₆ induced PP-EAE had robust (not quantitated) antibody deposition in the glomeruli [14].

To investigate the contribution of MOG₉₂₋₁₀₆ antibodies in EAE, hybridoma cell lines were generated from an A.SW mouse with progressive EAE. Two hybridoma cell lines producing MOG₉₂₋₁₀₆ antibodies were characterized. The antibodies were found to be of the IgM isotype and polyreactive, recognizing not only the initiating antigen, but also ubiquitously expressed antigens associated with systemic autoimmune disease such as double-stranded DNA (dsDNA) and histone H1a, as well as protein isolated from organs other than the CNS [14]. The MOG₉₂₋₁₀₆ reactive antibodies were also lymphocytotoxic *in vitro*, suggesting they contribute to systemic pathology. Injection of hybridoma cells producing MOG₉₂₋₁₀₆ antibody into naïve mice resulted in antibody deposition in glomeruli in kidney. All together, this demonstrates that MOG₉₂₋₁₀₆ reactive natural antibodies recognize a variety of antigens including several ubiquitously expressed antigens and that passive transfer of the antibodies recapitulated features of systemic autoimmunity that had also been observed in A.SW mice with progressive EAE [14].

In the current study, we examined whether the manifestations of systemic autoimmunity detected in A.SW mice with progressive EAE and naïve mice injected with MOG₉₂₋₁₀₆ antibody producing hybridomas were pathologic resulting in kidney dysfunction. To assess whether MOG₉₂₋₁₀₆ antibodies caused kidney dysfunction, we measured the extent of renal Ig deposition, cellularity of glomeruli, and changes in urea and creatinine in plasma and urine of mice sensitized with MOG₉₂₋₁₀₆ or injected with hybridoma cells. Mice with PP-EAE exhibited renal disease in the form of Ig deposition, glomerular hypercellularity and proteinuria. Adoptive transfer of hybridoma cells producing MOG₉₂₋₁₀₆ reactive antibodies

into immunocompetent mice resulted in renal pathology comprising Ig deposition in the kidney and proteinuria. However, transfer of hybridoma cells producing MOG₉₂₋₁₀₆ reactive antibodies into nude mice induced only mild renal pathology with Ig deposition in tubules and glomeruli in the kidney and increased urea in the serum, but proteinuria was not detected suggesting a possible role for mature T cells in disease.

A potential mechanism that could explain why injection of hybridoma cells producing MOG₉₂₋₁₀₆ reactive antibodies resulted in Ig deposition in the kidneys could be that antibodies purified from these hybridoma cell lines, A4ac and A4cd, also cross-react with dsDNA [14]. It is known that anti-dsDNA antibodies can cause kidney dysfunction (reviewed in [15]). In the current study, we determined that other hybridoma cell lines, which also cross-react with dsDNA, also resulted in Ig deposition in the kidneys. Therefore, both sensitization with MOG₉₂₋₁₀₆ and transfer of MOG₉₂₋₁₀₆ antibodies that cross-react with dsDNA are capable of inducing not only demyelination, but also renal disease. These data suggest that renal involvement could be immune mediated in MS.

Materials and methods

Mice and EAE induction

SJL/J and A.SW mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). BALB/c nude mice were purchased from the National Cancer Institute (Frederick, MD, USA). Four to six week old A.SW mice were immunized subcutaneously at the base of the tail with 100 nmol of MOG₉₂₋₁₀₆ peptide (DEGGYTCFFRDHSYQ) (Core Facility of the University of Utah, Salt Lake City, UT, USA) [16,17] in complete Freund's adjuvant (CFA) containing 2 mg/ml *Mycobacterium tuberculosis* H37 Ra (Difco Laboratories, Detroit, MI, USA), or with CFA alone. Mice were weighed and observed for clinical signs. Ataxic signs of EAE were assessed according to the following criteria: 0 = no clinical disease; 1 = mice turning their heads or bodies to one side with or without a waddling gait; 2 = mice leaning significantly to one side and falling while walking; 3 = mice continuously rolling by twisting their bodies or rotating laterally in a circle; 4 = mice can not stand but lie on their sides with or without rolling; and 5 = moribund state or death [13,18].

Hybridoma cells and injection

Anti-MOG₉₂₋₁₀₆ hybridoma cell lines, A4ac and A4cd, were produced from an A.SW mouse with MOG₉₂₋₁₀₆ induced progressive-EAE [14,19]. The anti-lipopolysaccharide (LPS) hybridoma cell line, XMMEN-OE5, was a kind gift from Dr. Moses Rodriguez (Mayo Clinic, Rochester, MN, USA). The anti-oligodendrocyte hybridoma cell line, O4, was a kind gift from Dr. Cornelia Bergmann (Lerner Research Institute, Cleveland, OH, USA). Sp2/0-Ag14 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Hybridoma cells were administered as previously described [14,20]. SJL/J and nude mice were injected intraperitoneally with 1×10^7 A4ac, A4cd, O4, XMMEN-OE5 or Sp2/0 hybridoma cells, monitored daily for weight change and development of ascites and euthanized using isoflurane at the indicated time points or one month after injection.

Serum IgG and IgM ELISAs

Blood was collected from mice by cardiac puncture at the time of sacrifice. Enzyme-linked immunosorbent assays (ELISAs) were performed to measure the amount of serum IgM and IgG. Ninety-six-well plates were coated with 10 µg/ml cyto-anti-mouse IgG (H + L) (The Binding Site, Birmingham, UK) in phosphate-buffered saline (PBS) and allowed to absorb overnight in a humidified box at 4°C. Nonspecific binding was blocked with 10% Cosmic calf serum (CCS) (HyClone, Logan, UT, USA) and 0.2% Tween 20 (Sigma-Aldrich, St.

Louis, MO, USA). Serial dilutions of sera were added to the plates and incubated for 90 minutes at room temperature. After washing, peroxidase-conjugated goat anti-mouse IgM (Stressgen Biotechnologies, Victoria, BC, Canada) or IgG(γ) (Caltag Laboratories, Burlingame, CA, USA) were added to the wells and incubated for 90 minutes at room temperature. Immunoreactive complexes were detected with *o*-phenylenediamine dihydrochloride (Sigma-Aldrich) and were read at 492 nm in a Titertek Multiskan Plus MK II spectrophotometer (Flow Laboratories, McLean, VA, USA).

Serum anti-MOG₉₂₋₁₀₆ IgG and IgM ELISAs

ELISAs were performed to measure the level of serum MOG antibody as described previously [13]. Briefly, 96-well flat-bottom Nunc-Immuno MaxiSorp™ plates (Nalge Nunc International, Rochester, NY, USA) were coated overnight with 10 μ g/ml MOG₉₂₋₁₀₆ peptide. After blocking with 10% CCS and 0.2% Tween 20, serial dilutions of sera were added to the plates and incubated for 90 minutes at room temperature. After washing, a peroxidase-conjugated anti-mouse IgG (γ) (Caltag Laboratories) or anti-mouse IgM (Stressgen Biotechnologies) were added for 90 minutes. Immunoreactive complexes were detected as described above.

Polyreactivity ELISA

Reactivity of the antibodies produced by the hybridomas to dsDNA (Sigma-Aldrich) and proteins isolated, as described previously [14], from mouse blood and liver was assayed by ELISA. Briefly, the antigens were coated onto Nunc-Immuno MaxiSorp™ 96-well plates (Nalge Nunc International) at a concentration of 10 μ g/ml in PBS and allowed to absorb overnight at 4°C in a humidified chamber. After blocking with 10% CCS and 0.2% Tween 20, serial dilutions of supernate from the hybridomas were added to the plates and incubated for 90 minutes. After washing, peroxidase-conjugated anti-mouse IgM was added for 90 minutes. Immunoreactive complexes were detected as described above.

Biochemistry

Twenty-four hour urine samples were collected using metabolic cages, with free access to food and drinking water. Serum was obtained as described above. Levels of urea and creatinine in plasma and urine were determined using the Quantichrome™ Urea Assay Kit (BioAssay System, Hayward, CA, USA) and the Quantichrome™ Creatinine Assay Kit (BioAssay Systems) according to the manufacturer's instructions. Levels of protein in urine were measured using either Uristix® urine dipsticks (Bayer Corporation, Elkhart, IN, USA) or the BCA method.

Histology

Mice were euthanized using isoflurane, exsanguinated and perfused with PBS followed by a buffered 4% paraformaldehyde solution and organs were removed. Tissues were then embedded in paraffin. Four-micrometer thick tissue sections were stained with hematoxylin and eosin.

Immunohistochemistry

IgM and C1q deposition were visualized using the avidin-biotin peroxidase complex (ABC) technique, with biotin-conjugated mouse IgM antibody (1:100 dilution, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA), biotin-conjugated donkey anti-mouse IgG (H+L) (1:200 dilution, Jackson ImmunoResearch Laboratories, Inc.) or biotin-conjugated mouse C1q antibody (1:1000 dilution, Dako Corporation, Santa Barbara, CA, USA). IgM and IgG deposition was visualized by immunofluorescent staining using biotin-conjugated goat anti-mouse IgM (1:100 dilution, Jackson ImmunoResearch Laboratories,

Inc.) or biotin-conjugated donkey anti-mouse IgG (H+L) (1:100 dilution, Jackson ImmunoResearch Laboratories, Inc.), followed by streptavidin fluorescein isothiocyanate (FITC) (1:50 dilution, BD Pharmingen, San Diego, CA, USA). Fluorescent images were collected and analyzed by laser-scanning confocal microscopy.

Renal pathology

Histologic assessments were performed on coded slides. Glomerular hypercellularity was assessed as other groups have described [21-23]. The total number of glomerular cell nuclei were counted in 20 randomly selected glomeruli per mouse and the average number of cells per glomerular cross-section (c/gcs) determined.

The extent of glomerular antibody deposition was analyzed by randomly selecting 20 glomeruli. Individual glomeruli were divided into four quadrants. Each quadrant containing Ig deposition was given a score of 1. The total number of positive quadrants was determined, then divided by the total number of quadrants and multiplied by 100 to give the percent involvement of Ig deposition.

Results

Renal Ig deposition, hypercellularity and proteinuria in mice with MOG₉₂₋₁₀₆ induced PP-EAE

Previously, we detected Ig deposition in the glomeruli in animals with PP-EAE compared to animals in the preclinical stage of the disease [14]. In the current study, we compared proteinuria, glomerular cellularity and quantitated the extent of antibody deposition in naïve A.SW mice and mice injected with MOG₉₂₋₁₀₆ emulsified in CFA or, as control, CFA alone. A.SW mice with PP-EAE had significantly more Ig deposition in the glomeruli compared to naïve A.SW mice ($P < 0.01$, t -test; Figure 1(A)-(D)). Mice injected with CFA alone also tended to have more Ig deposition in their kidneys than naïve mice, but the difference did not reach significance ($P = 0.12$, t -test). Complement C1q deposition did not correlate with Ig deposition (data not shown), suggesting that the renal pathology observed was not complement dependent. Antibody has been shown to alter the size and charge selectivity of the glomerular basement membrane in the absence of complement [24-26].

Naïve mice or mice injected with adjuvant alone had similar numbers of cells in their glomeruli (Figure 1 (F)). In contrast, hypercellularity was observed in the kidneys of mice with PP-EAE. Mice with PP-EAE had significantly more cells per glomerular cross-section in comparison to naïve mice ($P < 0.05$, t -test). Overall, the same trend was observed in proteinuria as was detected in cellularity of glomeruli and extent of Ig deposition (Figure 1 (E)). However, the difference between the groups did not reach statistical significance.

Transfer of MOG₉₂₋₁₀₆ hybridoma cells to naïve mice induces Ig deposition and proteinuria

Previously, we reported that mice injected with hybridoma cells producing MOG₉₂₋₁₀₆ antibodies contained more Ig in their kidneys than control mice at a single time point [14]. To determine whether the antibody found in the kidneys of mice was indicative of kidney dysfunction, a time course study was undertaken. SJL/J mice were injected with the MOG₉₂₋₁₀₆ reactive hybridoma cell line (A4cd), and were euthanized at weekly time points. Kidney function was assessed by measuring the levels of creatinine and urea in both plasma and urine, and protein in the urine. Urine was collected using metabolic cages. Blood and tissues were harvested from mice weekly. Total urine volume, protein and creatinine in both plasma and urine were measured. IgM deposition was confirmed by immunofluorescent staining in the glomeruli of the kidneys of mice injected with A4cd hybridoma cells in media (Figure 2 (A)), but not control mice injected with media alone (Figure 2(B)). IgM was

deposited in a linear pattern in the glomeruli. A linear pattern of Ig deposition in the glomeruli is the characteristic finding in human patients with Goodpasture's syndrome, in which antibodies target the glomerular basement membrane. In contrast, circulating immune complexes exhibit a granular pattern of deposition in the glomeruli [27]. Therefore, the linear pattern of Ig deposition in the glomeruli of mice injected with MOG₉₂₋₁₀₆ hybridomas suggests that the antibodies are recognizing intrinsic fixed antigens. Proteinuria was detected in mice at 5 weeks post-injection, and by 6 weeks, mice injected with MOG₉₂₋₁₀₆ hybridoma cells had significantly more protein in their urine compared to control mice ($P < 0.05$, *t*-test), indicating that kidney function was altered (Figure 2 (C)). Development of proteinuria corresponded to the time at which Ig deposition was detected in the kidneys. Proteinuria is one of the best indicators of kidney disease or renal involvement in systemic disease [20,28]. It can be detected as early in the disease course as the asymptomatic preclinical phase [28]. These results suggest that MOG antibody plays an important role in kidney disease in this model. MOG₉₂₋₁₀₆ hybridoma cell injected mice have increased amounts of MOG₉₂₋₁₀₆ antibodies in their serum compared to controls, but similar total amounts of IgM and IgG (data not shown). Serum IgM and IgG and proteinuria did not correlate, suggesting that an increase in Ig concentration is not the direct cause of the proteinuria.

Changes in creatinine clearance were also observed in SJL/J mice injected with the MOG₉₂₋₁₀₆ reactive hybridoma cells (Figure 2 (D)). Creatinine is produced at a constant level as a byproduct of muscle metabolism and filtered without reabsorption through the kidneys [28]. Creatinine levels in the serum and creatinine clearance are useful in monitoring changes in renal function, but are not the most reliable indicators of early kidney disease [29]. As much as 50% of glomerular surface area can be damaged before significant differences in serum creatinine levels and creatinine clearance are detected, due to compensation by increases in extrarenal metabolism and secretion by renal tubules [29]. Decreases in creatinine clearance were observed in mice injected with A4cd starting at 1 week after injection, but statistical significance was not reached during the observation period. This suggests that there were changes in kidney function, but that the disease was not severe enough to significantly alter creatinine clearance. Histological analyses revealed no significant cellular proliferation within the glomeruli of mice injected with the MOG antibody secreting hybridoma cells (Figure 2 (E)). However, the detection of Ig deposition in the glomeruli of the kidney and proteinuria provide evidence that the kidneys were damaged functionally.

Renal Ig deposition by hybridoma cells that produce antibodies cross-reactive with dsDNA

Purified MOG₉₂₋₁₀₆ antibodies, A4ac and A4cd, are natural IgM antibodies that react with a number of self and non-self antigens, including dsDNA. Although, the precise mechanism by which our MOG antibodies deposit in the kidney is unknown, reactivity against dsDNA is one plausible mechanism, since autoantibody responses against dsDNA have been associated with renal pathology in systemic autoimmune diseases, such as SLE (reviewed in [30]). This predicts that if IgM antibodies have cross-reactivity against dsDNA, injections of such antibodies (or hybridoma cells) should also result in Ig deposition in the kidney. To test this hypothesis, we used two other hybridoma cell lines producing antibodies that cross-react with dsDNA: 1) oligodendrocyte reactive natural IgM antibody O4 [31] and 2) anti-LPS antibody, XMMEN-OE5. While the polyreactivity of XMMEN-OE5 was not previously reported [32], we found that XMMEN-OE5 exhibited higher reactivity to dsDNA than A4cd or O4 (Figure 3 (B)). However, XMMEN-OE5 does not react with MOG₉₂₋₁₀₆ (Figure 3 (A)), or protein isolated from mouse blood (Figure 3(C)) or liver (Figure 3 (D)). As predicted, Ig deposition, without C1q deposition (data not shown), was detected in the glomeruli of the kidneys of mice injected with either O4 or XMMEN-OE5 (Figure 3 (E) –

(F)). Mice injected with a control hybridoma, 8.18C5, which does not exhibit cross-reactivity with dsDNA did not result in Ig deposition in the kidney (data not shown).

Renal Ig deposition and dysfunction after transfer of MOG₉₂₋₁₀₆ hybridomas to nude mice

It is possible that within the time frame of the experiment described above, the hybridoma cells are rejected in immunocompetent mice. Therefore, the experiment was repeated using nude mice. Nude mice were injected with the MOG₉₂₋₁₀₆ reactive hybridomas, A4ac and A4cd, or their non-secreting myeloma fusion partner, Sp2/0. The nude mice had to be sacrificed 9 days after injection with hybridoma cells, due to development of ascites in many of the mice. In contrast to immunocompetent mice injected with MOG₉₂₋₁₀₆ reactive hybridoma cells, nude mice had Ig deposition in their glomeruli even when the hybridoma fusion partner was injected (Figure 4 (A),(B)). Interestingly, less Ig deposition was observed in nude mice than was observed in immunocompetent mice injected with MOG₉₂₋₁₀₆ reactive hybridomas (Figures 2 (A) and 4(A),(C)). Due to detection of Ig in mice injected with the fusion partner, Ig deposition in the glomeruli of nude mice could not be used as an indicator of MOG₉₂₋₁₀₆ antibody cross-reactivity or binding in this experiment. However, all eight of the nude mice injected with the MOG₉₂₋₁₀₆ reactive hybridoma cells A4ac (Figure 4 (D),(F)) or A4cd (data not shown) had Ig in their tubules compared to none of the five mice injected with the fusion partner (Figure 4 (C),(D)), demonstrating that MOG₉₂₋₁₀₆ antibodies also cross-react with kidney antigens in nude mice. Similar Ig deposition was seen in the tubules of mice injected with A4ac and A4cd hybridoma cells.

Ig deposition and proteinuria were detected 5 weeks after injection of MOG₉₂₋₁₀₆ hybridoma cells into immunocompetent mice. Ascites or tumors in the peritoneal cavity were only detected in one mouse, requiring that the mouse be euthanized. In contrast, the absence of adaptive T cell responses in nude mice resulted in the development of ascites in all of the nude mice 9 days after hybridoma cell injection, resulting in a shorter observation period. Within the time frame of this experiment, no significant difference was observed in proteinuria between the groups of mice (data not shown). However, mice injected with A4cd had significantly more urea in their plasma than mice injected with the fusion partner ($P < 0.05$, *t*-test) (Figure 4 (G)) and significantly less creatinine in their plasma than mice injected with the fusion partner ($P < 0.01$, *t*-test) (Figure 4 (H)). Despite the lack of proteinuria, detection of Ig deposition and increased urea in the serum are suggestive of renal injury induced by transfer of MOG₉₂₋₁₀₆ hybridomas to nude mice.

Similar to the results observed in immunocompetent mice, MOG₉₂₋₁₀₆ hybridoma cell injected nude mice had increased amounts of antibodies reactive with MOG₉₂₋₁₀₆ and dsDNA in their serum compared to controls, but similar total levels of IgM and IgG (Figure 5 (A)-(D)). However, control nude mice had high serum anti-dsDNA antibody titers (Figure 5 (B)), which could explain the detection of Ig deposition in glomeruli in these mice.

DISCUSSION

Previously, we reported renal Ig deposition in mice with progressive EAE or naïve mice injected with hybridoma cells producing MOG₉₂₋₁₀₆ reactive natural antibodies [14]. In the current study, we confirm and extend these findings by demonstrating that the Ig deposition was indicative of kidney disease comprising proteinuria, glomerular hypercellularity and changes in urea and creatinine in serum and urine.

Mice with PP-EAE exhibited renal pathology, and had more Ig deposition, cells per glomerular cross-section and protein in their urine compared to naïve mice. Interestingly, mice injected with adjuvant (CFA) developed more Ig deposition in their kidneys and had increased protein in their urine compared to naïve mice, but less than that observed in mice

sensitized with MOG₉₂₋₁₀₆ emulsified in CFA. This suggests that activated T cells and adjuvant may be partially responsible for the kidney disease observed in mice with PP-EAE.

Adoptive transfer of hybridoma cells producing MOG₉₂₋₁₀₆ reactive antibodies into immunocompetent mice also resulted in renal pathology consisting of Ig deposition in the kidney and proteinuria. In contrast, transfer of hybridoma cells producing MOG₉₂₋₁₀₆ reactive antibodies into nude mice induced only mild renal pathology with Ig deposition in the tubules in the kidney and increased urea in the serum, but proteinuria was not detected. However, the observation period was much shorter, 9 days compared to 6 weeks, which may account for the difference in disease severity.

Interestingly, injection of other hybridoma cell lines producing antibodies that cross-react with dsDNA also resulted in Ig deposition in glomeruli. In addition, Ig deposition was detected in the glomeruli of nude mice that had high serum anti-dsDNA antibody titers, after injection of non-secreting myeloma cells. Thus, injection of hybridoma cells producing MOG₉₂₋₁₀₆ reactive antibodies could result in Ig deposition in the kidneys through the cross-reaction of A4ac and A4cd with dsDNA. Antibodies against dsDNA have been shown to react with and/or deposit in the kidney in several animal models of glomerulonephritis [20,33,34]. In addition, antibodies against dsDNA are used in the diagnosis of SLE, and anti-dsDNA antibodies have been isolated from the kidneys of SLE patients [33]. The mechanism by which antibodies against dsDNA lead to renal Ig deposition and tissue injury remains an area of active investigation. Molecular mimicry has been proposed to be the mechanism. Mostoslavsky et al. [20] demonstrated that pathogenic anti-DNA antibodies obtained from lupus prone mice cross-react with the glomerular protein α -actinin. The cross-reactivity was proposed to be due to structural similarity. A4ac and A4cd have previously been shown to cross-react with a large number of antigens including dsDNA [14], and MOG₉₂₋₁₀₆ contains the consensus sequence for the Ig superfamily [35], suggesting that molecular mimicry is a plausible mechanism for renal Ig deposition and injury in mice sensitized with MOG₉₂₋₁₀₆ or injected with MOG₉₂₋₁₀₆ reactive hybridoma cells.

Previously, it has been reported that mutation of three amino acids in the heavy chain of an anti-dsDNA antibody changed the pattern of Ig deposition in the kidney from predominantly glomerular to tubular [33]. It is possible that a similar mechanism is responsible for the shift in Ig deposition that we observed in nude mice, due either to mutation in the hybridoma cells or polymorphism in the antigen being recognized in the kidney due to strain differences, since the nude mice are on a BALB/c background.

Antibodies against dsDNA have been demonstrated to comprise a significant fraction of the intrathecal IgG in patients with MS. Anti-dsDNA antibodies have also been detected in brain plaques and CSF of patients with MS. Two anti-dsDNA antibodies obtained from patients with MS were also found to react with the surface of oligodendrocytes and neuronal cells [36]. This suggests that, antibodies which can cross-react with myelin protein and dsDNA exist in MS patients, and could potentially induce pathology in the kidneys of these patients.

Both sensitization with MOG₉₂₋₁₀₆ and transfer of MOG₉₂₋₁₀₆ antibodies are capable of inducing renal injury in addition to their effects in the CNS. The mechanism(s) through which this occurs has not been determined, but we hypothesize, based on our previous experiments, that it involves molecular mimicry and/or that cross-reactivity with dsDNA plays a role in renal pathology. To investigate this hypothesis we are currently attempting to identify the target antigen in the kidney. The data provided in this study has important implications for the development and/or administration of therapies such as altered peptide ligands or monoclonal antibodies, in which cross-reactivity must be considered. Our data

also suggests that renal involvement in MS should be further studied. This model will be useful in examining the development and/or coexistence of symptoms of systemic autoimmunity, such as renal disease, in diseases that have previously been considered organ-specific, like MS.

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References

1. Cohen, PL. Systemic Autoimmunity. In: Paul, WE., editor. *Fundamental Immunology*. Fifth Edition. Lippincott Williams & Wilkins; Philadelphia: 2003. p. 1371-99.editor
2. Calabresi PA, Austin H, Racke MK, Goodman A, Choyke P, Maloni H, McFarland HF. Impaired renal function in progressive multiple sclerosis. *Neurology* 2002;59:1799–801. [PubMed: 12473777]
3. Whitaker JN, Dowling PC, Cook SD. Immunofluorescent studies of the kidney in human neurologic disorders. *Journal of Neuropathology and Experimental Neurology* 1971;30:129–30. [PubMed: 5542510]
4. Daar AS, Fabre JW. Organ-specific IgM autoantibodies to liver, heart and brain in man: generalized occurrence and possible functional significance in normal individuals, and studies in patients with multiple sclerosis. *Clinical and Experimental Immunology* 1981;45:37–47. [PubMed: 7030539]
5. Matsiota P, Blancher A, Doyon B, Guilbert B, Clanet M, Kouvelas ED, Avrameas S. Comparative study of natural autoantibodies in the serum and cerebrospinal fluid of normal individuals and patients with multiple sclerosis and other neurological diseases. *Annales de l'Institut Pasteur Immunologie* 1988;139:99–108.
6. Tachovsky TG, Lisak RP, Koprowski H, Theofilopoulos AN, Dixon FJ. Circulating immune complexes in multiple sclerosis and other neurological diseases. *Lancet* 1976;2:997–9. [PubMed: 62267]
7. Tanphaichitr K. Multiple sclerosis associated with eosinophilic vasculitis, pericarditis, and hypocomplementemia. *Archives of Neurology* 1980;37:314–5. [PubMed: 6446277]
8. Achari AN, Trontelj JV, Campos DJ. Multiple sclerosis and myasthenia gravis. A case report with single fiber electromyography. *Neurology* 1976;26:544–6. [PubMed: 945496]
9. Esiri MM. Immunoglobulin-containing cells in multiple-sclerosis plaques. *Lancet* 1977;2:478. [PubMed: 70689]
10. Woyciechowska JL, Brzosko WJ. Immunofluorescence study of brain plaques from two patients with multiple sclerosis. *Neurology* 1977;27:620–2. [PubMed: 559966]
11. Aita JF, Snyder DH, Reichl W. Myasthenia gravis and multiple sclerosis: An unusual combination of diseases. *Neurology* 1974;24:72–5. [PubMed: 4129578]
12. Meloff K. Multiple sclerosis and polyarteritis (Letter to the Editor). *Archives of Neurology* 1980;37:786.
13. Tsunoda I, Kuang L-Q, Theil DJ, Fujinami RS. Antibody association with a novel model for primary progressive multiple sclerosis: Induction of relapsing-remitting and progressive forms of EAE in $H2^S$ mouse strains. *Brain Pathology* 2000;10:402–18. [PubMed: 10885659]
14. Peterson LK, Tsunoda I, Masaki T, Fujinami RS. Polyreactive myelin oligodendrocyte glycoprotein antibodies: Implications for systemic autoimmunity in progressive experimental autoimmune encephalomyelitis. *Journal of Neuroimmunology* 2007;183:69–80. [PubMed: 17197039]
15. Peterson, LK.; Fujinami, RS. Molecular mimicry. In: Shoenfeld, Y.; Gershwin, ME.; Meroni, P-L., editors. *Autoantibodies*. Second Edition. Elsevier Press; Philadelphia: 2006. p. 13-20.editors

16. Amor S, Groome N, Linington C, Morris MM, Dornmair K, Gardinier MV, Matthieu J-M, Baker D. Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. *Journal of Immunology* 1994;153:4349–56.
17. Papenfuss TL, Rogers CJ, Gienapp I, Yurrita M, McClain M, Damico N, Valo J, Song F, Whitacre CC. Sex differences in experimental autoimmune encephalomyelitis in multiple murine strains. *Journal of Neuroimmunology* 2004;150:59–69. [PubMed: 15081249]
18. Greer JM, Sobel RA, Sette A, Southwood S, Lees MB, Kuchroo VK. Immunogenic and encephalitogenic epitope clusters of myelin proteolipid protein. *Journal of Immunology* 1996;156:371–9.
19. Libbey JE, Peterson LK, Tsunoda I, Fujinami RS. Monoclonal MOG-reactive autoantibody from progressive EAE has the characteristics of a natural antibody. *Journal of Neuroimmunology* 2006;173:135–45. [PubMed: 16469392]
20. Mostoslavsky G, Fischel R, Yachimovich N, Yarkoni Y, Rosenmann E, Monestier M, Baniyash M, Eilat D. Lupus anti-DNA autoantibodies cross-react with a glomerular structural protein: a case for tissue injury by molecular mimicry. *European Journal of Immunology* 2001;31:1221–7. [PubMed: 11298348]
21. Dean EG, Wilson GRA, Li M, Edgton KL, O'Sullivan KM, Hudson BG, Holdsworth SR, Kitching AR. Experimental autoimmune Goodpasture's disease: a pathogenetic role for both effector cells and antibody in injury. *Kidney International* 2005;67:566–75. [PubMed: 15673304]
22. Kitching AR, Turner AL, Semple T, Li M, Edgton KL, Wilson GR, Timoshanko JR, Hudson BG, Holdsworth SR. Experimental autoimmune anti-glomerular basement membrane glomerulonephritis: a protective role for IFN- γ . *Journal of the American Society of Nephrology* 2004;15:1764–74. [PubMed: 15213264]
23. Tesch GH, Schwarting A, Kinoshita K, Lan HY, Rollins BJ, Kelley VR. Monocyte chemoattractant protein-1 promotes macrophage-mediated tubular injury, but not glomerular injury, in nephrotoxic serum nephritis. *Journal of Clinical Investigation* 1999;103:73–80. [PubMed: 9884336]
24. Boyce NW, Holdsworth SR. Anti-glomerular basement membrane antibody-induced experimental glomerulonephritis: evidence for dose-dependent, direct antibody and complement-induced, cell-independent injury. *Journal of Immunology* 1985;135:3918–21.
25. Boyce NW, Holdsworth SR. Direct antiGBM antibody induced alterations in glomerular permselectivity. *Kidney International* 1986;30:666–72. [PubMed: 3537458]
26. Couser WG, Darby C, Salant DJ, Adler S, Stilmant MM, Lowenstein LM. Anti-GBM antibody-induced proteinuria in isolated perfused rat kidney. *American Journal of Physiology* 1985;249:F241–F250. [PubMed: 3161341]
27. Alpers, CE. The kidney. In: Kumar, V.; Abbas, AK.; Fausto, N., editors. *Robbins' Pathologic Basis of Disease*. Seventh Edition. Elsevier Saunders; Philadelphia: 2005. p. 955-1021. editors
28. Nolan, CR.; McKinney, TD.; Forland, M. Urinalysis and renal function tests. In: Stein, JH., editor. *Internal Medicine*. 5th Edition. Mosby; St. Louis: 1998. editor
29. Anonymous. Clinical evaluation of genitourinary disorders. In: Beers, MH.; Berkow, R., editors. *The Merck Manual of Diagnosis and Therapy*. 17th Edition. Merck & Co., Inc.; Whitehouse Station: 1999. p. 1803-14. editors
30. Riboldi P, Gerosa M, Moroni G, Radice A, Allegri F, Sinico A, Tincani A, Meroni PL. Anti-DNA antibodies: a diagnostic and prognostic tool for systemic lupus erythematosus? *Autoimmunity* 2005;38:39–45. [PubMed: 15804704]
31. Asakura K, Miller DJ, Pogulis RJ, Pease LR, Rodriguez M. Oligodendrocyte-reactive O1, O4, and HNK-1 monoclonal antibodies are encoded by germline immunoglobulin genes. *Brain Research Molecular Brain Research* 1995;34:283–93. [PubMed: 8750831]
32. Asakura K, Miller DJ, Pease LR, Rodriguez M. Targeting of IgMk antibodies to oligodendrocytes promotes CNS remyelination. *Journal of Neuroscience* 1998;18:7700–8. [PubMed: 9742140]
33. Gaynor B, Putterman C, Valadon P, Spatz L, Scharff MD, Diamond B. Peptide inhibition of glomerular deposition of an anti-DNA antibody. *Proceedings of the National Academy of Science U S A* 1997;94:1955–60.

34. Putterman C, Diamond B. Immunization with a peptide surrogate for double-stranded DNA (dsDNA) induces autoantibody production and renal immunoglobulin deposition. *Journal of Experimental Medicine* 1998;188:29–38. [PubMed: 9653081]
35. Gardinier MV, Amiguet P, Linington C, Matthieu J-M. Myelin/oligodendrocyte glycoprotein is a unique member of the immunoglobulin superfamily. *Journal of Neuroscience Research* 1992;33:177–87. [PubMed: 1453482]
36. Williamson RA, Burgoon MP, Owens GP, Ghausi O, Leclerc E, Firme L, Carlson S, Corboy J, Parren PW, Sanna PP, Gilden DH, Burton DR. Anti-DNA antibodies are a major component of the intrathecal B cell response in multiple sclerosis. *Proceedings of the National Academy of Science U S A* 2001;98:1793–8.

Figure 1.

A.SW mice with progressive EAE exhibit Ig deposition and hypercellularity in kidneys, and increased protein in their urine compared to naïve mice or mice injected only with adjuvant. Kidney sections were immunostained for Ig deposition. (A) No Ig deposition was seen in glomeruli (G) or tubules (T) in the kidneys of naïve mice. (B) Some Ig deposition was detected in the glomeruli of the kidneys of mice injected with CFA alone. (C) Significant Ig deposition localized in the glomeruli of the kidney was observed in mice with progressive EAE. (D) The extent of glomerular antibody deposition was determined and expressed as the percent involvement of Ig deposition. A.SW mice that developed PP-EAE after sensitization with MOG₉₂₋₁₀₆ had significantly more Ig deposited in their kidneys compared to naïve mice. (E and F) A.SW mice with PP-EAE also developed proteinuria and glomerular hypercellularity. c/gcs = cells per glomerular cross-section, * = $P < 0.05$, ** = $P < 0.01$, t -test. Data are expressed as the mean + SEM.

Figure 2.

Naive mice injected with MOG₉₂₋₁₀₆ hybridoma cells (A4cd) develop Ig deposition and increased protein in urine compared to control mice. MOG₉₂₋₁₀₆ hybridoma cells were injected intraperitoneally into SJL/J mice to examine the timing of Ig deposition. Mice were killed at weekly intervals after injection and immunofluorescent staining for Ig deposition performed on kidneys. (A) Significant amounts of antibody deposition were detected in the glomeruli (G) of the kidneys of mice injected with A4cd hybridoma cells in media at 5 and 6 weeks after injection. (B) In contrast, no antibody deposition was detected in kidneys of media injected control mice at any time point. Images shown are representative images from the 5 weeks time point. (C) Mice injected with A4cd hybridoma cells developed proteinuria 5 weeks postinjection. (D) Mice injected with A4cd hybridoma cells tended to have reduced creatinine clearance at almost all time points compared to control mice. (E) No difference in the number of cells per glomerular cross-section (c/gcs) was observed. * = $P < 0.05$, t -test. Data shown are mean + SEM.

Figure 3.

Ig deposition in the kidney is induced by antibodies that cross-react with dsDNA. ELISA analyses showed that anti-MOG (A4cd) and anti-oligodendrocyte (O4) natural antibodies, but not the anti-LPS antibody (XMMEN-OE5), reacted with MOG₉₂₋₁₀₆ (A) and protein isolated from mouse blood (C) and liver (D), as compared with an isotype control. (B) In contrast, ELISA analyses demonstrated that all three antibodies reacted significantly with dsDNA, compared to the isotype control. Ig deposition was detected in glomeruli of mice injected with XMMEN-OE5 (E) and O4 (F), using anti-mouse IgG (H+L).

Figure 4.

In vivo binding of MOG antibodies in the tubules in the kidneys and increased urea and creatinine in the serum of nude mice injected with MOG₉₂₋₁₀₆ hybridoma cell lines. Nude mice were injected intraperitoneally with MOG₉₂₋₁₀₆ hybridoma cell lines, A4ac ($n = 3$) and A4cd ($n = 5$), to examine Ig deposition in the absence of hybridoma rejection. Control mice were injected with the hybridoma fusion partner, Sp2/0 ($n = 5$). Mice were killed 9 days after injection and immunostaining for Ig deposition performed on kidneys. Antibody deposition was detected in the glomeruli (G) of the kidneys of both mice injected with the hybridoma fusion partner (Sp2/0) (A and C) and mice injected with the MOG₉₂₋₁₀₆ hybridoma cells (B and D). In contrast, significant amounts of antibody deposition were detected in the tubules (T) of mice injected with MOG₉₂₋₁₀₆ hybridoma cells (B, D and F), but not in mice injected with the fusion partner (A, C and E). Images shown are from a mouse injected with the A4ac MOG₉₂₋₁₀₆ hybridoma cells. (G and H) Nude mice injected with A4cd hybridoma cells had significantly more urea ($P < 0.05$) and less creatinine ($P < 0.01$) in their serum compared to control mice. A4ac injected mice tended to have less urea and creatinine in their serum compared to control mice, but statistical significance was not reached. * = $P < 0.05$, ** = $P < 0.01$, t -test. Data shown are mean + SEM.

Figure 5.

ELISA analyses revealed increased serum MOG₉₂₋₁₀₆ and dsDNA IgM and similar total serum IgG and IgM in nude mice injected with MOG₉₂₋₁₀₆ hybridoma cells compared to control mice. Plates were coated with MOG₉₂₋₁₀₆ (A) or dsDNA (B) and serum antibody titer determined using peroxidase-conjugated anti-mouse IgM antibody. Alternatively, plates were coated with capture anti-mouse Ig, and serum levels of total IgM (C) and IgG (D) determined using peroxidase-conjugated anti-mouse IgM and IgG (γ) antibodies. Data shown are mean + SEM.