Monoclonal Anti-PLA2R and Recurrent Membranous Nephropathy: Another Piece of the Puzzle

Laurence H. Beck, Jr.

Department of Medicine, Renal Section, Boston University School of Medicine, Boston, Massachusetts

doi: 10.1681/ASN.2012101023

Many of the advances in our understanding of human pathophysiology have come both from experimentation in animal models and from direct observations of human disease. Membranous nephropathy (MN) is no exception; decades of work in the Heymann nephritis model demonstrate the role of the complement system in coupling the immune deposits that form beneath the podocyte foot processes to the sublethal cell injury that disrupts slit diaphragm and cytoskeletal architecture, leading to massive proteinuria. However, we now know that the target antigen in the rat model, megalin, is not present on human podocytes, and that the major antigenic target in human disease is instead the phospholipase A2 receptor (PLA2R). Moreover, whereas complement activation by the classic pathway is important in the rat model, IgG4, a subclass unable to activate this pathway, is the dominant moiety in human primary MN.

The research team of Ronco, Debiec, and colleagues has recently showed that antibodies to dietary cationic BSA are first to identify a human antigen, neutral endopeptidase, in fetomaternal alloimmune MN, and have more recently demonstrated that antibodies to dietary cationic BSA are responsible for another rare form of MN in infants. Their current work in this issue of JASN, in which they describe the intriguing case involving monoclonal anti-PLA2R leading to recurrent MN, is yet another example of how, in the words of Louis Pasteur, “la chance favorise seulement l’esprit préparé (chance favors only the prepared mind).”

The complement system is clearly involved in the pathogenesis of human MN, although the specifics are less well understood. Evidence for complement activation includes the presence of C3 within immune deposits, as well as the terminal complement components C5b-9 in both the glomerulus and the urine. There are three potential (and not mutually exclusive) pathways by which complement might be activated, all resulting in the generation of C5b-9. IgG or IgM-containing immune complexes bind and activate C1q leading to the cleavage of C4 and generation of the classic C3 convertase. The alternative pathway, stimulated by nonhost membranes such as bacterial surfaces, incorporates factor B, and not C4, into its C3 convertase. The third and less well known route to complement activation is by the lectin pathway. In this system, terminal sugar residues typically limited to microbial carbohydrates (but also found on IgGs in certain disease states) bind and aggregate mannose-binding lectin (MBL) and MBL-associated serine proteases (MASPs). Similar to C1qrs, the MBL/MASP complex cleaves C4 to initiate a process analogous to the classic pathway. The main histopathologic evidence for the lectin pathway is the absence of C1q and presence of MBL within deposits.

This distinction becomes important among the various forms of MN. Multiple studies show that in primary MN, IgG4 is the predominant subclass within subepithelial deposits, whereas other subclasses predominate in secondary forms of disease such as those associated with lupus or malignancy. Consistent with the relative abilities of the IgG subclasses to activate the classic complement pathway, C1q is often prominent in a fine granular capillary loop distribution in lupus-associated MN but is absent or very weak in primary MN. Although not typically examined during routine immunofluorescence of the native kidney, C4 has been demonstrated in immune deposits of primary MN, potentially implicating the lectin pathway. MBL can also be found in MN biopsies, and there is preliminary evidence that IgG4 anti-PLA2R autoantibodies with galactose-deficient side chains can bind MBL and activate this pathway.

Debiec and colleagues describe here an extraordinary case of MN, associated with a monoclonal anti-PLA2R autoantibody, which recurred in the kidney allograft 13 days after transplantation. Contrary to what one might expect, this mAb was not IgG4, but rather IgG3 with a κ light chain restriction. Consistent with this subclass restriction, the authors demonstrate the presence of C1q, C3, and C5b-9, but not MBL, in the biopsy specimen. Moreover, they determine that the same process had occurred in the native kidney years earlier. This monoclonal IgG was not found to be associated with a hematologic disorder, despite extensive investigation.

Glomerular deposits composed of intact monoclonal IgG can occur in type I cryoglobulinemia or immunotactoid GN; however, the absence of substructure in the deposits and normal serum complement levels exclude these entities in the present case. Another consideration would be proliferative GN with monoclonal IgG deposits (PGNMID), which is also commonly associated with monoclonal IgG3. Although PGNMID can be associated with primarily subepithelial deposits in a small percentage of cases, there are also focal proliferative features, which are absent here. The demonstration of the colocalization of IgG3 and PLA2R by confocal microscopy suggests that the monoclonal IgG3 anti-PLA2R
is not depositing nonspecifically due to physicochemical properties of the monoclonal IgG alone (compare with de Seigneux et al.13), but rather appears to be targeted to the subepithelial space by podocyte-expressed PLA2R.

The pathogenicity of anti-PLA2R autoantibodies has not yet been proved, due to the lack of appropriate animal models, but may be suggested by several observations from the emerging literature. The close association of anti-PLA2R antibody titers and clinical disease activity, the presence of PLA2R within immune deposits, the elution of anti-PLA2R antibodies from biopsy tissue, and genetic analyses that have implicated loci within or near the PLA2RI gene have all hint at direct pathogenicity. Conversely, the absence of anti-PLA2R in secondary MN and other glomerular diseases suggests that it is not merely a biomarker of the disease process or reflective of podocyte damage. In the present case, we will assume that the IgG3 in the deposits reflects the circulating monoclonal anti-PLA2R due to its colocalization with the antigen, although this has not been formally demonstrated by evaluating the antigenic specificity of the IgG3 eluted from the biopsy tissue.

Another factor that hints at direct pathogenicity is the high likelihood of disease recurrence in patients who are anti-PLA2R seropositive at the time of transplantation.14,15 Such circulating antibodies are conceptually similar to the putative permeability factor that leads to rapid onset of proteinuria in recurrent FSGS. Debiec and colleagues also note the similarity of the current case to the experimental model of passive Heymann nephritis, in which antimegalin antibodies are introduced, bind their target antigen in situ, and bring about disease in <1 week.

It should be noted that anti-PLA2R seropositivity at the time of transplantation does not guarantee recurrence,14 and should not be a contraindication to transplantation. Transplant immunosuppression, genetic factors in the HLA and PLA2RI loci, and other factors may have a large effect on the recurrence of MN.

Despite formal proof, the current case is highly suggestive of the monoclonal anti-PLA2R being the cause of disease. k-restricted IgG3 was the only IgG subclass found within the deposits of the native kidney, was present in the circulation at the time of transplantation, and was found associated with PLA2R in subepithelial deposits of the allograft only 13 days later. One could postulate the existence of other, more proximally pathogenic antibodies, but the subclass restriction observed in both native kidney and allograft would mean that they too would need to be IgG3- and k light chain-restricted, an unlikely possibility.

Despite our ability to find PLA2R within immune deposits soon after transplantation,14,16 the time course for the clinical development of recurrent MN is rather slow. As we know from experimental Heymann nephritis and from humans who have had serial protocol biopsies, subepithelial deposits increase in size over time, and likely need to reach a threshold at which complement activation can overwhelm local complement regulatory systems to cause podocyte damage. The earliest evidence of recurrent MN as detected in protocol biopsies reveals almost-undetectable electron-dense deposits despite the typical fine granular IgG deposits by immunofluorescence.17 These electron-dense deposits ultimately grow and are associated with increased podocyte foot process effacement and proteinuria. Interestingly, and not fully explained, C4d is found within deposits, whereas C3 is absent or weak, even in more advanced recurrent disease.

In the present case, daily urinary protein excretion increased from 1.85 g to 5.1 g at the point when the patient required treatment with rituximab. Subsequently, proteinuria decreased to 0.4 g in the setting of the disappearance of anti-PLA2R, which likely led to the cessation of new immune deposit formation and gradual restoration of the normal podocyte architecture. It should be noted that the anti-PLA2R titer had already declined from 1:300 at the time of the allograft biopsy to 1:10 before the decision was made to treat with rituximab. The immunosuppression routinely given for kidney transplantation may, at times, be sufficient to eliminate these antibodies. Therefore, in the absence of protocol biopsies and with the sole reliance on clinical features for detection of disease, a transient recurrent MN could be missed despite anti-PLA2R seropositivity at the time of transplantation.

In the end, this remarkable case brings up as many questions as it answers. Does the route to complement activation matter, because these various forms of MN all seem to lead to the same phenotype? Which IgG subclass is pathogenically more important in primary MN? Why did antibodies of other subclasses not arise in this case secondary to intra- or intermolecular epitope spreading? Not only must we expand our understanding of MN with new animal models, molecular expression data, and clinical trials, we will also need to cultivate our observational skills in these settings to find those pieces of the puzzle that have thus far been overlooked.

DISCLOSURES

None.

REFERENCES

Mast Cells: Subordinates or Masterminds in Autoimmunity?

Kathrin Eller and Alexander R. Rosenkranz
Clinical Division of Nephrology, Medical University of Graz, Graz, Austria


doi: 10.1681/ASN.2012101025

Mast cells have long been known as effector cells in allergic disorders. By binding IgE, they rapidly release a plethora of preformed cytokines, chemokines, bradykinins, and other highly active substances that mediate bronchospasm and urticaria.1 Over recent years, it has become clear that mast cells also regulate other immune cells such as dendritic cells, B cells, and T cell populations. Intriguingly, they appear to exert either pro- or anti-inflammatory effects depending on the surrounding milieu.1

In this issue of JASN, Gan and coworkers describe a pivotal role of mast cells in the regulation of autoimmunity in a murine model of ANCA vasculitis with necrotizing GN.2 Their findings support increasing evidence for an immunoregulatory, rather than an effector role of mast cells in inflammatory kidney diseases. Thus far, it has been described in experimental nephrotoxic serum nephritis that mast cells have protective functions.3,4 It was previously unclear, however, how these effects were mediated. Gan et al. now provide compelling evidence that IL-10 production by mast cells is essential for their immunosuppressive effects in a model of experimental ANCA vasculitis.2 By secreting IL-10, mast cells increased the number of regulatory T cells (Tregs) in the draining renal lymph nodes. Tregs are potent anti-inflammatory cells that have been shown to counteract the proinflammatory T cell populations such as TH1 and TH17 cells. A disrupted balance between the pro- and anti-inflammatory cell populations is regarded as a trigger for the development of autoimmune diseases such as inflammatory bowel disease, lupus erythematosus, and others,5 as well as inflammatory kidney diseases such as Goodpasture’s disease.6 Importantly, Gan et al. note that the increase in absolute numbers of mast cells and Tregs limits the TH17 response in vivo within the draining lymph nodes.2

In inflammatory kidney disease, immunoregulation is orchestrated within the lymph node. Here, mast cells, as well as Tregs, provide a zone of immunosuppression.3,7–9 After induction of ANCA vasculitis, Gan et al. found elevated numbers of both cell populations in the lymph node, in close proximity to each other.2 However, it is unclear whether mast cells and Tregs migrate, or rather proliferate, in the in vivo situation. The investigators hypothesize that mast cells migrate from the immunization site to the lymph nodes, but provided no direct experimental evidence to support this assumption. Alternatively, mast cells might change the phenotype of Tregs to induce IL-10–expressing Tregs. In addition, mast cell–derived IL-10 decreases infiltration and expression of costimulatory molecules of dendritic cells, thereby limiting the activation and proliferation of effector T cells such as TH1 and TH17 cells.1 Thus, it cannot be completely ruled out that the described immunoregulatory effect of mast cells in the experimental model of ANCA vasculitis is attributable to dendritic cells.

In addition, IL-10 has also been shown to inhibit innate immune cells such as neutrophilic granulocytes and macrophages in a model of chronic low-dose ultraviolet B irradiation.10 Both cell populations are known to play key roles in ANCA vasculitis.11 However, in a model of nephrotoxic serum


See related article, “Recurrent Membranous Nephropathy in an Allograft Caused by IgG3x Targeting the PLA2 Receptor,” on pages 1949–1954.