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Primary Membranous Nephropathy as a Model of Autoimmune Disease

Patrick Hamilton, Durga Kanigicherla and Paul Brenchley

Abstract

Membranous nephropathy is the most common cause of adult nephrotic syndrome worldwide with a significant health care burden. There has been a leap in our understanding of the disease mechanism over the last decade with a remarkably strong genetic component to the development of the disease and its strong association with high affinity antibody—in the form of anti-PLA2R autoantibody in the majority of cases, with a smaller proportion associated with anti-THSD7A autoantibody. New evidence is now providing confirmation of specific elements in the development of the disease pathogenesis, such as involvement of loss of peripheral tolerance. There is a striking correlation between disease activity and anti-PLA2R antibody levels, along with response to treatment; evidence points strongly to these antibodies being pathogenic. The development of membranous nephropathy therefore follows the well appreciated multi-hit step-wise path to autoimmune clinical disease. Given its strong genetic basis and putative pathogenic antibody the disease provides an invaluable model for understanding of autoimmunity. This chapter focuses on the most up to date knowledge of autoimmune membranous nephropathy and provides a paradigm for understanding the underlying disease mechanisms in autoimmunity.

Keywords: autoimmune disease, membranous nephropathy, nephrotic syndrome, anti-PLA$_2$R

1. Introduction

Autoimmune disease is a term that covers a range of conditions in which the immune system recognises the body as a foreign pathogen, and encompasses over 80 conditions affecting almost every organ [1]. Individually, many of these conditions are rare but taken together they represent a significant mortality and morbidity affecting approximately 9% of the population worldwide with the prevalence rising [1, 2]. Given that the majority of conditions have no cure and potentially require lifelong treatment, and that most patients are diagnosed during young adulthood or in middle age, the cost to healthcare systems is particularly significant.

Despite the variation of organs affected and clinical presentation, many of the conditions that appear distinct will share a common theme of disease pathogenesis. Underscoring many of the conditions is a genetic susceptibility that taken in concert with an environmental trigger sets off an immune cascade resulting in the

end organ damage and clinical signs and symptoms that bring the patient to the attention of their healthcare provider, the so-called multi-hit hypothesis. As science progresses and we gain greater insight into these disease processes, it is becoming more apparent that there are similarities in many of the conditions. At present, most management strategies attempt to globally restrict the immune system, a strategy that has been shown to help control the disease but comes with a significant side effect profile. Despite the accelerating knowledge we are gaining of the underlying pathogenesis, there remains a lack of directed novel therapies for patients at present, although in many conditions there are signs that this is changing.

Membranous nephropathy represents a particularly interesting basis to understand this process given its clear pathological classification, strong genetic contribution, putative pathogenic antibody and evidence for the loss of tolerance that is now emerging. In this chapter, we review the current understanding of autoimmune membranous nephropathy and use it as a basis for the understanding of autoimmune disease in general.

2. Background

Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults worldwide but despite this remains a rare disease. Incidence is estimated at 1.2 per 100,000 in European cohorts with a peak incidence in the fifth and sixth decades, although it can affect any age, and has a slight male preponderance [3]. The classical presentation of the disease is with nephrotic syndrome, that is, the tetrad of leg swelling, proteinuria and serum hypoalbuminemia, with or without hypercholesterolemia. A number of patients have also been known to present with venous thrombosis. This can be in the form of deep vein thrombosis (DVT) and, not uncommonly as the first presentation of the disease, with acute kidney injury (AKI) as a result of renal vein thrombosis. Hypercoagulopathy as a result of the loss of anti-thrombotic factors such as anti-thrombin III and plasminogen due to proteinuria, an increased level of factor VIII and fibrinogen, along with an increased platelet hyperaggregability has been noted in nephrotic syndrome whatever the cause. However, compared to other conditions that have a similar degree of proteinuria, MN has a relatively higher risk of venous thrombosis and its associated risks; the mechanism for this association has not been ascertained [4–6].

Clinically there are two distinct forms of MN, but these can be histologically very similar and difficult to differentiate. Both require very different treatment strategies and therefore distinguishing them is imperative. Primary MN accounts for the majority of patients (approximately 75–80%) and has now been shown to be an autoimmune disease. Secondary MN is caused by a multitude of causes including medications, systemic disorders and toxins, and its treatment is therefore aimed at the underlying trigger or condition [7].

It is one of the idiosyncrasies of MN that up to a third of patients if left untreated will achieve spontaneous remission within the first 2 years following diagnosis, and this potential for spontaneous remission has informed the current treatment options, especially for those patients without rapidly progressive renal decline [8]. The mainstay of treatment at present has a focus on the reduction of proteinuria with the use of an renin-angiotensin pathway blockade or immunosuppression if this fails [7]. It has also meant that for many studies, patients undergo three to 6 months of supportive care before they are eligible, in case any response to treatment seen is actually as a result of spontaneous remission. However, with the increasing use, understanding and monitoring of biomarkers such as anti-PLA$_2$R, treatments are likely to be less empiric in the future.

If patients do reach ESRD and receive a renal transplant, it has been well demonstrated that this can provide a dramatic improvement to not only life expectancy but also quality of life [9–11]. However, this comes with the risk of recurrence of MN following transplantation (up to 34% of patients) despite the judicial use of immunosuppression and can lead to the loss of the graft in up to 50% of these cases. There is some evidence to suggest that receiving a transplant from a living related donor increases the risk of recurrence, but this is far outweighed by the complications associated with remaining on dialysis [12]. Current practice therefore, is to attempt to match HLA antigens as closely as possible to reduce the reliance on immunosuppression to minimise rejection.

For many patients, MN remains a relapsing and remitting disease, requiring lifelong follow up under the care of specialists in tertiary care. Despite being a rare condition, its chronicity, current standard treatments and their associated side-effects, the risk of ESRD, and disease recurrence means it is a disease that has a significant impact on both a patient's quality of life and a healthcare system with finite resources.

3. Diagnosis

Recent advances in biomarker research for MN have shown promising results but at present diagnosis requires biopsy confirmation. Histologically the disease is characterised by thickening of the glomerular basement membrane and spikes seen on silver stain. Immunofluorescence almost universally shows coarse granular immunoglobulin IgG and complement C3 deposition on the capillary wall. Electron microscopy (EM) will show sub-epithelial immune complex deposition (**Figure 1**). It has become apparent over the years that the dominant IgG subclass found histologically (and for antibodies to PLA2R as described below) in primary MN is IgG4 [13–15]. This appears to differ from secondary MN where IgG1 predominates [16]. IgG makes up a significant proportion of serum protein in humans contributing approximately 10–20% of circulating proteins. It can be further subdivided into four subclasses with differing effects. IgG4 is the least abundant of these subclasses and is generally found in response to allergens or in response to repeated exposure to an antigen [17].

New research findings suggest that there may be a class switch involved in primary membranous nephropathy. Here it has been shown that in early MN (stage I of the Ehrenreich and Churg scale) the predominant subclass of antibody is IgG1 but as the disease progresses this changes so that IgG4 predominates [16].

4. Treatment

In primary MN, disease activity is still measured by proteinuria level and renal excretory function despite the advances in anti-PLA$_2$R research. Proteinuria level has been shown to be not only a marker for remission when it is low but also predicts progression to ESRD when increased. If proteinuria reduces through either spontaneous remission or with treatment, then the risk of CKD progression also falls. It is for this reason that the main focus of treatment in primary MN is concerned with control of proteinuria, with or without the use of immunosuppression, generally in the form of the Ponticelli regime (or calcineurin inhibitors if cyclophosphamide is not tolerated or is contraindicated). This regime of rotating high dose intravenous steroids and immunosuppression was first described in the mid-1980s and has been the recommended regime since [7, 18–20]. Despite its success in treating the condition, the Ponticelli regime comes with a significant side effect burden, including an increased risk of infection, osteoporosis, diabetes mellitus, weight gain,
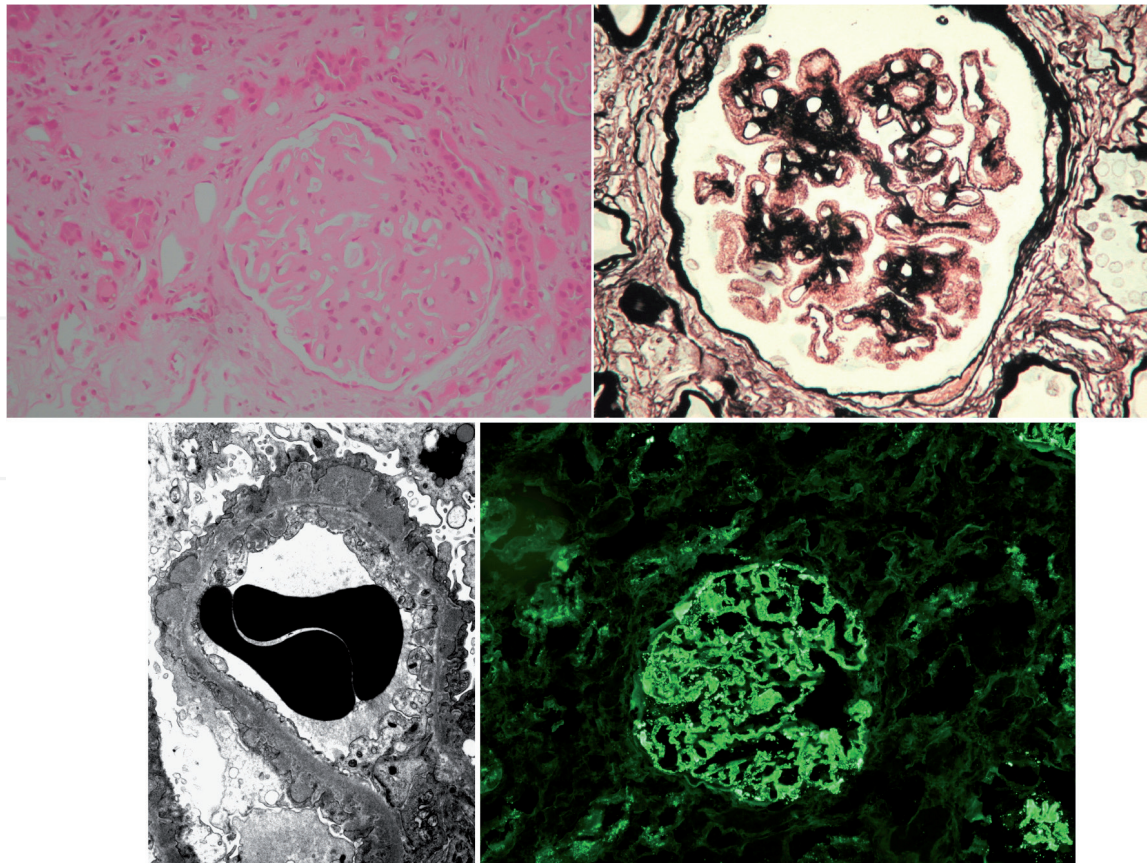
Figure 1.
Histological appearance of membranous nephropathy (a) haematoxylin and eosin stain (H&E) showing marked capillary loop thickening (b) silver staining showing spikes (c) electron microscopy of MN showing sub-epithelial immune complex deposition (d) immunofluorescence showing IgG deposition on the capillary wall. Figures courtesy of Dr. Lorna Williams, Consultant Histopathologist, Manchester University Hospitals Foundation Trust, UK.

haemorrhagic cystitis, infertility and malignancy [18]. It is this that has led many researchers to search for an alternative therapy including mycophenolate mofetil and tacrolimus but with variable evidence to show an improvement in outcomes.

Rituximab is a monoclonal antibody against CD20, found on the B-cells, which leads to a reduction in B-cell numbers and has been used extensively in cancer therapy and autoimmune diseases since its introduction in the 1990s. A number of case series and studies have shown the potential that Rituximab can have for primary MN, but so far there has only been one randomised controlled trial (RCT) [21–24]. Here it has been shown that compared to standard anti-proteinuric therapy, patients treated with rituximab show a greater reduction in anti-PLA$_2$R levels at month 3, followed by a later reduction in proteinuria, increase in serum albumin and are more likely to enter remission [24]. Combined with the high cost of the medication itself, its widespread use has been restricted in resource-limited, evidence-based healthcare systems, such as the NHS in UK [25]. The use of Rituximab therapy is currently under investigation in the MENTOR study in North America and via the Commissioning through Evaluation pathway run under the auspices of the National Institute for Health and Care Excellence (NICE) in the UK. It is possible that based on these two studies the use of Rituximab will become more ubiquitous in the near future.

5. Future novel treatments

The use of many of these medications comes with side effects that can be unpalatable to the patient and physician and the search for treatments with a reduced

side-effect profile is on-going. Treatments such as immunoadsorption (IA) allow for the controlled removal of antibodies without the side effects associated with immunosuppression. Immunoadsorption RCTs in MN though, are non-existent and certainly not in the anti-PLA$_2$R era. Immunoadsorption is a method of removing specific circulating immunoglobulins from the blood and has been shown to remove over 80% of circulating IgG with a single session adsorption of 2.5 plasma volumes, with albumin and antithrombin III almost unaffected [26]. With multiple sessions, this can rise to over 98% [27]. These are removed in the absorber through binding Peptide-GAM. Using two columns per machine, one regenerating whilst the other is removing antibodies; the process can occur indefinitely until the required level of antibody has been removed.

Post IA it appears that autoantibodies can be slow to re-emerge. Use in dilated cardiomyopathy for the removal of β_1-adreno-receptor autoantibodies (β_1-AAB) has shown that only a small minority of patients (0% in the first year and 15% by 3 years) will show an increase in significant β_1-AAB autoantibodies [28, 29].

To our knowledge, there has only been one publication using immunoadsorption for the treatment of MN [30]. In 1999, Esnault et al. successfully used IA for the treatment of various aetiologies of Nephrotic syndrome including four patients with MN [31]. Here they showed that not only is the procedure safe but that there was a significant improvement in proteinuria in all patients with membranous nephropathy. The main side effect in this group of patients was headache, which resolved without sequelae. Since that time the treatment has been used in numerous other autoimmune conditions including Focal Segmental Glomerulosclerosis (FSGS) [32], systemic lupus nephritis (SLE) [33, 34], ANCA-associated small vessel vasculitides [35, 36], Anti-glomerular basement membrane antibody disease [37] and in renal transplantation [38–40].

In conditions such as SLE, the use of immunoadsorption can dramatically reduce the level of circulating immune complexes and autoantibodies leading to clinical improvement in even severe life-threatening SLE. These results have been shown with as little as two sessions within 3 days and repeated every 3 weeks if patients remain with active disease [33]. With the current understanding of primary MN's autoimmune process, the use of IA could provide the ability to rapidly remove the pathogenic antibodies leading to remission. Current IA machines can remove the different classes such as IgG4 with an increased specificity but cannot differentiate further than that. If IA is proved to work for primary MN, it may be possible to develop an IA column that is specific only for anti-PLA$_2$R, therefore allowing for an even more targeted and personalised treatment.

6. Autoimmune membranous nephropathy

Until recently autoimmune (or primary) MN was known as idiopathic MN as its cause remained unclear. It was generally a diagnosis of exclusion, once a patient had biopsy confirmation of MN and all causes of secondary MN had been ruled out. It was for a long time postulated to be an autoimmune disease, but the target antigen in humans remained elusive. In the late 1970s, work on the Heymann Nephritis rat model of experimental MN showed that circulating IgG antibodies could bind to the podocytes [41–43]. The target antigen was found to be megalin, but this was not present on human podocytes, so the search for the target antigen continued. It was not until 2009, almost 40 years later that this was discovered. Here Beck et al. used western blotting with MN patient sera, to show that antibodies bound to a 185 kDa protein band from glomerular extracts. This band was only seen in the primary MN group and not seen in normal patients or other proteinuric conditions including

patients with secondary MN. Using mass spectrometry this band was found to contain the M-type phospholipase A2 receptor 1 (PLA2R) [15]. Since then, the increased interest and research into MN has led to the discovery of a second minor target antigen in thrombospondin type-1 domain containing 7A (THSD7A) [44].

7. M-type phospholipase A2 receptor 1

The landmark paper by Beck et al. describing the discovery of autoantibodies to PLA2R found on human podocytes transformed our understanding of the MN disease process. Here was evidence that for the majority of patients with MN, the condition was, as had been postulated, an autoimmune disease [15].

PLA2R is a transmembrane receptor for Phospholipase A2, a protein from the mannose receptor family, one of four described in humans; Endo180, DEC205, Mannose Receptor (MR) and PLA2R [45–47]. As with all the mannose receptor family, the transmembrane glycoprotein has an extracellular component, in the case of PLA2R, this is made up of an N-terminal ricin rich domain, a fibronectin type II domain and 8 C-type lectin domains (CTLDs) [48]. In the kidney, it is found almost exclusively on the podocytes, but it has also been found on neutrophils and in the lung [49, 50]. Its function in the kidney remains unknown, however, and how the anti-PLA$_2$R antibodies alter its normal function leading to proteinuria, if indeed that is what is part of the process, also remains unknown [15, 51].

The predominant antibody to PLA2R is IgG and in particular IgG4, which is the major component of immune complex deposition in primary MN [13, 14]. These immune complexes appear to form in the kidney with the IgG4 antibodies and the PLA2R antigen being co-localised, giving further evidence for the role of PLA2R in the disease process [52, 53]. The fact that the complexes form in situ in the kidney may explain why some patients with biopsy-proven MN and clinical evidence for the disease are serum anti-PLA$_2$R negative. Debiec and Ronco showed in 2011 that there were a number of patients who were serum anti-PLA$_2$R negative but had detectable PLA2R in glomerular deposits. They did also find a few patients with no PLA2R in the glomerular deposits but who were serologically positive [54]. We know that anti-PLA$_2$R antibodies have a high affinity for PLA2R in the podocytes and it may be that a certain level of deposition is required to overload the system before the anti-PLA$_2$R antibodies become serologically detectable [48].

Much of the excitement surrounding anti-PLA$_2$R is due to its apparent pathogenicity with the resultant potential for use as a biomarker and as a treatment target. Several studies have provided evidence for its pathogenicity showing that a high titre correlates with disease activity. For patients who go into remission either spontaneously or through the use of immunosuppression, the anti-PLA$_2$R level falls months before this becomes clinically apparent with a fall in proteinuria. If a patient relapses, this again is predated by a rise in antibody titres [55–60].

Outcomes can also be predicted with high titres predicting a worse outcome in regards to renal function and an improved outcome with low titres [55]. If treatment does not result in antibody negativity, then they are left with a high risk of relapse [51, 57]. Ruggenenti et al. have shown similar results with a reduction in anti-PLA$_2$R levels strongly predicting remission and increasing titres following this, predicting relapse [59].

With the increasingly strong evidence for the involvement of anti-PLA$_2$R antibodies in the pathogenesis of primary MN, the focus has now shifted to trying to understand the antigen and its interaction with the autoantibody. Work carried out in Manchester has now determined the major epitope on the PLA2R antigen

that is recognised by the anti-PLA$_2$R antibodies. Four different sized fragments of extracellular PLA2R (full-length N-C8, N-terminus to C-type lectin domain (CTLD) 3 (N-C3), N-terminus to CTLD2 (N-C2) and a ricin rich domain) were used to investigate the reactivity of human anti-PLA$_2$R autoantibodies. It was found that the major epitope was located in the N-C3 region of the receptor. The antibodies were also found to bind with an equal affinity to the four different fragments, confirming the existence of a single epitope. The epitope itself is a 31-mer peptide made up of the beta-1 and beta-3 strands and encompassing the beta-2 strand [48].

Leading on from this the Manchester team also constructed a 3D model of the structure of the immune complex incorporating the extracellular N-C8 PLA2R and the autoantibody with the binding site [48]. This work has been further confirmed by Kao et al. who found that the dominant epitope is in the N-terminal region as well, in particular in the region from the ricin rich domain through the fibronectin-like type to the CTLD1 [61].

8. Thrombospondin type-1 domain-containing 7A

The fact that anti-PLA$_2$R antibodies are found in up to 80% of patients with primary MN raises a number of possibilities. It is known that some patients with secondary membranous can develop malignancies years after the diagnosis of MN and it may be that these patients fall into this category. Whether these patients represent a cohort who have two separate conditions and it is coincidental that one is known to cause the other is still up for debate. A second possibility is that there are more pathogenic antigens leading to the formation of autoantibodies than previously thought. In fact, for a small number of patients with primary MN, this seems to be the case.

Using western blotting, Thomas et al. discovered a glomerular protein of 250 kDa in patients with anti-PLA$_2$R negative biopsy-proven membranous nephropathy. This corresponded to THSD7A, a protein found in the podocyte foot processes [44].

They went on to show that the predominant antibody to this antigen was IgG4 in keeping with a diagnosis of primary MN, and on histological staining, in a similar fashion to anti-PLA$_2$R, the immune complexes were co-localised with the antigen. Levels of the antibody were shown to correlate with disease activity, being higher in active disease and lower as the clinical manifestations of the disease improved. Interestingly, there appeared to be no statistical significance in the clinical presentation or demographics between the anti-PLA$_2$R positive and the anti-THSD7A positive patients, except for a slightly higher number of women in the anti-THSD7A group, although this is believed to be due to the small numbers involved.

This evidence suggests that for a minority of primary MN patients, approximately 2.5–5% in this study, a second unrelated and discrete antigen is involved with the pathogenesis of the disease [44]. Whether this all represents a separate disease and whether there are other minor antigens still to be discovered remains unknown as does the major epitope in THSD7A. However, for PLA2R, in addition to the major epitope in the CysR domain, evidence from the work of Fresquet et al. on the identification of the major epitope of PLA2R, showed that 10% of anti-PLA$_2$R positive sera reacted with an epitope at CTLD4-8. This suggests that there may be a further, as yet unidentified, antibody to this minor epitope [48]. This idea of epitope spreading has been suggested by Lambeau et al. who have defined additional epitopes in CTLD1 and CTLD7 domains [62].

9. The multi-hit hypothesis

9.1 Genetic association

Why some patients develop an autoantibody to PLA2R is still an unknown, but it does appear to have a strong genetic component. The first clue to the genetic basis of the disease was the discovery of the association with Human Leucocyte Antigen (HLA)—DR3 followed closely by the identification of familial clustering in 1984 [30, 63, 64]. Following the discovery of the PLA2R antigen, researchers studying Korean and Chinese populations investigated the association of a number of single nucleotide polymorphisms (SNPs) known to be associated with PLA2R. They both found that a polymorphism at rs35771982 was significantly associated with primary MN. Interestingly, this polymorphism is located on CTLD1, in the region that was later found to contain an epitope in the antigen [48, 65, 66].

The major Genome-Wide Association Study (GWAS) in MN of 556 patients (French, Dutch and British) revealed two major loci of allelic association. The first is not unexpectedly on chromosome 6p21 within HLA-DQA1 gene, and the second is on chromosome 2q24 containing PLA2R1. For patients who were homozygous for these alleles, their odds ratio for having primary membranous nephropathy was 78.5 [67]. This work has recently been validated in a study using genotype and HLA imputation alongside a GWAS in 323 patients with primary MN. Here the association of HLA-DQA1 and PLA2R1 with primary MN was confirmed, without detecting any other novel signals [68].

How these genetic markers modulate the risk of developing MN is unknown. The idea that the genetically restricted class II presentation of PLA2R peptides to affect the class switch to high-affinity IgG anti-PLA$_2$R is a theory that remains to be tested.

9.2 Environmental trigger

Indicative of the rapid pace of research into primary MN since the discovery of the PLA2R antigen, we now have not only the clinical correlation of the antibody with disease activity but also the major epitope on the antigen and evidence for the genetic polymorphism located in the antigen itself. This, however, does not completely explain the development of the disease. The polymorphisms described in these studies are actually variants that are common to the general population. It seems likely that, similar to other autoimmune diseases such as IgA nephropathy, primary MN is a multi-hit disease. A patient with the polymorphism has a genetic predisposition but to develop the disease needs an external trigger.

Fresquet et al. have shown that an amino acid sequence which is part of the dominant epitope in the CysR region of the PLA$_2$R antigen is also found in the cell wall of some species of clostridia [48]. Further searches using the Basic Local Alignment Search Tool (BLAST) [69] has shown this peptide sequence is found in a number of other common pathogens such as Pseudomonas and *Saccharomyces cerevisiae*.

There is now also emerging evidence implicating air pollution in the development of autoimmune MN. A recent large study in China investigating the emerging trends of glomerulopathy based on renal biopsies in relation to air pollution noted a rise in the incidence of MN in all age ranges and in all regions, this is in contrast to other glomerular disease investigated which all remained the same. It was more prevalent in areas with the highest air pollution and the long-term average was found to be associated with a significantly increased risk of autoimmune MN.

9.3 Loss of tolerance

What is not known at present is the risk of developing autoimmune MN if you have the genetic predisposition, only that you are more likely to have the risk alleles if you have autoimmune MN. What remains elusive is how a patient's immune system converts from an advantageous defence against common pathogens to a pathogenic entity in itself. A characteristic of autoimmune MN is the heterogeneity shown in prognosis and its waxing and waning nature over time. A proportion of patients will undergo a phenomenon of spontaneous remission, and in patients with a more severe phenotype, it is not unusual for them to follow a relapsing and remitting course. Many patients, when they first come to medical attention, will describe self-limiting episodes many months or years prior to their diagnosis that is likely to be nephrotic states and the first signs of the disease. This suggests that far from being a continuously progressive immunological process, particularly in light of the pathogenicity of the autoantibody, that there may be natural mechanisms at play attempting to maintain a balance. Work in other autoimmune diseases such as autoimmune thyroiditis has proven the existence of antigens capable of maintaining a population of natural T Regs and thereby keeping pathogenic antibodies suppressed [70].

As the technology evolves, flow cytometry is becoming an ever more powerful tool for the study of the immune system. A recent study using patients enrolled in the GEMRITUX trial showed that patients had lower proportions of IgD− and IgD+ memory B cells, T Regs and a higher proportion of naïve B cells at baseline compared to healthy donors [71]. In this study by Rosenzwajg et al., patients who responded to treatment were observed to have a lower proportion of T Regs at baseline compared to those who did not respond to treatment. They also noted that in patients with no response to treatment, there was no increase in T Regs following treatment, however in patients who went on to respond, there was a significantly higher proportion of T Regs at day 8 compared to baseline [71].

This is similar to work currently being undertaken in our lab (unpublished) in which flow cytometry was used to model the immune system following treatment with immunoadsorption [72]. In our cohort, we also found that there was a lower proportion of IgD+ memory B cells in the patient group but a similar level of IgD− memory B cells albeit with a much larger range. For the Naïve B cells and T Regs, the medians were very similar between the patients and control group but with a much larger range in the patient cohort. One of the striking differences between our patient group and the control group at baseline is that there does not seem to be any statistical difference in PLA$_2$R positive B cells, with a number of volunteers in the control group showing a relatively high proportion of these cells. This seemingly counterintuitive result, in fact, appears to add weight to the importance of loss of tolerance in the disease process.

Given the shared sequence of amino acids (SVLTLENC), it could be expected during the development of normal natural immunity to a range of pathogens, developing IgM antibodies to this linear peptide sequence is common, entirely normal and beneficial to the host. The risk of developing an autoimmune pathology only arises then, if a patient has the genetic makeup (pathological alleles of DQA1 and PLA2R) required to present PLA$_2$R T cell peptides to their immune system. Only with the permissive genetic background and continued exposure to the pathogen or environmental trigger, causing immune processing of PLA$_2$R, will class switching occur from IgM to IgG, and therefore allowing the development of pathogenic high-affinity antibodies. In our PLA$_2$R panel, the healthy control group showed a significant level of PLA$_2$R positive B cells. A current on-going and unpublished project being carried out in our lab is the development of an IgM anti-PLA$_2$R

ELISA. Although it cannot be proven in the current flow cytometry experiment, it would appear to suggest that there is a high likelihood that the B cells seen in the healthy population may, in fact, be IgM positive B cells as opposed to being IgG positive.

A further interesting dimension to immune regulation and loss of tolerance that needs further study is the role that T reg cells play and how they are a potential mechanism for the suppression of pathogenic antibodies. The relapsing and remitting nature of autoimmune membranous nephropathy and the phenomenon of spontaneous remission indicates that at some level there must be an immune mechanism capable of suppressing the anti-PLA_2R antibodies, much like that found in autoimmune thyroiditis. Another on-going study, again unpublished, in our lab has identified a number of healthy controls without the prerequisite HLA-DQA1 or PLA2R1 genes needed to develop autoimmune MN, who have a detectable level of circulating soluble PLA_2R using mouse anti-PLA_2R as the capture antibody. There is the potential that these circulating soluble-PLA_2R antigens are active in maintaining a functioning level of T Regs to suppress class switching and downregulate the pathogenic antibody level. If natural T Regs did indeed have a role in keeping the pathogenic IgG anti-PLA_2R antibodies suppressed, the expectation would be that in times of active disease the levels would be low. The opposite would also be true with high levels in times of remission or just before remission or response to treatment. The T cell panel used for the patient cohort does start to show a pattern of T Regs change over time, a pattern that appears to support the theory above, especially when taken in the context of antibody level. At week 4 follow up, the T Regs level has dropped to their lowest point, this is also at the same time point at which the anti-PLA_2R is at its highest. The proportion of T Regs then show an increase at both week 10 and week 16 follow up, just as the antibody level is decreasing.

10. Summary

Autoimmune MN has experienced a step change in our understanding of the disease pathogenesis since the discovery of the anti-PLA_2R autoantibody in 2009 [15], however, there is much that still remains unknown. Despite the advances seen over the last decade, the management of the disease remains an empirical treatment based on a regimen first introduced over two decades ago. There is as yet no disease-specific therapy or alternative to glucocorticoids and immunosuppression in mainstream use.

As with all autoimmune diseases, the eventual clinically apparent symptoms are the end result in a journey of multiple steps, the so-called multi-hit hypothesis. We know that there is a strong genetic component in the development of the disease, with patients homozygous for both the HLA-DQA1 and PLA2R1 genes are almost 80 times more likely to develop the disease than patients who do not [67]. What we still do not know is whether the possession of these genes in itself guarantees the development of the disease. It is likely that a further trigger (likely environmental) is required to progress to the disease state.

Development of the normal natural immunity requires the production of antibodies, including IgM, to linear peptides in a whole range of epitopes. With this beneficial protective immunity, circulating IgM antibodies to the PLA_2R p28mer peptide can, in fact, be a normal occurrence. The presence of these antibodies in patients without the genetic predisposition to the disease would just be an expected variant of normal. It is in those patients who do have the genetic predisposition to developing the disease, that the presence of IgM antibodies with the ability to recognise the p28mer will have the potential to progress to the disease state to generate

a high-affinity IgG response. Once this occurs, and there is recognition of the podocyte PLA_2R epitope there begins a positive reinforcement with ever-increasing affinity. The exact nature of how patients eventually develop a pathogenic IgG antibody remains elusive. However, there is now tentative emerging evidence showing that in a control group of healthy volunteers and a patient group with active disease there is a PLA_2R antigen positive B cell population in both. This is coupled with an on-going unpublished study showing a level of circulating anti-PLA_2R IgM antibodies in these normal healthy patients. This requires further work, but it is the first evidence for an antibody class switch in autoimmune MN.

There is also data showing that as the anti-PLA_2R antibody rises in the weeks following treatment, there is a reduction in the natural T Regs. Following this, as the level of T Regs starts to rise there is a corresponding fall in the antibody level. Taken in tandem with unpublished work that is on-going showing a measurable level of circulating soluble PLA_2R in healthy controls, this would appear to show that a similar process to autoimmune thyroiditis is taking place in autoimmune MN.

There do remain a number of important questions in relation to the disease pathogenesis though; how does the anti-PLA_2R attaching to the epitope causes the damage we see? Despite strong circumstantial evidence suggesting its pathogenicity, direct evidence is currently lacking. Can the antibody titre supplant the need for a renal biopsy? How many patients who have the genetic predisposition eventually go on to develop the disease and is there a way to predict which patient does? And are there more autoantibodies associated with the development of autoimmune membranous nephropathy.

Current understanding of the role anti-PLA_2R plays in the pathogenesis has now led many to envisage a greater role for its use in clinical practice. Not only is it increasingly being used for disease monitoring and for prognosticating treatment response but it is also becoming a necessary tool for diagnosis. This has the distinct prospect of drastically altering the current diagnostic pathway and ultimately a patients quality of life. A number of groups are now actively investigating the feasibility of serum anti-PLA_2R negating the need for a renal biopsy, not only reducing time to diagnosis but also avoiding the need for an invasive procedure.

The hope is that by understanding the pathway of disease in this and other autoimmune conditions, new safer and more efficacious treatment options will be available for patients in the future. This is particularly pertinent given the increasing incidence of autoimmune diseases worldwide and the increased burden on patients and healthcare systems.

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