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# Membranous Nephropathy – The start of a paradigm shift

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## Abstract

**Purpose of review—**Primary membranous nephropathy is a common glomerular disease characterized by sub-epithelial immune deposits that has become the prototype of an autoimmune glomerular disease. The purpose of this review is to highlight recent advances regarding the pathogenesis of membranous nephropathy as well as potential new therapies.

**Recent findings—**The discovery of two major podocytes antigens: neutral endopeptidase (NEP), involved in rare cases of neonatal membranous nephropathy, and the M-type phospholipase–$A_2$ receptor 1 ($PLA_2R1$) the first antigen discovered in adults, have been major "breakthroughs" in our understanding of the pathogenesis of human membranous nephropathy. Anti-$PLA_2R$ antibodies appear to predict activity of the disease as well as response to therapy. Pediatric and adult cases of membranous nephropathy occurring in the presence of circulating cationic bovine serum album (BSA) and anti-BSA antibodies have also been described raising the possibility that food antigens may be involved in the development of membranous nephropathy. Moreover, the results of genetic susceptibility have become available. Exciting progress has also been made in the treatment of this disease including therapy with ACTH and Rituximab.

**Summary—**Understanding disease pathogenesis is crucial in guiding patient evaluation and designing appropriate therapy. Recent discoveries have helped to elucidate the pathophysiology of membranous nephropathy and may facilitate a more patient-specific treatment approach in these patients.

### Keywords

ACTH; cationic bovine serum albumin; membranous nephropathy; $PLA_2R$ antibodies

## Introduction

Membranous nephropathy is a common immune-mediated glomerular disease characterized by the presence of immune deposits on the epithelial side of the glomerular capillary wall. It remains the leading cause of nephrotic syndrome in Caucasian adults.[1] Until recently, most of our understanding of the pathogenic mechanisms came from experimental models in rats, i.e. the Heymann nephritis model.[2,3] In this model, megalin is the podocyte antigen involved but megalin is neither expressed in human podocytes nor detected in the subepithelial deposits in patients with idiopathic/primary membranous nephropathy. Thus, for years the membranous nephropathy target in human podocytes remained elusive. Thanks to modern technology, major advances have occurred in our understanding of the autoimmune processes involved in the development of human membranous nephropathy. A number of podocyte antigens, namely neutral endopeptidases (NEP), M-type phospholipase

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$A_2$ receptor ($PLA_2R$), aldose reductase (AR), and superoxide dismutase (SOD) 2 have been identified as targets for autoantibodies in patients with membranous nephropathy. Non-podocyte circulating antigens, i.e. cationic bovine serum albumin (BSA) responsible for childhood forms of membranous nephropathy have also been described. The presence of some antibodies appears to correlate with disease activity and response to treatment. Genetic studies are elucidating predisposing factors for development of the disease. Although in most patients the disease progresses relatively slowly, approximately 40% of patients eventually develop ESRD.[4] Because of its frequency, it remains the 2nd or 3rd most common type of primary glomerulonephritis resulting in end stage renal disease.[5] Available immunosuppressive therapies are at least partially successful in reducing proteinuria in membranous nephropathy, but their use is controversial and all are associated with a significant adverse effects and a high relapse rate, thus tempering their use. (reviewed in [6]) This review will highlight the most recent findings in the pathogenesis of the disease as well as potential new therapies for patients with membranous nephropathy.

## Anti-neutral endopeptidase antibodies

The initial proof that circulating antibodies against a podocyte protein could cause membranous nephropathy in humans came from Debiec and colleagues who first described the case of a patient with neonatal membranous nephropathy due to the transplacental transfer of circulating anti-neutral endopeptidase antibodies to the fetus.[7] Neutral endopeptidase (NEP) is a membrane bound enzyme that is able to digest biologically active peptides and is expressed on the surface of human's podocytes, syncytiotrophoblastic cells, lymphoid progenitors, and other many epithelials cells and polymorphonuclear leukocytes. Mothers with truncating mutations of the metallomembrane endopeptidase (MME) gene fail to express NEP on cell membranes. NEP-deficient mothers, who were immunized during pregnancy, were able to transplacentally transfer nephritogenic antibodies against NEP to her children causing membranous nephropathy in the newborn.[8] The fact that rabbits injected with the maternal IgG form from these mothers also developed membranous nephropathy was another proof that the disease was related to circulating anti-NEP antibodies, and demonstration of a human counterpart to Heymann nephritis.[9]

## $PLA_2R$ autoantibodies

The discovery that antibodies to the M-type phospholipase $A_2$-receptor ($PLA_2R$) are present in 70 to 82% of the patients with primary membranous nephropathy has revolutionized the field of membranous nephropathy.[10] The $PLA_2R$ is a transmembrane receptor belonging to the mannose receptor family and a receptor for the secreted phospholipase A2, a lipolytic enzyme that cleaves the fatty acid bond of membrane glycerophospholipids.[11] A common functional feature of this family of receptors is their ability to undergo endocytosis and thus involved in the internalization of extracellular ligands. Sera from patients with primary membranous nephropathy contained IgG4 antibodies that specifically recognized $PLA_2R$, but these antibodies are not present in the serum of healthy controls, in patients with secondary causes of membranous nephropathy, or other glomerular and autoimmune diseases.[10,12] Levels of anti-$PLA_2R$ have been found correlate strongly correlated with the disease activity: disappearance of the antibody is associated with remission of proteinuria while reappearance of the antibody heralds a relapse of nephrotic syndrome. [10,13,14] How the presence of these antibodies lead to the development of proteinuria is unknown.

Monitoring anti-PLA2R levels may be also helpful to monitor response to therapy. Hoxha et al., treated 5 patients with membranous nephropathy with rituximab; in 2 of them disappearance of anti-PLA2R from circulation anticipated a complete or partial remission of

proteinuria.[12] Patients who failed to clear anti-PLA2R from circulation did not achieve remission of proteinuria. Similarly, we have recently reported on our experience with the use of rituximab in 35 patients with primary membranous nephropathy treated with 2 to 4 doses of rituximab.[13] Pretreatment samples from 25 of 35 (71%) patients contained antibodies against anti-$PLA_2R$, and these autoantibodies declined or disappeared in 17 (68%) of these patients within 12 months after rituximab. Those who demonstrated this immunologic response fared better clinically with 59% and 88% of the patient attaining complete or partial remission of proteinuria at the end of 12 and 24 months, respectively. This compared with 0% and 33% among those patients with persistent anti-$PLA_2R$ levels. Changes in antibody levels preceded changes in proteinuria. One subject who relapsed during follow-up had a concomitant return of anti- $PLA_2R$.[13] (Figure 1)

Further studies are needed to explain the potential discrepancies between circulating anti-$PLA_2R$ antibodies and detectable $PLA_2R$ in glomerular deposits in some patients. Debiec and Ronco evaluated the presence of $PLA_2R$ autoantibody in the serum and $PLA_2R$ in glomerular deposits in 42 consecutive patients with primary membranous nephropathy.[15] In 21 patients, anti-$PLA_2R$ antibodies were present in circulation and had $PLA_2R$ in glomerular deposits. However, 3 patients with high levels of circulating anti-$PLA_2R$ antibodies did not have detectable $PLA_2R$ in glomerular deposits. These cases suggest that antibodies were not nephritogenic or that epitopes were poorly accessible at the time of kidney biopsy. On the other hand, 18 patients had no detectable circulating anti-$PLA_2R$ antibodies, although 10 of them had $PLA_2R$ in glomerular deposits. Debiec and Ronco suggest that these apparently discordant findings might be due to rapid clearance of antibodies from the circulation but deposition in glomeruli or patients with persistent proteinuria due immunologically inactive disease but with irreversible glomerular ultrastructural changes.[15]

The recognition that proteinuria may persist despite the absence of immunological disease activity but as a consequence of an altered/remodeled glomerular basement membrane due to longstanding disease is of great clinical relevance. In this situation, further immunosuppression would be unnecessary and potentially harmful, and management should be conservative. Alternatively, the persistent proteinuria in the presence of circulating anti-$PLA_2R$ could be due to ongoing immunological disease and continued, increased, or altered immunosuppressive therapy should be considered.[13] Taken all together, these observations suggest that detection of circulating anti-$PLA_2R$ antibodies and $PLA_2R$ in biopsy samples and quantification of circulating anti-$PLA_2R$ levels may provide a tool for monitoring disease activity and treatment efficacy in patients with membranous nephropathy.

The role of anti-$PLA_2R$ antibodies has also been evaluated in recurrent and de novo membranous nephropathy after kidney transplantation.[16] Anti-$PLA_2R$ antibodies were present in 5 of 10 patients with recurrent membranous nephropathy, but in none of the 9 patients with de novo membranous nephropathy. Some patients with ESRD due to membranous nephropathy and circulating anti-$PLA_2R$ antibodies at the time of kidney transplant did not develop recurrence. We have a similar experience in our own transplant population in which positive anti-$PLA_2R$ antibodies at the time of transplant do not predicting recurrence of membranous nephropathy. Because anti-$PLA_2R$ antibodies are not always associated with recurrence of the disease, the presence of these antibodies should not preclude kidney transplantation in patients with ESRD secondary membranous nephropathy and positive anti-$PLA_2R$ antibodies.

## Antibodies to superoxide dismutase 2 (SOD2), aldose reductase (AR) and α-enolase

Using a combined proteomic approach Prunotto et al. identified specific IgG4 antibodies against the cytosolic proteins SOD2, AR, and α-enolase in both serum and glomeruli of patients with primary membranous nephropathy but not from biopsies of patients with other glomerular diseases or normal kidney.[17] It is unclear what the role of these antibodies is in the pathogenesis of primary membranous nephropathy. As opposed to NEP and $PLA_2R$, these are cytosolic proteins and are not present or minimally expressed on normal podocyte membranes but are “neo-expressed” in glomeruli of patients with membranous nephropathy. As in the case of anti- $PLA_2R$ antibodies, the predominance of IgG4 in the glomerular immune deposits supports the concept of an isotype-specific mechanism. Mechanisms of translocation of these intracellular molecules have been proposed as way of explaining the development of these autoantibodies. Preliminary in vitro data showed an increase of SOD2 expression on podocyte plasma membrane after treatment with hydrogen peroxide, suggesting that oxidative stress may drive glomerular expression of SOD2, whereas induction of AR seems less specific.[17] It is also possible that antibody spreading occurs, whereby the development of a particular autoantibody (e.g. anti-NEP, anti-$PLA_2R$, etc.) induces the expression of other antigens that, in turn, form targets for the development of additional autoantibodies.[18]

Most recently, the same group also identified glomerular α-enolase as an additional target for autoantibodies in patients with membranous nephropathy.[19] Alpha-enolase is one of the most abundant cytosolic protein, is particularly expressed in tubular kidney cells but it is absent in normal glomeruli. IgG4 antibodies against α-enolase were present in circulation, were eluted from microdissected glomeruli, and co-localized with C5b-9 (membrane attack complex) in sub-epithelial deposits from kidney biopsies of patients with membranous nephropathy. Antibodies against α-enolase have been reported in patients with primary and secondary membranous nephropathy [20] as well as in patients with a number of other autoimmune diseases including lupus erythematosus, ANCA associated vasculitis and inflammatory bowel diseases[21–25] and whether these antibodies are simply a reflection of a nonspecific/molecular epitope spreading immune response in membranous nephropathy awaits further research.

## Antibodies to Bovine Serum Albumin

Debiec and colleagues recently evaluated a cohort of 9 children and 41 adults with primary membranous nephropathy and found high levels of circulating anti-bovine serum albumin antibodies, of both IgG1 and IgG4 subclasses, in 4 children and 7 adults with membranous nephropathy.[26] These patients also exhibited high levels of circulating bovine serum albumin but without an increase on immune complex levels in circulation. Bovine serum albumin (BSA) immunopurified from these 4 children’s serum differed from BSA purified from adult patients by migrating in the basic range of pH, whereas BSA from adults migrated in neutral regions and similar as native bovine serum albumin. In children who were negative for anti-$PLA_2R$ antibodies but had both high levels of circulating cationic BSA and BSA-specific antibodies, BSA colocalized with IgG in subepithelial immune deposits. IgG1 and IgG4 eluted from kidney-biopsy specimens showed reactivity that was specific for BSA.

The mechanism of BSA-induced membranous nephropathy in these patients is unclear. Human exposure to BSA is common (e.g. cow’s milk) and antibodies to BSA are common in the general population.[27] A variety of modern food processing conditions could induce protein-modifications affecting the proteolysis of BSA as well as facilitating the absorption

and passage into the bloodstream. The relative elevated PH in infants (pH 3 or 4 versus pH 2 in adults) and immaturity of the gastrointestinal tract could be make children more susceptible to absorb not digested or only partially digested BSA, especially in the setting of childhood gastroenteritis. In this context, positively charged circulating cationic BSA could become attached to the negatively charged glomerular endothelial glycocalyx and heparan sulphate proteoglycans in the GBM acting as a target for the deposition of anti-BSA IgG and *in situ* formation of immune-complexes.(Figure 2) In animal models, cationic form of BSA can induce membranous nephropathy ("planted" antigen model).[28–30] The studies by Ronco and Debiec suggest that the "planted" antigen model can also be applied to human disease.[26]

In patients with BSA-mediated membranous nephropathy, the antibodies predominantly targeted the BSA peptide 147–161 (containing two linear epitopes not present in human albumin and without cross reactivity to podocyte proteins), whereas controls with high anti-BSA antibodies but no membranous nephropathy had a broader spectrum of peptides reactivity. Debiec et al. suggest that the psychochemical properties of the BSA (e.g. charge; BSA modification during food processing/digestion) together with the amount of circulating BSA as well as a predominant T-helper type 2 (Th2) immune response resulting in production of IgG4 are the conditions necessary for the development of membranous nephropathy.[31]

The four children with membranous nephropathy had both high levels of anti-BSA antibodies as well as BSA in circulation, and similar findings were seen in four of the seven adults with membranous nephropathy. BSA could specifically be detected in glomerular immune deposits only in patients who had both circulating cationic BSA and anti-BSA antibodies suggesting that both are required for the development of the disease.[26] Levels of anti-BSA IgG1 and IgG4 antibodies and circulating cationic BSA correlated with disease activity: high in patients with nephrotic range proteinuria and low in patients in remission.

Further studies are needed to explain the origin of circulating cationic BSA. BSA immuno-purified from the serum of children migrate in the basic range of pH, whereas the BSA from adult patients migrated in the neutral region as native BSA. BSA colocalized with IgG immune deposits only in four children with circulating cationic BSA, but in none of the 18 adults patients with membranous nephropathy for whom biopsy specimens were available, implying that only cationic BSA can induce membranous nephropathy. On the other hand, positive $PLA_2R$ staining was detected in 14 of the 20 adult biopsy specimens again pointing to a different pathogenic process in adults with membranous nephropathy. Why only antibodies against BSA amino acid residues 147–161 are associated with membranous nephropathy? Is genetic susceptibility the additional hit that triggers membranous nephropathy? Antibodies against other regions of BSA have been reported in patients with rheumatoid arthritis and multiple sclerosis but these patients do not have an associated membranous nephropathy.[32,33] Whether or not dietary proteins could play a role in other cases of membranous nephropathy is unknown, but in children, the diagnosis of membranous nephropathy should raise the possibility of BSA-induced membranous nephropathy.

## Genetic susceptibility - the HLA-DQA1 and $PLA_2R1$ risk alleles

Interaction of genetic susceptibility and environmental factors could play a role in the development of glomerular diseases such as IgA nephropathy and primary membranous nephropathy.[34–37] Using genome-wide association studies (GWAS) Stanescu and a group of international collaborators recently linked single-nucleotide polymorphisms (SNPs) in the genes encoding M-type phospholipase $A_2$ receptor 1 ($PLA_2R$) and HLA complex class II

HLA-DQ alpha chain 1 (HLA-DQA1) in Caucasian populations with membranous nephropathy.[38] Although the risk for primary membranous nephropathy was higher with the HLA-DQ1 allele than with the $PLA_2R1$ allele, it adds support to the findings of positive anti-$PLA_2R$ antibodies in the majority of patients with membranous nephropathy.[10] For person who are homozygous for both risk alleles, the odds ration for developing membranous nephropathy is close to 80, with additive increase in the odds ration, depending on the combination of genotypes.[38]Although these findings do not prove causality, they suggest that genetic background plays a significant role in the predisposition of the primary membranous nephropathy, as previously noted in cases of familial primary membranous nephropathy.[39,40] The HLA region of the reported association spans a 6-Mb interval, extending far beyond the linkage-disequilibrium block of HLA-DQ1 raising the possibility that that several independently associated variants account for this signal.[41] Further work is required to confirm causative variants within the genomic regions, the causative link between the presence of the HLA-DQA1 risk allele and $PLA_2R1$ autoantibodies as well as identify specific gene-environment interactions that “trigger” membranous nephropathy.

## NEW THERAPIES

### Adrenocorticotropics hormone

Berg and colleagues were the first to report that adrenocorticotropics hormone (ACTH) lowered the albumin excretion and improved GFR in patients with membranous nephropathy.[42] Subsequent case series strengthened this observation.[43,44] A randomized controlled study in 32 patients with membranous nephropathy found similar remission rates in patients treated with ACTH compared to a 6-month course of alternating corticosteroid and cyclophosphamide.[45] These studies were conducted using a synthetic version of ACTH (Synacthen®; not available in the US), but a retrospective case series of 11 patients with treatment-resistant membranous nephropathy using a natural, highly-purified ACTH gel formulation (H.P. Acthar gel®), reported similar encouraging results. [46]. The exact mechanism by which ACTH mediates its effects in proteinuria is not completely understood, but is likely independent of its induction of cortisol production, as its production remains low and there is concomitant evidence that steroids alone do not affect the outcome of the disease.[47] ACTH is derived from the pro-opiomelanocortin (POMC) precursor. POMC is proteolytically cleaved by endopeptidases to yield various polypeptide fragments with varying physiological activity such as ACTH and α-melanocyte-stimulating hormone (α-MSH). The melano-corticotropic receptor (MCR1) is located on various cells, including B cells, T cells, antigen presenting cells and human podocytes. In a recent study, treatment with ACTH, α-MSH or MS05, a specific MCR1 agonist, showed similar but significant reduction in proteinuria in rats with passive Heymann nephritis.[48]. These results suggest that ACTH mediates its effects via α-MSH interaction on MCR1 on podocytes, and may explain why patients who are resistant to previous immunosuppressive therapies respond to ACTH. A randomized, placebo-controlled, study using Acthar® Gel in treatment-resistant patients with membranous nephropathy and nephrotic syndrome is currently underway. (ClinicalTrials.gov Identifier: NCT01386554)

### Rituximab

Previous studies have suggested that selective B-cell depletion with rituximab can induce remission of proteinuria in approximately 70% of the patients with v.[4,49] We recently extended these observations in a study involving 20 patients (11 failures to prior therapy) with membranous nephropathy and proteinuria >5g/24h who received rituximab ($375mg/m^2 \times 4$), with retreatment at 6 months regardless of proteinuria response.[50] Baseline proteinuria of 11.9±4.9g/24h decreased to 4.2±3.8g/24h and 2.0±1.7g/24h at 12 and 24 months, respectively ($p<0.001$) while creatinine clearance increased from 72.4±33 at

baseline to 88.4 ±31.5 ml/min/1.73m$^2$ at 24 months (p=0.02) (Figure 3). Of 18 patients who completed 24-months follow up, 4 were in complete remission, 12 were in partial remission (CR + PR = 80%), 1 had a limited response (>50% drop in P but >3.5g/24h) and 1 patient relapsed. Similar observation have been reported in a matched-cohort study compared two-year outcomes of 11 consecutive patients with primary membranous nephropathy who received rituximab as second line therapy for persisting nephrotic syndrome or relapsing disease.[51] A multicenter randomized control study comparing the use of Rituximab versus cyclosporine in the treatment of membranous nephropathy is currently on the way. (ClinicalTrials.gov Identifier: NCT01180036)

## Conclusion

As stated by Dr. Richard Glassock in his summation remarks at the 2nd International Conference on Membranous Nephropathy in Bergamo, Italy, in May 2011 “The field of membranous nephropathy is now in the early stages of what Thomas Kuhn called a “paradigm shift”. After years of relative stasis, and as a result of major breakthroughs we now find the field in a state of disequilibrium where the accelerated rate of new information generated greatly exceeds the discard rate of older information and the total information has expanded markedly”. We now know that auto-immunity accounts for 70–80% of the cases of membranous nephropathy and new auto-antibodies are been discovered, including antibodies against common food products. Further research will be needed to elucidate the trigger (s) for the production of these antibodies (molecular mimicry, activation of auto-reactive B/T cells, others) as well as to understand how they disrupt podocyte function. Genetic susceptibility is certainly involved in the development of membranous nephropathy as demonstrated by the linkage of these patients to HLA-DQ gene loci and to specific $PLA_2R$ SNPs. Promising new therapies are being evaluated by two important ongoing clinical trials. When commercially available, quantification of antibodies (e.g. anti-$PLA_2R$ antibodies) may help monitoring disease activity and response to therapy more efficiently than proteinuria alone. Soon, we should be able to reach the goal of “personalized” therapy in patients with membranous nephropathy.

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**KEY POINTS**

- Membranous nephropathy has become the prototype of an autoimmune glomerular disease.
- A number of autoantibodies have been described in these patients, including antibodies to common food products.
- Some of these autoantibodies, for example anti-$PLA_2R$, may help in monitoring disease activity as well as response to therapy.
- Genetic susceptibility appears to be involved.
- Novel approaches to treatment with the use of ACTH and rituximab are being tested currently.

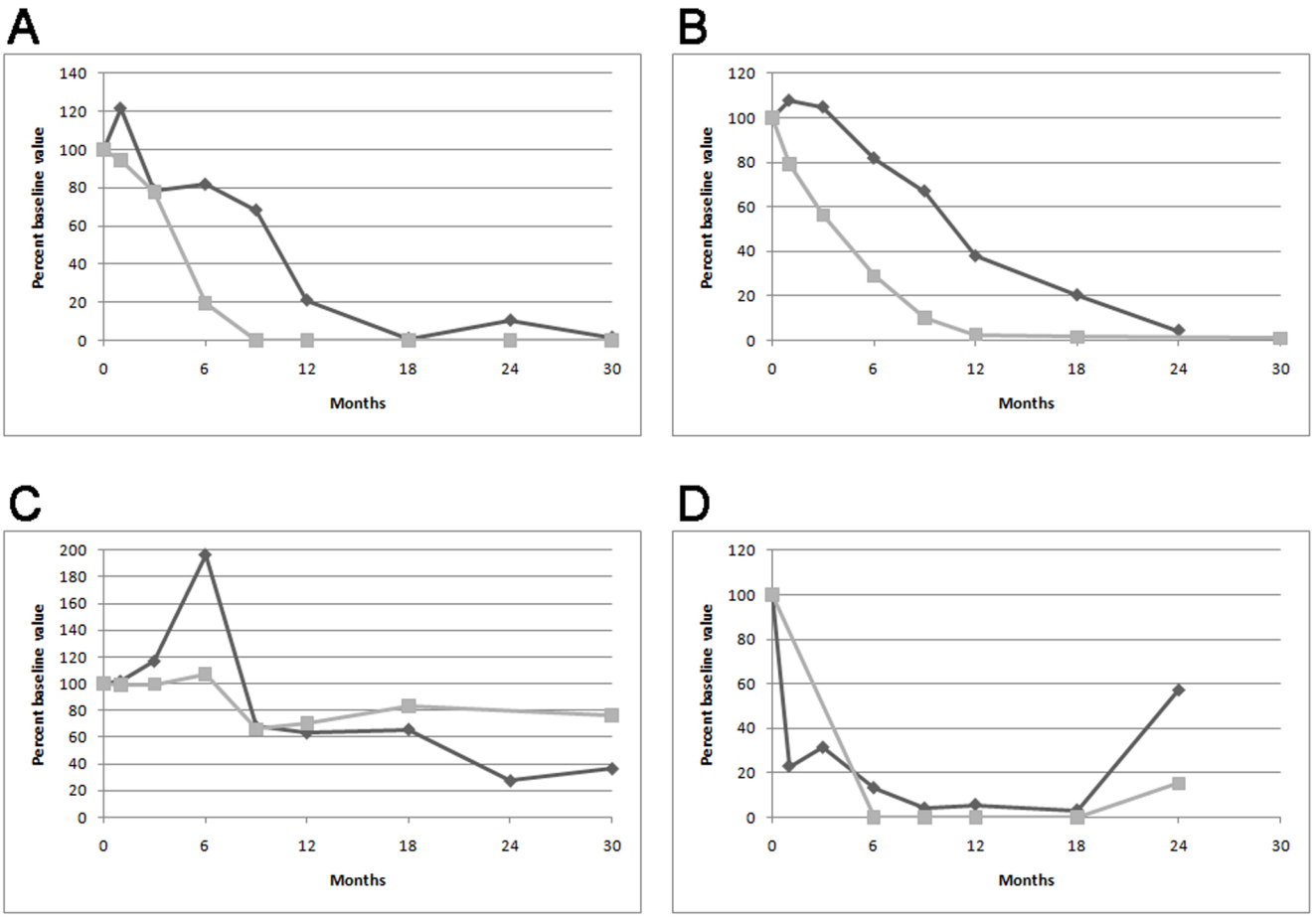


**Figure 1.**

Representative plots of anti-$PLA_2R$ (gray squares) and proteinuria (black diamonds) versus time following initial RTX treatment. Values are plotted as percent of baseline value. Panel (a) and (b) depicts the typical reduction and disappearance of anti-$PLA_2R$ followed by resolution of proteinuria exhibited by the majority of patients. Panel (c) is representative of patients in whom anti-PLA2R did not substantially decline following treatment and the associated with persistence of proteinuria. Panel (d) depicts the single patient whose anti-$PLA_2R$ level returned with relapse of his disease after having initially disappeared. (From Beck et al. [13] with permission)

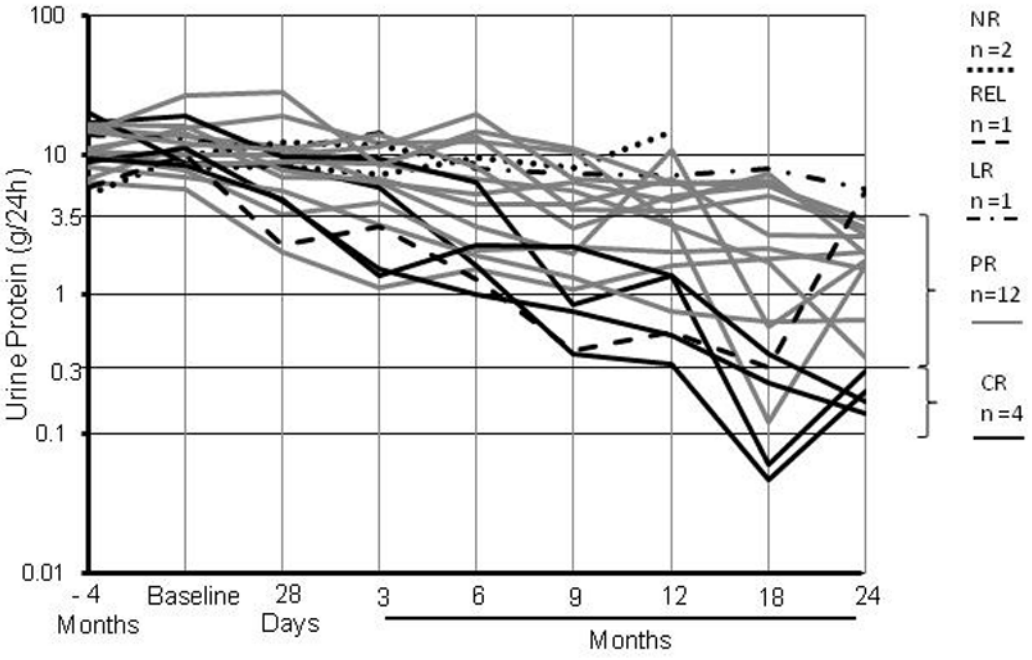


**Figure 2.**

Longitudinal effect of rituximab on proteinuria (log transformed). CR (complete remission) defined as proteinuria (P) less than 0.3 g/24h; PR (partial remission) defined as reduction in P of greater than 50% and final P less than 3.5g but greater than 0.3 g/24h; LR (limited response) defined as reduction in P of greater than 50% and final P greater than 3.5g/24h; NR (no response), a reduction in P of less than 50%; REL, relapse. (From Fervenza et al. [50] with permission)