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Pregnancy in a Patient With Primary Membranous Nephropathy and Circulating Anti-PLA₂R Antibodies: A Case Report

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Abstract

There is little information about pregnancy outcomes in patients with active membranous nephropathy (MN), especially those with circulating autoantibodies to M-type phospholipase A₂ receptor (PLA₂R), the major autoantigen in primary MN. Herein, we present what we believe to be the first known case of successful pregnancy in a 39-year-old woman with PLA₂R-associated MN. In the year prior to pregnancy, the patient developed anasarca, hypoalbuminemia (1.3–2.2 g/dl), and proteinuria (29.2 g/d). Kidney biopsy revealed MN with staining for PLA₂R, and the patient was seropositive for anti-PLA₂R autoantibodies. She did not respond to conservative therapy and was treated with intravenous rituximab (2 × 1 gram). Several weeks after presentation, she was found to be 6 weeks pregnant and was closely followed without further immunosuppressive treatment. Proteinuria remained in the 8–12 g/d range. Circulating levels of anti-PLA₂R declined but were still detectable. At 38 weeks, a healthy baby girl was born, without

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Supplementary Material

Figure S1: Immunofluorescence staining of PLA₂R in the patient's biopsy specimen.

Figure S2: Electron micrograph of the patient's biopsy specimen.

Figure S3: Western blot of maternal and fetal serum against recombinant PLA₂R.

Figure S4: Western blotting for anti-PLA₂R in placental tissue.

Note: The supplementary material accompanying this article (doi:_____) is available at www.ajkd.org

Supplementary Material Descriptive Text for Online Delivery

Supplementary Figure S1 (PDF). Immunofluorescence microscopy of the patient's biopsy specimen.

Supplementary Figure S2 (PDF). Electron micrograph of the patient's biopsy specimen.

Supplementary Figure S3 (PDF). Methods for Western blotting for cell-expressed recombinant PLA₂R.

Supplementary Figure S4 (PDF). Western blotting for anti-PLA₂R in placental tissue.

proteinuria at birth or at her subsequent 6-month postnatal visit. At the time of delivery, the mother still had detectable circulating anti-PLA₂R of immunoglobulin (Ig) G1, IgG3, and IgG4 subclasses, although at low titers. Only trace amounts of IgG4 anti-PLA₂R were found in the cord blood. Potential reasons for the discrepancy between levels of anti-PLA₂R in the maternal and fetal circulation are discussed.

Index words

membranous nephropathy (MN); nephrotic syndrome; pregnancy; M-type phospholipase A₂ receptor (PLA₂R); autoantibody; placenta; rituximab; immunoglobulin (Ig) G subclass

Pregnant patients with autoimmune disease may deliver newborns with a spectrum of clinical manifestations due to the transplacental passage of circulating autoantibodies. Pregnant patients with lupus or myasthenia gravis can deliver babies with corresponding disease in the neonate^{1, 2}. Neonatal membranous nephropathy (MN) not associated with congenital infection was first described in 1990 and attributed to the passive transfer of maternal antibodies to putative renal antigens³. More than a decade later, Debiec and colleagues identified the first antigen involved in such cases as neutral endopeptidase (NEP), a metalloprotease present on the surface of the podocyte and involved in the proteolytic regulation of vasoactive peptides⁴. Debiec et al described a mother with a mutation preventing expression of NEP who expressed anti-NEP antibodies due to fetomaternal alloimmunization from a previous miscarriage; these antibodies were to cross the placenta and cause subepithelial deposits in the fetal kidney of subsequent pregnancy. The M-type phospholipase A₂ receptor (PLA₂R) was later identified as the major autoantigen for primary MN in adults⁵. Little literature exists about pregnancy outcomes in patients with nephrotic syndrome due to primary MN, with no data available about pregnancy in PLA₂R-associated disease. Herein, we present what we believe to be the first known case of pregnancy in a patient with PLA₂R-associated MN who was seropositive for anti-PLA₂R autoantibodies throughout the course of her pregnancy.

Case Report

A 39-year-old multiparous woman with morbid obesity presented for work-up of severe nephrotic syndrome several months before her current pregnancy. She had been treated for resistant hypertension and lower extremity edema during the past year, but her proteinuria had been overlooked. At presentation, her serum creatinine level was 1.52 mg/dL (corresponding to an estimated glomerular filtration rate [eGFR] of 46 ml/min/1.73 m² as calculated by the IDMS-traceable 4-variable MDRD [Modification of Diet in Renal Disease] Study equation), serum albumin, 1.5 g/dL, and 24-hr urine protein, 29.2 g. Kidney biopsy specimen revealed features typical of primary MN with additional strong staining for the PLA₂R antigen within immune deposits (Figure S1). Many of the subepithelial deposits were completely surrounded by new basement membrane material (Figure S2) and 35% of the parenchyma showed evidence of tubular atrophy and interstitial fibrosis, suggesting some element of chronicity to this process. Based on a commercially available ELISA (Euroimmun US), the patient was seropositive for anti-PLA₂R antibodies, with a titer of 125

RU/ml. Despite the maximum level of conservative therapy (lisinopril 40 mg twice daily, and increasing doses of torsemide, simvastatin, and warfarin [initiated for severe hypoalbuminemia]), she failed to respond and was therefore treated with rituximab (2 intravenous doses of 1 gram each separated by 2 weeks).

Several weeks after these infusions, the patient was found to be 6 weeks pregnant. Lisinopril, simvastatin, and warfarin were immediately stopped, and her hypertension was reasonably well controlled with carvedilol, amlodipine, and torsemide. She thereafter was closely followed by both the renal and maternofetal medicine services. Following the rituximab doses, her circulating anti-PLA₂R titer declined throughout the course of pregnancy (Figure S3 and Figure 1), but her urinary protein-creatinine ratio remained high, at 8–12 g/g.

Toward the end of her pregnancy, the patient developed hypertension up to 190/110 mm Hg, with signs of fetal distress that prompted delivery by caesarian section. At 38 weeks, a healthy baby girl was born, without proteinuria at birth (or at her subsequent 6-month postnatal visit). The mother continued to have massive proteinuria and therefore was administered one additional course of rituximab therapy. She eventually went into partial clinical and full serological remission as evidenced by the improvement in her proteinuria, serum albumin, and total cholesterol levels. Her kidney function has stabilized with a creatinine of 1.55 mg/dl (eGFR, 45 ml/min/1.73 m²). Anti-PLA₂R antibodies were undetectable in her latest test.

Discussion

In order to investigate the presence of anti-PLA₂R antibodies in the maternal and fetal blood, we performed western blotting of human glomerular extract using maternal serum samples and cord blood serum collected at the time of delivery. Secondary antibodies specific for the different human immunoglobulin (Ig) G subclasses (The Binding Site Group Ltd) were used to qualitatively determine the relative proportions of IgG1, IgG3, and IgG4 anti-PLA₂R (Figure 2). IgG1 from both maternal and cord blood serum produced matching banding patterns in HE, with the notable exception of PLA₂R, which was detected only by the maternal serum. Fetal concentrations of IgG1, IgG3, and IgG4 are known to be at least equal to those in the maternal circulation during the last trimester⁶, so the apparent difference in anti-PLA₂R was unexpected. However, the cord blood serum was found to be very weakly positive for the IgG4 subclass of anti-PLA₂R.

The absence of proteinuria in the newborn was surprising, as our assumption---based on the literature---was that the maternal anti-PLA₂R antibodies that were present throughout the entire pregnancy would have transferred to the fetus. It is clear from western blotting that, at the time of birth, there was a large discrepancy between the levels of anti-PLA₂R in the maternal and fetal circulation (Fig 2). Although we have no conclusive answers for this discrepancy, we will discuss several possibilities that might explain this difference as well as the infant's absence of kidney disease.

The placenta acts as a biologic modulator that regulates disease expression in the newborn⁷. Its role is not limited to its barrier function⁸ but rather extends to a more complicated transplacental Fc-receptor–dependent transport system that is influenced by antibody subclass and avidity^{9, 10}. However, it is known that IgG, including IgG4, is normally efficiently transported across the placenta⁶. As PLA₂R is known to be present in placental tissues^{11, 12}, it is possible that in our patient the placenta may have acted as an immunoadsorbant, sequestering the antibodies before they were able to reach the fetal circulation. We sought to examine this possibility by acid elution of IgG from the patient's placental tissue to determine if there was detectable reactivity to PLA₂R. Although total IgG was successfully eluted, we were not able to find any reactivity to human PLA₂R by western blotting (Fig S4). Although this suggests that there was no major sequestration of anti-PLA₂R in the placenta, we note that the amount of the placental sample available for testing was small, and thus we cannot exclude sequestration in other, unsampled portions of the placenta.

An alternate hypothesis is that the anti-PLA₂R was, in fact, transferred to the fetal circulation, but that it was rapidly sequestered in the developing fetal kidneys, with an increasing mass of potential antibody binding sites. The recently measured high affinity of anti-PLA₂R for an epitope in the amino-terminal cysteine-rich domain of the protein is supportive of the kidney acting as a “sink”¹³. Decreasing maternal titers and distribution among the expanding mass of antigenic targets on the fetal podocytes could have yielded immune deposits too small to amount to clinical disease. There was no clinical indication to biopsy the infant at birth, though had we done so, it is possible that miniscule subepithelial deposits might have been present. Protocol or clinically indicated biopsies that occur very early after kidney transplantation into anti-PLA₂R-seropositive recipients demonstrate that small deposits can be detected without clinically significant proteinuria^{14, 15}. Additionally, PLA₂R-containing immune deposits can be demonstrated prior to the emergence of detectable circulating anti-PLA₂R in the recipient¹⁶.

Variability in the dominant IgG subclass can also affect disease phenotype, as recently shown in anti-NEP-associated neonatal MN, in that IgG1 anti-NEP had a more deleterious effect than the IgG4 subclass¹⁷. The absence of cord blood IgG1 anti-PLA₂R in the present case may therefore explain the lack of clinical disease. Finally, as opposed to the generation of maternal antibodies directly against a fetal alloantigen, the autoimmune process involved in the targeting of antibodies to PLA₂R may be dependent on an acquired change or “second hit” in maternal PLA₂R that had not yet occurred in the fetal kidney.

In summary, although the outcome of pregnancy in our case was favorable, potentially due to the course of rituximab administered before it was known that the patient was pregnant, we are hesitant to suggest that pregnancy is universally safe in nephrotic patients with immunologically active PLA₂R-associated MN. In fact, we might have predicted a worse outcome had the mother had higher or increasing titers of anti-PLA₂R during the pregnancy, particularly if there had been co-dominance of a complement-fixing IgG subclass. Therefore, we recommend close attention to the immunologic course of MN in the mother, frequent assessments of the mother and fetus throughout the pregnancy, and careful monitoring of the infant in the perinatal period.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Watson RM, Lane AT, Barnett NK, Bias WB, Arnett FC, Provost TT. Neonatal lupus erythematosus. A clinical, serological and immunogenetic study with review of the literature. *Medicine (Baltimore)*. 1984; 63:362–378. [PubMed: 6390081]
2. Tzartos SJ, Efthimiadis A, Morel E, Eymard B, Bach JF. Neonatal myasthenia gravis: antigenic specificities of antibodies in sera from mothers and their infants. *Clin Exp Immunol*. 1990; 80:376–380. [PubMed: 1695559]
3. Nauta J, de Heer E, Baldwin WM 3rd, ten Kate FJ, v d Heijden AJ, Wolff ED. Transplacental induction of membranous nephropathy in a neonate. *Pediatr Nephrol*. 1990; 4:111–116. [PubMed: 2397175]
4. Debiec H, Guignonis V, Mougenot B, Decobert F, Haymann JP, Bensman A, et al. Antenatal membranous glomerulonephritis due to anti-neutral endopeptidase antibodies. *N Engl J Med*. 2002; 346:2053–2060. [PubMed: 12087141]
5. Beck LH Jr, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med*. 2009; 361:11–21. [PubMed: 19571279]
6. Saji F, Samejima Y, Kamiura S, Koyama M. Dynamics of immunoglobulins at the feto-maternal interface. *Rev Reprod*. 1999; 4:81–89. [PubMed: 10357095]
7. Rocha-Alvarez R, Friedman H, Campbell IT, Souza-Aguiar L, Martins-Castro R, Diaz LA. Pregnant women with endemic pemphigus foliaceus (Fogo Selvagem) give birth to disease-free babies. *J Invest Dermatol*. 1992; 99:78–82. [PubMed: 1607681]
8. Chaouat G, Kolb JP, Wegmann TG. The murine placenta as an immunological barrier between the mother and the fetus. *Immunol Rev*. 1983; 75:31–60. [PubMed: 6226590]
9. Sennhauser FH, Balloch A, Macdonald RA, Shelton MJ, Robertson DM. Maternofetal transfer of IgG anti-Escherichia coli antibodies with enhanced avidity and opsonic activity in very premature neonates. *Pediatr Res*. 1990; 27:365–371. [PubMed: 2188203]
10. McNabb T, Koh TY, Dorrington KJ, Painter RH. Structure and function of immunoglobulin domains. V. Binding, University of immunoglobulin G and fragments to placental membrane preparations. *J Immunol*. 1976; 117:882–888. [PubMed: 956658]
11. Ancian P, Lambeau G, Mattéi MG, Lazdunski M. The human 180-kDa receptor for secretory phospholipases A2. Molecular cloning, identification of a secreted soluble 10 form, expression, and chromosomal localization. *J Biol Chem*. 1995; 270:8963–8970. [PubMed: 7721806]
12. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. *Science*. 2015; 347:1260419. PLA2R1 expression data accessed from <http://www.proteinatlas.org/ENSG00000153246-PLA2R1/tissue/placenta> on. [PubMed: 25613900]
13. Fresquet M, Jowitt TA, Gummadova J, Collins R, O'Cualain R, McKenzie EA, et al. Identification of a major epitope recognized by PLA2R autoantibodies in primary membranous nephropathy. *J Am Soc Nephrol*. 2015; 26:302–313. [PubMed: 25288605]
14. Blosser CD, Ayalon R, Nair R, Thomas C, Beck LH Jr. Very early recurrence of anti-Phospholipase A2 receptor-positive membranous nephropathy after transplantation. *Am J Transplant*. 2012; 12:1637–1642. [PubMed: 22390840]

15. Rodriguez EF, Cosio FG, Nasr SH, Sethi S, Fidler ME, Stegall MD, et al. The pathology and clinical features of early recurrent membranous glomerulonephritis. *Am J Transplant.* 2012; 12:1029–1038. [PubMed: 22233329]
16. Kattah A, Ayalon R, Beck LH Jr, Sethi S, Sandor DG, Cosio FG, et al. Anti-phospholipase A₂ receptor antibodies in recurrent membranous nephropathy. *Am J Transplant.* 2015; 15:1349–1359. [PubMed: 25766759]
17. Vivarelli M, Emma F, Pellé T, Gerken C, Pedicelli S, Diomedi-Camassei F, et al. Genetic homogeneity but IgG subclass-dependent clinical variability of alloimmune membranous nephropathy with anti-neutral endopeptidase antibodies. *Kidney Int.* 2015; 87:602–609. [PubMed: 25565308]

Figure 1.

Clinical course of disease around the time of pregnancy. Plotted on the left axis is UPCR (values are from random collections, with the exception of the initial value of 29.2 g, which is a 24h collection); the right axis is serum albumin. In addition, anti-PLA₂R levels are indicated by squares; ELISA titers in RU/ml are labeled for each point. Time points on the x axis refer to time in relation to estimated date of conception. Arrows indicate the timing of rituximab (RTX) administration, given as 2×1 g doses in early pregnancy and after pregnancy. Bars represent timing of lisinopril and tacrolimus administration.

Figure 2.

Western blotting of native human glomerular extract (HGE; a source of native PLA₂R) was performed with maternal serum (0.4 months pre-pregnancy and at the time of delivery) and serum derived from cord blood at the time of delivery. Left panel: IgG1, IgG3, and IgG4 subclasses of anti-PLA₂R were individually detected with subclass-specific secondary antibodies. Cord blood recognizes identical non-specific bands in HGE as does maternal serum at delivery, with the notable exception of PLA₂R (arrow). A positive-control lane (not shown) in which recombinant PLA₂R was electrophoresed was used to identify the position of the PLA₂R band. Right panels: Subclass-specific detection of native PLA₂R (HGE) in maternal vs. cord blood serum. Times indicated are duration of exposure of the western blot exposure to film. Cord blood produces a weak gG4 anti-PLA₂R band in the 30- and 90-second exposures.