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**Towards a bona fide animal model of PLA2R1-associated
membranous nephropathy: one step beyond**

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Towards a bona fide animal model of PLA2R1-associated membranous nephropathy: one step beyond

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Abstract

The major form of membranous nephropathy (MN) is characterized by autoantibodies to phospholipase 1 receptor (PLA2R1). The study by Tomas et al. describes the first animal model where human PLA2R1 (hPLA2R1) is ectopically expressed in mouse podocytes. Intriguingly, the transgenic mice spontaneously develop anti-hPLA2R1 antibodies and MN-like features, including immune deposits and nephrotic syndrome. The model raises questions about the spontaneous production of anti-hPLA2R1 antibodies and the additional steps to establish a bona fide animal model of MN.

Main text

Membranous nephropathy (MN) is a rare autoimmune kidney disease, yet a major cause of nephrotic syndrome in non-diabetic adults, with about 10,000 new cases per year in Europe¹. The disease is defined by specific glomerular lesions with thickening of the glomerular basement membrane due to granular immune complex deposits consisting of IgGs, antigens and complement components, leading to effacement of podocyte foot processes, heavy proteinuria and kidney failure. Autoantibodies against the phospholipase A2 receptor 1 (PLA2R1) are found in about 70% of MN patients, identifying PLA2R1 as the major autoantigen¹. Establishing an animal model of PLA2R1-associated MN is crucial to better understand the pathophysiological mechanisms of the disease. However, unlike in humans, PLA2R1 is not expressed in mouse podocytes. A murine model of PLA2R1-associated MN thus requires ectopic expression of PLA2R1 in mouse podocytes as a primary step.

In 2020, Meyer-Schwesinger et al. described a model of transgenic mice overexpressing mouse PLA2R1 (mPLA2R1) in podocytes (Figure 1A)². The mice were healthy and had a normal kidney function, and developed MN-like features only upon passive injection of rabbit anti-mPLA2R1 antibodies, leading to the formation of subepithelial immune complexes, complement activation and proteinuria². However, injection of patients' anti-PLA2R1 autoantibodies was ineffective. This first "mPLA2R1 transgenic model" was thus "a model of the model", representing an interesting passive animal model of "mPLA2R1-associated MN induced by anti-mPLA2R1 antibodies", requiring additional studies using PLA2R1 and anti-PLA2R1 of human origin².

The study by Tomas et al. in this issue represents an important step forward in the direction of establishing a mouse model of PLA2R1-associated human MN³. By using the same strategy as for mPLA2R1², the authors succeeded in expressing human PLA2R1 (hPLA2R1) in mouse podocytes. Surprisingly, without any treatment, all the transgenic animals quickly and spontaneously develop a massive production of anti-hPLA2R1 antibodies starting at week 4, immediately followed by a full-

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3 blown MN-like disease with the presence of hPLA2R1-IgG immune complexes in the glomeruli, a
4 structural alteration of the podocytes and the induction of a nephrotic syndrome (Figure 1B).
5 Furthermore, the circulating antibodies had the same features as patients' autoantibodies, recognizing
6 conformational epitopes in the same domains. No circulating hPLA2R1 was detected. The immune
7 causality of the observed phenotype was established by showing the absence of production of anti-
8 PLA2R1 antibodies and signs of MN-like disease in hPLA2R1 transgenic mice bred with Rag2-deficient
9 mice, which lack mature and functional B and T cells, and this despite high expression of hPLA2R1 in
10 podocytes in the double transgenic mice. This study thus represents an important step in our
11 understanding of PLA2R1-MN pathophysiology, proving the causality between the production of anti-
12 hPLA2R1 antibodies and their pathogenic effects when the same human antigen is expressed in
13 podocytes. Interestingly, this study was performed on a BALB/c background which is known to be more
14 Th2-prone than the commonly used C57BL/6 background, suggesting the possible contribution of
15 genetic factors, paralleling the situation observed in patients' cohorts with strong genetic associations
16 between HLA, PLA2R1 and other immune-related genes¹.

17
18 The study by Tomas et al. raises however several questions, starting with the fulgurant and auto-
19 inducible character of the model, which was likely unexpected by the authors. Specifically, what is the
20 nature of the anti-hPLA2R1 immune response: Is it truly auto-immune like in MN patients, alloimmune
21 like in NEP-associated MN⁴ or rather due to a "simple" hetero-immunization (also called xeno-
22 immunization) against a human neoantigen ectopically expressed in a cell-specific manner in mice? To
23 the best of our knowledge, this is a case of hetero-immunization against hPLA2R1. On one hand, in the
24 Tomas study, the ectopic expression of hPLA2R1 at the surface of mouse podocytes is driven by the
25 NPHS2 podocin promoter and should start as early as 8.5 dpc, as shown previously for β -galactosidase
26 expression in the original study using the same podocin promoter². On the other hand, the expression
27 of hPLA2R1, as it was designed on purpose, is highly specific to podocytes, without expression in other
28 kidney cells nor in the thymus³. This strongly suggests the absence of central tolerance to hPLA2R1.
29 Furthermore, the anti-hPLA2R1 antibodies did not react against mPLA2R1, showing the absence of an
30 autoimmune response³. In further contrast, in the previous study by Meyer-Schwesinger et al.², the
31 overexpression of mPLA2R1 in podocytes did not lead to formation of anti-mPLA2R1 antibodies,
32 because of the endogenous expression of mPLA2R1 in the thymus, maintaining tolerance. It should
33 also be noticed that mPLA2R1 and hPLA2R1 have an overall protein identity of only 75%, and in line
34 with their very different binding properties⁵, hPLA2R1 exhibits to the mouse immune system a highly
35 distinct protein surface containing species-specific conformational epitopes, as observed by Tomas et
36 al.³. Finally, because of its role in cellular senescence⁶, it cannot be excluded that the overexpression
37 of hPLA2R1 may induce podocyte cytotoxicity by itself or upon binding of anti-PLA2R1 antibodies,
38 further contributing to the immune response and pathogenicity. Together, these observations
39 converge to a scenario where the sole ectopic expression of hPLA2R1 at the surface of podocytes
40 serves as a neoantigen continuously presented to the immune system and without acquired central
41 tolerance in the thymus, leading to the fast and massive production of circulating anti-hPLA2R1
42 antibodies. The circulating antibodies in turn immediately target the same hPLA2R1 antigen on the
43 surface of podocytes, culminating in the rapid formation of immune deposits and heavy proteinuria
44 (Figure 1B). With such features, this hPLA2R1 transgenic model is probably the fastest ever observed
45 animal model of MN, which was likely not expected as such. As a consequence of the strong and
46 chronic immunization over weeks from the onset of hPLA2R1 expression in podocytes, some animals
47 developed ascites as early as the age of 5-6 weeks, and all animals were severely compromised by the
48 age of 12 weeks, requiring termination of the experiments³.

49
50 This specific hPLA2R1 scenario can be extended to a more general one, where the mouse podocyte
51 would serve as an antigen presenting cell for any "non-mouse antigen" expressed at the podocyte
52 surface and sufficiently distinct from the mouse ortholog protein to induce a hetero-immunization
53 response towards the "podocyte neoantigen", in the absence of a tolerogenic system in the thymus of
54 transgenic mice. In line with this scenario, we identified two studies of transgenic mice where a "non-
55 mouse" antigen was specifically expressed in podocytes by using the podocin promoter, and which in
56 turn induced podocyte injury by an unknown mechanism, but in fact possibly due to the production of
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3 antigen-specific pathogenic antibodies. In the first study consisting of two articles in a row, it is striking
4 to note that the specific expression of the human AGE receptor OST48 in podocytes, but not its
5 ubiquitous expression (see the previous article cited in ref 7) which is likely associated to central
6 tolerance, led to effacement of podocyte foot processes, endoplasmic reticulum stress and decline in
7 kidney function⁷. In the second study, rat nephrin was overexpressed in podocytes in mice deficient or
8 not for mouse nephrin, and the authors observed heavy proteinuria only in transgenic mice deficient
9 for mouse nephrin, likely because of loss of tolerance to the endogenous mouse nephrin⁸. Although
10 none of these studies analyzed the presence of antibodies to the respective human OST48 or rat
11 nephrin antigens, the observed phenotypes are consistent with the production of pathogenic
12 antibodies that would target the antigens on the podocyte surface and induce an MN-like disease,
13 similar to what is nicely shown in the study by Tomas et al. for hPLA2R1 (figure 1B).

14
15 With both the immune and pathogenic phases likely occurring in the kidney, this hPLA2R1 transgenic
16 model seems to fulfill the so-called definition of MN as an “organ-specific” disease. However, is this
17 the case for human MN? Some recent hypotheses suggest that the etiology of human MN may be
18 associated to dysregulation of expression of hPLA2R1 in the lung, associated with environmental
19 factors such as pollution¹. The model may also be useful to test the so-called “kidney-as-a-sink”
20 hypothesis and study the kinetics of production of circulating antibodies versus those bound to
21 podocytes.

22
23 Another important question raised by this model is its usefulness in therapeutic testing. This model is
24 clearly not representative of the situation in human patients where anti-hPLA2R1 antibodies seem to
25 be present years before diagnosis and the clinical signs of MN⁹. The fast and strong immune reaction
26 to hPLA2R1 will make difficult the fine dissection of the underlying pathophysiological mechanisms
27 and will limit the therapeutic window of intervention which is extremely narrow in time.

28
29 In conclusion, although this novel hPLA2R1 transgenic model represents a net progress in the
30 understanding of the mechanisms at play in PLA2R1-associated MN, we still need a bona fide animal
31 model that would recapitulate the multiple steps of the autoimmune and pathogenic phases of the
32 disease, in short from etiology to a bona fide autoimmune response to complement-mediated
33 podocyte injury¹. The next models of PLA2R1-associated may be obtained by further manipulating the
34 mouse genome by creating m/hPLA2R1 knock-in mice, by gene promoter targeting or acute
35 overexpression of hPLA2R1. Finally, this novel transgenic model may be useful to provide a more
36 general warning message to the kidney community, and even beyond, to avoid expressing a “non-
37 mouse” surface antigen in a podocyte or in a cell-specific manner, that would lead to the aberrant
38 production of antibodies which might be pathogenic to the cells expressing the corresponding antigen,
39 in a way different from autoimmunity and alloimmunity, but likely due to hetero-immunization.

40 41 42 43 **Disclosure**

44 FB declares no competing interests. G.L. receives royalties through CNRS related to the patents
45 “Diagnostics for membranous nephropathy” and “methods and kits for monitoring membranous
46 nephropathy” on PLA2R1 and THSD7A, respectively.

47 48 49 **Legend to Figure 1**

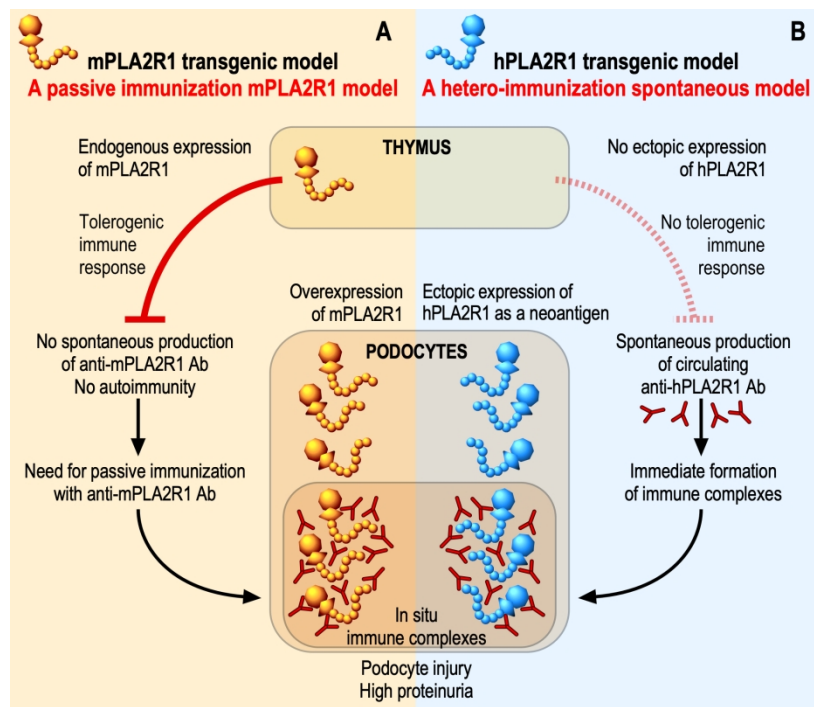
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51 **Major differences between the mouse and human PLA2R1 transgenic models of human MN.** In the
52 passive mPLA2R1 transgenic mouse model (**panel A**), the endogenous mPLA2R1 is expressed in the
53 thymus, leading to an effective tolerogenic immune response that inhibits the production of anti-
54 mPLA2R1 circulating (auto)antibodies (Ab), and hence preventing an autoimmune reaction. Passive
55 hyperimmunization by injection of exogenous anti-mPLA2R1 antibodies is needed to form in situ
56 immune complexes with mPLA2R1 overexpressed in podocytes and to induce MN-like features
57 including podocyte injury and proteinuria. In the hPLA2R1 transgenic mouse model (**panel B**), as the
58 expression of hPLA2R1 is driven by the promoter of podocin, a gene specifically expressed in
59 podocytes, there is no ectopic expression of hPLA2R1 in the thymus. In this condition, the absence of
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3 tolerogenic immune response allows the spontaneous and massive production of circulating anti-
4 hPLA2R1 antibodies which bind to hPLA2R1 ectopically expressed in podocytes and induce a full-blown
5 disease with MN-like features.
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8 9 **References**

10 **Due to strict constraints in the number of references, we apologize that some seminal works could**
11 **not be cited. The readers will find the related references in reference 1.**

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Legend to Figure 1

Major differences between the mouse and human PLA2R1 transgenic models of human MN. In the passive mPLA2R1 transgenic mouse model (panel A), the endogenous mPLA2R1 is expressed in the thymus, leading to an effective tolerogenic immune response that inhibits the production of anti-mPLA2R1 circulating (auto)antibodies (Ab), and hence preventing an autoimmune reaction. Passive hyperimmunization by injection of exogenous anti-mPLA2R1 antibodies is needed to form in situ immune complexes with mPLA2R1 overexpressed in podocytes and to induce MN-like features including podocyte injury and proteinuria. In the hPLA2R1 transgenic mouse model (panel B), as the expression of hPLA2R1 is driven by the promoter of podocin, a gene specifically expressed in podocytes, there is no ectopic expression of hPLA2R1 in the thymus. In this condition, the absence of tolerogenic immune response allows the spontaneous and massive production of circulating anti-hPLA2R1 antibodies which bind to hPLA2R1 ectopically expressed in podocytes and induce a full-blown disease with MN-like features.

212x148mm (300 x 300 DPI)