Advances of the experimental models of idiopathic membranous nephropathy (Review)

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Abstract. Idiopathic membranous nephropathy (IMN) is one of the main types of chronic kidney disease in adults and one of the most common causes of end-stage renal disease. In recent years, the morbidity of IMN among primary glomerular diseases has markedly increased, while the pathogenesis of the disease remains unclear. To address this, a number of experimental models, including Heymann nephritis, anti-thrombospondin type-1 domain-containing 7A antibody-induced IMN, cationic bovine serum albumin, anti-human podocyte antibodies and zymosan-activated serum-induced C5b-9, have been established. This review comprehensively summarized the available animal and cell models for IMN. The limitations and advantages of the current models were discussed and two improved models were introduced to facilitate the selection of an appropriate model for further studies on IMN.

Contents

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1. Introduction

Chronic kidney disease (CKD) is becoming a serious public health problem because it is related to an increased risk of cardiovascular disease and mortality (1-4). As one of the main types of CKD, membranous nephropathy (MN), is an autoimmune disease characterized by diffuse deposition of immune complexes under glomerular epithelial cells (GECs), with diffuse thickening of the glomerular basement membrane (GBM) (5,6). According to primary diseases, MN can be categorized into idiopathic membranous nephropathy (IMN) and secondary membranous nephropathy (SMN), while IMN accounts for approximately 80% of the total incidence of MN (7). In recent years, the morbidity of IMN in China significantly increased from 6.48% between 1997 and 1999 to 22.79% between 2009 and 2011 (8-11). The clinical prognosis of IMN varies greatly. Among untreated patients, approximately one third undergo spontaneous remission, one-third progress to end-stage renal disease over 10 years and the remainder develop non-progressive CKD. In short, approximately two-thirds of cases progress to CKD (12-15). Thus far, no curative therapies have been achieved for CKD or IMN (16,17), which may be attributed to unclear mechanisms. Therefore, the establishment of ideal model for IMN is important for mechanism research and a number of studies have tried to establish new models (18-21). This review comprehensively discussed the available animal and cell models for IMN. The limitations and advantages of the current models were discussed and two improved models were provided. Overall, this review could enable the selection of an appropriate model for studies on IMN and further the current understanding of human IMN.

2. Rat models of IMN

Heymann nephritis model. Heymann nephritis, also known as homologous immune complex nephritis, is a widely used model of MN (22). On the basis of whether the antibodies

are autologous or homologous or not, Heymann nephritis is divided into active and passive nephritis models (23). In 1959, Heymann *et al* (24) used autogenous or homologous rat homogenized proximal tubular brush border in immune rats to develop a model of nephritic syndrome, known as active Heymann nephritis (AHN). AHN is characterized by granular glomerular capillary wall deposits of rat immunoglobulin G (IgG) and subepithelial electron-dense deposits after 3-4 weeks. It has been demonstrated that 30-80% of AHN rats developed proteinuria within 8-10 weeks after immunization (25). Another study demonstrated that injected rats with resistance to the proximal tubule brush border antigen (Fx1A) antibodies also showed IgG, C3 and C5b-9 depositions under the glomerular epithelium and a significant level of proteinuria (26). This is termed passive Heymann nephritis. It indicates that the subepithelial deposits are formed by the circulating antibodies combining with the intrinsic antigen in the glomerulus rather than by the circulating immune complex. In this model, subepithelial electron-dense deposits should be detectable after 3-5 days of injecting anti-Fx1A and rats developed persistent proteinuria after about 7-10 days (27).

The pathogenesis of Heymann nephritis has been a controversial issue for a long time. At present, its antigen, mainly megalin (gp330), is believed to exist in the brush border of the proximal convoluted tubule and epithelial cell membrane of the glomerulus (24,28-30). In animal models, active immunization with megalin resulted in immune complex deposition under the epithelium of the glomerulus, without activation of C3 or C5b-9 and proteinuria. However, when the animals were injected with antibodies against megalin monoclonal antibody and complement regulatory proteins such as cluster of differentiation (CD)59 and CR1-related gene/protein Y at the same time, pathological proteinuria occurred (31). These studies confirm that the membrane attack complex (MAC; C5b-9) formed by the activation of the immune complex is the main inflammatory mediator of Heymann nephritis and is closely related to the production of pathological proteinuria (32,33). Furthermore, although megalin is expressed in human podocytes (34,35), it is not detected in the glomerular subepithelial immune complex and no circulating anti-megalin antibodies are found in patients with IMN (36). In addition, the complement pathway and subclass of IgG in this model are still unknown (37-39). Therefore, it is not equivalent to human IMN.

Anti-dipeptidyl peptidase IV model. Dipeptidyl peptidase IV (DPP IV) is identified as a major antigen (gp108) of Fx1A (40), which is mainly expressed on the brush borders of renal tubules, intestinal microvilli and glomerular capillary loops. After injecting rabbit anti-DPP IV in the rats, the rabbit IgG was deposited in the glomerular capillary loops for 4-8 h and proteinuria occurred within 8 h. After 2 days, proteinuria peaked and then rapidly decreased. To find the target antigen, serum DPP IV-depleted rats were used and similar results were obtained. The results suggest that DPP IV located along the glomerular capillary wall plays an important role in the induction of proteinuria (40). Compared with the model induced by gp330, this method induced the activation of a urinary protein that appears transiently, no deposition of C3 and faster disappearance of IgG (41). Taken together, these efforts reveal the target antigen in MN and the pathogenesis of the kidney disease model.

3. Mouse models of IMN

Thrombospondin type-1 domain-containing 7A-associated MN model. Thrombospondin type-1 domain-containing 7A (THSD7A), which was identified in 2014, is one of the target podocyte autoantibodies in IMN (42). These receptors are type I transmembrane glycoproteins consisting of three regions, namely the transmembrane domain, short intracellular C-terminal tail and large extracellular domain (43,44). In the human glomerulus, THSD7A is commonly expressed in foot processes, with observation of 250 kDa proteins (45) in non-reducing states. The intrinsic antigen respectively binds to anti-THSD7A IgG from the serum of the patient with MN, forming immune complexes *in situ* along with the glomerular filtration barrier (GFB) (46).

Previous findings have demonstrated that THSD7A is strongly expressed in murine podocyte (47). This has enabled the establishment of a model of THSD7A-related MN, which is closer to human IMN (Fig. 1). Tomas *et al* (20) injected human anti-THSD7A antibody-containing serum in mice and demonstrated that human anti-THSD7A autoantibodies can specifically bind to mouse (m)THSD7A. The mice manifest significant albuminuria around day 3 and until day 70. The researchers observed a granular pattern on the subepithelial aspect of the GFB, huIgG-mTHSD7A colocalization and immune complex deposition. In addition, immunofluorescence staining analyses revealed complement C3 accumulations within the subepithelial granular pattern. Consistent with the above-mentioned results, electron microscopic images showed electron-dense deposits with a strictly subepithelial localization and extensive foot process effacement around the local regions.

In the following year, a heterologous model of THSD7A-associated MN was introduced (48). First, the researchers generated rabbit anti-THSD7A antibodies by co-immunization with a combination of mTHSD7A and human (h)THSD7A cDNAs. Then, the mice were injected with anti-THSD7A IgG purified from rabbit serum. The mice that received rabbit IgGs developed severe nephritic syndrome and the albumin-to-creatinine ratio increased gradually during the entire observation period of 14 days. The mice presented the same histopathological changes as the abovementioned THSD7A-associated model, with the only differences being the unknown IgG hypotype and undetected C3 (Table I).

Anti-aminopeptidase A (APA) model. APA is a hydrolase present in the kidney of mice and is mainly located on the podocyte membrane and the brush border of proximal tubules. The model was established by injecting anti-APA monoclonal antibodies in mice (49). The visible antibodies, belonging to the IgG1 subclass, are widely distributed in the GEC membrane and proximal tubular S1 and S2 brush borders. The dose-dependent proteinuria lasted for 16 days and podocyte foot processes fused but lacked activation of the complement system. Subsequently, another study demonstrated that injection of anti-APA monoclonal antibody can cause two slit-pore-associated proteins, CD2-associated

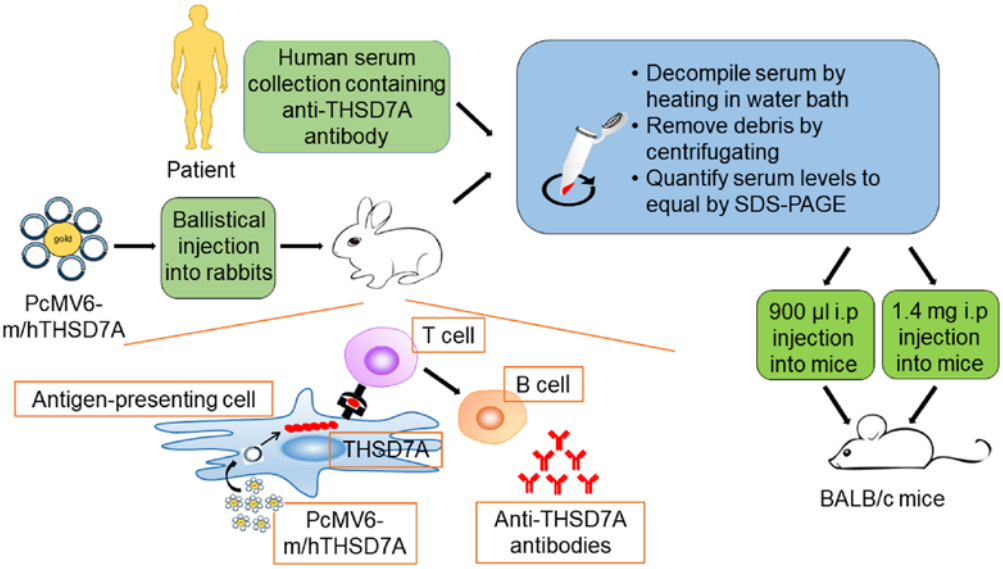

Figure 1. Establishment of two kinds of animal models of THSD7A. The antigen-presenting cell of the skin takes up pCMV6-m/hTHSD7A, which leads to the production of the full-length mTHSD7A and hTHSD7A in the antigen-presenting cell. Then, the encoded proteins are presented to the T cell through the peptide MHC, inducing the proliferation and differentiation of T cells, which produce helper T lymphocytes. With the assistance of helper T lymphocytes, B cells are activated, producing antibodies against THSD7A in rabbits. THSD7A, thrombospondin type-1 domain-containing 7A; m, mouse; h, human.

protein (CD2AP) and podocin structural alteration, which may be the underlying mechanism of proteinuria (50).

Cationic bovine serum albumin model. According to the negative characteristic of the GBM, injecting rabbits with cationic bovine serum albumin (C-BSA) daily (pI>9.5) intravenously induced proteinuria and nephrotic syndrome approximately 2 weeks later (51). Immunofluorescence showed IgG, C3 and other granule depositions in the GBM (52). Electron microscopy revealed subepithelial electron-dense deposits and irregular GBM thickening may occur in the later stages of the disease. To examine the mechanism of this model, C-BSA was injected in the renal artery to block the blood circulation after rinsing the rabbit kidney thoroughly with saltwater and anti-BSA antibodies were subsequently injected. Under immunofluorescence, immune complex particle sample distribution was observed along the glomerular capillary wall, which provided evidence that the pathogenesis of the C-BSA model did not involve circulating immune complex deposition in the glomerulus but immune complex deposition *in situ* (53). Chen *et al* (54) believed that this model is due to the C-BSA deposited in the GBM, which is full of negative charge and the corresponding antibody (principally IgG1), produced by its own form of immune complex *in situ*, activates the complement pathway. Debiec *et al* (55) found bovine serum albumin in subepithelial immune deposits in children with MN, demonstrating C-BSA binding to the anionic glomerular capillary wall and subsequent formation of IC *in situ*. They hypothesized that C-BSA may be one of the main pathogenic targets in childhood MN, especially in children aged <5 years.

Anti-α3NC1 mouse model. The non-collagen 1 domain of human α3 (IV) collagen (α3NC1) is a normal constituent of the GBM. Zhang *et al* (56) hypothesized that owing to its higher isoelectric point (pI~9), it may be conducive to the formation of GBM deposition, similar to the mechanism of C-BSA. Zhang *et al* (56) used mice immunized with α3NC1 to develop clinical and histopathological features of IMN. The extent and quality of autoimmunity against α3NC1 and T-cell responses are critical to the severity of nephropathy (57). Compared with other models, the prominent advantage is that regulation of gene expression is possible since mice are used. Furthermore, the model can be developed rapidly and reliably. Luo *et al* (58) found that factor B-null mice lacked glomerular deposition of C3 and C5b-9 and did not develop albuminuria. Albuminuria was reduced but not completely abolished in the C5-deficient mice. It indicates that the alternative pathway is necessary for pathogenic complement activation. Only the DBA/1 mice are associated with a high success rate for mouse modeling and other mice have a certain degree of resistance to α3NC1 and cannot be used for developing such models (59).

Anti-podocyte nephritis model. The anti-podocyte nephritis (APN) (60) model is created by injecting murine podocytes as antigens in allogeneic animals to produce antibodies; the immune serum is then injected back in the murine animal. This model can show large amounts of proteinuria and deposition of immune complexes. The deposited immune complex is mainly IgG, but the subclass may be different from that in humans and the amount of C3 deposition is minimal (61). The uses of podocyte antibodies produced in rabbits and mice are different. Antibodies produced by rabbit immunization do not affect mice and produce immune complexes by themselves, one needs to preimmunize mice with Freunds adjuvant. However, the monoclonal antibody produced by goat immune podocytes can cause nephrotic syndrome and epithelial immune complex formation. Through isolation of immunoreactive goat IgG and mass spectrometric analysis, the target antigen in the APN model was found to be Annexin 3 and researchers found a positive Annexin 3 expression in the renal tissue of patients (62). However, confirming whether

Table I. Similarities and differences between human and rabbit IgGs of THSD7A-associated MN.

Feature	Human IgG associated model	Rabbit IgG associated model
Similarities		
Animal	BALB/c mice	BALB/c mice
MN histomorphology	Granular subepithelial IgG deposition, subepithelial immune deposits, broadening of podocyte foot processes	More pronounced and extensive
Proteinuria	Significant proteinuria from day 3	Massive proteinuria from day 3, nephrotic syndrome
Initiation reaction	huIgG-mTHSD7A	Rabbit IgG-mTHSD7A
Differences		
Source of serum	Human with MN	Rabbit without MN
Injection	Whole serum from the patient	Purified IgG from rabbit
IgG in mice	huIgG, mIgG	Rabbit IgG
Complement C3	Present	Absent
Nephritic syndrome	Unpublished data	Serious
Application	Rodents studied	Not all rodent species
Establish	Comparatively simple	Comparatively complex

THSD7A, thrombospondin type-1 domain-containing 7A; MN, membranous nephropathy; IgG, immunoglobulin G.

this is another antigen of IMN as M-type phospholipase A2 receptor (M-PLA2R) requires further research (63).

4. Limitations and advantages of the animal models

Owing to different preparation methods, the abovementioned models all have limitations or advantages. Active Heymann nephritis is an autoimmune disease model and more closely resembles human membranous nephropathy, thus, it is more suitable for the study of the immune mechanism. However, it usually has large individual variability, which makes it unsuitable for studies of pharmacodynamics and injury (25). The passive Heymann nephritis model is widely used as it is relatively stable and its pathological changes are similar to human MN. The anti-DPP IV model is different from the pathological manifestation of human IMN due to C3 deficiency (47). The THSD7A-associated MN model is comparable to human IMN as a result of the common THSD7A antigen in mice and humans, similar to the complement activation pathways (lectin pathway) (64) and the common subclass of IgG (IgG4). However, the detection rate of THSD7A is approximately 3% in all patients with IMN (65,66). This model may not represent the pathogenesis and all pathological changes of IMN. Further, it is similar to the Heymann nephritis model, which is induced by the binding of antigens and antibodies. The C-BSA model is a new tool for IMN study that has a relatively low cost, simple operation and wide application. In addition, the degree of pathological changes is related to the dose; thus, models of different stages of IMN can be developed. The limitations of the model include the high mortality due to several tail vein injections and dosage. A unified standard to establish a model that reflects conditions in young children is imperative. As in the anti-aminopeptidase A, anti-α3NC1 mouse and APN models, although these models show different pathogenesis that of human IMN and are currently underutilized, they can still be used to examine the pathogenesis of IMN. For example, a study suggested that the incidence of IMN is closely associated with the lungs (67), as in the anti-α3NC1 mouse model.

5. Cell models of IMN

Anti-THSD7A antibody induced podocyte model. Except for the animal model of THSD7A-related MN, *in vitro* results suggested that anti-THSD7A antibodies may also directly influence the integrity of podocyte (Fig. 2). Immortalized cultured murine or human podocyte cell lines do not express THSD7A at detectable protein levels and thus primary GECs are used. Exposure of GECs to anti-THSD7A antibody-containing serum results in the binding of huIgG to the cell membrane and a change in the cytoskeletal organization, accompanied by altered focal adhesions. When the model was recreated in 293 cells, the results suggested a direct influence of anti-THSD7A antibodies on the cytoskeletal organization (20).

Zymosan-activated serum (ZAS)-induced podocyte model. Research on zymosan began in the 1940s. It is a non-specific immune system stimulatory substance, existing in the yeast cell wall (68). Some researchers used zymosan to assemble C5b-9 complement complexes, which were developed to interfere with mouse serum to obtain ZAS. Subsequently, ZAS was applied to stimulate podocytes to develop the MN model *in vitro* (69-71) (Fig. 3).

Zymosan can promote rapid C3 cleavage in the alternate pathway (AP) through assembly and protection of the amplification convertase on its surface. It is different from the classic complement pathway (CP), in which the antigen-antibody complexes bind and convert C1 to its activated state on cell

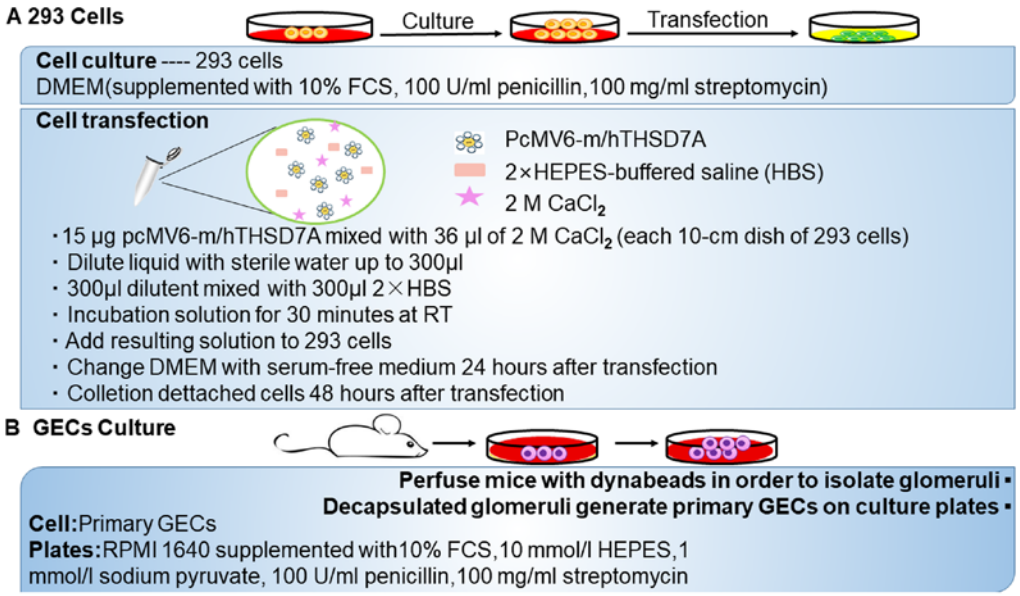

Figure 2. Development of two kinds of cell models of THSD7A. (A) The method of 293 cell culture and transfection. (B) The method for primary glomerular epithelial cell culture. THSD7A, thrombospondin type-1 domain-containing 7A.

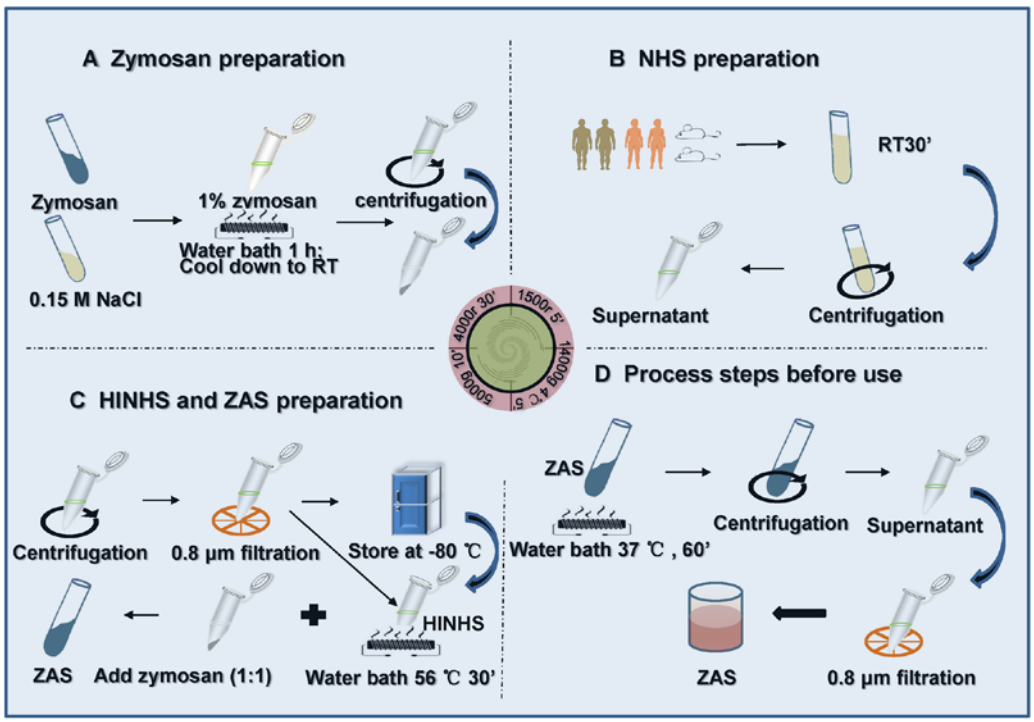

Figure 3. Preparation method of ZAS. (A-D) After 1 h exposure of podocytes to ZAS at different dilutions, the release rate of lactate dehydrogenase was determined to identify the optimum concentration of ZAS. NHS, normal human serum; HINHS, heat-inactivated normal human serum; ZAS, zymosan-activated serum.

membranes (72). However, the formation of C5b-9 by zymosan induces antibody-independent complement activation in the fluid phase via the AP, which differs from that assembled on cell membranes via the CP (73,74). Zymosan, one of the major activators of the AP, provides a contact surface that initiates the complement cascade process by directly activating C3 in serum without apparent utilization of C1, C4 or C2 (72). Subsequently, the AP surface-bound C3 and C5 convertases assembled on zymosan particles initiate the production of the C5b-9 complex (75,76).

To assemble sublytic C5b-9 MAC in the fluid phase, normal human serum is treated with zymosan as previously reported (70,77). Then, the zymosan particles are removed from the serum sample by centrifugation. In addition, the output rate of C5b-9 can be measured using an anti-human C5b-9 monoclonal antibody, which can specifically bind with the C5b-9 complex and then be removed from serum by immunoprecipitation, as previously described (70). The alteration in the membrane integrity of podocytes could be determined by measuring the release of the intracellular enzyme lactate dehydrogenase (76).

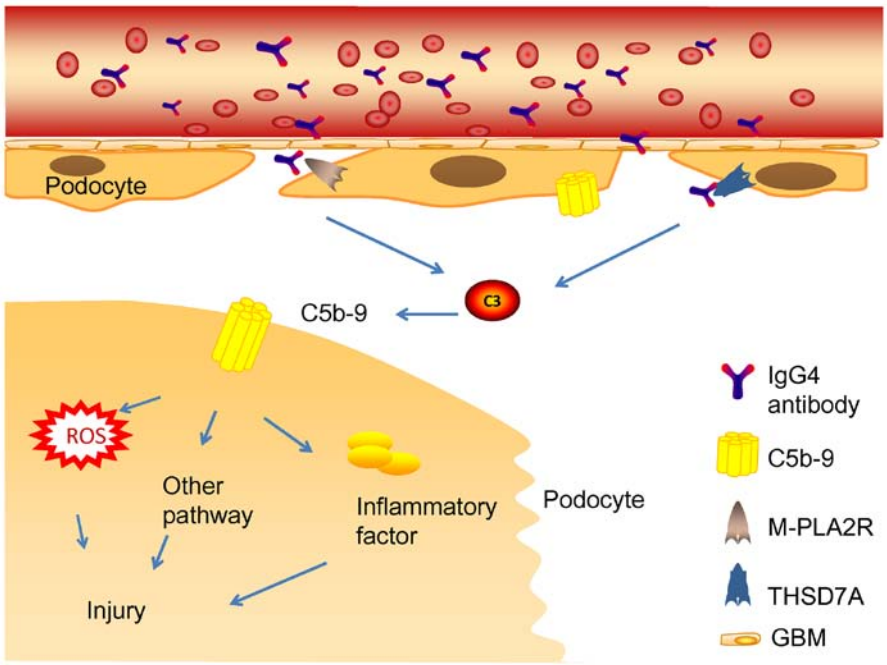

Figure 4. Mechanism of human membranous nephropathy. The combination of autoantibodies and antigens exposed in the podocytes activated the complement system, forming the membrane attack complex (MAC, C5b-9) *in situ* and caused podocyte injury and proteinuria. MAC, membrane attack complex; ROS, reactive oxygen species; IgG, immunoglobulin G; M-PLA2R, M-type phospholipase A2 receptor; THSD7A, thrombospondin type-1 domain-containing 7A; GBM, glomerular basement membrane.

6. Limitations and advantages of the cell models

Experiment *in vitro* is the indispensable method to examine podocyte damage. The anti-THSD7A antibody and ZAS-induced podocyte model could simulate the mechanism of C5b-9 or anti-THSD7A antibody damage to podocytes. They have insufficient levels of evidence and cannot fully simulate the pathogenesis of human IMN. Compared with the ZAS-induced podocyte model, as THSD7A is an autoantigen can be detected in patients with MN, anti-THSD7A model can more accurately reappear the pathogenesis of MN. In addition, according to practical experience, the source of serum required for the preparation of the ZAS model is relatively extensive. For example, human, rat and mice sera can all be used. However, the physiological status of the blood sample will affect the C5b-9 output rate (70,72).

7. Pathogenesis of IMN: From model to human

At present, the pathogenesis of IMN is remains to be elucidated. A number of studies have demonstrated the pathogenesis of immunological damage (78-80). IMN is believed to be an autoimmune disease. As a result, research on IMN has shifted from verifying the Heymann nephritis hypothesis to further studying immune mechanisms. The current research on IMN mainly focused on complement activation, the specific antigen, IgG4 and gene.

Glomerular subepithelial immune complexes play a vital role in the pathogenetic mechanism of MN (14). The activation of the complement system induces the production of MAC (C5b-9). Complement system activation and MAC formation cause inflammatory mediator release and podocyte damage (81) (Fig. 4). Generally, immune complexes are the major substances that activate the CP (82). Nevertheless, it remains undiscovered as a pathway to complement system activation by IgG4 immune complexes in phospholipase A2 receptor 1 (PLA2R1) and THSD7A-associated MN (83-85). IgG4 could hardly activate the CP because of its low affinity to C1q or Fcγ receptors (86). Such studies support the hypothesis that the lectin pathway may be the main initiation pathway (87). In line with this, mannose-binding lectin (MBL) deposition has been detected in the subepithelial space of the glomerular capillaries (88). MBL may bind to specific carbohydrates present in PLA2R or THSD7A, which leads to complement system activation (89,90). Patients with C4 deficiency also develop IMN, while the classic and lectin pathways cannot be activated in patients with C4 deficiency. In addition, deposition of factor B in IMN has been reported, which appears to be an indirect evidence of the activation of the AP pathway (91).

Thus, the activation pathway of complement is still unclear (Fig. 5). The development of models does not contribute to the activation pathway of complement. Furthermore, the complement pathways of most models are unclear (Table II). An AP is essential to complement activation in the anti-α3NC1 mouse model via factor B knockout (58). The human anti-THSD7A-related mouse model provides an opportunity to use genetic intervention in complement activation. In addition, it might be useful to omit complement activation and attack podocytes with MAC (C5b-9).

The next focus is antigen exposure in human MN. Several reported cases of MN might aid our understanding of the research question. One example is the case of a 40-year-old female patient with THSD7A-associated MN concomitant with mixed adenoneuroendocrine carcinoma of the gallbladder. THSD7A was detected in her gallbladder tumor, which corresponded to the immunohistochemical test result. No anti-THSD7A

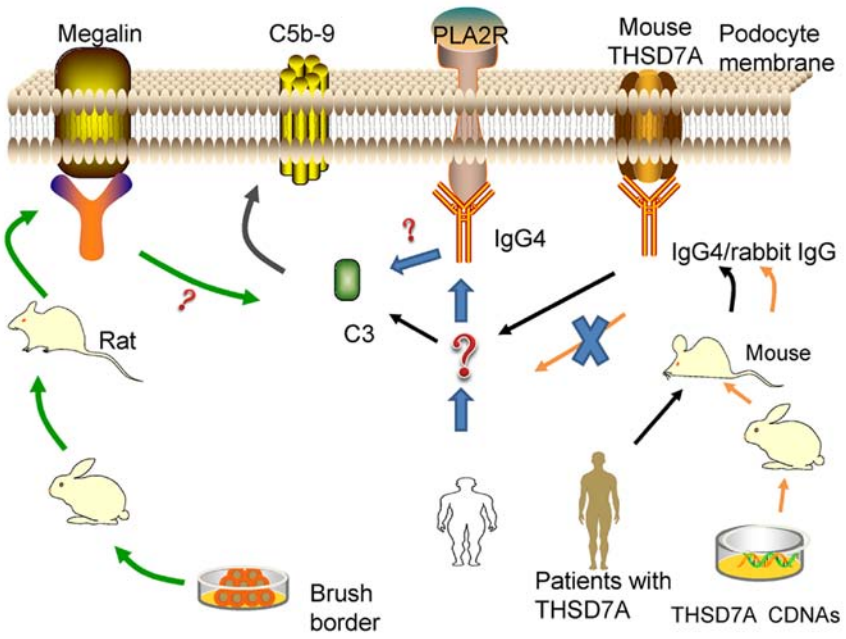

Figure 5. Pathogenesis of the human IMN, passive Heymann nephritis and THSD7A-associated models. The common pathogenic mechanism is the combination of autoantibodies and autoantigens activating the complement system and then inducing the production of a complement membrane complex (C5b-9) to damage podocytes. However, the activation pathway of complement is unclear and no complement was involved in the heterologous model (rabbit) of THSD7A-associated MN. IMN, Idiopathic membranous nephropathy; THSD7A, thrombospondin type-1 domain-containing 7A; MN, membranous nephropathy; PLA2R, phospholipase A2 receptor; IgG, immunoglobulin G.

antibodies were detected in the plasma and her urinary protein level decreased significantly after chemotherapy (92). Another example is that of two patients with THSD7A-associated MN accompanied by angiolymphoid hyperplasia with eosinophilia. THSD7A expression outside the kidney may be vital to the pathogenesis of IMN (93). These cases seem to indicate that antibodies produced outside the kidney help with the initial recognition of antigens. However, the aforementioned cases have limitations. Electron microscopy observation results were not reported in the articles, so whether the diagnosis of SMN is consistent is uncertain. In addition, a cross-sectional study (94) found a significant correlation between the incidence of IMN and exposure of particulate matter of <2.5 ($PM_{2.5}$). In areas with severe air pollution, the incidence of IMN increased significantly. PLA2R can be found in neutrophils and the lung (95,96). In addition, PLA2R and THSD7A are disparate paths to the same disease (97). Thus, the authors of the present review may hypothesize that an unidentified extrarenal cause exists in IMN or SMN.

The authors of the present review hypothesized that tumor tissues overexpress THSD7A, or PLA2R is expressed in neutrophils and lung cells, which leads to complement system activation and MAC formation (92,93,98). However, the current models do not help us answer the question of antigen production. Most current models were established by injecting antibodies produced heterogeneously. This approach is not helpful for examining the original antigens in human IMN. To clarify whether an undiscovered mechanism exists, a more appropriate model with PLA2R-expressing podocytes should be established.

Finally, the reason that IgG4 is the most common antibody deposited in the subepithelial immune complex in most human IMN types remains unclear. IgG4 is related to inflammation and autoimmune disorders (99), but it is widely found in IMN. A previous study suggested that IgG4 could have a partially protective effect by acting on IgG1 or IgG3 (100). Another study suggested that IgG autoantibodies transform from IgG1 to IgG4 during disease progression (101). Probably owing to the different antigens or ways antibodies are induced, only few known models detect IgG4 (Table II). IgG4 is the main antibody of the THSD7A-associated MN model. Whether purified anti-THSD7A IgG4 can form immune deposits in this model will likely advance IgG4 research

Evidence of the significant correlation between specific genetic factors and IMN is accumulating (102,103). The human leukocyte antigen (HLA) gene has a strong gene correlation with the *PLA2R* gene and the interaction may be the basis of autoimmune injury (104). Although no relevant study has described the polymorphism of the *THSD7A* gene, the combined application of *THSD7A* and *PLA2R* gene tests is helpful to further examine the correlation between the IMN pathogenesis and genes, providing an important value for the establishment of the genetic model of MN.

8. Potential future models

Future rodent model with C5b-9 attacks. In human MN, three complement activation pathways have been reported, but reports about each pathway are conflicting. The common feature is that complement activation induces assembly of the MAC (C5b-9) on podocytes, which is necessary for sublethal damage to the renal intrinsic cells (22). Therefore, establishing a model with MAC (C5b-9) podocytes may avoid the disadvantages of the difference in pathways between the models and human MN (Fig. 6). First, sublytic C5b-9 is assembled *in vitro* and then incubated with microbubbles that carry cationic

Table II. Comparison of different models/patients.

Model/patient		Species	Target antigen	Identification in patients	Stability of proteinuria	Complement pathway	IgG	Type of IgG	C3	C5b-9
IMN		Human	PLA2R, C-BSA THSD7A, NEP			Unclear	Detected	Mainly IgG4	Detected	Detected
Heymann nephritis	Active	Rat	Megalin (GP330)	No antibody or antigen of megalin detected	Easy to recover	Unknown	Detected	Unknown	Detected	Detected
	Passive	Rat			Relatively stable	Unknown	Detected	Unknown	Detected	Detected
Anti-DPP IV model		Rat	gp108	Not found	Instable	Nonactivated	Not found	Not found	Not found	Not found
C-BSA model		Rabbit/mice	C-BSA	Young children, especially below five years old (childhood)	High mortality	Unknown	Detected	IgG1/IgG4	Detected	Detected
Anti-α3NC1 model		DBA/1 mice	α3NC1	A self-target	Stable antigen for pulmonary hemorrhagic nephritic syndrome	Alternative Pathway	Detected	mIgG1 more than mIgG2a and mIgG2b	Detected	Detected
Anti-APN model		Mice	Annexin-3	Positive expression of Annexin-3 in the renal tissue	14 days	Unknown	Detected	Maybe different from humans	Detected (minimal)	Not found
Anti-APA model		BALB/c mice	APA	Not found	16 days	Nonactivated	Detected	Unknown	Not found	Not found
THSD7A-associated model		Mice	THSD7A	Adults	Remained elevated until day 70	May be the lectin pathway	Detected	IgG4	Detected	Unknown
ZAS-induced model		Podocyte	Zymosan	Not found	-	The alternative pathway	-	-	Detected	Detected

IMN, idiopathic membranous nephropathy; PLA2R, phospholipase A2 receptor; C-BSA, cationic bovine serum albumin; α3NC1, non-collagen 1 domain of human α3 (IV) collagen; THSD7A, thrombospondin type-1 domain-containing 7A; NEP, neutral endopeptidase; IgG, immunoglobulin G; ZAS, zymosan-activated serum.

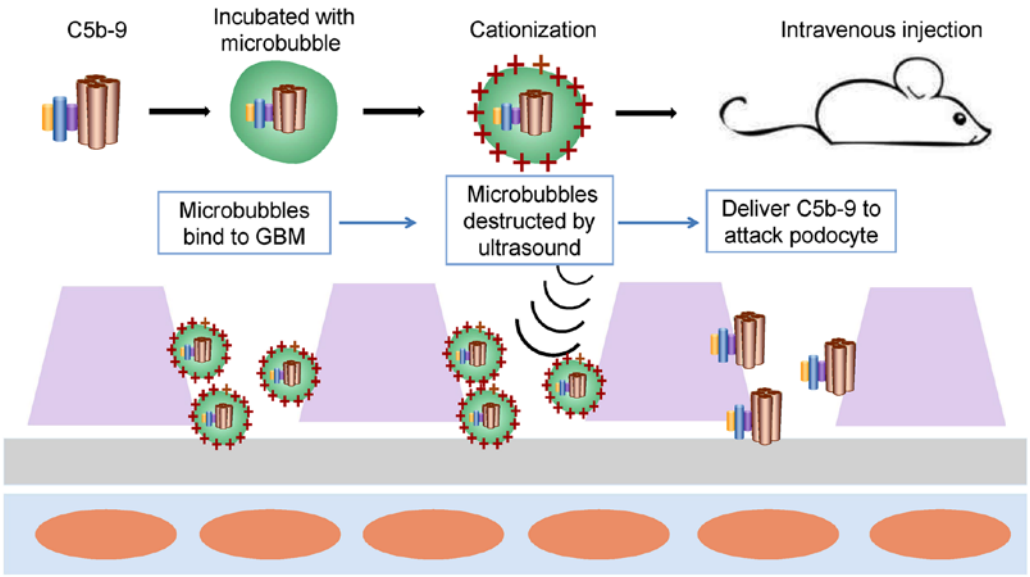

Figure 6. Future rodent model with C5b-9 attacks. First, sublytic C5b-9 is assembled *in vitro* and then incubated with microbubbles that carry cationic charges by using the ultrasonic targeted microbubble destruction technology (103,104). Then, the suspension of microvesicles is injected immediately in the mice intravenously. Microvesicles are likely to contact with the glomerular basement membrane and podocytes as a result of carrying cationic charges. Ultrasonic sensors are used to scan the kidney repeatedly and when microbubbles are observed traveling into the kidney, the ultrasonic pressure will be increased to destroy the microbubbles, delivering C5b-9 to attack podocytes. GBM, glomerular basement membrane.

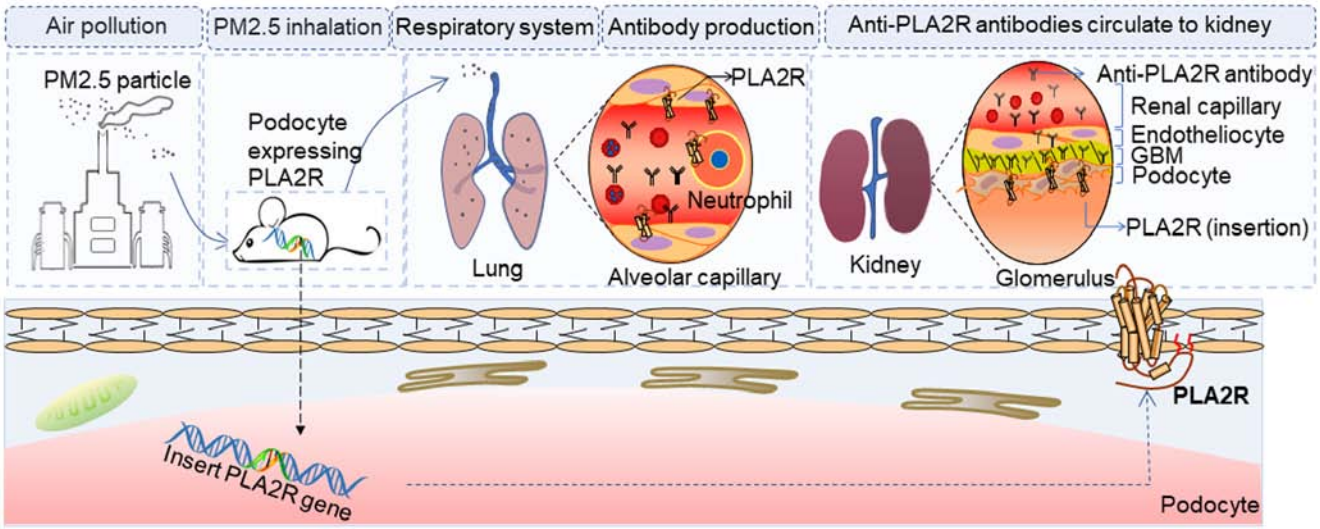

Figure 7. Future rodent model with podocytes expressing PLA2R. A rodent model with podocytes expressing PLA2R was established by gene insertion. Using PM 2.5 to stimulate mice with podocytes expressing PLA2R may induce a lung or systemic inflammatory response and then expose the PLA2R in the lung or neutrophils. Furthermore, antibodies circulate in the blood and finally bind to the podocytes, eventually causing podocyte injury due to the immunoreaction. PLA2R, phospholipase A2 receptor; PM, particulate matter.

charges. The specific steps follow the protocol provided by ultrasonic-targeted microbubble destruction (105,106). Then, the suspension of microvesicles is injected immediately in mice through the tail vein. Microvesicles are likely to contact the GBM and podocytes because they carry cationic charges. Ultrasonic sensors are used to scan the kidney repeatedly and when microbubbles are observed traveling into the kidney, the ultrasonic pressure will be increased to destroy the microbubbles, delivering C5b-9 to attack podocytes.

Future rodent model with PLA2R-expressing podocytes. PLA2R is a glycoprotein constituent located on the podocytes of healthy humans (107,108). It is a type I transmembrane receptor and a member of the mannose-receptor family (109). It was identified in immune deposits in 70-80% of patients with IMN, but PLA2R was not detected in the glomeruli of healthy rodents or rabbits (63). According to the PubMed genetic database (Gene ID: 18779, https://www.ncbi.nlm.nih.gov/gene/?term=18779), mice express PLA2R in the kidneys. Thus, establishing a rodent model with podocytes expressing PLA2R may contribute to the advancement of PLA2R-related human MN (Fig. 7).

Subsequently, the anti-PLA2R antibody can be injected to induce IMN. In addition, as mentioned previously, several

extrarenal factors may activate the autoimmune system to produce anti-PLA2R antibody. Previous findings have shown that PLA2R-deficient mice had significantly expressed inflammatory cells around the airways as compared with wild-type mice (110). Thus, using $PM_{2.5}$ to stimulate mice with podocytes expressing PLA2R, may expose PLA2R in the lung or neutrophils and then generating antibodies to bind to podocytes.

An ideal model is the one that will contribute to understand the pathogenesis of IMN, so as to improve the diagnosis and therapy. It is worth noting that increasing evidence has shown the traditional Chinese medicine (TCM) is beneficial for both antifibrosis therapy in CKD (111-113) and treating primary disease such as IMN (114-116). However, their mechanisms have not been elucidated. TCM could bring a new understanding of the pathogenesis of IMN.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

HXJ and ZF wrote the main parts of this manuscript; ZBZ, CHX, WZ and JG wrote other parts and designed the figures; BLL and YW improved the language of the manuscript; YNL and WJL conceived the structure and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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