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The Pathogenesis of Nephrotic Syndrome: A Perspective from B Cells

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Abstract

Background: Nephrotic syndrome is a special type of chronic kidney disease, the specific pathogenesis of which remains unclear. An increasing number of studies have suggested that B cells play an important role in the pathogenesis of nephrotic syndrome. ***Summary:*** Idiopathic nephrotic syndrome is a common kidney disease in children. While previously believed to be primarily caused by T-cell disorders, recent research has shifted its focus to B cells. Studies have shown that B cells play a significant role in the pathogenesis of NS, potentially even more so than T cells. This article provides a comprehensive review of the involvement of B cells in the development of idiopathic nephrotic syndrome. ***Key Messages:*** B cells are involved in the pathogenesis of nephrotic syndrome by producing autoantibodies and various cytokines.

Plain Language Summary

Nephrotic syndrome has a high incidence and complex pathogenesis. The traditional view is that T-cell disorder is the main cause of nephrotic syndrome, but more and more studies have shown that the pathogenesis of nephrotic syndrome is closely related to B-cell disorders. This paper offers a comprehensive and systematic analysis and summary of previous articles on nephrotic syndrome and finally provides a detailed introduction to the role of B cells in the pathogenesis of nephrotic syndrome. Abnormalities in each stage of B-cell differentiation and development will lead to B-cell dysregulation and eventually lead to nephrotic syndrome. This paper makes a systematic review from the following aspects: Traditional presentations on pathogenesis of nephrotic syndrome, evidence of B-cell involvement in pathogenesis of nephrotic syndrome, causes of B-cell dysregulation, involvement of T cells in B-cell dysregulation, autoantibodies produced by B cell, the role of these autoantibodies in pathogenesis of nephrotic syndrome, the role of B-cell-associated cytokines and signaling pathways in pathogenesis of nephrotic syndrome, and novel therapeutic pathways that target dysregulated B cells.

Introduction

Idiopathic nephrotic syndrome (INS) comprises a range of clinical syndromes stemming from increased permeability of the glomerular basement membrane due to various causes, leading to substantial protein loss in

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both plasma and urine. The key clinical features include significant proteinuria, hypoalbuminemia, hyperlipidemia, and edema. The incidence of INS in children across different races and regions ranges from 1.15 to 16.9 per 100,000 individuals [1]. The primary pathological classifications are minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS). INS can be further categorized into steroid-sensitive nephrotic syndrome (SSNS) and steroid-resistant nephrotic syndrome (SRNS) based on the response to glucocorticoids. Although the exact etiology of INS remains incompletely understood, previous studies predominantly examined T-cell abnormalities and glomerular permeability factors. However, contemporary perspectives increasingly emphasize the pivotal role of B cells in INS pathogenesis. This article delves into the alterations in B-cell phenotypes in INS, elucidates the mechanisms through which B cells contribute to INS pathogenesis, and discusses the implications of B cells in both the pathogenesis and treatment strategies of INS.

T Cell and Glomerular Permeability Factor

There have been many studies on the pathogenesis of INS. The traditional view is that T-cell disorders are the main cause of INS. The measles virus can greatly inhibit cellular immunity, leading to a reduction in the original nephrotic syndrome symptoms in patients with INS following infection [2]. Cyclosporine, a potent immunosuppressant that selectively inhibits T cells, exhibits significant efficacy in alleviating clinical symptoms among patients with INS [3]. Our previous investigations on the immune cell profiles of children with INS revealed an imbalanced proportion of Th1/Th2 cells, excessive elevation of Th17 cells, and a notable increase in both CD8+ T cells and effector CD8+ T cells [4].

Hoyer et al. [5] discovered that in the weeks following kidney transplantation in patients with SRNS, there is a recurrence of the disease accompanied by significant proteinuria. They postulated the presence of a substance in the body fluids of these patients, now known as the glomerular permeability factor, which can induce glomerular damage. Yoshizawa et al. [6] injected concentrated supernatant of peripheral blood mononuclear cells from patients with MCD into rats and observed a significant increase in urinary protein at 8 h postinjection. This study revealed that peripheral blood mononuclear cells produce a glomerular permeability factor in MCD patients, leading to alterations in glomerular permeability and resulting in massive proteinuria. Similarly, studies on FSGS have identified a pathogenic circulating factor, elevated soluble urokinase-type plasminogen activator receptor (suPAR). suPAR is found in two-thirds of primary FSGS patients and mediates the activation of podocyte β3 integrin, resulting in podocyte depletion and proteinuria both before and after transplantation [7]. Later, circulatory permeability factors such as CASK, which are secreted by monocytes and M2 macrophages, were found [8].

B Cells in Nephrotic Syndrome

The levels of sCD25 and sCD23, which serve as activation markers for T and B cells, respectively, were observed to increase during the relapse stage of SSNS and decrease during the remission stage [9]. These synchronized abnormalities at different disease stages suggest that INS is not solely caused by T-cell abnormalities but may also involve B cells. In patients with primary nephrotic syndrome, B cells remain active throughout the disease course. CD20 is expressed in all B-cell subpopulations, and rituximab (RTX), a chimeric monoclonal antibody targeting CD20, can effectively deplete B cells in the body. It has been widely used to treat various autoimmune diseases and may also benefit nephrotic syndrome patients [10]. A retrospective study demonstrated significant remission in patients with multidrug-resistant nephrotic syndrome before reaching the end-stage renal disease requiring transplant therapy when immunoglobulin immunoadsorption was combined with agents that deplete B cells, providing strong evidence for the involvement of B cells and immunoglobulin in the pathogenesis of nephrotic syndrome [11].

B cells arise from pro-B cells in the bone marrow. In the bone marrow, the functional rearrangement of immunoglobulin gene fragments, predominantly composed of immunoglobulin heavy chain (IgH) and light chain (IgL), occurs to generate a B-cell repertoire capable of recognizing various antigens. Eventually, immature B cells with IgM molecules on their surfaces are formed. After completing early development in the bone marrow, transitional 1 (T1) B cells can migrate to the spleen and evolve into transitional 2 (T2) B cells. In the spleen, T2 B cells transform into B1 cells, follicular B cells, and marginal zone (MZ) B cells [12]. B1 cells can rapidly differentiate into short-lived plasma cells that secrete natural IgM in a T-cell-independent fashion [13]. Follicular B cells are predominantly located in the follicles of the spleen and lymph nodes and constitute the main B-cell subgroup in both humans and mice. Although they

are mature B cells, they remain inactive, and their activation necessitates the assistance of CD4+ T cells. MZ B cells are located in the marginal region of the spleen and other lymphoid tissues, are exposed to a considerable number of pathogens, and serve as the first line of defense against blood pathogens [14]. MZ B cells are the sole type of B cell that depends on Notch2 signaling for proliferation and plays a protective role in podocyte injury in doxorubicin-treated mice, which will be described in detail later. Plasma cells are also known as antibody-secreting cells. When mature B cells are stimulated by antigens, they differentiate into plasma cells with the support of antigen-presenting cells and T cells, which can produce a large quantity of autoantibodies against the antigen. Additionally, some mature B cells differentiate into memory B cells, which have a long lifespan, and when the body encounters the antigen for the second time, these cells redifferentiate into plasma cells that can secrete antibodies, generating a large number of high-affinity antibodies against the antigen.

The differentiation and development of B cells are regulated by a variety of factors. For example, the B-cell activating factor BAFF, which is highly dependent upon when transitional B cells develop into follicular B cells and MZ B cells, or the CD40 and CD40 receptors during the differentiation of B cells into plasma cells. Abnormalities in these factors are the causes of B-cell dysregulation. A recent single-cell sequencing study analyzed all peripheral blood immune cells in children with INS and revealed that genes encoding BCRs in the B cells of INS patients, activation-related genes, and immunoglobulin-related genes were highly expressed. The autoantibodies that cause podocyte injury in the blood of INS patients may be due to autoreactive clones of extrafollicular B cells [15]. BAFF (also known as BLys or TNFSF13B), a member of the TNF family, is an important molecule that regulates B-cell differentiation and activation. BAFF is involved in a variety of B-cell-related autoimmune diseases, such as systemic lupus erythematosus and multiple sclerosis [16–18]. BAFF is expressed in podocytes, and a high level of BAFF in the glomeruli of children with nephrotic syndrome can predict the deterioration of renal function [19]. BAFF is elevated in the plasma of adult MCD patients and is positively correlated with the percentage of plasma cells [20]. In membranous nephropathy, the BAFF level is greater in patients who are positive for anti-PLA2R antibodies than in antibody-negative patients. After 6 months of immunosuppressive therapy, plasma BAFF levels decreased more significantly in patients without negative antibody results than in those without negative antibody results [21]. B-cell activating factors are closely related to the onset and course of nephrotic syndrome and may play an indirect pathogenic role in the pathogenesis of nephrotic syndrome by facilitating B-cell differentiation into plasma cells and stabilizing B-cell development.

Initial B-cell activation requires two signals, one from BCR-associated molecules and the other from helper T cells, known as T-cell-dependent activation. After CD40L on the surface of follicular T helper cells binds to CD40 on the surface of B cells and cross-links with BCR, B cells are activated and proliferate, initiating B-cell-mediated immune and antibody responses [22]. The tph subgroup was found to be activated in the circulation of INS patients, but the expression of cd40l on tph cells was reduced [23]. In another study, MCD patients who went into remission on RTX treatment had a significant reduction in follicular T helper cells [24]. Abnormal T-cell subsets can act on antibodies through the process of B-cell differentiation and development, which is one of the reasons for B-cell dysregulation in INS patients.

B cells exhibit a diverse range of activation stages within the body, and the subpopulation and proportion of B cells undergo constant fluctuations during different stages of INS pathogenesis. During disease onset, there is an increase in total B cells, including transitional B cells and memory B cells, which also increase during remission. The notable increase in the number of transitional B cells in patients with hormone-sensitive nephrotic syndrome renders these cells valuable biomarkers for early screening of SSNS [25]. Multiple studies have demonstrated that memory B cells play a crucial role in disease recurrence and can serve as predictors of INS disease progression. The population of CD19+CD138+B cells, which represents the major subset responsible for antibody production, exhibited an increase in both new and recurrent nephrotic syndromes and displayed a positive correlation with urea nitrogen levels in newly diagnosed patients. Conversely, CD27+ memory B cells were found to be elevated in relapsed patients and showed a positive association with 24-h proteinuria levels [26]. In FRNS/SDNS patients, complete depletion of B cells occurs 1 month after RTX infusion, followed by gradual reconstitution of memory B-cell populations 9 months later, indicating disease recurrence [27]. Further investigations revealed that recurrence in nephrotic syndrome patients treated with RTX and orfazumab was linked to the presence of circulating memory B cells at the time of anti-CD20 monoclonal antibody infusion rather than to the timing or type of anti-CD20 monoclonal antibody used [28].

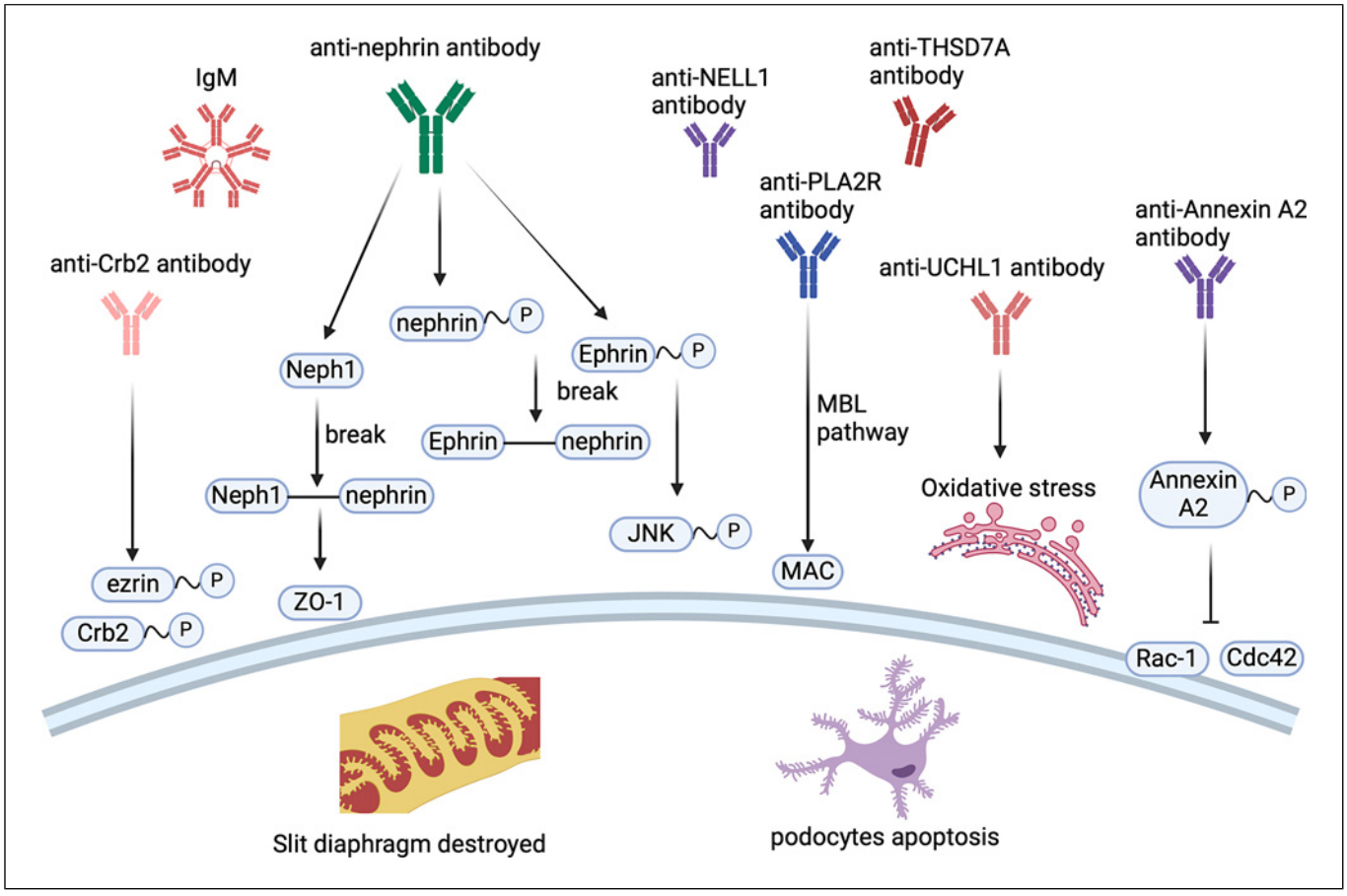

Fig. 1. Mechanisms of autoantibody pathogenesis: various pathways leading to slit diaphragm and podocyte damage. A diverse array of circulating autoantibodies, which elicit damage through various pathways, has been identified in nephrotic syndrome patients. The binding of anti-Crb2 antibodies to Crb2 protein triggers the phosphorylation of Crb2 and ezrin. Interactions between anti-nephrin and anti-Neph1 antibodies disrupt the association between nephrin and ZO-1, leading to a rapid reduction in glomerular ZO-1 levels. Additionally, the anti-nephrin antibody can bind to both nephrin and ephrin-b1, inducing phosphorylation. Ephrin-b1 phosphorylation results in enhanced JNK phosphorylation, followed by the downregulation of ephrin-b1 accompanied by the regulation of ephrin-b1 and nephrin. Alterations in glycosylation patterns due to IgG4 anti-PLA2R antibody activity and complement activation via the lectin pathway may target Neph1 and synaptopodin in podocytes for antibody-mediated injury. Elevated levels of glomerular ubiquitination caused by autoantibodies against UCHL1 lead to damage through oxidative stress. Autoantibodies targeting annexin A2 induce hyperphosphorylation at tyrosine 24, affecting the Rho signaling pathway while downregulating the expression and activity of Rac1 and Cdc42. Furthermore, the involvement of anti-NELL1 and anti-ThSD7A antibodies as well as IgM is implicated in this process; their ultimate targets are podocytes and slit diaphragms, resulting in podocyte apoptosis and destruction of the slit diaphragm, ultimately leading to disruption of the filtration barrier and causing proteinuria.

Autoantibodies

As shown in Figure 1 and Table 1, the conventional perspective holds that micropathologic nephropathy is characterized by extensive fusion of podocyte foot processes without the deposition of immune complexes, as observed via electron microscopy. However, a recent retrospective study revealed the presence of budded ballooning clusters, which were identified in Bowman's space, in the glomeruli of patients with MCD. These clusters, known as ball-shaped reactive fragments of foot processes, exhibited dot staining for IgG, C3, kappa, and lambda. This staining pattern suggests the deposition of immune complexes in MCD [29]. Furthermore, budded ballooning cluster has also been observed in patients with primary FSGS, primary membranous nephropathy, amyloidosis, and thrombotic microangiopathy. This research revealed the presence of circulating antibodies in

Table 1. Different autoantibodies in nephrotic syndrome

	Renal localization	Animal model	Mechanism of action	References
Anti-nephrin antibody	Glomerular slit diaphragm	Proteinuria occurs in passively immunized mice	Interacts with Neph1 and disrupts the interaction between Neph1 and ZO-1; interacts with ephrin-b1, promoting the phosphorylation of JNK, leading to the structural destruction of the septum pellucidum	[30–33]
Anti-UCHL1 antibody	Renal tubule, tubulointerstitial cells, and injured podocytes	Proteinuria occurs in passively immunized mice	Causes damage through oxidative stress	[34–37]
Anti-PLA2R antibody	Podocytes	Proteinuria occurs inactively immunized mice	The glycosylation pattern of anti-pla2r antibody igg4 changes, activating complement through the lectin pathway, highlighting podin and Neph1 as targets of complement-induced podocyte injury	[38, 39]
Anti-Crb2 antibody	Podocytes and glomerular parietal epithelial cells	Proteinuria occurs in actively immunized mice	Involved in the trafficking of proteins in the septum pellucidum, phosphorylation of glutamic acid affects kidney development	[40–42]
Anti-annexin A2 antibody	Podocytes	Proteinuria occurs in passively immunized mice	Autoantibodies against annexin A2 lead to hyperphosphorylation of annexin A2 tyrosine 24 and act on the Rho signaling pathway, triggering cytoskeletal rearrangement by downregulating the expression and activity of Racl and Cdc42	[43–45]

patients with MCD, FSGS, and other glomerular diseases leading to podocyte disappearance and proteinuria, providing more compelling evidence. Indeed, previous studies have investigated the presence of circulating autoantibodies in the bloodstream of these individuals.

The presence of anti-nephrin autoantibodies was initially detected in 29% of patients with MCD. The levels of this antibody increase during the acute phase of the disease and decrease during remission, closely correlating with the progression of the illness [30]. Subsequently, Shirai et al. [46] compared the levels of anti-nephrin autoantibodies among patients with different types of nephropathy and observed that the antibody levels in membranous nephropathy and lupus nephritis patients were similar to those in normal individuals. However, in patients with FSGS, especially recurrent FSGS, the antibody levels were significantly greater and correlated with proteinuria after transplantation. The colocalization of intraglomerular IgG with nephrin was observed to transfer from the slit septum into the podocytes during FSGS recurrence. Therefore, anti-nephrin antibodies may be implicated in the pathogenesis of nephrotic syndrome.

When C57BL/6J mice were injected with polyclonal anti-nephrin antibodies, they exhibited transient reductions in urine output and weight gain, along with an elevated urea nitrogen/creatinine ratio and severe hypoalbuminemia. Pathological examination revealed segmental glomerulosclerosis in the mice. Electron microscopy revealed a decrease in the number of podocytes, the disappearance of foot processes, and the deposition of IgG and complement C3 within the glomeruli [47].

The development of an animal model for nephrotic syndrome induced by an anti-nephrin antibody further substantiates the pathogenicity of the nephrin antibody. Subsequent studies have investigated pathogenesis, revealing that both nephrin and ephrin-b1 are integral components of the glomerular slit diaphragm [31]. Syndrome exhibited a significant decrease. In rats, the administration of an anti-nephrin antibody resulted in the binding of phosphorylated nephrin and ephrin-b1, leading to enhanced phosphorylation of JNK, followed by the downregulation of ephrin-b1 accompanied by dysregulation of ephrin-b1 and nephrin, destruction of the original slit diaphragm structure, and barrier dysfunction [32]. Phosphorylated JNK also induces podocyte injury and apoptosis through its downstream molecules [48, 49]. Another target for interaction with the nephrin antibody is Neph1. The interaction between the anti-nephrin antibody and the anti-Neph1 antibody in vivo disrupts the correlation between Neph1 and ZO-1, resulting in a

rapid reduction in glomerular ZO-1 levels and disordering glomerular filtration function [33].

Anti-UCHL1 antibodies were detected in the serum and urine of patients with INS, and UCHL1 antibody levels were significantly correlated with proteinuria. Furthermore, these levels were significantly greater during disease relapse than during remission [34]. UCHL1 is a multifunctional protein that regulates the cellular ubiquitination process and regenerates free ubiquitin in vivo. It is predominantly expressed in the brain and other nervous systems and plays a role in various neurodegenerative diseases, such as Alzheimer's disease [50], Parkinson's disease, neuroendocrine cancer, and brain injury [51], making it a potential molecular target for these conditions [52, 53]. In healthy human renal cortex tissue, UCHL1 is mainly expressed in distal tubules, density maculae, and nerve fibers; its expression in glomeruli is minimal. However, primary FSGS glomeruli exhibit three distinct patterns of UCHL1 expression [35]. In mouse models, UCHL1 is primarily expressed in the nerve endings of renal arterioles, tubular interstitial endothelial cells and nonvascular tubular interstitial cells. Additionally, podocytes from mice older than 2 years also express UCHL1 [36]. In vitro studies have shown that undifferentiated podocytes mainly express UCHL1 in their cytoplasm and frontal membrane [35]. Jamine et al. [34] demonstrated that the injection of purified anti-UCHL1 antibodies into mice resulted in significant proteinuria and hypoalbuminemia after 72 h; electron microscopy revealed the disappearance of the foot process.

UCHL1 knockout mice showed no significant changes in kidney ultrastructure [36], but the UCHL1 knockout rat model developed proteinuria after 1 week, accompanied by the disappearance and shrinkage of the foot processes. Glomerular extracellular matrix-interacting proteins (fibronectin, laminin, integrin β3) and endoplasmic reticulum stress-related proteins (BiP and ATF4) were increased in FSGS patients compared with controls [37], and immunohistochemistry showed that glomerular ubiquitin was also increased in FSGS patients and membranous nephropathy patients with high UCHL1 expression [35]. UCHL1-induced podocyte damage may be mediated by oxidative stress. Much of the research on the pathogenesis of UCHL1 is based on gene knockout experiments focusing on the role of the UCHL1 protein in glomeruli. The deposition of immune complexes in nephropathy and the identification of a significant number of circulating antibodies suggest an independent pathogenic pathway for these antibodies. However, further investigations into the pathogenesis of UCHL1 antibodies are needed.

Crb2 is expressed in the podocytes and parietal epithelial cells of human glomeruli, where it colocalizes with synaptopodin [40, 54]. Whole-exon sequencing of SRNS patients revealed a mutation in CRB2, which was associated with resistance to cyclosporine and RTX [54, 55]. Podocyte-specific Crb2 knockout mice developed proteinuria at 2 months and FSGS at 6 months, accompanied by injury to podocyte processes and decreased expression of podocyte-associated proteins [40, 56]. Crb2 promotes the development of foot processes and slit membranes in the glomeruli [57] and plays a role in trafficking slit membrane-associated proteins. Moller et al. [41] proposed an intriguing hypothesis suggesting that proteinuria resulting from crb2 deficiency may be attributed to the disruption of slit diaphragm protein trafficking, leading to the loss of Crb2-SD-targeted phenotypes. Transport abnormalities of the nephrin protein have also been documented in Finnish congenital nephrotic syndrome caused by defective mutations in the NPHS1 gene [58]. Furthermore, potential interactions between crb2 and nephrin might collectively impact foot process and slit diaphragm function. Injection of Crb2 protein into mice revealed that circulating anti-Crb2 antibodies form immune complexes with renal Crb2 protein, which subsequently deposit in the kidney. Both active immunization-induced proteinuria and kidney injury are associated with the deposition of immune complexes consisting of Crb2 protein-CRB2 antibodies within the kidney, resembling manifestations observed in human MCD and FSGS patients [42], which provides strong evidence that circulating crb2 antibodies cause nephrotic syndrome. In vitro experiments demonstrated that crb2 antibody binding to surface-expressed podocytes induced phosphorylation events involving both the crb2 and ezrin proteins; however, the precise mechanism underlying kidney injury caused by circulating crb2 antibodies remains elusive, necessitating further animal experimentation for elucidation.

Compared to those in MCD and FSGS patients, a large number of autoantibodies have been detected in patients with idiopathic membranous nephropathy (MN). PLA2R is a prevalent autoantibody in idiopathic membranous nephropathy, with a positive rate exceeding 70% in patients, and the antibody level correlates with disease activity. Immunofluorescence analysis of renal biopsy samples revealed particle-mode fluorescence of PLA2R in glomerular podocytes, which was colocalized with IgG4 [38]. PLA2R triggers podocyte injury through the complement pathway. Injection of anti-PLA2R antibody into

mice expressing PLA2R in podocytes resulted in the presence of IgG and complement C3 within podocytes after 7 days, accompanied by upregulation of SOD1 [39]; this injury was attenuated when C3 was knocked out in mice expressing PLA2R in podocytes [59], and similar findings were observed in vitro in cultured podocytes. The glycosylation pattern of anti-PLA2R IgG4 antibodies changed, activating complement via the lectin pathway; Neph1 and synaptopodin were identified as potential targets for antibody-mediated injury to podocytes [60]. The addition of rapamycin to cultured podocytes prevented PLA2R-induced injury, suggesting that activation of the mTOR pathway may also contribute to podocyte damage caused by PLA2R [61]. THSD7A is an additional autoantibody found in membranous nephropathy patients, with a detection rate of 2–3%. Unlike PLA2R, THSD7A exhibits linear expression within the glomerulus [62]. It serves as a conserved component of podocytes located on the foot process near the septum pellucidum and is potentially involved in regulating the filtration barrier through mechanical mechanisms [63]. Furthermore, there are also antibodies associated with malignant tumors in membranous nephropathy, such as NELL1 [64], and antibodies related to secondary membranous nephropathy, such as EXT1/EXT2 [65]. The presence of multiple antibodies has been identified in membranous nephropathy, with one or more antibodies being detected in the serum of patients [66]. These antibodies play a role in inducing injury through distinct pathways and are associated with various disease subtypes.

Annexin A2 is expressed in human glomerular epithelial cells and has the ability to bind calcium, phospholipids, and F-actin, which play crucial roles in cytoskeleton rearrangement [43]. Through tyrosine phosphorylation, annexin A2 can interact with RhoGTPase as a molecular switch to influence cell adhesion and movement [44]. Our previous studies revealed that the prevalence of autoantibodies against annexin A2 in patients with MCD and nonhereditary FSGS is 17.8%. These autoantibodies induce the overphosphorylation of tyrosine 24 on annexin A2, leading to the modulation of the Rho signaling pathway by downregulating Rac1 and Cdc42 expression and activity. This triggers cytoskeleton rearrangement, resulting in podocyte dysfunction and ultimately causing proteinuria and INS [45]. Additionally, we employed two-dimensional electrophoresis combined with mass spectrometry to screen for autoantibodies targeting podocytes or endothelial cells in children with nephrotic syndrome. The overall positive rate for podocyte autoantibodies was 66%, while it was 89% for endothelial cell autoantibodies [67, 68]. There is a dose-response relationship between podocyte autoantibodies and proteinuria; only when these antibodies reach a certain threshold do they cause proteinuria, where levels above this threshold are positively correlated with urinary protein content. Effective hormone or immunosuppressive treatment can reduce the level of these autoantibodies to below the threshold value. The discovery of these autoantibodies further enhances our understanding of the immune mechanisms underlying INS pathogenesis. Along with other clinical manifestations associated with nephropathy, autoantibody-mediated damage to the glomerular filtration barrier through various pathways targeting podocytes leads to proteinuria. The pathogenic mechanism of each antibody needs to be thoroughly investigated, elucidating its specific pathogenic pathway and identifying appropriate targets for intervention to effectively treat the disease. These are the current research objectives of our group.

In addition to IgG, activation of IgM and complement has also been observed in FSGS patients. Patients with deposition of IgM and complement C3 in the glomerulus exhibit increased proteinuria and glomerulosclerosis, which are independent risk factors associated with the primary outcome of FSGS [69–71]. Peritoneal B1-a cells produce natural IgM and specific IgM antibodies in FSGS patients can bind to epitopes on injured glomerular endothelial cells, thereby activating complement through the classical pathway and causing secondary glomerular injury [72]. However, some researchers have found no significant difference in kidney injury between mice lacking c1q in the classical pathway and control mice after Adriamycin (ADR) administration. This finding suggested that the classical complement pathway may not be relevant to the pathogenesis of ADR-induced FSGS [73]. These contradictory results may be attributed to the inability of doxorubicin-treated mice to fully simulate FSGS, and further studies are required for validation. However, the generation of the membrane attack complex, which plays a crucial role in mediating kidney injury, ultimately stems from the activation of the three complement pathways. Knocking out cd59a, the primary regulator of membrane attack complex, can exacerbate kidney injury and mortality in mice [73, 74].

B-Cell-Associated Cytokines and Signaling Pathways

As shown in Figure 2, the mRNA expression of IL-4 and IL-13 is upregulated in patients with nephrotic syndrome, the source of which remains unknown [75].

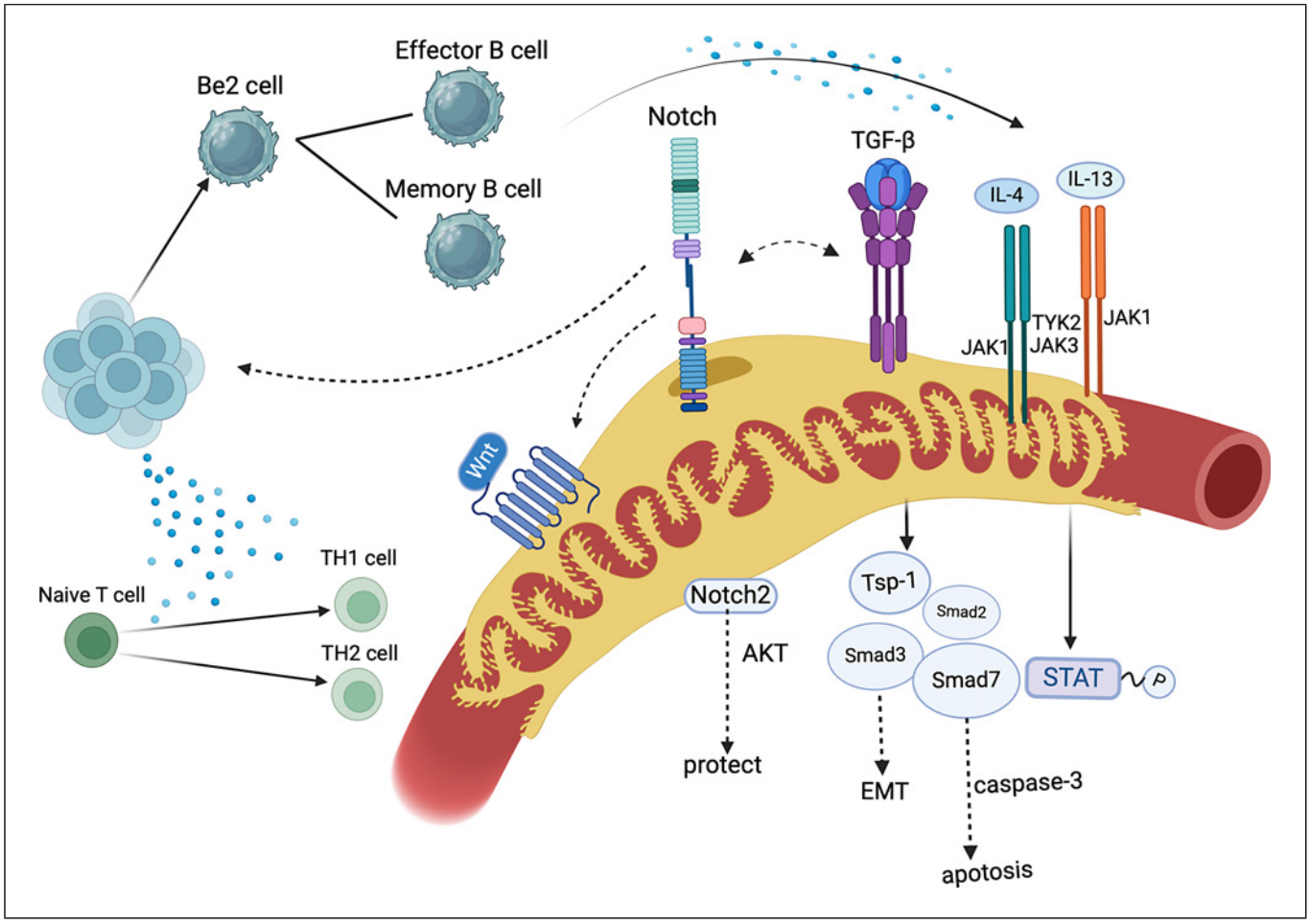

Fig. 2. Cytokines produced by B cells and signaling pathways affected by B cells can differentiate into various subsets, such as effector B cells and memory B cells. A specific subset of effector B cells can influence the differentiation of naive CD4+ T cells into TH1 and TH2 cells by producing polarizing factors such as IL-4. The Notch gene is expressed at different stages of B-cell development and has the potential to impact the terminal differentiation of B cells and may stimulate mature B cells to differentiate into antibody-secreting cells. Notch2 also protects against apoptosis through the AKT pathway. Downstream molecules of TGF-β, such as tsp-1 and smad2/3, were found to be expressed in the glomeruli of FSGS patients. TGF-β induces the upregulation of Btg2, promoting podocyte EMT through the Smad3 pathway. Additionally, interaction with Smad7 can induce podocyte apoptosis through a caspase-3-independent pathway. Furthermore, B cells can also produce cytokines such as IL-4 and IL-13, which activate the tyrosine kinases JAK1 and JAK3 by binding to IL-4Rα and IL-13Rα on the surface of glomerular endothelial cells, subsequently activating the transcription factor STAT6 for signal transduction. EMT, epithelial mesenchymal transition.

Although T cells are capable of independently producing IL-4, a recent study demonstrated that B cells also have the ability to produce cytokines. This not only stimulates T-cell differentiation but also leads to local activation of B cells and cytokine production, which directly causes proteinuria in mice. In vitro experiments demonstrated that B cells can differentiate into distinct effector subsets, thereby generating Th1- and Th2-related cytokines. One particular subset of effector B cells can modulate the differentiation of naive CD4+ T cells into TH1 and TH2 cells by secreting polarizing factors such as IL-4. Upon stimulation by effector TH cells and antigens, B cells can also produce specific immunomodulatory cytokines, including IL-2, IFN-γ, IL-12, and IL-4 [76]. Following activation by polarized TH2 cells, B cells transform into Be2 cells, which are subsequently injected into mice, resulting in proteinuria within 24 h. Moreover, proteinuria was not induced when IL-4-deficient B cells were used, and circulating levels of IL-4 could not be detected in the serum, indicating that local activation of the B-cell population and the release of IL-4 were responsible for the development of proteinuria. The renal pathology

observed in these mice resembled that of mice fed a human MCD diet. Immunohistochemistry revealed that IL-4 treatment significantly increased pSTAT6 expression in podocytes and other glomerular cells, which was suppressed by facitinib. A subset of MCD patients also exhibited weakly positive pSTAT6 staining [77]. IL-4 and IL-13 activate the tyrosine kinases JAK1 and JAK3 through binding to IL-4Rα and IL-13Rα on the surface of glomerular visceral endothelial cells, subsequently leading to activation of the transcription factor STAT6 for signal transduction [78, 79].

The Notch signaling pathway is an extremely conserved cell communication mechanism in living organisms. In mammals, Notch encodes four homologous receptors, namely, Notch1-4, which activates downstream transcription factors through CSL-DNA binding proteins by binding to the Notch ligands Jagged1, Jagged2, Delta-like1, Delta-like3, and Delta-like4 on adjacent cells and ultimately exerting biological effects [80, 81]. On the one hand, the notch signaling pathway plays a regulatory role in B-cell differentiation. The Notch gene is expressed at various stages of B-cell development, and its delta ligand is present in bone marrow B-line cells. The Notch signaling pathway has the potential to impact the terminal differentiation of B cells and may stimulate mature B cells to differentiate into antibody-secreting cells through a noncanonical RBP-Jk-independent pathway [82, 83]. Notch1 can also upregulate its expression in mature B cells by activating the BCR, LPS, and CD40 pathways, thereby promoting further B-cell activation. In mouse models, Notch signaling in peripheral B cells can also enhance BCR signaling, impacting biological effects regulated by the BCR [84]. Additionally, mature B cells expressing the active form of Notch1 can also boost Treg and Th2 cell responses in an IL-33-dependent manner, contributing alongside T cells to the onset of INS [85].

On the other hand, the Notch signaling pathway also exerts a distinct influence on nephrotic syndrome. Notch is involved in podocyte maturation in normal kidneys [86] and interacts with podocytes in nephropathy. Podocyte-specific expression of notch1 and notch2 was found in the kidneys of patients with FSGS and MCD and is related to proteinuria levels [87]. Abnormal activation of notch signaling was found in human and mouse models of collapsed FSGS and ADR-treated mice [88, 89]. Mice expressing the bioactive intracellular product of notch receptor proteolysis (NOTCH-IC) in podocytes develop proteinuria and progressive glomerulosclerosis at 2 weeks after birth [90]. Unlike Notch1, Notch2 plays a protective role in the process of kidney injury in ADR-treated mice. After ADR injection, the use of jagged1 antagonists aggravated proteinuria and podocyte injury, while the use of a Notch2-activating monoclonal antibody significantly reduced podocyte injury and proteinuria levels [89]. Notch2 protects cultured podocytes from apoptosis through the AKT pathway in vitro. Sirt6 may regulate Notch in podocytes. The glomerular basement membrane was thickened in the SIRT6-knockout mice, and the expansion of the podocyte foot processes disappeared. Reduced sirt6 levels lead to increased H3K9ac levels in the Notch1 and Notch4 promoter regions, resulting in increased Notch transcription [91]. In addition, in an experiment on podocyte injury induced by yeast-activated serum (ZAS), Sirt6 improved podocyte injury by blocking RAS signaling through the Wnt1/β-catenin pathway [92]. In addition, the expression of jagged1 and notch1 in TGF-β1-cultured podocytes increased, indicating that jagged1 may be a downstream target of TGF-β1. Increased expression of jagged1 leads to notch activation, while increased expression of notch also leads to an increase in TGF-β1, and there is a positive interaction between these two molecules [93].

Podocyte depletion and apoptosis are the primary pathological mechanisms observed in various human chronic kidney diseases and animal models of nephropathy, wherein TGF-β plays a crucial role. By employing RT–PCR to detect the gene expression of TGF-β in renal biopsy tissues from FSGS patients, it was found that the expression of the TGF-β gene exceeded 90% [94], while the expression of its downstream molecules tsp-1 and smad2/3 in the glomeruli of FSGS patients was significantly greater than that in normal controls [95]. Numerous studies have focused on investigating the interaction molecules and mechanisms involved in TGF-β-induced podocyte injury. In vitro experiments with cultured podocytes revealed that TGF-β interacts with Smad7 to induce podocyte apoptosis through a caspase-3-independent pathway [96]. The addition of TGF-β upregulated the expression of Wnt1 and β-catenin, which could be completely inhibited by sb431542, a potent inhibitor of ALK-5. Following the translocation of TGF-β in vivo, Wnt/β-catenin acts as a downstream pathway, leading to further podocyte injury and proteinuria [97]. ADR-treated mice, a common animal model of FSGS, exhibited phosphorylation of the downstream targets of the mTOR pathway, namely, TGF-β, Smad3, and S6RP, on the first day after ADR treatment. The use of SB431542 and rapamycin inhibited the phosphorylation of these molecules and the loss of proteins such as nephrin, podocin, and wt-1 in the glomerulus, significantly alleviating proteinuria and glomerulosclerosis in mice [98].

TGF-β can also induce podocyte apoptosis through the cyclin-dependent kinase inhibitor p21 and the inhibition of PINCH-1-ILK-α-parvin complex formation [99, 100]. In ADR-treated mice, the mRNA and protein levels of the B-cell translocation gene Btg2 were significantly upregulated. Btg2 colocalized with podocin in the mouse glomerulus. The addition of ADR to podocytes cultured in vitro resulted in epithelial mesenchymal transition, which was enhanced in Btg2-overexpressing podocytes and weakened in Btg2-knockdown cells. TGF-β induces Btg2 and promotes podocyte epithelial mesenchymal transition through the Smad3 pathway, which is one of the mechanisms by which ADR causes FSGS in mice [101].

Predictive Treatment

B-cell CD20 depletion has been shown to be effective in the treatment of nephrotic syndrome. RTX, a human-mouse chimeric CD20 monoclonal antibody, depletes all b-cells after 1 month of use, leading to clinical remission [27]. However, failure to improve nephrotic syndrome after FSGS kidney transplantation has also been reported [102]. The second generation of anti-CD20 antibodies, represented by the humanized monoclonal antibody olaparib, has a broader binding site and higher affinity. Low-dose olaparib can be used in patients who are resistant to RTX and is effective in treating steroid-dependent or frequently relapsing nephrotic syndrome [103].

Recently, precision cell therapy has shown potential in treating autoimmune diseases. CAR-T-cell therapy uses TCRs to graft antigen-antibody-specific binding sites onto T cells, making them specific for killing. CD19 CAR-T-cell therapy has induced remission in patients with autoimmune diseases such as systemic lupus erythematosus, idiopathic inflammatory myositis, and systemic sclerosis [104]. AQP4-IgG is a pathogenic antibody in the serum of patients with relapsed/refractory neuromyelitis optica spectrum disorder. The use of B-cell maturation antigen-targeted CAR-T-cell therapy in neuromyelitis optica spectrum disorder patients has safely reduced the level of pathogenic antibodies [105]. The chimeric autoantibody receptor CAAR-T cells are a therapy similar to CAR-T cells and are designed to express T cells with extracellular domains of autoantibodies, target B cells that produce pathogenic autoantibodies, and specifically eliminate pathogenic B cells without affecting the total B-cell count or function. This approach has achieved specific B-cell depletion in a mouse model of muscle-specific tyrosine kinase myasthenia gravis by expressing T cells that express anti-MuSK autoantibodies (MuSK-CAART) [106]. As previously mentioned, pathogenic autoantibodies are involved in nephrotic syndrome. T cells modified with known autoantibodies to specifically eliminate pathogenic B cells may be an effective method for the precise treatment of nephrotic syndrome.

Conclusion

The discovery and pathogenicity studies of B-cell autoantibodies have provided strong evidence for the involvement of B cells in the pathogenesis of nephrotic syndrome. In addition, B cells can also produce cytokines that directly or indirectly contribute to the pathogenesis of nephrotic syndrome through signaling pathways. Specific targeted therapy against B cells offers a new direction for the precision treatment of nephrotic syndrome.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Shifan Zhu wrote the manuscript, and Jiayu Zhang, Langping Gao, Qing Ye, and Jianhua Mao revised the manuscript.

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