Review Article

Do Foxp3⁺ Regulatory T Cells (Treg Cells) Play a Role in the Immunopathogenesis of Primary/Idiopathic Minimal Change Disease?

Susan Swee-Shan Hue,¹ Sufi Muhammad Suhail,² Jason Chon Jun Choo,² Nurhashikin Yusof,³ Alwin Hwai-Liang Loh,⁴ and Francisco Salcido-Ochoa³

¹ Tregs and HLA Research Force and Department of Pathology, Singapore General Hospital, The Academia, 20 College Road, Singapore 169856

² Renal Medicine Department, Singapore General Hospital, The Academia, 20 College Road, Singapore 169856

³ Tregs and HLA Research Force and Renal Medicine Department, Singapore General Hospital, The Academia, Level 3, 20 College Road, Singapore 169856

⁴ Department of Pathology, Singapore General Hospital, The Academia, 20 College Road, Singapore 169856

Correspondence should be addressed to Francisco Salcido-Ochoa; francisco.salcido.ochoa@sgh.com.sg

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Minimal change disease constitutes a major cause of nephrotic syndrome. It is regarded as a non-immune-complex mediated primary glomerulopathy and pathogenetically is characterised by podocyte injury and effacement of foot processes; therefore, it is also classified as a type of podocytopathy. T cell dysfunction with increased levels of a soluble glomerular permeability factor has been proposed to play a major role in the pathogenesis of minimal change disease. It has been therefore suggested that a dysfunction of regulatory T cells, the orchestrators of immune homeostasis, could be implicated in perpetuating T cell activation in this condition. However, the actual contribution of regulatory T cell dysfunction in the immunopathogenesis of primary minimal change disease is still largely unclear. We here propose a theoretical model based on the available evidence.

1. Introduction

The glomerulus is the functional unit of the kidney responsible for blood filtration, which is the first step in blood purification and the production of urine. Normal functioning of the glomerulus requires the integrity of all three major components of the glomerular filtration barrier, that is, endothelial cells, glomerular basement membrane (GBM), and the visceral epithelial cells or podocytes. Podocytes have cellular projections, or foot processes, which interdigitate with each other and wrap around the capillary loops, leaving spaces in between referred to as slit diaphragms. The slit diaphragms serve as a permeable selective barrier with sieving function allowing filtration of fluid and small solutes while preventing large-molecular-weight plasma components and anionic serum proteins like albumin from passing

into the urine. Many glomerular disease processes cause damage and subsequent disruption to the glomerular filtration barrier, eventually leading to pathological glomerular permeability to proteins. If proteinuria exceeds 3.5 g/1.73 m²/day and is accompanied by hypoalbuminaemia, oedema, and/or hyperlipidaemia, the resultant clinical picture is referred to as nephrotic syndrome.

Primary minimal change disease (MCD) is a distinct histopathological entity that typically presents with idiopathic nephrotic syndrome. But some patients may develop MCD secondary to use of nonsteroidal anti-inflammatory drugs or a lymphoproliferative disorder. Light microscopy in MCD typically reveals very minor changes such as podocyte enlargement or ectatic capillary loops (Figure 1). At times, diffuse mesangial hypercellularity can also occur. On immunofluorescence, no immunoglobulin deposition is

FIGURE 1: Histopathological changes in minimal change disease. (a) Glomerulus showing minor changes from a patient with minimal change disease. Capillary loops are mildly ectatic (asterisks) and a few podocytes are slightly enlarged (black arrows). There is no significant mesangial expansion or hypercellularity (red arrows) (periodic acid schiff, original magnification $\times 600$). (b) Electron microscopy showing extensive effacement of podocyte foot processes (black arrows) from the same patient. Inset: normal, intact foot processes (uranyl acetate and lead citrate).

encountered, unless one is dealing with the controversial variant IgM nephropathy. Therefore, the pathological diagnosis of MCD requires evaluation of ultrastructural changes by electron microscopy. The presence of diffuse effacement or “fusion” of the podocyte foot processes is characteristic (Figure 1). Electron dense immune-complex deposits are absent. Detachment of podocytes from the GBM can be seen, as is the aggregation of cytoskeletal filaments at the base of epithelial cells. Since podocyte injury is the central mechanism in its pathogenesis, MCD has been classified, along with other forms of glomerular disease, as a podocytopathy [1].

Although non-immune-complex mediated, numerous immune (and nonimmune) alterations have been implicated in the pathogenic mechanisms of this podocytopathy. T cell dysfunction along with the presence of a circulating glomerular permeability factor (GPF) is long held to be the main factor implicated in triggering podocyte injury in primary MCD [2]. It is therefore theoretically plausible that a dysfunction in the regulatory T cell (Treg cell) population, which is widely recognised as the master moderator of immune responses [3, 4], could be implicated in perpetuating the uncontrolled T cell activation and immune injury in this condition. In this review, we discuss the potential role of T cells, in particular Treg cells, in the immunopathogenesis of MCD, and propose a pathophysiologic model with Treg cells participating in different stages of the disease process.

2. Regulatory T Cell Biology

Many subsets of T cells have been described with the ability to maintain immune homeostasis by promoting peripheral tolerance to self-antigens and controlling pathogenic inflammatory responses by modulating the activation and function of other immune cells. Chief among these are the Foxp3^+ Treg cells which are widely regarded to be the central players in the mediation of immune responses. Foxp3 , a fork-head box transcription repressor factor, appears to be the master controller of Treg cell development and function

in humans as exemplified in children with the immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome [5], who carry dysfunctional mutations in the Foxp3 gene. These children display reduced number and impaired function of all lineages of Foxp3^+ Treg cells, leading to a disease manifestation characterised by T cell overactivation, lymphoproliferation, autoimmunity, allergies, and childhood death [5]. Of note, Treg cell dysfunction has also been described in many autoimmune and hyperimmune disorders, but contrary to the IPEX syndrome, in which there is a primary defect in the Foxp3 gene leading to impaired development of all lineages of Foxp3^+ Treg cells, these immune disorders are more likely to have isolated deficiencies in selected Foxp3^+ Treg cell clones specific for certain antigens and/or are associated with selective defects in Treg cell subset differentiation, their migration capacity, or their suppressive capacities on different immune cell targets. We believe that the same may be extrapolated to MCD.

Foxp3^+ Treg cells can be divided into two main subsets with different origins (thymic and peripheral Tregs), which in turn can display different activation statuses and migration capacities (naïve versus activated/memory), and likely have distinct roles in health and disease [12–14]. Thymic Foxp3^+ Treg cells derive from the thymus and are naturally committed to immunoregulation. They acquire their immunoregulatory capacities while being positively selected by self-peptide: major histocompatibility complex (MHC) molecules expressed in the thymus [15, 16]; therefore, they are believed to contribute to the maintenance of self-tolerance in peripheral tissues. Upon exiting the thymus, naïve thymic Foxp3^+ Treg cells, expressing selectin CD62L and the chemokine receptor CD197 [17], traffic to the secondary lymphoid organs where they modulate the activation state of antigen-presenting cells (APCs) bearing self-peptide: MHC complexes, thereby preventing the activation as well as differentiation of emerging autoreactive T cell clones [18, 19]. Upon antigen-specific activation, thymic Treg cells acquire the increased capacity to migrate to the peripheral tissues,

TABLE 1: Studies supporting Treg cell involvement in the immunopathogenesis of minimal change disease.

Reference	Disease	Population	Immunopathogenic findings
[6]	MCD or FSGS	38 children	More infiltrating T cells when compared to controls but reduced number of infiltrating Foxp3 ⁺ T cells in both MCD and FSGS patients
[7]	INS	41 children	Decreased numbers of peripheral CD39 ⁺ Foxp3 ⁺ Treg cells and impaired ATP catabolism
[8]	MCD	21 Adults	Similar number of Foxp3 ⁺ Treg cells compared to controls but reduced suppressive function during active disease
[9]	MCD	25 adults	Increased circulating Th17 cells over Treg cells when compared to controls, which correlated with severity of proteinuria. Ratios reverted upon steroid therapy in most patients
[10]	INS	36 children	Increased circulating Th17 cells over Treg cells when compared to controls, together with increased intrarenal IL-17 expression
[11]	MCD + IPEX	5-year-old boy	The occurrence of MCD in IPEX syndrome, where Treg cells are deficient, suggests a possible pathogenic association

FSGS: focal segmental glomerulosclerosis; INS: idiopathic nephrotic syndrome; IPEX: immunodysregulation polyendocrinopathy X-linked syndrome; MCD: minimal change disease.

thereby also being able to prevent autoaggression at the sites of inflammation [13]. Hence, it is thought that the impaired development or function of thymic Treg cells is a major factor contributing to loss of self-tolerance [4] and to the development of autoimmune disorders.

In contrast to thymic Foxp3⁺ Treg cells, peripheral Foxp3⁺ Treg cells differentiate in secondary lymphoid tissues or inflamed sites from non-Treg cells in response to foreign antigens or altered self-antigens under unique conditions of antigen presentation [4]. Their induction is driven by the concomitant activation of effector T cells (Teff cells), which produce the required IL-2 for peripheral Treg cell differentiation [20, 21], in the presence of other permissive cytokines. Peripheral Foxp3⁺ Treg cells seem to complement the role of thymic Treg cells by controlling the immune responses to foreign antigens and self-antigens not expressed in the thymus, thereby preventing not only autoimmunity but also chronic inflammation and inappropriate inflammatory responses [13, 18, 19, 22]. Peripheral Treg cells display an activated memory-like phenotype and express chemokine receptor CD196, which equip them with migratory capacity to inflamed tissues. This property allows them to colocalise with IL-17-producing CD4⁺ Th17 cells, a type of Teff cells involved in the propagation of proinflammatory responses, which also use the same mechanism (i.e., CD196 expression) to migrate to inflamed tissues [23–25].

Of special interest, a more differentiated subset of peripheral Foxp3⁺ Treg cells, the CD39⁺Foxp3⁺ Treg cells, appears to have increased survival advantage, prime regulatory capacities when compared to other Treg cells, and could be the natural counterplayers of Th17 cells [23–26].

Mechanistically, antigen-specific T cell receptor ligation is essential for Treg cell activation, but their suppressive action appears to be non-antigen-specific [27]. Treg cells mediate immunoregulation by using a myriad of cellular and humoral mechanisms on different target immune cells [28], exerting their functions by modulating the activation, differentiation, and effector functions of Teff cells and APCs, or inducing their apoptosis [4, 28]. Among the different immunosuppressive armamentaria deployed by Treg cells,

the surface expression of CD39 and other ATP-catabolising enzymes may play a major role in their suppressive action mediated by CD39⁺Foxp3⁺ Treg cells. It has been demonstrated that intracellular ATP released upon cell damage can bind to purinergic inotropic receptors on different immune and epithelial cells and triggers proinflammatory responses [24, 25]. In this context, it is likely that the ATPase function of CD39 on CD39⁺Foxp3⁺ Treg cells might take part in the catabolism of ATP into anti-inflammatory metabolites like adenosine, which subsequently could inhibit Teff cell function or induce apoptosis of Teff cells at inflamed sites by acting on adenosine receptors [24, 25].

3. Role of Regulatory T Cells in Primary Minimal Change Disease

Association of MCD with atopy and haematological malignancies, as well as remission of MCD following T cell suppression by pharmacological means (ciclosporin, mycophenolic acid analogues, steroids, or cyclophosphamide) or after measles infection, has been long suggested as evidence of T cell involvement in the pathogenesis of this podocytopathy [2]. Since the strength of this evidence is relatively weak, especially when other nonimmunological factors have been also implicated in the pathogenesis of MCD [29], the definite role of T cells and the immune system in general in the pathogenesis of MCD has not gained universal approval in the medical/scientific community. Nonetheless, given the likely involvement of a GPF, a putative cytokine in the pathogenesis of MCD [30, 31], and the many recent studies demonstrating an imbalance between Treg cells and their counterparts, the Th17 cells in MCD patients (Table 1), we believe that Foxp3⁺ Treg cells could plausibly play a part in the pathogenetic mechanisms of proteinuria in this primary podocytopathy. In addition, further evidence shows that patients with MCD indeed display reduced numbers and functional impairments in Foxp3⁺ Treg cells, including the CD39⁺Foxp3⁺ Treg cell subset (Table 1). All these encouraging observations have rendered renewed interest in further deciphering the roles

of immune system in the pathogenesis of MCD. However, the precise roles played by different Teff cells (Th1, Th2, or Th17 cells) and by Treg cells and the potential mechanisms in which T cell dysregulation contributes to podocyte injury are still largely unclear.

4. Immunopathogenic “Injury Loop” in Primary Minimal Change Disease

Based on the evidence presented above suggesting a role for Foxp3⁺ Treg cell dysfunction in MCD and on the existing theories [32] suggesting molecular links between Treg cells and podocyte injury, we would like to propose a theoretical “injury loop” model for the immunopathogenesis of MCD (Figure 2). However, it should be emphasised that the initial trigger leading to the T cell dysregulation in MCD or whether such dysregulation is the initial pathogenic pathway or a consequence of other intrinsic or extrinsic factors causing podocyte injury still remains unknown. Also, the fact that other nonimmunological factors (infections, allergens, environmental toxins, drugs, or genetic predisposition) are likely to play a part in the pathogenesis of MCD should not be neglected. In our model of Treg cell dysfunction, the overproduction of cytokines and the aberrant expression of costimulatory molecules play a pivotal role in the initiation and/or perpetuation of podocyte injury. In this model of dysregulation, if adequate glomerular autoregulatory mechanisms or immunoregulatory mechanisms take place, the “injury loop” may be terminated, restoring glomerular immune homeostasis.

4.1. “Treg/Teff Imbalance” Phase. We speculate that excessive amounts of cytokines, having GPF activity, are produced by dysregulated Teff cells in response to some unknown antigenic stimuli and triggering events. This dysregulated function of Teff cells may be a consequence of impaired normal homeostatic mechanisms attributable to numerical or functional imbalance between Foxp3⁺ Treg cells, possibly peripheral Treg cells, and their counterpart Th17 cells [6–10] or other Teff cells (Figure 2(a)). Candidate cytokines for this GPF activity include IL-13 [30] and IL-8 [31], which might be produced by different subsets of Teff cells, but other soluble factors could be also implicated. These cytokines could be produced in response to different antigenic stimuli occurring systemically or, in a more limited fashion, locally. The most plausible scenario is the activation of Teffs in the secondary lymphoid tissues or in the inflamed sites in response to a microbial infection elsewhere, leading to the systemic production of cytokines, which eventually reach the glomeruli via the bloodstream. On the other hand, the contribution of interstitial Teff cells is likely to be less significant since it is known that the T cell infiltrate in MCD is typically scarce [6] or nonexistent. Nonetheless, glomerular endothelial cells can potentially function as APCs as they have been found to express MHC class II molecules in MCD patients [33]. Thus, the possibility that Teff cells can be activated or restimulated by antigen presented by the glomerular endothelial cells while circulating within the vicinity of the glomerular loops cannot

be excluded. The loss of negative charges from the glomerular barrier, a characteristic early change in MCD, which may in itself be a consequence of the cytokine effects on glomerular endothelial cells, could further facilitate the filtration of the overproduced cytokines into the urinary space, causing podocyte injury.

4.2. “Podocyte Injury” Phase. Upon reaching the urinary space, these cytokines appear to have direct toxic effects on podocytes and to cause further alteration to the integrity of the glomerular filtration barrier [2] (Figure 2(b)), although the molecular pathways involved are yet to be defined. It is possible that podocyte injury releases ATP to the extracellular compartment, where it displays proinflammatory capacity, contributing to further podocyte injury in a vicious cycle. In the presence of depleted or dysfunctional activated CD39⁺Foxp3⁺ Treg cells, immunoregulation and catabolism of ATP are impaired [7], and this injurious process is further amplified.

4.3. “Injury Loop” Phase. Under the influence of this abnormal cytokine milieu and/or elevated “injury molecules” such as ATP, podocytes upregulate MHC class II molecules [33] and T cell costimulatory molecules like CD80 [34, 35] (Figure 2(c)), which are characteristic of APCs. Although it is tempting to speculate that podocytes could act as APCs and thus provide another means to perpetuate Teff cell activation, in the context of MCD, it is our opinion that Teff cells are unlikely to reach the urinary space to interact directly with podocytes, particularly in the absence of GBM disruption. A more plausible alternative mechanism in the amplification of Teff cell activation may be attributed to the role of CD80 expression on podocytes. Pronounced expression of CD80 on podocytes with increased urinary excretion of CD80 has been demonstrated in patients with MCD [34, 35]. In addition, the incubation of cultured podocytes with sera from patients with MCD in relapse is able to induce the expression of CD80 on podocytes [36], which further reinforce the potential pathogenic role of podocyte CD80 synthesis and expression, as well as supporting the role of a circulating pathogenic factor in MCD. It is tempting to speculate that these shed (thus soluble) CD80 molecules could possibly cross the GBM and diffuse back to the bloodstream, especially if produced in large amounts, reaching Teff cells circulating locally or those residing in the draining secondary lymphoid tissue. If this indeed would occur *in vivo*, soluble CD80 molecules could then bind to its receptor, CD28 on Teff cells, and sustain the activation of Teff cells with continuous secretion of GPFs. In the absence of appropriate glomerular autoregulatory mechanisms or in the presence of a dysfunctional Foxp3⁺ Treg cell population, this process would remain unchecked in a pathogenic “injury loop” with persistent proteinuria and nephrotic syndrome.

4.4. “Restoration of Homeostasis” Phase. As per conventional immune responses, great part of the immune regulation of the Teff cells in MCD might take place in secondary lymphoid tissues, where they are activated. It is difficult to

FIGURE 2: Dysfunctional Treg cells promoting a pathogenic "injury loop" in minimal change disease. (a) A numerical or functional imbalance between Foxp3^+ Treg cells and Teff cells in response to undefined antigenic stimuli leads to systemic (e.g., at the lymph nodes) or local overproduction of cytokines, which could reach podocytes through filtration. (b) Direct toxicity of cytokines on podocytes further increases glomerular permeability and the release of proinflammatory molecules such as ATP. (c) If intrinsic or Treg cell-dependent regulatory mechanisms fail, persistent injury to podocytes leads to expression and shedding of CD80 molecules. Soluble CD80 molecules could diffuse back into the bloodstream and bind to CD28 on Teff cells, distantly or locally, sustaining continuous activation and synthesis of cytokines in a podocyte "injury loop." (d) If instead CD39^+ Foxp3^+ Treg cells manage to control Teff cells through the secretion of suppressive cytokines and soluble CD152 and by initiating extracellular ATP catabolism through CD39 on their surface, immune balance is restored and the "injury loop" is terminated.

conceive that immunoregulation takes place in the interstitium of the kidney as mentioned before since MCD is characterised by none or minimal inflammation and T cell infiltrate. The significance of such infiltrate, when present, is unknown and currently under investigation (our work is in progress). Nonetheless, it is possible that certain degree of the immunoregulatory cross-talk occurs around the glomerular interphase, between circulating cells and their products in the bloodstream with the glomerular endothelium and the podocytes in the urinary space. In this respect, CD80 can play a dual role, not only favouring T cell activation but also facilitating immunomodulation. CD80 can bind to CD152 (CTLA-4), which is a negative costimulatory molecule related to CD28 [37]. CD152 appears to have a role in switching off T cell responses at later stages of the immune response, and it is also a crucial molecule for the immunosuppressive function of Foxp3⁺ Treg cells [37]. Ligation of CD80 by soluble CD152 produced by Treg cells is known to inhibit CD80 expression on APCs [38]. Therefore, soluble CD152 or other immunoregulatory cytokines like TGFβ or IL-10 secreted by Foxp3⁺ Treg cells, other regulatory T cells or even podocytes [32], could potentially cross the glomerular barrier and bind to CD80 on podocytes, turning off its expression, thus terminating the “injury loop,” bringing back homeostasis (Figure 2(d)). The observation of low serum and urinary levels of CD152 in MCD patients during relapse and returning to higher levels during remission supports this hypothesis [35]. This mechanism could be partially responsible for the spontaneous remission observed in some MCD patients. In others, pharmacological immunosuppression is required to suppress T cell activation and the synthesis of GPFs and to allow Foxp3⁺ Treg cell-dependent mechanisms to act more efficiently.

5. Implications of the “Injury Loop” Model on the Therapy for Minimal Change Disease

Immunosuppressive drugs used for the induction of remission and the prevention of relapses in MCD could be crucial factors to determine the restoration of homeostasis or the perpetuation of the “injury loop.” If Foxp3⁺ Treg cell dysfunction indeed plays an important role in the initiation or perpetuation of the “injury loop” and if promoting Treg cell function could reverse this process, the differential effects of immunosuppressive drugs upon different immune cell types should be considered and studied in more detail. The data on the effects of immunosuppressive drugs on Treg cells in patients with MCD is very limited. Ciclosporin is used in MCD with the aim of modulating the effector arm of the immune system, potentially reducing the synthesis of GPFs, as well as for its effect on podocytes stabilising the structure of the slit diaphragm [29]. However, studies on kidney transplantation have demonstrated that ciclosporin has negative effects on the development and function of Treg cells [39], which might be counterproductive. On the other hand, drugs like sirolimus or everolimus seem to favour Treg cell development and function [39], but their value in proteinuric disorders is limited as proteinuria is one of their common side effects. The effects of other drugs like cyclophosphamide on Treg cells are less understood. Cyclophosphamide can

deplete unspecifically several immune cells, creating a lymphopenic environment, which has been shown to promote the consequent expansion of Treg cells in patients undergoing combined kidney and bone marrow transplantation [40]. In theory, this homeostatic proliferation of Treg cells could also happen when cyclophosphamide is used in MCD and be beneficial. However, cyclophosphamide provides significant toxicity and is used in selected cases. Other drugs like steroids, mycophenolic acid analogues, and azathioprine seem to have no significant deleterious effects on Treg cells or to deplete them unspecifically in a dose-dependent fashion [41, 42].

Although the choice of immunosuppression could alter the balance between Treg cells and T_H17 cells, aiding in the restoration of immune homeostasis and terminating the “injury loop,” the evidence presented here is not yet compelling enough as to suggest that promoting the immunoregulatory network mediated by Treg cells is the answer to target this primary podocytopathy, but our model serves as food for thoughts in opening new avenues for research.

6. Conclusion

Primary MCD is an important cause of idiopathic nephrotic syndrome in children and adults, having podocyte injury as a central part in its pathogenesis. The treatment of MCD has remained a clinical challenge. Although immunosuppressive therapy is often effective, it has potential toxicity often posing long-term hazards to patients. Hence, better understanding of the underlying pathogenesis could lead to the development of novel diagnostic approaches and more targeted therapies, thus reducing the burden of renal disease. Although the role of Foxp3⁺ Treg cells in the pathogenesis of MCD is being slowly unraveled in the literature, we are still very much at the fledgling stage in this journey of discovery. Nevertheless, with the exciting ongoing research efforts worldwide, we foresee newer diagnostic approaches, treatment guidelines, and prognostic indicators on the horizon, where the focus of therapies does not just rest on broad spectrum nonspecific measures but also on more targeted immune strategies potentially involving Treg cells.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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