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Abatacept and Glomerular Diseases: The Open Road for the Second Signal as a New Target is Settled Down

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Abstract: Glomerulopathy is the third most important cause of kidney disease. Proteinuria is the hallmark of glomerular damage, and a marker of progression of kidney disease, cardiovascular morbidity and mortality. Strategies to reduce proteinuria are partially successful, and despite proteinuria management, renal disease may still progress. Immunosuppression to treat glomerulopathies is non-specific, partially effective and presents side-effects. It is critical to find safe drugs with specific podocyte molecular targets. Podocytes contain a complex array of proteins. Lymphocyte activation antigen B7-1 (CD80) is located on antigen presenting cells modulating CD4+ and CD8+ T cells by interacting with co-stimulator CD28, a glycoprotein located on T-cells, or with cytotoxic T-lymphocyte protein 4 (CTLA-4) co-inhibitor. Normally, podocytes do not express B7-1. However, certain glomerulopathies are associated with an increase on the surface of podocytes of B7-1, which reduces the ability of podocytes to attach to the surrounding glomerular basement membrane, favouring podocyturia and proteinuria. When the B7-1-CTLA-4 interaction takes place, the immune response is abrogated, while a B7-1-CD28 coupling leads to T cell activation. Abatacept binds to B7-1 by blocking the CD28 or potentiating the CTLA-4 signals. In B7-1 positive podocytes, abatacept may be a specific tool to decrease proteinuria. Selected patents are also briefly presented in this review.

Keywords: Abatacept, B7-1, CD28, CD80, CTLA-4, glomerulopathy, podocyte, proteinuria.

INTRODUCTION

Glomerulopathy is the third most important etiologic entity that causes end-stage kidney disease. In addition, diabetic nephropathy and hypertension, the two most frequent etiologies that lead to chronic renal replacement therapy, are also main causes of secondary glomerulopathy [1]. In any case, the glomerulus is always affected. Different degrees of proteinuria accompany the diverse causes of glomerulonephritis, and the higher its amount, the higher the risk of progression to kidney failure [2-5]. Proteinuria may be due to many causes, but independently of the etiology, the glomerular filtration barrier, composed by the podocyte, the glomerular basement membrane and the endothelial cell, is indefectibly affected [6-8]. This could be the result of local or systemic insults, mainly due to immunological, metabolic, or hemodynamic factors [9, 10]. In primary and many secondary causes of glomerulopathies, either primary or secondary, the immune system is always involved.

In the case of secondary causes of glomerular diseases due to metabolic or hemodynamic derangements as diabetic nephropathy and hypertension, the immune system is also involved [11]. Tissue injury in glomerular diseases is mediated by both the innate and adaptive immune response [9]. In this regard, the main components of the innate limb of the immune system that play a main role in glomerulonephritis are neutrophils, macrophages, dendritic cells, toll-like receptors and the complement system. The adaptive components of the immune system are composed by B cells and the production of antibodies, and T cells and the production of cytokines and lymphokines [9]. Different mechanisms that share many of the above mentioned components of the immune system participate in each glomerulopathy, to which metabolic and hemodynamic alterations may add on, and aggravate the structure of the glomerular filtration barrier [9-11]. Consequently, as glomerulopathies progress, proteinuria tends to increase and the glomerular filtration rate to decrease [12]. With respect to the different types of glomerular diseases, some pathophysiological considerations will be concisely made.

In minimal change disease, the complement system is not involved and no autoimmune features are present [9]. However, elevated levels of B7-1 have been found in the urine in subjects with active disease [13, 14]. In primary focal and segmental glomerulosclerosis (FSGS), the complement system is quiescent. Primary forms of the disease can be secondary to genetic causes, to elevated levels to circulating factors that increase the permeability of the glomerular filtration

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membrane to albumin [14]. It appears that in certain situations podocytes express B7-1 molecules on their surface that stimulate the interaction with T cells and the effacement of foot processes take place, causing proteinuria [15].

In primary membranous nephropathy, it has recently been found that the main cause of this disease is an antibody directed against the receptor of phospholipase A2, located on the surface of podocytes and explaining the characteristic localization of subepithelial deposits that characterizes membranous nephropathy [16]. The classic pathway of the complement system is involved; the presence of antibodies and the autoimmune features of membranous disease cannot discard the involvement of B and T cells, and the eventual participation of B7-1 positive podocytes in the pathogenesis of this entity [9, 16].

On the contrary, despite IgA nephropathy is a mesangioproliferative disease with profound autoimmune features in which Toll-like receptors and the alternate and lectin pathways are involved, it seems that abatacept can worsen its clinical course [17]. However, the presence of B7-1 positive podocytes has not been assessed.

Rapidly progressive crescentic glomerulonephritis is classically classified into three main diseases: Firstly, anti-glomerular basement membrane glomerulonephritis, characterized by acute necrotizing glomerular lesions and crescents, is due to the production of antibodies against components of the α chain of type IV collagen [9]. The complement system and both B and T cells are involved in this disease [9]. The presence of B7-1 positive podocytes or the employment of abatacept has not been yet considered. Secondly, pauci-immune glomerulonephritis is characterized by anti-neutrophil cytoplasmic antibodies mainly directed against neutrophil myeloperoxidase or proteinase-3 ezymes [9]. It is characterized by focal necrotizing glomerular lesions and the presence of crescents. Neutrophils, Toll-like receptors, the alternative pathway of complement, B and T cells participate [9]. Finally, in immune-complex glomerulonephritis focal lesions with crescents are due to diffuse mesangial depositions of immune complexes formed by components of the classical pathway of complement plus immunoglobulins. Components of the innate and adaptive limbs of the immune system are present in the pathogenesis of this entity.

Primary immune-complex membrano-proliferative glomerulonephritis (previously called type I glomerulonephritis) is an entity in which neutrophils, the classical pathway of complement, B and T cells play a critical role in its development [9]. Again, B7-1 podocytes have not been assessed. C3 glomerulopathy encompasses two different entities, dense deposit disease and C3 nephropathy [65]. In both diseases, the alternative pathway of complement is the main cause of the disease and they differentiate each other morphologically mainly due to the immunofluorescent distribution of the C3 component of complement and on the ultrastructural features [9]. The complete absence of immunoglobulins on glomeruli makes these diseases unlikely to be related to B7-1 positive podocytes, suggesting B cells not being stimulated by previously activated T cells.

CTLA-4 fusion proteins have been proposed to be evaluated as potential tools for the prevention and treatment of diabetic nephropathy [18, 19]. Diabetic nephropathy develops in 20% to 40% of diabetic patients, and is the leading cause of end-stage renal disease in the United States [20]. Multiple mechanisms contribute to the development of glomerular disturbances, in which fibrotic and hemodynamic cytokines, oxidative stress, advanced glycation products and genetic interactions take place [20, 21]. It has also been suggested that the innate immune system plays an important role in the pathogenesis of diabetic nephropathy. Monocytes from diabetic type 2 patients with diabetic nepropathy present elevated concentrations of B7-1 compared to controls, supporting the suspicion that there may be a potential benefit for assessing abatacept in diabetic nephropathy [22].

Finally, in lupus nephritis all kind of immunoglobulins and C3 are localized in mesangial areas (classes I and II), or in subendothelial (classes III and IV) and/or subepithelial (class V) spaces. Irrespective of the class, in lupus nephritis neutrophils, Toll-like receptors, the classic pathway of complement, and B and T cells all participate [9]. As it will be discussed later, the advantage of abatacept for the treatment of lupus nephritis appears to offer some benefits [23-25].

In general, there are many interventions available to reduce proteinuria, albeit in general they are partially successful. Moreover, despite an eventual disappearance of proteinuria after pharmacological interventions, chronic kidney disease progression may still continue, due to the fact that proteinuria may be indicating a significant damage to the glomerulus in conjunction with medullar interstitial fibrosis and tubular atrophy. The backbone of glomerular disease therapy relies mainly on adequate body weight, blood pressure and metabolic control, reduced salt intake, tobacco discontinuation, and tailored immunosuppression [26]. Despite these interventions, glomerulopathies lead a considerable amount of individuals to dialysis. Immunosuppression, mainly based on steroid therapy alone or combined with other drugs as cyclophosphamide, mycophenolate, azathioprine, cyclosporine, tacrolimus, and more recently rituximab, basiliximab and eculizumab, can be employed with different degrees of based evidence in certain glomerulopathies [27-29]. However, the success is variable and the side effects not infrequent [1, 27-29].

In this regard, the assessment of new therapies is mandatory. Although evaluated in a small amount of patients and in a few number of glomerulopathies, abatacept has been shown to be a promising agent to take into consideration [15, 23-25]. Abatacept best studies mechanism of action consists on competing with CD28 present on T cells for the binding to B7-1 or B7-2 on antigen presenting cells [30] Fig. (**1**). To the present time, the inhibition of CD28 action on T cells [30] accomplished by abatacept has only been studied in

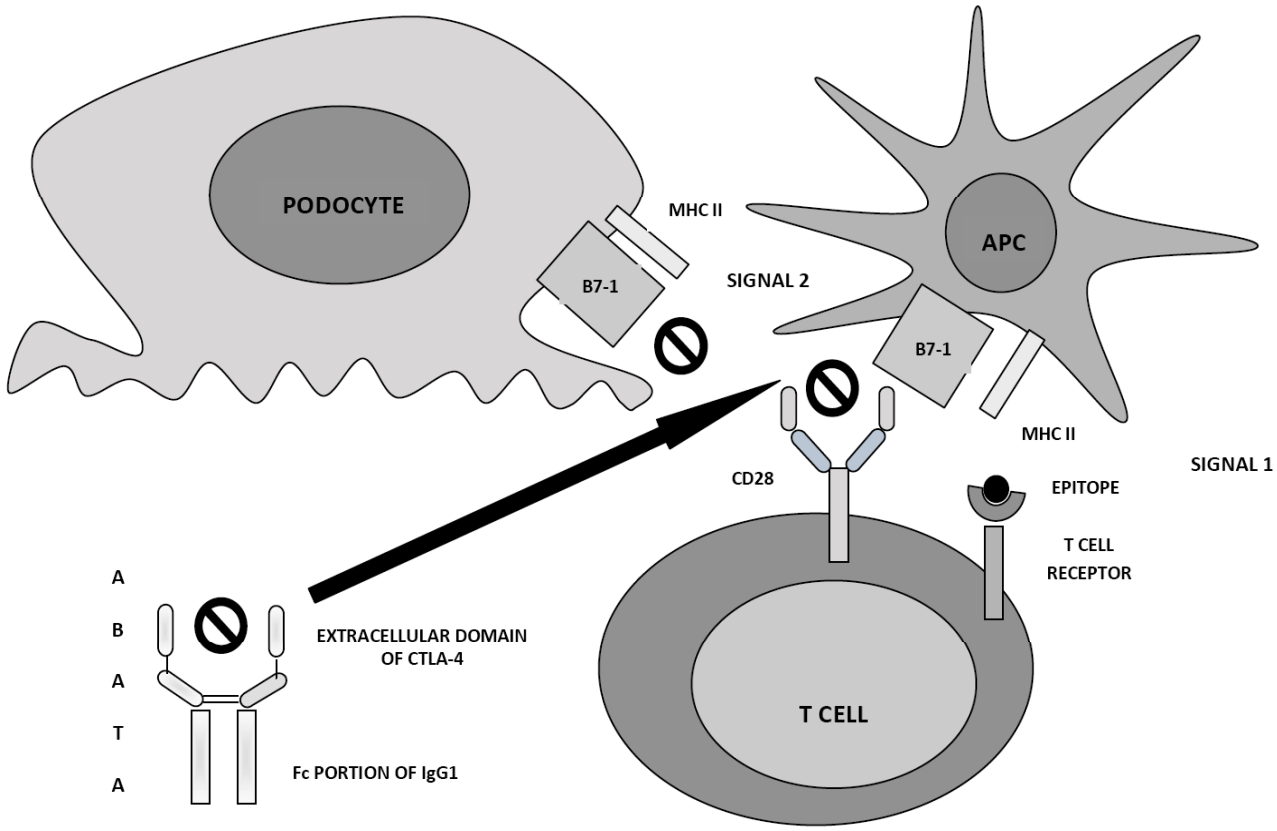

Fig. (1). Abatacept is composed of the extracellular ligand binding domain of CTLA-4 bound to the Fc portion of IgG1. The extracellular domain binds to B7-1 in antigen-presenting cells (APC) and in injured podocytes, inhibiting the co-stimulatory (second) signal from APC to CD4+ T cell. This abrogates the activation and proliferation of T cells and as a consequence of B cells. Symbol: ⃠: Blocking effect.

primary FSGS and in lupus nephritis, and with a small number of patients [15, 23, 24]. It appears that abatacept is capable of reducing proteinuria in glomerulopathies in which B7-1 positive podocytes are present and side effects are scant [15, 31-34]. Finally, in the different types of glomerulonephritis the identification of the B7-1 molecule in the podocyte has not been yet assessed. Therefore, it would be interesting to deepen the study of the presence of the B7-1 molecule on podocytes and the potential response to abatacept in the different B7-1 positive glomerular diseases.

CURRENT PROBLEMS IN THE MANAGEMENT OF PROGRESSION AND TREATMENT OF GLOMERULOPATHIES

Proteinuria is not only the hallmark of glomerular basement membrane disease due mainly to podocytopathies, insults to the fenestrated glomerular endothelium, hyperfiltration or glomerular scarring, but is also a marker of progression of chronic kidney disease and a surrogate of cardiovascular morbidity and mortality [2-5, 35]. Notwithstanding the etiology, it is critical to reduce proteinuria. Albeit many dietetic and pharmacological approaches are available, it is evident that these interventions are only partially successful [26]. In addition, proteinuria is not easily managed, and reductions to less than 0.5 g/day are not easily accomplished. Finally, despite significant proteinuria reduction, the glomerular disease still progresses. Many reasons account for this situation, as additional chronic tubulointerstitial and glomerulosclerosis, local hypoxia, secondary hypertension, hyper filtration, podocytopenia and ongoing cytokine-mediated inflammation and oxidative stress [36]. Even after kidney transplantation, some primary glomerulopathies and diabetic nephropathy itself can recur in the graft, eventually leading to graft failure and loss. With the exception of rapidly progressive glomerulonephritis, glomerular diseases carry a slowly progressive, insidious and relentless clinical course that many times present symptomatic just at progressed stages, when the situation is virtually impossible to revert.

In addition, nephrologists lack of useful biomarkers in terms of kidney function at preclinical stages in reference to glomerular disease progression. Creatinine is the currently employed molecule to assess kidney function, but presents several pitfalls. At clinical practice, there are no routinely available urinary biomarkers of glomerular damage that can antedate to proteinuria, that can identify the molecular culprit of a podocytopathy, or that can foretell a response to a pharmacological approach. Important advances have been achieved in the last decade with respect to specific biomarkers in certain glomerulopathies, as the identification of the antibody to the podocytic phospholipase A2 receptor antigen, the soluble urokinase-type plasminogen activator receptor (suPAR) with its role in various glomerulopathies (albeit

its exact role is yet to be determined), or the measurement of plasmatic or urinary globotriacylceramide or lyso-3 globotriaosyl ceramide in Fabry disease, among others [14,16, 37-40].

Due to the shortage of specific biomarkers availability in glomerular diseases, not only is an exact diagnosis based on pathophysiological backgrounds not even feasible, but also most of the currently employed treatments lack of accurate targets [41]. Pharmacological approaches to treat glomerulopathies are based mainly on immunosuppression, are non-specific, partially affective and carry a considerable burden of side-effects. It is therefore critical to find drugs with specific targets, based on molecular pathophysiological pathways of disease, and with the highest safety profile [42, 43].

In recent years the podocyte has been rediscovered. Besides its architectural function, the podocyte has been shown to play relevant roles in glomerular basement membrane synthesis and maintenance, in interacting with adjacent podocytes and neighbour endothelial cells, in immunosurveillance and in cytokine and growth factor release [44]. It has also been demonstrated that the podocyte is a highly differentiated cell with a set of both cytoplasmic and membrane-bound proteins with very specific functions [44]. Finally, a genetic or acquired alteration in one of these molecules can result in a podocytopathy that can aggravate the function of other contiguous proteins and result in heavy proteinuria [45]. Once a podocyte is detached and lost in the urine, it cannot be replaced. When a single glomerulus loss represents over 20-30% of its podocyte population, which normally accounts for 400 to 500 podocytes, a situation of "no return" is generated and that glomerulus is committed to sclerose [46, 47]. Any reduction at podocyturia rate is mandatory to prevent chronic disease [46, 48, 49].

In this regard, the potential role the B7-1 molecule may play in the pathogenesis of the above mentioned glomerulopathies it yet to be assessed. The possibility of measuring B7-1 levels in the urine may turn this molecule as a useful biomarker for the diagnosis and follow-up of subjects with B7-1 positive glomerulopathies. Finally, the possibility that abatacept could interfere in the early phases of the immune response in a specific manner supports the need for assessing the role the CD28-B7-1 interaction plays in the glomerular disease universe Fig. (**1**), Fig. (**2**).

THE SECOND SIGNAL

Normal podocyte architecture and shape are necessary for the kidney to accomplish one of its functions: A normal filtration process. Podocyte dysfunction, injury, and loss are frequent relevant factors for the development of chronic kidney disease, including systemic factors, glomerular and podocytic paracrine mediators [50-53]. It has recently been shown that in a small population of patients with biopsy proven primary FSGS that displayed the expression of the B7-1 molecule in podocytes, responded satisfactorily to abatacept infusion [54, 55]. Abatacept achieved partial or complete reductions of proteinuria, suggesting that B7-1 could be employed as a trustable biomarker in therapy and follow-up of some glomerular diseases [15].

The mere expression of B7-1 is injurious to podocytes and disturbs slit diaphragm function [52, 53]. However, podocytes do not express this ligand in normal conditions Fig. (**3**). In this regard, at first glance it would appear that in certain glomerulopathies podocytes would behave as antigen presenting cells, in which the B7-1 molecule is constitutively expressed [15]. B7-1 is a 53 kDa membrane associated protein that under abnormal conditions is localized exclusively in podocytes, but can also be found in renal tubules [52, 56]. It is better known for its role in the immune system as a co-stimulatory receptor involved in T-lymphocyte activation [56] Figs. (**1** & **2**). B7-1 activation by puromycin in cultured podocytes has been found to attenuate expression of nephrin and results in foot process effacement and retraction [57]. The ability of B7-1 to regulate podocytes' filtering capacity is also shown when lipopolysaccharide (LPS) is injected in mice, resulting in increased B7-1 expression and proteinuria, while proteinuria does not occur in mice that are knockouts for B7-1 [52] Fig. (**3**).

Therefore, within the glomerulus B7-1 may modulate the immune mediated injury to podocytes [58]. T cells need two signals to be stimulated for activation. The first signal comes from the coupling of the antigen presenting cell and the T cell receptor via de major histocompatibility complex. This first signal is considered as the antigen-specific signal. The antigenic structure is processed by the antigen presenting cell (neutrophil, macrophage or dendritic cell). Therafter, the epitope of the engulfed antigen is presented in the major histocompatibility complex II. Finally, the CD4+ T cell identifies the antigen by the T cell receptor [59] Fig. (**2**). This signal alone leads to anergy or tolerance [60]. The second signal required for T cell activation is named the co-stimulatory or accessory signal is also provided by the antigen presenting cell in which CD28 is located in T cells, and lymphocyte activation antigens B7-1 or B7-2 (also known as CD86) are the ligands in the antigen presenting cell [61], Figs. (**1** & **2**). B7-1 modulates the action of CD4+ and CD8+ T cells through the alternatively coupling with the surface glycoprotein CD28 co- activator, constitutively located on T cells, or the CTLA-4 co-inhibitor, located on CD4+ and CD8+ T cells after stimulation [62, 63]. As mentioned, in normal conditions podocytes do not express the B7-1 molecule in its cellular membrane. However, various rodent models of glomerular diseases have been found to present an increase of the molecule B7-1 in the surface of podocytes [64-67]. In this respect, the podocyte would act as an antigen presenting cell to T cells, which would then activate other T cell populations as well as B cell, triggering antibody synthesis Fig. (**1**). This is the initial step of immune-complex formation, a key event in the pathophysiology of glomerulonephritis. Although B7-1 and B7-2 co-stimulation are equivalent at inducing the production of interleukin-2 (IL-2), interferon (IFN) γ, IL-2 receptor α and IL-2 receptor γ chains, B7-2 more effectively

co-stimulates IL-4 and Tumor necrosis factor-beta (TNF-β) production, whereas B7-1 more effectively promotes granulocyte-macrophage-colony stimulating factor (GM- CSF) synthesis [68]. "In T cells, binding of B7-1 to T cell receptors triggers the migration of protein kinases, and actin-binding proteins" [69, 70]. This immunological reaction among T cells leads to "a rearrangement of the T cell actin cytoskeleton, activating protein tyrosine kinases" [69, 71-74].

These findings may portrend relevant implications for the important role the second signal may be playing at the initial steps of the immune response involved in glomerulopathies. In this regard, here are at least two implications related to the podocyte expression of B7-1. First, the role of podocytes as antigen presenting cells in abnormal conditions; second, B7-1 positive podocytes present a diminished skill to adhere to the contiguous glomerular basement membrane through the β1 integrin [53, 75]. In T cells, B7-1 links to CD28 or to CTLA-4 by its extracellular domains, while in podocytes the intracellular portion of B7-1 blocks β1-integrin activation [76, 77] Fig. (**3**). Podocytes expressing B7-1 modify their morphology and function, promoting podocyte migration by the inactivation of β1 integrin and causing the detachment of their foot processes from the glomerular basement membrane, podocyturia and eventually proteinuria [14, 78, 79]. This is a result of the interaction between T cells and podocytes through B7-1 and B7-2; inhibiting β1-integrin activation in podocytes by abatacept could be a potential mechanism that could explain the underlying antiproteinuric action of the drug [15]. Podocyte foot processes (FPs) surround "the capillary loops that are anchored to the glomerular basement membrane (GBM) through α3β1 integrin and α-/β-dystroglycans" [80-82]." α3β1 integrin acts as a receptor for laminin", a component of the GBM [49]. In podocytes, α3β1 integrin is located in the basal part of FPs**.** Podocyte cells with α3 integrin deficiency present morphological resemblances to those with FPs effacement [83, 84].

In murine glomerular endothelial cells, the synthesis of B7-1 can be increased "by warm ischemia/reperfusion" [85]. In podocytes, the expression of B7-1 is remarkable in individuals with lupus nephritis, in models of minimal change disease, and in nephrin knock-out mice [52]. According to Reiser, this evidence suggests that B7-1 could be considered as a 'podocyte stress marker' [53]. B7-1 can be detected and measured in the urine and may be a potential biomarker of podocyte injury. Urinary levels of B7-1 in patients with relapsed minimal change disease are higher versus the levels found in patients with minimal change disease in remission**,** lupus (with or without proteinuria), other glomerulonephritis (FSGS, membrano-proliferative glomerulonephritis, IgA nephropathy, and membranous nephropathy), and healthy control patients [56, 86]. Data from a second study by the same group showed that urinary B7-1 concentrations were increased in individuals with minimal change disease in relapse when compared to patients with minimal change disease in remission or those with FSGS [87]. Additionally, the level of urinary B7-1 mRNA was found to be enhanced in patients with glomerular kidney disease compared to that of healthy subjects [88, 89].

Promising data thus presents for the utility of urinary B7-1 as a biomarker of podocytopathy; however, the fact that B7-1 can also be derived from tubular epithelium reduces confidence in its specificity [56]. This urinary biomarker could be modified with abatacept both in transplantation and in glomerulonephritis [90-93]. Moreover, this intervention with abatacept has shown new molecular pathway insights about the "effect of blocking CD28 and CTLA-4 on antigen-specific T-cell responses" [94]. In this regard, "cell death pathways are remarkably involved in T-cell tolerance caused by CD28 and CTLA- 4 blockade" [94]. "CD28 and CTLA-4 blockade inhibits naïve antigen-specific CD4+ T-cell responses but does not completely control the expansion of antigen-specific CD8+ T-cell responses" [95, 96]. Moreover, CD8+ memory T-cell responses are, mainly independent of CD28 during memory immunity [64, 65]. However, certain CD4+ memory T-cell subsets are resistant to the "CD28 and CTLA-4 blockade in a model of transplantation suggesting that subjects with a baseline elevated precursor concentration of auto reactive or alloreactive T cells may be resistant to the approach of blocking CD28 and CTLA-4 molecules" [97, 98].

THE SECOND SIGNAL AND THE IMMUNE SYSTEM

In glomerulopathies there exists an intrincate and complex interaction between the innate immune system, mainly represented by the complement system, toll-like receptors (TLRs), dendritic cells, neutrophils, monocytes and Natural Killer cells, and the adaptive immune system, represented by B and T cells and their subtypes [9]. For the purpose of this article, we will focus on TLRs and its interaction with antigen presenting cells and B and T lymphocytes and their relationship with B7-1. TLRs are trans-membrane proteins with leucine-rich constituents, engaged in the recognition of pathogen-associated molecular patterns [99]. "TLRs are present on many immune cells (including macrophages, neutrophil granulocytes, mast cells, dendritic cells and T and B lymphocytes)" [100, 101]. Various kinds of TLRs interact with specific noxious molecules [102-104]. While "TLR3 recognizes double-stranded RNA, TLR4 identifies LPS of Gram-negative bacteria" [100, 105-107]. The coupling of ligands to TLRs stimulates various signaling pathways, inducing the synthesis of inflammatory mediators, mainly cytokines, chemokines and IFNs [105]. "TLRs activate nuclear factor Kappa b (NFκB)" [108, 109]. In turn, IFN-γ induces the synthesis of TLR4 in mesangial cells and down-regulates them in monocytes [110], Fig (**2**).

In addition, stimulation of TLRs is involved in the increase surface concentration of co-stimulators on antigen presenting dendritic cells, linking both limbs of the immune system: The innate and the adaptive systems [105, 111]. In antigen presenting cells, LPS is the most important factor that augments the expression of B7-1 via TLR-4 signaling

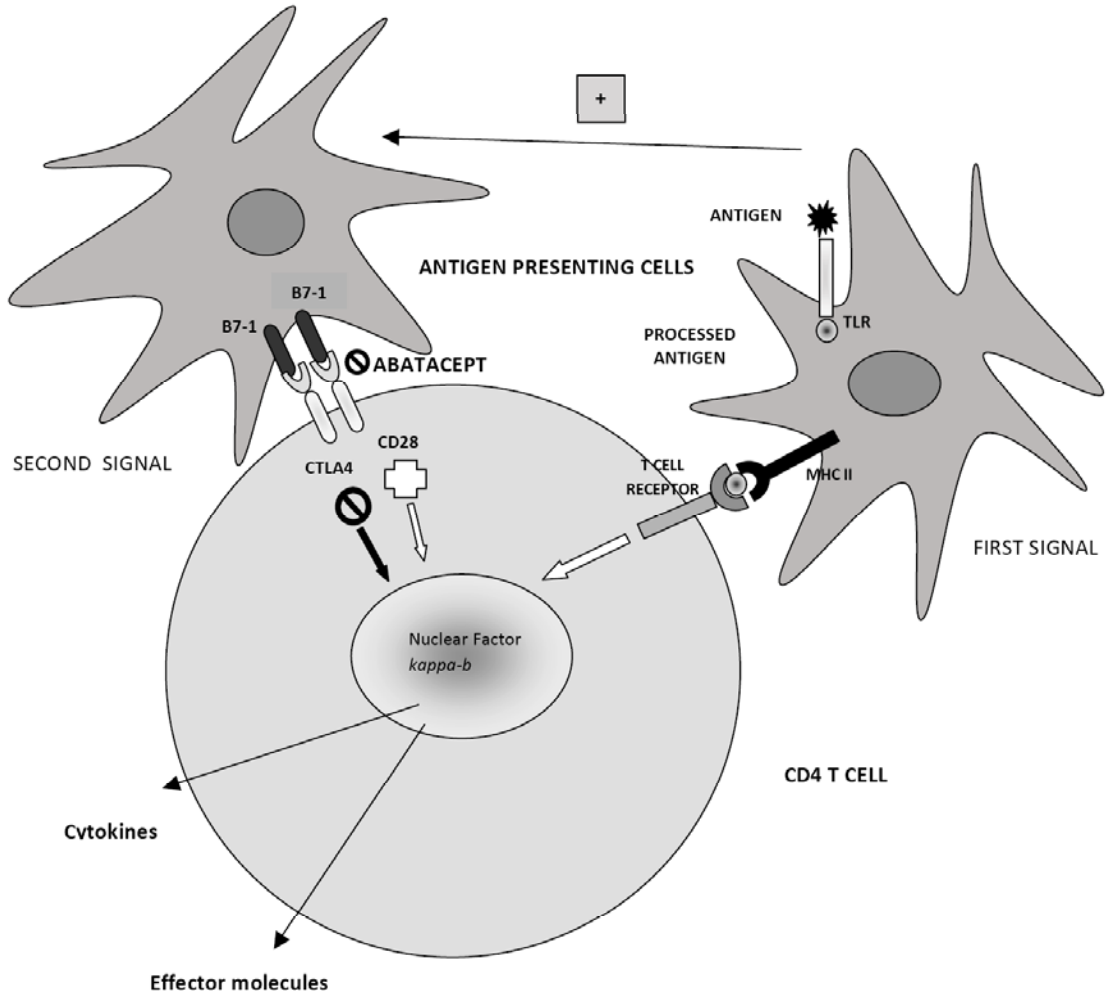

Fig. (2). First and second signal of CD4 T cell activation.The first signal begins with the identification of antigen epitopes by Toll-like receptors (TLR) in an Antigen Presenting Cell. Once processed, the epitope is bound to a Major Histocompatibility Complex Class II molecule and presented to the T cell receptor (TCR), which in turn internalizes the stimulation signal to the nucleus where nuclear factors of aactivation are synthesized. The activated Antign Presenting Cell in turn activates other similar cells and the second signal is triggered. A B71-CD28 turns on the simulatory pathway, and the secretion of cytokines and stimulatory molecules, while a B7-1-CTLA4 coupling inhibits the T cell activation. Symbols: ⊘: Blocking effect; ⬡: Stimulatory effect.

[112]. Podocytes identify "LPS by TLR-4, which causes the reorganization of the kidney-filtration apparatus: the appearance of podocyte FP effacement and proteinuria" [52] Fig. (**3**). "This process needs an increase in number of the co-stimulatory molecule B7-1" [52]. "Podocyte B7-1 modifies the actin cytoskeleton of podocytes and modulates slit diaphragm disposition" [52]. These actions do not depend of T and B cells, suggesting a new role for B7-1.

Normally, podocytes contain the "LPS receptor TLR-4 and its co-receptor CD14 and respond to LPS with the upregulation of B7-1" [52] Fig. (**3**). As already mentioned, in podocytes B7-1 may indicate the existence of an independent T cell pathogenetic mechanism for the disarray of the glomerular filtration apparatus [52]. But, why do podocytes express B7-1? The rise of B7-1 concentration in podocytic cells caused "by LPS may be a phylogenetically well-preserved mechanism" [113], which could be triggered by Gram-negative sepsis. In this regard, transient proteinuria has been encountered in Gram-negative sepsis [113]. This transient proteinuria may represent a normal response to dispose circulating pathogen- associated molecules. Thus, Reiser *et al*. have proposed according to their findings a new role for podocyte B7-1 in the generation of proteinuria, which presents the B7-1 molecule with a different role from its already known function in the co-stimulation signal [52].

However, TLR4 ligands are capable of causing kidney damage by augmenting inflammation, independent of the adaptive limb of the immune system [114]. "Albeit TLR4s located in renal cells and in circulating leukocytes contribute equally to the glomerular influx of neutrophils, the activity of TLR4s expressed in leukocytes is classically associated with the severity of tissue injury" [115, 116] and proteinuria Fig. (**3**). Another link between TLRs and B7-1 is that B7-1 podocyte expression correlates "with the severity of lupus nephritis and primary FSGS" [52]. An increase of podocytic B7-1 could add another triggering mechanism for the development of proteinuria as it may alter the

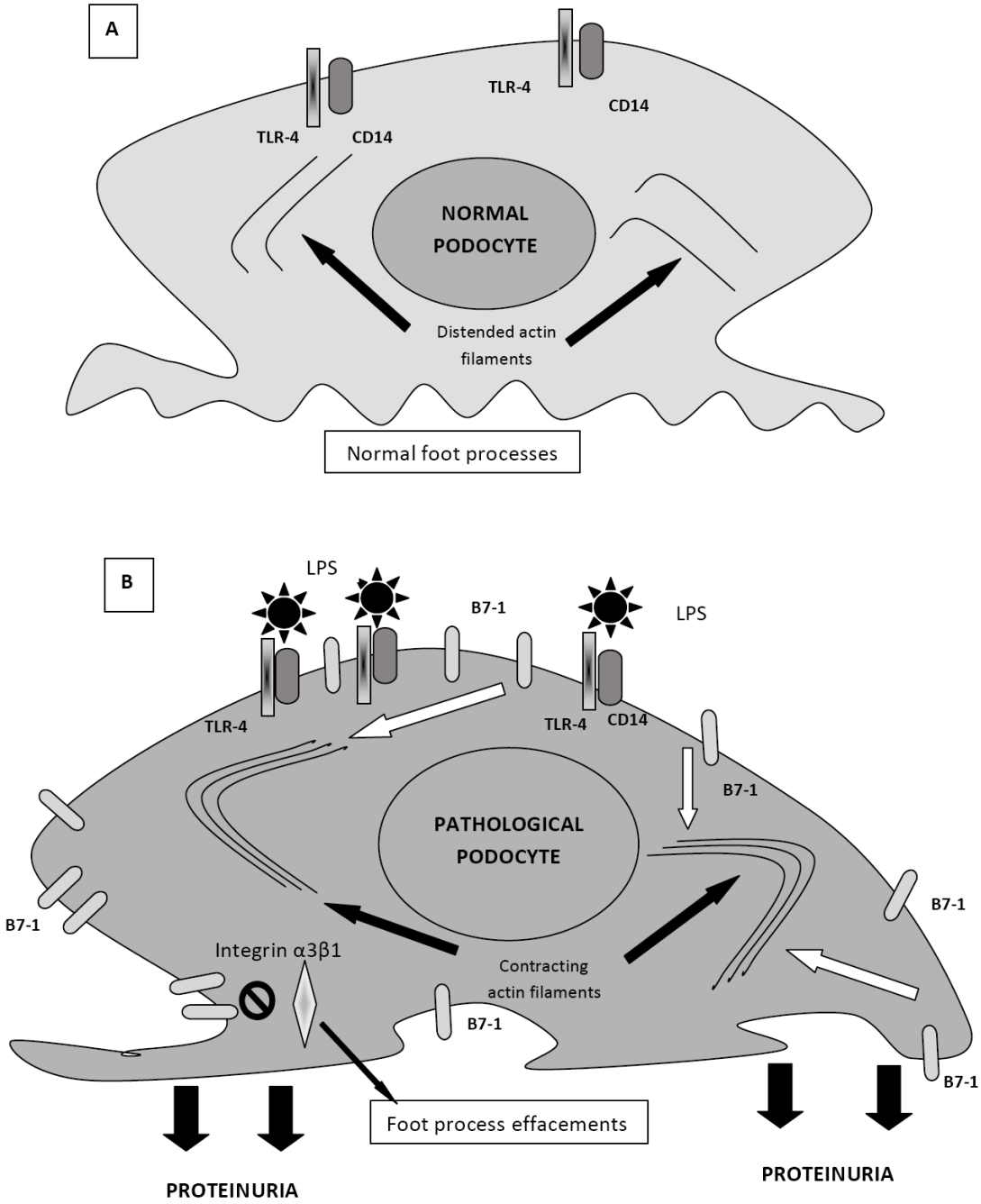

Fig. (3). 3A: A normal podocyte expresses Toll-like receptor 4 and its co-ligand, CD14. **3B**: Toll-like receptor 4 acts as a receptor for lipopolysaccharide (LPS) under abnormal conditions, and the expression of B7-1 is triggered. In turn, B7-1 stimulates the reorganization and contraction of actinin filaments, leading to podocyte contraction and foot process effacement. B7-1 also inhibits α3β1 integrin, leading to foot process effacement. As a consequence, the glomerular basement becomes denuded causing proteinuria.

glomerular basement membrane structure. Finally, LPS signaling through TLR4 reorganizes the podocyte actin cytoskeleton, podocyte contraction and proteinuria [52]. Another possibility is that circulating permeability factors, such as hemopexin or suPAR (soluble urokinase-plasminogen activator inhibitor receptor) could increase B7-1 expression via TLR-4 activation and could increase the risk for the development of proteinuria [14]. Thus, B7-1 expression on podocytes can be considered as a factor that can lead to proteinuria, by altering the shape of podocytes.

Recently, Shimada *et al.* have reported elevated urinary levels of B7-1 [56] and an increased expression of B7-1 in podocytes of patients with minimal change disease [56, 87]. The mechanism for the induction of B7-1 in minimal change disease could be due to TLR-4 activation [52]. Other TLR ligands similarly induce B7-1 and podocyte phenotype modification through an NF- KB -dependent pathway. Interestingly steroids can block the upregulation of B7-1 on the podocyte, giving light to another mechanism of action steroids play in glomerular diseases [109, 117, 118]. The over-expression of

TLR4 in transgenic mice is also enough to induce lupus-like autoimmune disease [119]. However, in minimal change disease podocyte TLR3 signaling has a role that is independent of B7-1 [120]. TLR3 can be activated by viral dsRNA. This activation results in transient proteinuria with focal FP fusion and with B7-1 synthesis in podocytic cells and B7-1 urinary excretion, as it occurs in minimal change disease. However, in humans minimal change disease is generally associated with nephrotic range proteinuria and persists unless corticosteroid therapy is started. Ishimoto has proposed that the persistence of proteinuria may be caused by the inability of the subject with minimal change disease to decrease podocyte B7-1. An important modulator that could be involved in this setting is CTLA-4, which is also expressed in podocytes, and can down-regulate B7-1 on antigen- presenting cells [17, 121]. T regulatory cells (Tregs) are abnormal in minimal change disease with impaired secretion "of IL-10 and transforming growth factor beta (TGF-β)" [120, 122].

Finally, "TLRs that identify nucleic acids may worsen immune complex glomerulonephritis by three different mechanisms. First, viral double-stranded RNA stimulates TLR3 to facilitate the synthesis of pro- inflammatory molecules by mesangial cells, macrophages and dendritic cells" [123, 124] "Secondly, in the presence of other cofactors, TLR7 could stimulate B cells, leading to increased autoantibody production and glomerular immune complex deposition" [124]. "Finally, TLR3, TLR7 and TLR9 may cause the transformation of dendritic cells to antigen-presenting cells, thereby stimulating selective production of pro-inflammatory cytokines, chemokines and type I IFN by renal monocytes" [90]. The stimulation of mesangial TLR3 by viral RNA could support inflammatory mediators release that may be involved in cell proliferation and apoptosis [116, 125]. Whether B7-1 and TLR3 and TLR4 are related in the pathogenesis of glomerulopathies needs further investigation, as well as the pharmacological manipulation of these molecular interactions. Although not yet studied with any potential relationship with the B7-1 molecule, several TLRs are involved in the development of certain glomerulonephritis [126]. For example, TLR2 has been associated with a murine model of crescentic glomerulonephritis, in which T cells and immunoglobulins play an important role [127]. Interestingly, TLR4 ligands can cause renal injury by triggering inflammatory pathways, independently of the adaptive limb of the immune system [128].

THE RATIONALE FOR ABATACEPT EMPLOYMENT IN GLOMERULOPATHIES

There is certain evidence showing that abatacept may be useful in the therapy of B7-1 positive glomerulopathies. "Abatacept (CTLA-4Ig) is a recombinant fusion protein with an extracellular domain of human CTLA-4 and a modified fragment of the Fc domain of human IgG1 [42, 43, 54, 55,129-134] Fig. (**1**). It accomplishes its effect by competing with CD28 for the coupling to B7-1 or B7-2" [30]. "By impeding CD28 recruitment on T cells and plasma cells" [30], abatacept hinders pathways involved at least in primary FSGS and in lupus nephritis [15, 23, 24, 129]. A therapeutic blockade of the second signal pathway employing an immunoglobulin fusion protein that ligates to B7-1 or B7-2 and interfering the stimulatory CD28 or potentiating inhibitory CTLA-4 signals has been tested both in autoimmunity and in transplantation [129-131].

Podocyte B7-1 expression has been encountered "in genetic, drug-induced, immune-mediated, and LPS - induced experimental renal diseases with nephrotic syndrome" [52,132-138]. Abatacept efficiently and specifically blocks this molecular interaction. As mentioned previously, it has been reported that in podocytes with B7-1 positive expression and proteinuria due to primary FSGS, abatacept has successfully decreased proteinuria [134]. Moreover, abatacept has secondarily decreased proteinuria in subjects with rheumatoid arthritis, a labeled indication of the drug. Abatacept binds B7-1 with a 20-fold higher affinity than CD28 and even presents a better inhibitory effect than anti-B7 antibodies [139].

Recently, Yu *et al*. administered abatacept in 1 or 2 intravenous (iv) doses of 10mg/kg to four subjects "with recurrent FSGS post kidney transplant and to one patient with primary FSGS" [15]. The patients suffering from recurrent FSGS underwent concurrent plasmapheresis. Albeit the main drawback of this report is the very low number of patients, these subjects reported a 10 to 48 month remission. Due to a beneficial therapy, B7-1 staining of renal biopsies of subjects with glomerulopathies was assessed and the B7-1 expression in podocytes was documented. In the non-transplant patient with primary FSGS therapy with abatacept 10mg/kg on day 1, 15 and 30 and every month and was associated with partial remission and proteinuria decrease at 12 months. Several hypotheses for this response could be proposed: Abatacept may be capable of modulating the immune response by interacting with B7-1 and CD28 co-stimulation consequently decreasing leukocyte derived circulating factors as suPAR and, consequently protecting podocytes from contraction [14]. Secondly, abatacept may bind to podocyte B7-1, modifying its intracellular downstream functions in relation to actin and integrin roles in podocyte contraction [2, 14, 129-131]. Also, plasmapheresis could have removed a circulating factor and this clearance caused remission, independent of podocyte B7-1 expression and/or abatacept infusion. [14, 53]. Finally, abatacept could play a role in podocyte TLRs signals through B7-1 interaction (mainly through TLR-9) or independent of B7-1 [140,141]. This mechanism could be for instance via the endogenous calprotectin system, composed of TLR4 agonists S100A8/S100A9 and present in monocytes [142, 143]. These proteins have been shown to play critical roles in LPS-induced sepsis, vasculitis and certain glomerulonephritis [144, 145]. To my knowledge, this hypothesis has not been explored in this field.

Although the podocyte B7-1 pathway appears to play an important role in some glomerular entities, these initial clini-

cal results suggest targeting this pathway requires more studies with randomized controlled trials. As commented by Haraldsson, the relevance of distinguishing B7-1 positive from B7-1 negative glomerulopathies could foretell the response to abatacept [146]. However, immunohistochemical detection of B7-1 is technically difficult on paraffin tissue [147]. This statement is calling the attention that improved techniques must be developed for routine widespread use.

With respect to IgA nephropathy and abatacept, it has been reported that in a subject with rheumatoid arthritis (a labeled indication of abatacept), the initiation of abatacept, worsened hematuria and proteinuria. A kidney biopsy disclosed mesangial IgA deposition with necrosis and crescents. Abatacept was stopped and proteinuria 6 resolved after steroids therapy. As the authors state, the short term between abatacept induction and the clinical manifestations of IgA nephropathy, and its improvement after abatacept discontinuation, supports the hypothesis that CTLA4-Ig may act as a relevant factor in the pathogenesis of IgA nephropathy [17]. Either B7-1 or B7-2 is associated with renal tissue damage of IgA nephropathy. B7- 2 is widely located in glomeruli, in the periglomerular area, and in the interstitium surrounding the tubules, while B7-1 is just found in tubular cells. This characteristic distribution was also seen in extracapillar glomerulonephritis [148]. In addition, the number of B7-2 positive cells located in glomeruli, in the periglomerular area, and in the interstitium surrounding the tubules rised with the progression of renal histologic damage. Tubules that express B7-1 also displayed a trend to augment with kidney damage and were associated with the population of T cells that surround tubular T cells. B7-1 is synthesized "at low levels on resting monocytes and dendritic cells but not on resting B cells, while B7- 2 is expressed on resting monocytes and dendritic cells but not on resting B cells" [149, 150]. "Their expressions can be induced to high levels on activated B cells, activated macrophages, and dendritic cells" [150, 151]. The majority of B7-2 positive cells in the kidney biopsies of IgA nephropathy are monocyte or macrophages. Wu *et al* have shown that IN IgA nephropathy B7-1 was not expressed on monocytes and macrophages. Thus, B7-1 and B7-2 activate T cells in IgA nephropathy; while monocytes and macrophages are the major antigen presenting cells expressing B7-2 to stimulate T cells; tubular epithelial cells can express B7-1 and could activate interstitial T lymphocytes, while the expression of B7-1/B7-2 is linked to kidney function at the time of renal biopsy [152].

Finally, with respect to lupus nephropathy, treatment with abatacept can either abrogate or revert lupus nephritis [23-25]. Many hypotheses could explain the potential molecular targets of abatacept: The stimulation of naïve T cells, that needs B7-1/B7-2 coupling with CD28 on T cells [61]. "Lupus nephritis caused by the stimulation of naïve cells, which is impeded by abatacept, or by memory cells, which are not directly affected?" [23] Another possible hypothesis explaining abatacept usefulness in lupus is related to direct effects on plasma cells [23]. Another possibility could be the role TLRs and calprotectin play in lupus nephritis, probably associated to B7-1 coupling, due to the good response to abatacept [24,116, 145]. Moreover, Wofsy *et al.* assessed the efficacy and safety of 52-week therapy with abatacept against placebo, while receiving mycophenolate mofetil and glucocorticoids in subjects with lupus class III or IV nephropathy [24]. In general, the safety profile for abatacept in lupus nephritis was similar to that of mycophenolate mofetil and steroids alone, with the exception of a higher frequency of herpes zoster infections. In subjects with class III or IV lupus nephritis who were receiving background mycophenolate mofetil and glucocorticoids, abatacept administration correlated with a satisfactory safety profile with a better profile in anti-dsDNA antibody and complement concentrations. In those with nephrotic- range proteinuria, greater reductions in proteinuria were reported with abatacept group. These encouraging results should support further assessment of abatacept for the treatment of lupus nephritis [23, 24].

SAFETY PROFILE OF ABATACEPT

With respect to the safety of abatacept, it has been demonstrated to be safe as a monotherapy or in combination with methrotexate or steroids [153,154]. In one study, the most common adverse events include peri-infusional complications, with a frequency of 29% when compared to 31% [153,154], infections with a frequency of 18% vs 16% found with placebo, and included mild episodes of cellulitis, or septic arthritis, pneumonia, or neoplasms with a frequency similar to placebo and included in 1-year follow up: Two cases of basal cell carcinoma, one bladder cancer and one non-specified cancer against one endometrial cancer, one squamous cell carcinoma and one melanoma in the placebo group [153,154]. In another study, the rate of serious adverse events was 16.3/100 patient years. Patients in the abatacept group discontinued the drug due to adverse events (4% versus 2%) that occurred in the placebo group, The rate of serious infections was 11 cases (3%) and 2 cases (1%) in abatacept and placebo groups, respectively. The incidence of serious infections were 4.3/100 patient years. Two deaths due to infections (pulmonary aspergillosis and sepsis in abatacept group and pneumonia and sepsis in the placebo group). Malignancies in abatacept group: One large B cell lymphoma of the thyroid in the first year and fourteen neoplasms reported in 2 years of follow-up: Six basal cell carcinomas, 2 squamous cell carcinomas, 2 cases of lung cancer and 2 cases of lymphoma, 1 endometrial cancer and 1 myelodysplastic syndrome. versus one endometrial carcinoma in the placebo group during the 1-year. Six patients (1%) demonstrated antibody reactions to abatacept [155,156].

ONGOING TRIALS

According to the information offered on-line by the United States National Institutes of Health, 121 trials with abatacept are either in the phases of recruiting, active, completed or withdrawn [157]. With respect to active ongoing trials and glomerulonephritis, only one study has been identi-

fied: NCT01714817, which is in the recruiting phase, and is engaged in studying abatacept to treat lupus nephritis. It is a phase 3 randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of abatacept versus placebo on a background of mycophenolate mofetil and corticosteroids in the treatment of subjects with active class III or IV lupus nephritis [158].

CONCLUSION

In summary, abatacept is a promising agent to be appropriately tested in glomerulopathies, a field that in general lacks of specific treatments according to the pathophysiology of these diseases, in part due to the lack of specific biomarkres. B7-1 positive glomerulopathies open the road for a new paradigm in the interpretation of glomerular diseases, which if proven would certainly be of benefit to a vast population.

CURRENT & FUTURE DEVELOPMENTS

The real and potential role abatacept may play in the field of glomerular diseases is not known to date. One interesting field of development that could explain in forthcoming years in more detail and depth the relationship between B7-1 and glomerulonephritis may be the study of differentiation of induced pluripotent stem cells to generate renal cells with podocyte features [159-162]. In this respect, as podocytes are highly specialized cells with a limited capacity to divide and to grow in culture, once detached and lost in the urine are impossible to replace, despite the chance of recovery in urinary samples [46, 47, 163]. Therefore, the reprogramming of adult cells to generate induced pluripotent stem (iPS) cells with elevated proliferative and differentiation capacities represents a major advance for medical applications. Induced pluripotent stem cells should contribute to unravel the mechanisms involved in the pathogenesis of glomerulopnephritis, to screen novel therapies, and to replace damaged or disappeared cells to repair kidneys. “However, due to the complexity of the developmental processes and kidney structure, there have been few successful reports showing differentiation of pluripotent cells to kidney progenitors” [159].

In summary, the aberrant expression of B7-1 molecules on damaged podocytes could be just a mere marker of disease or a critical relevant molecule when aberrantly present on injured podocytes, contributing to histologic damage and proteinuria. Although the data is scant, the few encountered encouraging results suggest that B7-1 is more than just a marker of podocyte injury or adaptation to the burden imposed by a certain insult, suggesting that abatacept could be useful in blocking the second signal of the immune response in certain glomerulopathies that express B7-1 on podocytes surface. In this respect, randomized controlled trials should be mandatory for the assessment of abatacept in glomerular diseases. Moreover, B7-1 role in the differential diagnosis and eventual response to abatacept needs further and deeper investigation, but it will align treatment to pathophysiological pathways, making therapy regimes more specific. The histologic techniques to identify B7-1 in tissue samples must be improved and standardized in order to pave the road in this important chapter of clinical nephrology. B7-1 employment as a plasmatic or urinary biomarker deserves further development.

CONFLICT OF INTEREST

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