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Review Article

Novel therapies in lupus nephritis associated with regulatory T cells

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Abstract: Lupus nephritis is a major clinical manifestation of systemic lupus erythematosus. Corticosteroids and cytotoxic agents are standard of care for lupus nephritis treatment, but are associated with considerable morbidity and suboptimal outcomes. Recently, growing evidence confirmed that regulatory T cells exert a protective effect in lupus nephritis. Drugs development targeting the regulatory T cells would be helpful in providing novel therapeutic strategies for lupus nephritis. In this review, we will discuss the relationship between lupus nephritis and regulatory T cells and introduce some novel agents acted on regulatory T cells directly or indirectly.

Keywords: Lupus nephritis, regulatory T cell, novel agent, therapy

Introduction

Lupus nephritis (LN) is a frequent and serious complication of systemic lupus erythematosus (SLE), and is associated with considerable morbidity and even mortality [1]. There are obvious differences in the risk of lupus nephritis across certain ethnic groups. The LN cumulative incidence is lower in Caucasians (14.3%) with SLE compared with Asians (55%), African-Americans (50.5%) and Hispanics (43.1%) [2, 3]. In China, the geographical distribution appears to be a risk factor for the incidence of LN, for the proportion ratio of biopsy proven LN in all renal disease or in secondary glomerular disease significantly increased with decreasing latitude from the north to the south part of China [4].

The pathogenesis of lupus nephritis remains an active field of investigation, with much knowledge gained, but many questions still left to resolve. Generally, autoantibodies to components of chromatin including double-stranded DNA (dsDNA), histones, nucleosomes and formation of immune complexes (ICs) in glomeruli [5-7] are central in the pathogenesis of lupus nephritis. Ribosomal RNA-processing protein 8 (RRP8) and spermatid nuclear transition protein 1 (TNP1) are two novel LN-associated autoantigens. Circulating anti-RRP8 and anti-TNP1 autoantibodies deposited in glomeruli, may be useful for prediction of LN in subsets of SLE patients who are negative for anti-dsDNA antibodies [8]. B cells are often seen as the central cause of disease pathogenesis, which they induce by promoting autoimmunity and secreting autoantibodies [9, 10]. T cells are considered critical for the helping of B cells in the production of autoantibodies [11]. The toll-like receptor 9 (TLR-9) has been implicated in experimental and human SLE. TLR-9 signaling plays a protective role by modulating the activity of regulatory T cells (Tregs) in the MRL model of murine lupus [12]. The genetic variation of TLR-9 has an impact on the severity of lupus nephritis in the Indian population [13]. Binding of Hyaluronan (HA) to CD44 regulates leukocyte infiltration, secretion of inflammatory mediators, and clearance of apoptotic cells processes that dictate the severity of lupus nephritis [14].

Corticosteroids and cytotoxic agents are standard of care for LN treatment, but are associated with considerable morbidity and suboptimal outcomes. Recently, there has been excitement surrounding the development and implementation of biologics and small molecules such as Histone deacetylase inhibitors (HDACi) panobinostat [15], Fc Receptor I for IgA (FcαRI) [16] for the treatment of lupus nephritis. Tregs are a specialized subset of T cells that function to control the immune response. Forkhead-winged helix protein-3 (Foxp3) has been described as a key control gene for both development and function of Tregs. Recently, growing evidence confirmed that Tregs were impaired in their suppressive function and/or percentage of the $CD4^+$ population in lupus nephritis [17-19]. Depletion of $CD4^+CD25^+$ Tregs exacerbated the development of LN, while the expansion of Tregs was protective against LN in mice [20]. One study aim at evaluating the safety, tolerability, and the effect of 3 different doses of Treg therapy in adults with skin involvement of their lupus is currently recruiting participants (ClinicalTrials. gov). Thus, development of novel agents targeting the Tregs would be useful in providing new therapeutic strategies of lupus nephritis. Here, we will discuss the effects of Tregs in lupus nephritis and introduce some novel agents aimed at or associated with Tregs.

Role of $CD8^+$ Tregs in LN

$CD8^+Foxp3^+$ Treg cells were first identified in human tonsils, show a Treg phenotype with high CTLA-4 and CD45RO and low CD127 and CD69 expression, but mostly CD25 negative. Tonsillar $CD8^+Foxp3^+$ Treg cells express high levels of IFN-γ, TNF-α and IL-17A [21, 22]. $CD8^+Foxp3^+$ T cells could be induced in vitro from naïve $CD8^+$ T cell via the TCR or anti-CD3-mAb [21, 23], the induced $CD8^+Foxp3^+$ T cells are predominantly $CD25^{high}$. Also, $CD8^+$ Treg cells could be induced by stimulating naïve $CD8^+$ T cells in the presence of TGF-β and retinoic acid (RA), and low-dose IL-2 could dramatically expand and activate $CD8^+$ Treg cells [24, 25]. MiR-335 can directly regulate Foxp3 expression through binding to its target site. Either miR-9 or miR-155 alone could down regulate the CTLA-4 expression through direct and specific binding to their target sites [26]. $CD8^+$ Treg lineage is essential for the maintenance of self tolerance and prevention of murine autoimmune disease included lupus [21, 27-31].

$CD8^+$ Treg cells are associated with lupus nephritis and disease activity [27, 32]. After intravenous methyl-prednisolone (IVMP) pulse therapy in forty patients with active class III/IV childhood LN with heavy proteinuria, the number of $CD8^+$ Treg cells increased and renal histopathological and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score improved. IVMP-treated $CD8^+CD25^+$ Treg cells directly suppressed $CD4^+$ T proliferation and induced $CD4^+CD45RO^+$ apoptosis [22]. Upon in vitro activation, $CD8^+Foxp3^+$ Treg cells can inhibit T cell proliferation directly [21], the suppressive ability of $CD8^+CD25^+$ Treg cells is associated with $CD4^+$ Treg cells [33-37], but the specific mechanism is still uncertain and further studies are required.

Role of $CD4^+CD25^+$ Tregs in LN

$CD4^+CD25^+$ Tregs constitute 5-10% of peripheral blood $CD4^+$ T cells in normal naïve mice and humans [38]. Growing evidence confirmed that $CD4^+CD25^+$ Tregs naturally occurring and express Foxp3, which plays an important role in maintaining self-tolerance and preventing organ-specific autoimmunity. Many studies showed that patients with SLE had significantly decreased the percentage of Tregs in peripheral blood in association with disease activity [39-42]. Foxp3 mRNA expression is significantly reduced in new-onset SLE than healthy controls [43]. These results suggest that Tregs might play a specific pathogenic role in SLE. One study investigated the variations of Tregs $Foxp3^+$ in the kidney biopsies inflammatory infiltrate of different LN classes. The ratio of $FoxP3^+/CD3^+$ cells was significantly lower in patients with lupus nephritis class IV. The data demonstrated a decrease of $Foxp3^+$ Treg cells in the inflammatory infiltrate of LN, particularly during the most active phases of LN, as observed in the course of an IV class nephritis [19].

Toll-like receptors (TLRs) are a group of innate pattern recognition receptors that recognize multiple classes of microbial molecules, which are called pathogen associated molecular patterns (PAMPs), including lipopeptides, lipopolysaccharides, and nucleic acids [44]. TLR activation can either enhance or inhibit Treg cells

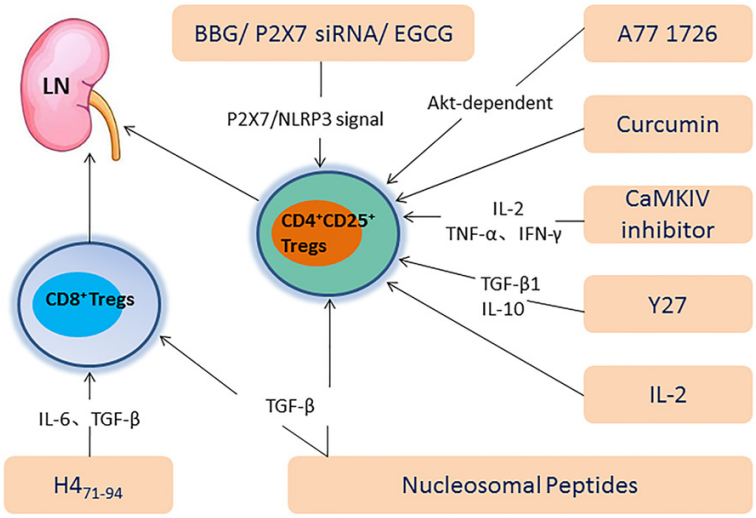

Figure 1. Summary of agents aimed at regulatory T cells: Nucleosomal Peptides induce $CD8^+$ and $CD4^+CD25^+$ regulatory T cell through upregulating TGF-β; $H4_{71-94}$ induces stable $CD8^+$ Treg cells by decreasing IL-6 and increasing TGF-β; BBG/P2X7 siRNA/EGCG increase Treg cell activity by inhibiting P2X7/NLRP3 activation; A77 1726 augmente $CD4^+Foxp3^+$ Tregs through an Akt-dependent mechanism; Y27 increases suppressive capability of Tregs by elevating TGF-β1 and IL-10 production; CaMKIV inhibitor increases the expression of FoxP3 by decreasing IL-2, upregulating TNF-α and IFN-γ; Curcumin and IL-2 effect $CD4^+CD25^+$ Tregs; then these agents have a therapeutic effect on LN.

suppressor function [45-50]. TLR2 activation on Tregs can induce Treg cells proliferation and result in a temporary loss of suppression [45, 51]. TLR4 activation can enhance the proliferation of Treg cells and increase their suppressor activity [51]. TLR7 ligand imiquimod-treated mice have worsened LN marked by tissue damage and strong CCL2 expression in inflammatory cell infiltrates [52]. Activation of TLR7 exacerbated lupus nephritis by modulating the abnormally costimulatory phenotype of dendritic cells and functions of Treg cells in MRL/lpr mice [53]. Also, TLR7 stimulation with imiquimod, which is reported to increase the inhibitory effects of Treg cells in C57BL/6 mice [54].

Therapeutics associated with tregs

The purpose of treatment of lupus nephritis is to induce remission, prevent disease recurrence, and preserve kidney function while minimizing side effects from medication. The current standard of care is suboptimal, and it's necessary to develop new agents which specifically target mechanisms involved in the pathogenesis of LN. More and more experiments indicate that Treg cells involved in the pathogenesis of LN, and manipulate the number or activity of Treg cells might be a promising strategy for treating lupus nephritis.

Nucleosomal peptides

Nucleosomes from apoptotic cells provide the major immunogens for the pathogenic Th and B cells in lupus [55, 56]. $H1'_{22-42}$, $H2B_{10-33}$, $H3_{85-102}$, $H4_{16-39}$, and $H4_{71-94}$ [57-59] are five major autoepitopes for lupus nephritis-inducing Th cells in murine and human lupus. These peptide epitopes are cross-reactively recognized by autoimmune Th cells and B cells. Peptides accelerate lupus nephritis upon immunization, but they delay or even reverse disease upon administration at high doses intravenously or intranasally and at low doses subcutaneously [57, 58, 60]. Very low-dose tolerance therapy in SNF1 mice induced $CD8^+$ and $CD4^+CD25^+$ regulatory T cell subsets and prevented the local inflammatory damage in kidneys. Both $CD4^+CD25^+$ and $CD8^+$ Treg cells are effective in suppressing lupus autoimmunity upon adoptive transfer in vivo [61]. These adaptive Treg cells suppressed IFN-γ responses of pathogenic lupus T cells to nucleosomal epitopes and reduced autoantibody production by inhibiting nucleosome-stimulated T cell help to nuclear autoantigen-specific B cells. Subcutaneously administered low-dose peptide tolerance induces tolerogenic plasmacytoid DC (pDC), which upregulated TGF-β not IL-10 and plays a critical role in generating potent Treg cells [62].

Kang et al. found that compared with the cocktail of peptides, $H4_{71-94}$ monotherapy more effectively delayed nephritis development, reduced autoantigen-specific Th1 and Th17 responses and frequency of T_{FH} cells in the spleen by inducing stronger $CD8^+$ Treg cells [63]. Competition for autoantigen processing and presentation when a mixture of peptides is used may explain the superiority of monotherapy [64]. $CD4^+CD25^+$ Treg cells might function as

inducer of Th17 cells or that these Treg cells themselves might differentiate into Th17 cells, as peptide-induced $CD4^+CD25^+$ Treg cells could not suppress nucleosome-specific Th17 response [64, 65]. $H4_{71-94}$ could induce stable Treg cells by decreasing IL-6 and increase TGF-β production by DCs, which in turn induced Smad-3 phosphorylation (TGF-β signal) in target autoimmune $CD4^+$ T cells. All those indicated that nucleosomal-histone peptide epitopes are suitable for Ag-specific tolerance therapy of lupus nephritis.

Curcumin

Curcumin is a polyphenolic compound obtained from the rhizomes of the plant *Curcuma longa*. The medical value of curcumin is well recognized as it has antioxidant, anti-inflammatory and antitumor activities [66-68]. Recent studies have confirmed that curcumin has the potential use in the treatment of animal autoimmune disease models such as allergic asthma and experimental autoimmune encephalomyelitis through regulating $CD4^+CD25^+$ Treg cells [69, 70]. Also, short term turmeric supplementation can decrease proteinuria, hematuria, and systolic blood pressure in patients suffering from relapsing or refractory lupus nephritis [71]. These findings suggest that curcumin may have a therapeutic effect on LN. Animal experiments further confirmed the protective effects of curcumin in LN [20]. It has revealed the protective effects of curcumin, at least in part, be attributed to $CD4^+CD25^+$ Tregs that curcumin could up-regulate Foxp3 expression. While depleted Tregs in NZB/W mice, the beneficial effects of curcumin disappeared. However, the present study is limited to the relation between Tregs and curcumin, it is still necessary to elucidate the specific immune-modulating signaling mechanism of curcumin in LN.

P2X7 antagonist

The purinergic receptor P2X7 is an extracellular ATP-gated plasma membrane ion channel receptor and is expressed in a wide variety of immune cells, but very little expression in normal kidney tissue [72]. In renal biopsies of patients with LN, the expression of P2X7R was increased in glomeruli and some tubules [73]. It was showed that the activation of P2X7 by ATP inhibited the suppressive potential and stability of Tregs [74]. Also, the inactivation of P2X7R protected Tregs from the deleterious effect of endogenous P2X7R ligands [75].

Blockade of the P2X7 signaling pathway effectively prevented the development of LN by inhibiting the formation of NLRP3 inflammasome [76]. The selective P2X7 antagonist Brilliant Blue G (BBG) could suppress renal expression of NLRP3 ASC and caspase1-p20, furthermore reduced the renal and serum levels of IL-1β. Short-term treatment with P2X7 siRNA had effects on MRL/lpr mice that were similar to those of BBG. It inhibited P2X7/NLRP3 activation, reduced the production of IL-1β and IL-17, decreased the ThI7:Treg cell ratio, and decreased the levels of circulating anti-dsDNA antibodies [76]. It's suggested that inhibition of P2X7 signaling effectively ameliorated LN by inhibiting NLRP3 inflammasome activation. Epigallocatechin-3-gallate (EGCG) is a major bioactive polyphenol present in green tea with antioxidant and free radical scavenging activities, has been reported to have anti-inflammatory effects by inhibiting NF-κB-mediated inflammatory responses in vivo [77]. Tsai PY et al. confirmed EGCG could alleviate renal lesions through enhancing splenic Tregs activity, increase renal nuclear factor E2-related factor 2 (Nrf2) and glutathione peroxidase activity, and reduce renal oxidative stress, NF-κB activation and NLRP3 mRNA/protein expression. It's clearly demonstrated that EGCG had prophylactic effects on lupus nephritis, which highly associated with its effects of enhancing the Nrf2 antioxidant signaling pathway, decreasing renal NLRP3 inflammasome activation, and increasing systemic Treg cell activity [78]. Therefore, development of pharmacologic inhibitors targeting the P2X7/NLRP3 signaling pathway would be helpful in providing novel therapeutic strategies for lupus nephritis. In view of the complexity of the pathogenesis of LN, targeting of the P2X7/NLRP3 pathway should be a part of the multitarget approach in this disease.

Others

A77 1726, the active metabolite of leflunomide, effectively inhibits the development of murine LN and attenuates the generalized autoimmune features. The therapeutic effects of A77 1726 are mediated, at least in part, by significantly augmenting $CD4^+Foxp3^+$ regulatory T

cells which suppress pathogenic IL-17-producing double negative (DN) T cells through an Akt-dependent mechanism [79].

Y27, a novel derivative of 4-hydroxyquinoline-3-formamide, prevented the development of murine systemic lupus erythematosus-like diseases. Y27 could boost the suppressive capacity of $CD4^+CD25^+$ Tregs in C57BL/6 mice assessed in vitro by the mixed lymphocyte reaction. However, Y27 did not affect the percentage of $CD4^+CD25^+Foxp3^+$ T cells. Y27 increased suppressive capability of Tregs, at least partially, for elevated TGF-β1 and IL-10 production, other mechanisms involved being still further investigated [80].

Calcium/calmodulin-dependent protein kinase IV (CaMKIV), a serine/threonine kinase expressed in T cells [81], plays a central role in the development of autoimmunity in the MRL/lpr mouse by decreased IL-2 production [82]. Silencing of CaMK4 in T cells from patients with SLE increases the expression of FoxP3 upon stimulation in the presence of TGF-β [82]. Treatment of MRL/lpr mice with KN-93, a small molecule inhibitor of CaMKIV, resulted in amelioration of both nephritis and skin disease. KN-93 treatment affected primarily TNF-α and interferon-γ (IFN-γ), suggesting a possible central role of CaMKIV in murine lupus [83].

IL-2 is critically required for maintaining the homeostasis and competitive fitness of Treg cells [84]. IL-2 deficiency is associated with Tregs defects in patients with SLE, and can be corrected with low doses of IL-2. The restoration of endogenous mechanisms of immune tolerance by low-dose IL-2 therapy, thus, proposes a selective biological treatment strategy, which directly addresses the pathophysiology in SLE [85]. A Pilot-Study with low-dose hrIL-2 for the treatment of lupus (Clinical Trials) is currently recruiting participants. This clinical study will confirm the efficacy and safety of low dose IL-2 treatment of SLE associated with selective modulation of $CD4^+$ T cell subsets.

Conclusion

In summary, Treg cells exert a protective effect in lupus nephritis, with much knowledge gained. However, the majority of these studies reported either reduced number or impaired function of circulating Treg cells in LN, the specific underlying mechanisms are still needed to investigate. Some novel agents aimed at Treg cells may have a therapeutic effect on LN (**Figure 1**) and provide a need for alternative therapeutic options. However, this only the beginning of a set of more rigorous studies those need to be performed before the field moves in these directions. For the complexity and number of factors involved in disease, combination therapy targeting both kidney inflammation and intra and extrarenal autoimmunity is still recommended.

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Disclosure of conflict of interest

None.

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