Autoimmunity Reviews 20 (2021) 102980

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Review

Interplay of immune and kidney resident cells in the formation of tertiary lymphoid structures in lupus nephritis

Simin Jamaly [a,b,*], Mehrdad Rakaee [c,d], Reza Abdi [d], George C. Tsokos [b], Kristin Andreassen Fenton [a]

[a] Department of Medical Biology, Faculty of Health Science, UiT The Arctic University of Norway, N-9037 Tromsø, Norway
[b] Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
[c] Department of Clinical Medicine, UiT The Arctic University of Norway, Tromsø, Norway
[d] Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

ARTICLE INFO

Keywords:
Systemic lupus erythematosus
Lupus nephritis
Tertiary lymphoid structures
Kidney resident cells,
Interstitial inflammation

ABSTRACT

Kidney involvement confers significant morbidity and mortality in patients with systemic lupus erythematosus (SLE). The pathogenesis of lupus nephritis (LN) involves diverse mechanisms instigated by elements of the autoimmune response which alter the biology of kidney resident cells. Processes in the glomeruli and in the interstitium may proceed independently albeit crosstalk between the two is inevitable. Podocytes, mesangial cells, tubular epithelial cells, kidney resident macrophages and stromal cells with input from cytokines and autoantibodies present in the circulation alter the expression of enzymes, produce cytokines and chemokines which lead to their injury and damage of the kidney. Several of these molecules can be targeted independently to prevent and reverse kidney failure. Tertiary lymphoid structures with true germinal centers are present in the kidneys of patients with lupus nephritis and have been increasingly recognized to associate with poorer renal outcomes. Stromal cells, tubular epithelial cells, high endothelial vessel and lymphatic venule cells produce chemokines which enable the formation of structures composed of a T-cell-rich zone with mature dendritic cells next to a B-cell follicle with the characteristics of a germinal center surrounded by plasma cells. Following an overview on the interaction of the immune cells with kidney resident cells, we discuss the cellular and molecular events which lead to the formation of tertiary lymphoid structures in the interstitium of the kidneys of mice and patients with lupus nephritis. In parallel, molecules and processes that can be targeted therapeutically are presented.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with impressive clinical heterogeneity which presents with manifestations from multiple organs. A multitude of pathogenic pathways have been identified originating from genetic, epigenetic, hormonal, environmental and immunoregulatory factors and all converge at causing inflammation of tissues and organ damage [1,2]. Every aspect of the innate and adaptive immune response has been reported to be involved in patients with SLE and they contribute to the expression of the disease in distinct subsets of patients. The presence of a plethora of autoantibodies has typified the disease while the production of those directed against nuclear antigens, small nuclear ribonucleoproteins, double-stranded DNA (dsDNA), and nucleosomes represent the hallmark of the disease [1,3]. Autoantibodies form soluble immune complexes (IC) with autoantigens (such as nucleosomes) released in abundance in the circulation of patients with SLE, that may deposit at basal membranes at various organs including the kidney and initiate inflammation. Autoantibodies may bind directly to kidneys antigens and form *in situ* IC as is typified by cationic anti-dsDNA antibodies which bind to the glomerular basement membrane [4–6]. In parallel the excessive production of cytokines including type I interferon (IFN), interleukin (IL)-17 and IL-23 further advance immune cell abnormalities or act directly on kidney resident cells to cause damage [7]. Last, but not least, autoreactive T cells infiltrate in kidney where they may form tertiary lymphoid structure (TLS) and cause organ damage.

* Corresponding author at: Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Center for Life Science, CLS 921, 3 Blackfan Circle, Boston, MA 02115, USA.
E-mail address: simin.jamaly@uit.no (S. Jamaly).

https://doi.org/10.1016/j.autrev.2021.102980
Received 23 July 2021; Accepted 31 July 2021
Available online 27 October 2021

Autoantibody or IC deposition within the kidney, along the action of cytokines and the infiltration of immune cells contribute to the development of kidney inflammation in patients with SLE which manifests as lupus nephritis (LN) with significant morbidity and mortality [8,9]. After an update on the interaction of resident and immune kidney cells we will discuss in detail the formation of TLS in the renal interstitium and its effect on kidney function.

2. Kidney resident cells

2.1. Podocytes

Podocytes are specialized cells on the visceral side of the Bowman's capsule that surround glomerular capillaries. They are part of the glomerular filtration machinery and are critical for maintaining renal function [10]. They express unique proteins including synaptopodin, nephrin [11], podocin [12] and Wilms' tumor protein [13], all of which are essential in the maintenance of their structure and function [14]. Genetic or acquired defects in the expression of key podocyte molecules leads invariably to their detachment and the development of renal failure [15]. Podocyte injury is notable in people with LN and accounts for the development of proteinuria and glomerular damage [16,17].

Podocytes are known to produce and express components of the complement pathway which along with the deposition and activation of complement from the circulation contributes to podocyte injury. Inhibition of the complement pathway has been entertained in clinical trials to treat people with LN [18]. Further, podocytes express all Toll-like receptors (TLR) and Nod-like receptor protein-3 (NLRP3) and caspase-1 [19]. Homocysteine activates NLRP3 inflammasomes in the podocytes of lupus-prone mice and patients with LN [20] and its suppression decreases proteinuria, histologic renal lesions, and podocyte foot-process effacement [16] suggesting that NLRP3 can be targeted therapeutically.

Podocytes from lupus-prone mice and people with LN express increased levels of major histocompatibility molecules along with the costimulatory molecules CD80 and CD86 which are considered markers of cell injury but simultaneously they may activate passerby lymphocytes and contribute to their accumulation in the renal parenchyma. Reversely, breaches in Bowman's capsule, in human crescentic glomerulonephritis may allow CD8+ T cells to reach the glomerular tuft and podocytes and cause their destruction [21].

Podocytes express the neonatal Fc receptor (FcRn)which enables the transfer of IgG from the capillary to the urine space. IgG from patients with LN enters podocytes using FcRn and causes upregulation of calcium/calmodulin-dependent protein kinase IV (CaMK4) which phosphorylates 14–3-3 β, the scaffold protein of synaptopodin, which upon its release is degraded. Synaptopodin is important in the maintenance of podocyte structure [22]. In parallel, CaMK4 activates NFkB which suppresses the expression of nephrin, an important protein of the split diaphragm, by promoting the function of the transcriptional repressor SNAIL [23]. IgG from patients with active LN causes the upregulation of CaMK4 by virtue of being under-galactosylated [23]. Global deletion of *Camk4* in MRL*lpr* lupus-prone mice effectively suppresses LN [24]. More importantly, podocyte-targeted delivery of a CaMK4 inhibitor suppresses all elements of LN and obviates the deposition of IC [22], suggesting that maintenance of the structure and function of podocytes by suppressing the activity of CaMK4 IC are not deposit. This line of information bespeaks to importance of local factors in the development of organ damage and the value of cell/organ-specific delivery of drugs to limit organ damage in autoimmunity.

2.2. Mesangial cells

Mesangial cells and mesangial matrix make the renal corpuscle's vascular pole, and are important in removing aggregated proteins and small IC from the basement membrane [19]. They are involved in the pathogenesis of LN as mesangial cell proliferation and mesangial matrix are present invariably in the LN kidneys [25]. Mesangial cells express Toll-like receptors (TLRs) [25,26], and when stimulated with a TLR3 ligand (dsRNA) they produce type I IFN [25] – a cytokine which is claimed to be important in the pathogenesis of SLE [25,27].

Antibodies to dsDNA bind to mesangial cells and activate inflammatory and fibrotic pathways, particularly those which involve the mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) signaling pathways, leading to the production of proinflammatory cytokines [27,28]. Mesangial cells secrete interleukin (IL)-6, which on its own can drive the development of glomerulonephritis [29]. CaMK4 is requisite for the proliferation of mesangial cells and the production of cytokines. Specifically, mesangial cells from the lupus-prone MRL*lpr* mice which lack genetically CaMK4, do not proliferate in response to platelet-derived growth factor and they do not produce IL-6 [30].

2.3. Renal tubular epithelial cells

Renal tubular epithelial cells are involved in the pathophysiology of LN. They secrete pathogenic cytokines, including type I IFN [31] and B-cell activating factor (BAFF) [32], both of which have significant roles in the development of SLE (Fig. 1). Furthermore, renal tubular epithelial cells from LN patients express the costimulatory molecule B7-H4, suggesting that they can activate T cells. Addition of anti-dsDNA antibodies to renal tubular epithelial cells in culture leads to the sequential upregulation of tumor necrosis factor (TNF) α, IL-1β, and IL-6 [33], which suggests the cells contribute to the inflammatory processes in the tubulointerstitium in LN [34]. Kidney tubular epithelial cells express apoptotic endonucleases [35] which apparently, when activated through yet unknown mechanisms, can cause cell death [36]. More recently, it was shown that tubular epithelial cells can produce CXCL12 in response to IL-23 to promote interstitial and its genetic deletion only in these cells limited glomerulonephritis in lupus-prone mice [7].

BAFF, is a well-established B cell growth and differentiation factor which helps auto-reactive B cells to survive and escape peripheral tolerance [37,38]. BAFF blockade with an antibody (Benlysta) has been approved to treat with SLE [39] and LN [40]. BAFF is also expressed by tubular epithelial cells of people with proliferative LN and the levels of expression correlate with the histopathology-defined activity index [32]. BAFF may promote further differentiation of B cells which are present in the interstitial space of kidneys from patients with LN [41]. Further, BAFF has been claimed to promote the formation of TLS in the kidney by increasing the number of T cells positioned inside the glomeruli and increase inflammation in mice [42] which may explain the therapeutic effect of Benlysta in patients with LN [40]. Benlysta targets B cells maturation and signaling by inhibiting B cells survival, and reducing differentiation to Ig-producing plasma cells in patients with LN [43].

2.4. Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent progenitor immunomodulatory cells present in all tissues [44]. They seem to have a role in dendritic and T-cell suppression [45]. Previous studies have shown that when concentrations of proinflammatory cytokines are low, MSCs may have immunostimulatory potential [45,46]. MSCs are detectable within the pelvis wall and TLS in the kidneys of lupus-prone mice [47]. Stimulation of MSCs with inflammatory cytokines leads to the expression of TNF-α, IL-1β, CCL19 and ICAM [47]. Although unclear, MSCs seem to have a role similar to that of lymphoid tissue organizer (LTo) cells and that resident tissue specific MSCs function like lymphoid tissue inducer (LTi) cells. They can reprogram and start an early inflammatory cascade by interacting with T cells [47]. MSC differentiation and immune cell accumulation causes an expansion of lymphatic vessels and therefore the formation of TLS [48].

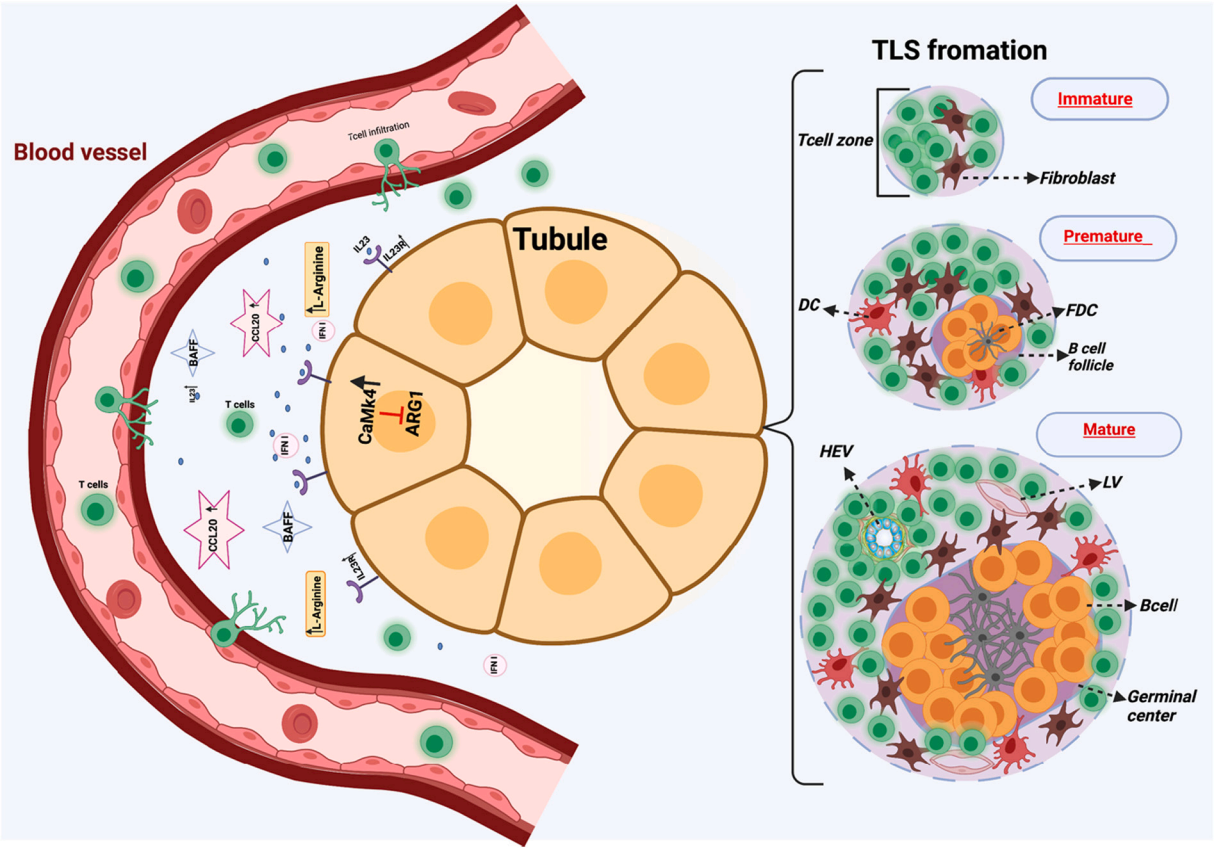

Fig. 1. Tertiary lymphoid structures in the kidney. Renal tubular epithelial cells secrete cytokines, including type I IFN, B-cell activating factor (BAFF), chemokine CCXL20 and IL23 which can attract lymphocytes to the kidney interstitium..IL-23 acts on tubular epithelial cells to increase the expression levels of CamK4 which suppresses the production of arginase 1, that leads to increased concentration of arginine in the tubulointerstitial environment which is necessary for the local growth of lymphocytes. Lymphocyte infiltration leads to tertiary lymphoid structure (TLS) formation that can be classified in three development phases. Immature TLS contain mainly T cells and fibroblasts. Premature TLS contains follicular dendritic cells (FDC), mature dendritic cells (DC) and small B cell follicle but lack of germinal center. Mature TLS has prominent FDSs, High endothelial venules (HEVs), lymphatic vessels (LVs) and germinal center. Created with BioRender.com.

2.5. Macrophages

Resident macrophages in the kidney normally are seen in the interstitium surrounding the glomeruli [49]. Peripheral monocytes after entry to kidney tissue and differentiation to macrophages act as main players in inflammation, injury, and fibrosis in acute and chronic kidney disorders [50]. $CD16^{+}$ or $CD14^{+}$ macrophages are recruited to injured kidneys in the presence of cytokines and chemokines [50]. Several subtypes of macrophages (M1, M2a-c) [51] have been recorded present in LN tissues with unknown origin and function [49,50,52]. In general, it seems that if resident macrophages are exposed to endosomal TLR ligands and damage-associated molecular pattern molecules (DAMPs) [53], they transit from a resolution phase to an inflammatory phase. During the inflammation phase, macrophages switch their phenotype to M1 and express Ly6C/Gr1 and secret proinflammatory cytokines [54,55]. In contrast, during the repair or resolution phase they polarize into the M2 phenotype [56,57].Therefore, it seems that macrophages have dual functionality and display high plasticity during the course of kidney disease.

3. Tertiary lymphoid structures

The term ‘tertiary lymphoid’ was introduced by Picker and Butcher [58] to explain extra-lymphoid sites in non-lymphoid tissues. TLS have been referred to in many ways, including tertiary lymphoid organs, tertiary lymphoid tissues and ectopic lymphoid structures. The accumulation of lymphocytes in peripheral non-lymphoid tissues, and the degree to which they become organized, varies according to the type and duration of antigenic inflammatory stimuli [59]. As a result, lymphoid aggregates range from loose collections, comprising a few T or B cells, to organized tissues displaying the hallmarks of TLS [60–63].

TLS are composed of a T-cell-rich zone with mature DCs next to a B-cell follicle with the characteristics of a germinal center surrounded by plasma cells. The minimal attributes needed to form functional TLS are not known, but TLS is defined as a lymphoid aggregate with organized stromal components consisting of follicular dendritic cells (FDCs) and fibroblastic reticular cells (FRCs), and characteristically, with high-endothelial venules (HEVs) and lymphatic vessels (LVs) [64,65]. A definition based on these criteria will exclude aggregates of B or T cells in response to inflammation lacking differentiated stromal compartments (Box 1).

TLS develop in various kidneys pathologies, including IgA nephropathy [66], early-stage IgG4-related tubulointerstitial nephritis [67], acute kidney injury [68,69], cancer [70], pyelonephritis [71], transplantation and LN [41,72,73]. In lupus-prone mice, TLS are found close to the pelvic wall, next to large arteries and veins [74]. In autoimmune diseases such as rheumatoid arthritis, Sjogren's syndrome, multiple sclerosis, diabetes, Hashimoto's thyroiditis, primary sclerosing cholangitis and primary biliary cirrhosis and myasthenia gravis, TLS may enable the *in situ* generation of autoreactive T and B cells and the production of autoantibodies that perpetuate the pathogenic process [63,70,75,76].

3.1. Crosstalk of kidney's immune cells with tertiary lymphoid structures

T cells maintain immune homeostasis under physiologic conditions

Box 1
: Markers of cell populations in TLS

- T cells: CD3 pan marker, CD8 or CD4-expressing subpopulations, oriented towards T helper 1 cell phenotype and CD^{4+} T regulatory cells $(FOXP3)^{+}$.
- B cells: CD20, B220 and the proliferation marker Ki67.
- Plasma cells: CD269. Mist 1.
- Macrophages: CD68.
- Follicular dendritic cells (FDCs): CD21.
- Dendritic cells (DCs): DC-$LAMP^{+}$, MIDC-8^{+}.
- High endothelial venules (HEVs): peripheral node addressin (PNAd).
- Lymphatic vessels: Lymphatic Vessel Endothelial Receptor 1 (LYVE1).

TLS localization

- TLS are mainly found in the medulla of kidneys.

TLS identification

- Haematoxylin and eosin (H&E) staining: H&E imaging is performed routinely in laboratories as a component of diagnostic. TLS can be easily detected in H&E slides/images. Expertise is required for a reliable assessment.
- Periodic acid-Schiff (PAS) staining: PAS staining is used for analyzing the glomerulus and alteration in vessels in kidney biopsies, in which local immune cell infiltration as well as TLS can be recognized.
- Multiplex immunohistochemistry (mIHC; chromogenic, immunofluorescent): mIHC is a precise staining method to identify different cell populations and evaluate the maturity in TLS.
- Gene expression signature: Transcriptomics data for TLS assessment.

and promote tolerance against self-antigens. In autoimmune kidney disorders malfunction of T-cell tolerance to autoantigens can lead to the generation of autoantibodies, inflammation, immune cell infiltration and development of different types of nephritis [77,78].

T cells may infiltrate the kidney tissue either because they have been activated in the periphery and express adhesion molecules or they may be naïve and become activated after they enter the kidney parenchyma by podocytes or tubular epithelial cells as discussed above. Activated cells express adhesion molecules such as CD44 which, when associated with phosphorylated esrin/rodesin/moiesin [79], binds to its ligand hyaluronic acid, the synthesis of which is increased in the kidneys of lupus-prone mice [80]. Since esrin/rodesin/moiesin is phosphorylated by Rho kinase, inhibition of its activity limits entry of T cells into the kidney [81]. Similarly, inhibition of hyaluronic acid synthesis decreases the entry of T cells in to the kidneys of lupus-prone mice [80] Interestingly, the numbers of $CD3^{+}CD44^{+}$ cells in the peripheral blood of people with SLE correlate with kidney disease activity [82].

The majority of the cells in TLS are $CD3^{+}$ T cells [74] and they include cytotoxic granule-expressing $CD8^{+}$ T cells and $CD4^{+}$ T cells that display a T_{H1} cell phenotype and $CD4^{+}$ T_{reg} cells [83–86]. It is assumed that mature dendritic cells (DCs) present antigen to $CD4^{+}$ T cells in the T-cell zone of TLS [87], but DC-$LAMP^{+}$ DCs have also been detected in the germinal centers, suggesting they have a role in antigen presentation to B cells [88]. B cells organize into germinal centers with plasma cells. B-cell areas contain $CD21^{+}$ FDCs whereas T cell areas contain MIDC-8^{+} DCs [74].

Double-negative (DN) T cells are defined by the presence of T-cell receptor (TCR) $\alpha\beta^{+}$ and the absence of CD4 and CD8 molecules. They are expanded in the peripheral blood of patients with SLE, provide help to B cells to produce autoantibody [89] and produce IL-17 [90]. It seems that they derive from $CD8^{+}$ T cells [91,92] in response to stimulation with autoantigen and in the presence of IL-23 [93]. Mechanistically, the *CD8* locus is shut off though epigenetic modifications imposed by the repressor cAMP response-element modulator α (CREMα) [94]. More interestingly, DN T cells are present in the kidneys of patients with LN and they produce IL-17 [90] pointing to their direct contribution to kidney inflammation.

The T_H17 subset of αβ T cell are defined by the expression of lineage determining transcription factor RORγt. They promote autoimmune response in humans and mice by producing granulocyte-macrophage colony-stimulating factor (GM-CSF), IFNγ and IL-17, −21 and − 22 [95,96]. T_H17 cells express C—C motif chemokine receptor type 6 (CCR6) and are recruited to the kidney by C—C motif chemokine 20 (CCL20), which is produced by mesangial cells after stimulation by IL-17 (also produced by neutrophils or γδT cells) [97] and by TEC exposed to IL-23 [7]. T_H17 cells present in the kidney secret IL-17 and promote inflammation by generating TLS [98], promote B-cell activation and loss of tolerance [99,100].

Follicular T-helper (T_{FH}) cells are $CD4^{+}$ T cell that express the transcription factor BCL6 and C-X-C motif chemokine receptor type 5 (CXCR5). These cells migrate into germinal centers in response to C-X-C motif chemokine 13 (CXCL13). They also express three surface receptors, including the inducible T-cell costimulatory, CD40L, PD-1, and produce IL-21 to advance B-cell activation and differentiation of B cells into memory B cells and plasmablasts [101–103].

Levels of circulating T_{FH} cells are increased in people with autoimmune diseases including SLE and studies in lupus-prone mice have confirmed they pathogenic role [102]. In SLE patients, the frequency of a subset of these cells – extrafollicular T_H cells – correlates with levels of anti-dsDNA antibody and the quantity of plasmablast B cells. Extrafollicular T_H cells define a $CCR6^{+}$ subset, which expresses CXCR5 but not BCL6 and can secrete IL-17 and facilitate immunoglobulin production by B cells [103,104].

T_{reg} cells are $TCR\alpha\beta^{+}Foxp3^{+}CD4^{+}$ T cells that develop in the thymus or the periphery. They display suppressive activity and control most immune responses through various mechanisms [105]. One of their best known roles in the kidney is the secretion of the anti-inflammatory cytokine IL-10 [106]. Some of the molecular events that lead to the dysfunction of T_{reg} cells have been elucidated. The molecules involved include protein phosphatase 2A (PP2A), mammalian target of rapamycin complex 1 (mTORC1), phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase, dual-specificity protein phosphatase (PTEN), and calcium/

calmodulin-dependent protein kinase IV (CaMK4). Targeting these may rescue or suppress T_{reg} cell function and modulate kidney inflammation [107].

Analysis of T-cell clonotypes from various tissues of LN mice, as well as from the peripheral blood or kidneys of patients with SLE, has revealed expansion of a restricted subset of TCR repertoire, indicating a response to the defined number of autoantigens [93]. This repertoire remains stable for months or years [108,109]. In SLE patients, the clones in the peripheral blood are different from those in the kidney [110], suggesting that naive T cells are activated independently in the periphery and the kidney.

Local production of antibodies is critical in TLS formation. Interestingly, the kidney immune system-related TLS gene profile in lupus-prone mice is similar to that in lymph nodes during active stages of LN [74]. TLS provide the T- and B-cell survival factors IL-7 and BAFF which recruit lymphocytes and favor the interaction between T and B cells in a confined environment [111]. Local B-cell activation has been demonstrated in TLS by the expression of Activation Induced Cytidine Deaminase (AICDA), an enzyme responsible for class switch recombination and somatic hypermutation [112] and active proliferation. Local differentiation of autoreactive plasma cells has also been observed [113].

In a model of pristane-induced murine SLE, B cells proliferate and class-switch within the TLS, and Sm/RNA antibody-producing plasma cells and plasmablasts produce locally autoantibodies [114,115]. Moreover, kidneys from LN patients contain germinal center-like structures containing FDCs. These centers may have a role in active local tissue-specific immune responses [41]. Identifying the events that initiate TLS formation should advance our understanding of kidney damage in patients with LN. BAFF and serum autoantibody levels correlate with TLS formeation in the kidneys [42] . Reducing the levels of BAFF reduced T-cell numbers in the glomeruli and prevented LN and the formation or maintenance of TLS [42].

Furthermore, the presence of FDCs, or the binding of IgG-ICs to FcγRIIB, may provide a source of intact antigen [41,116,117] for expansion and activation of B cells, and the production of lymphotoxin-α1β2, which may further promote the development of TLS [118,119]. Alternatively, $CD11b^{+}$ myeloid cells secrete high level of BAFF and enhance the chemotactic ability of B cells by modulating chemokine-induced signaling [120], thus leading to cell aggregation and compartmentalization of TLS. Since increased levels of BAFF amplify local T-cell activation [121], they may promote the activity of T_{FH} and prolong *in situ* germinal center responses of kidney TLS. Further, in patients with LN, only cell-to-cell interactions between T_{FH} cells and B cells induce high levels of Bcl-6 and IL-21 in the interstitium [122]. This may represent the effect of BAFF which promotes the expression of ICOSL on activated B cells [123] and induces the formation of T_{FH} cells [124,125]. Thus, renal TLS may form in LN due to hematopoietic cell infiltration into the kidney, but high BAFF levels are required to form or maintain correctly compartmentalized TLS. This suggests that the high level or prolonged production of BAFF may be a key event in the formation of compartmentalized TLS.

3.2. Molecular cues for the formation of tertiary lymphoid structures

CXCL13 which is produced by fibroblastic stromal cells, is a crucial chemokine for B and lymphoid-tissue inducer (LTi) cells. Mice lacking CXCL13 do not form lymph nodes except for the facial, cervical and mesenteric ones [126]. Induction of TLS formation [127] in mice can be accomplished by overexpressing CXCL13 driven by the rat insulin promoter (RIP), which is active in the pancreas and the kidney. This leads to the formation of TLS that are characterized by segregated B- and T-cell zones, the presence of conventional DCs, and a dense network of stromal cells and high endothelial venules (HEV)-type blood vessels [128]. CXCL12 (or stromal cell-derived factor 1 (SDF1)) is expressed by stromal cells of the bone marrow and is critical in bone-marrow hematopoiesis and B-cell development [129]. CXCL12 is expressed by HEVs in secondary lymphoid organs (SLO) and acts as an essential B-cell recruitment chemokine, while the T cells are mostly unresponsive [130] (Table 1).

Tubular epithelial cells from lupus-prone mice can express IL-23 receptors and produce the chemokine CCL20 which can attract lymphocytes to the kidney interstitium (Fig. 1) [7]..Further, IL-23 acting on tubular epithelial cells can suppress the production of arginase 1 which catabolizes arginine and therefore leads to increased concentrations of arginine which is necessary for the local growth of lymphocytes [7]. Through the first mechanism tubular epithelial cells are capable to produce proinflammatory chemokines to attract lymphocytes which can be activated locally. Through the second mechanism, tubular epithelial cells can display immunosuppressive capacities which can be obviated in the presence of IL-23 and possibly other stimulants.

CCL19 and CCL21 are expressed by HEVs and some stromal cells. They are the ligands for CCR7 present on T cells, DCs and LTi cells. *Plt* mice that lack the *CCL19* gene and CCL21 expressed by lymphoid tissue in lymphatic vessels revealed a critical role for CCR7 and CCL19/CCL21 in T-cell homing. In the RIP-overexpression model, CCL21 proved to be more effective than CCL19 in inducing TLS formation [131,132]. However, even with CCL21 overexpression there is no clear formation of B-cell follicles [131]. CCL28 has a role in the recruitment and homing of B and T cells and promotes adaptive immune responses [133–135]. Signals from the interaction between CCL28 and CCR3/CCR10 drive these processes and attract various immune cells from the local neighborhood [135,136]. Recently, the recruitment of T_{reg} cells by CCL28 has been observed, demonstrating that it has a role in the modulation of the immune system, maintaining tolerance to self-antigens, and preventing the development of autoimmune diseases [137,138] (Table 1).

Members of the TNF superfamily (TNFSF), namely TNFα, lymphotoxin (LT) α and β, and their signaling receptors TNFRI/II and LTβR, were suggested to promote the formation of TLS. Also, ectopic expression of TNFα or LTα, but not LTβ, under the control of RIP led to the formation of TLS [139,140]. The most substantial effect was seen when LTα and LTβ were co-expressed, resulting in invasive leukocyte accumulation in the pancreatic islets, and significantly larger TLS than those formed in LTα transgenic mice [139]. TNFR-I is the fundamental regulator of lymphoid tissue organogenesis and germinal-center formation,

Table 1
Molecular cues for the formation of TLS.

Gene name	TLS related function in kidney	Reference
CXCL12	Mainly secreted by HEV; B-cell recruitment	[129,130]
CXCL13	Mainly secreted with fibroblastic stromal cells; B and LTi cells chemotaxis; cells and vessels regulation in TLS, proliferation and formation in TLS	[126–128]
IL4	Stimulation of T cells, induced expression of LTαβ	[47]
IL6	Perivascular accumulation of B cells and mature plasma B cells	[146]
IL7	Stimulation of $CD4^{+}$T cells, induced expression of LTαβ	[131]
IL17	Increase the inflammatory and homeostatic chemokine production	[147]
IL23	Secreted with tubular epithelial cells; Suppresses the production of arginase 1; local growth of lymphocytes	[7]
CCL19 AND CCL21	Mainly secreted with HEVs and some stromal cells; Ligands for CCR7 on T cells, DCs and LTi cells; T-cell homing and inflammatory response	[131,132]
CCL28	Homing of B and T cells; promotes adaptive immune responses	[133–138]
TNF superfamily	Leukocyte accumulation, germinal-center formation	[139–145]
BAFF	Increases T-cell-driven cytokines such as IL-17, IL-4 and IFNγ, Promote accumulation of T_{FH} cells	[42,124]

HEV: High endothelial venules; LT_i: lymphoid-tissue inducer; LT: Lymphotoxin; TNF: Tumor necrosis family; BAFF: B-cell activating factor; $T_{FH:}$ T follicular helper cells; DC: Dendritic cells.

rather than TNFR-II [141], and it mediates LTα-induced pancreatic TLS [142]. Activation of TNFR-I and LTβR has also been implicated in aortic TLS, in which aberration of LTβR signaling leads to the suppression of CCL21 and CXCL13 expression, with the consequence of reduced HEV formation and disrupted TLS development [143,144] (Fig. 2).

While an effect of LTα, alone or with LTβ, appears clear, the role of TNFα is disputed. In some inflammatory diseases, including those involving TLS, TNFα has an anti-inflammatory activity [144]; insulitis in NOD mice and lupus in New Zealand mice improve after injection of TNFα [144,145].

Transgenic overexpression of IL-6 and IL-6R leads to perivascular accumulation of B cells and mature plasma B cells [146]. IL-1β produced by MSCs is overexpressed in lupus-prone mice and may contribute to TLS formation [47]. Stimulation of T cells with IL-4 or IL-7 induced expression of LTαβ; IL-7 was most potent for $CD4^+$ T cells [131]. The IL-17 gene family is vital in defense against pathogens and has been implicated in various chronic inflammatory scenarios. Like members of the TNFRSF, IL-17 receptor signals through NF-κB and IL-17 T cells are induced by IL-6, TGFβ and IL-23, but inhibited by IL-27. IL-17 is, therefore, an essential mediator for lipopolysaccharide-induced iBALT [147]. IL-7R is expressed by LTi cells and along with CXCR5) IL-7 promotes their formation in SLOs [126].

BAFF may promote tissue injury by affecting the quality and quantity of T-cell-driven cytokines such as IL-17, IL-4 and IFNγ. Increased levels of BAFF in the kidneys may prompt glomerular damage by invading T cells inside the glomeruli, or by inducing the formation of T_H17 cells. It is unclear whether the position of the T cells is a parallel or codependent process that promotes glomerulonephritis and tubulointerstitial nephritis. It has been shown that blocking T-cell co-stimulation [148] or neutralizing IFNγ and IL-4 [149,150], leads to an improvement or delay in renal pathology. Comparably, T-cell infiltration and aggregation have been found in kidney biopsies from SLE patients [151]. Immune-cell infiltration into tubulointerstitial areas in SLE is associated with LN [41], suggesting that the position of T cells within the kidney is vital in the disease.

3.3. Vessels in tertiary lymphoid structures

TLS are similar to lymph nodes in terms of structure, vasculature, cellular composition and chemokine profile. Immune cells include T- and B-cell zones and antigen-presenting cells, including FDCs and mature DCs. The vessels in TLS mainly divide to lymphatic and blood vessels (Fig. 3).

Renal lymphatic vessels (LVs) are considered part of the interstitium because they do not have a basement membrane, and they are blind-ended and lack pericytes [152]. Lymphatic capillaries express PROX-1, LYVE-1, CCL21, podoplanin, VEGFR-2 and VEGFR-3 [153]. The lymphatic vessels of TLS express lymphatic markers such as LYVE-1, PROX-1, podoplanin (in both mice and humans) and D2–40 (in humans) [154], as reported by various studies of chronic kidney rejection [155,156], cardiac allografts [157], transgenic mouse models [158] and a mouse model of age-related primary Sjögren's-like disease [159]. Nevertheless, there is still much to be elucidated.

It is not known whether TLS vessels carry out the same functions as those in lymph nodes. It seems that they contribute to fluid drainage, but this has not been fully explored. It is also not known whether LVs carry antigen and cells within the TLS and cells away from TLS, like afferent and efferent vessels in lymph nodes. That TLS LVs frequently contain cells [159,160], suggests they have a role as transporters through the expression of CCL21, which interacts with CCR7-expressing cells. However, LVs in some TLSs accumulate cells, suggesting that they do not facilitate cellular drainage and have impaired efferent function.

Lymph node resident cells express sphingosine-1 phosphate (S1P) and its interaction with the S1P1 receptor on lymphocytes is important for their egression from the lymph nodes. FTY720 (fingolimod) is an S1P1 agonist that causes its internalization and accumulation of lymphocytes in lymph nodes [161], thus functions as an immunosuppressant. When NOD mice with pancreatic TLS are treated with FTY720, they do not go on to develop islet destruction and diabetes [162]. FTY720 inhibits disease progression only at the time that the mice exhibit TLS [163]. Their pancreatic TLS were associated with high insulitis scores after FTY720 treatment, indicating that cells are trapped

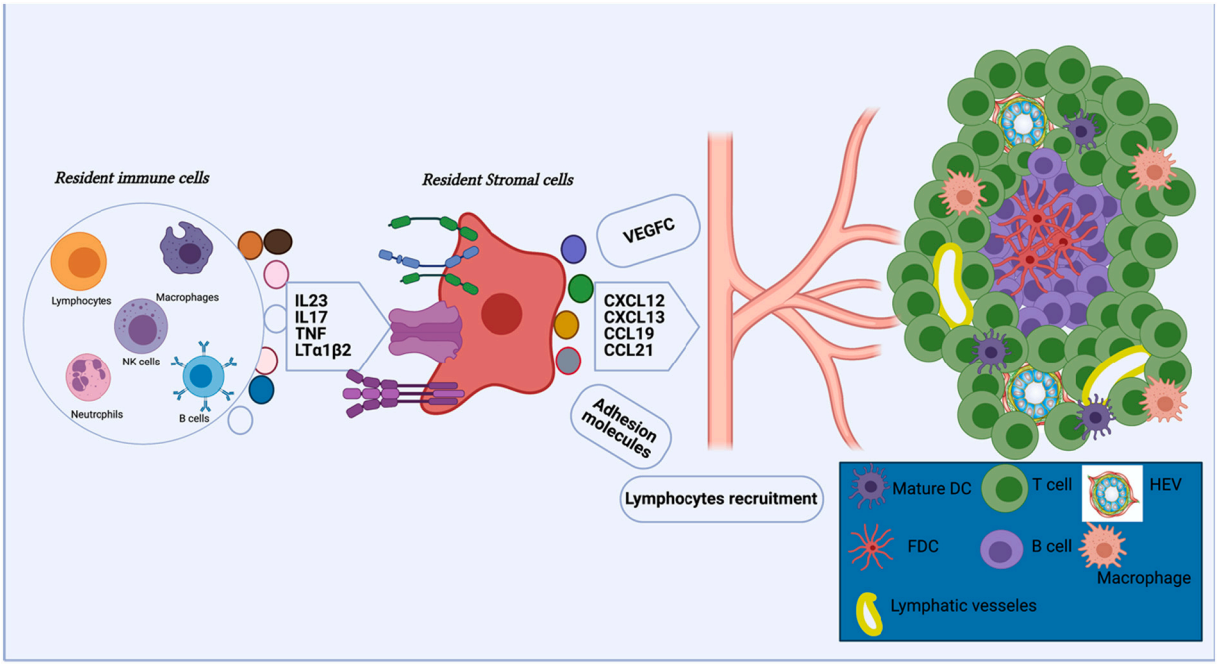

Fig. 2. Schematic demonstration of TLS formation: During chronic inflammation kidney resident immune cells release inflammatory cytokines and interleukins which act on resident stromal cell. Stromal cells release vascular endothelial growth factor (VEGFC), adhesion molecules (ICAM-1) and chemokines that recruit immune cells and generate high endothelial venules (HEV) and lymphatic vessels. Mature TLS consists of follicular dendritic cells (FDCs) and a germinal center (GC) of proliferating B cells, surrounded by T cells, HEVs and mature dendritic cells (DC). Created with BioRender.com.

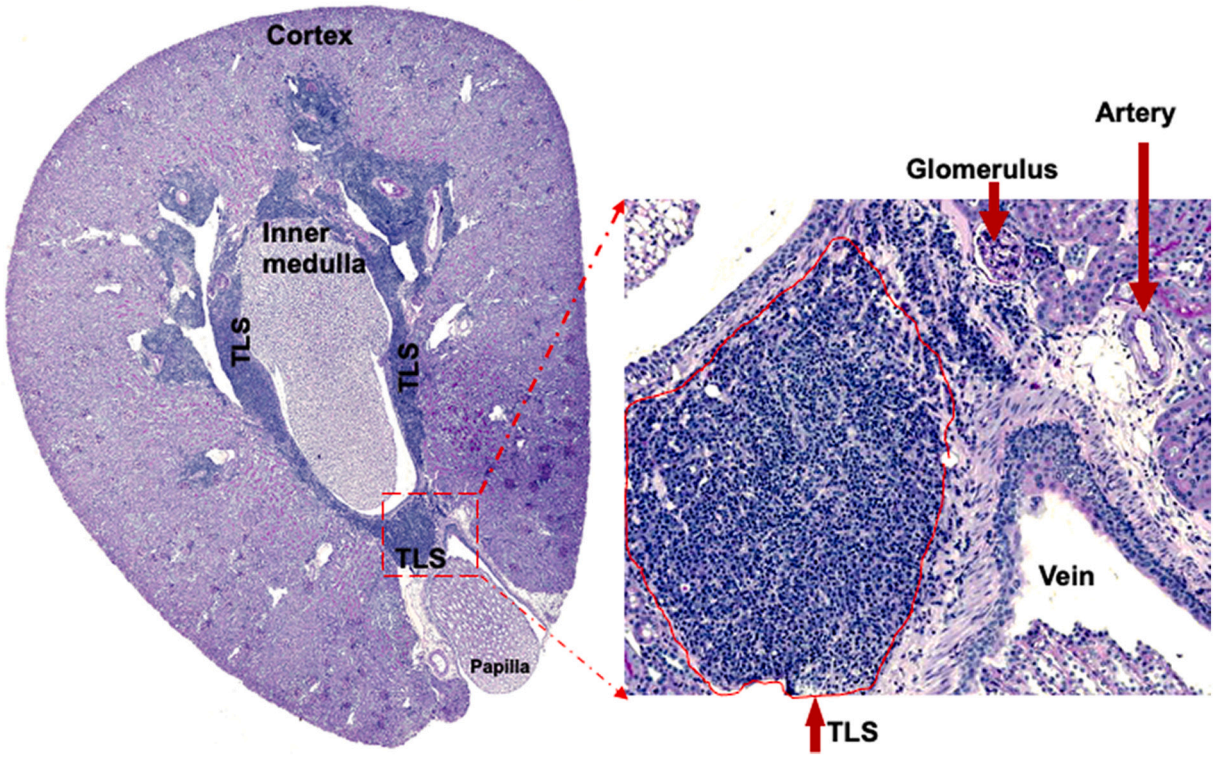

Fig. 3. Tertiary lymphoid strucures (TLS) in the kidney of the MRL.*lpr* lupus-prone mouse: The section is from a 12-week old MRL.*lpr* mouse stained with Periodic Acid-Schiff. TLSs appear as dark blue areas mostly in the inner medulla or the cortex and close to arteries and veins.

within them. Islet destruction and diabetes occurred within days of stopping FTY720 treatment [162,164]. Thus, it seems that the S1P gradient affects lymphocyte trafficking in TLS LVs. It is possible that fingolimod may force TLS to dissolve in patient and mice with lupus.

LVs transport soluble or cell-associated antigens into lymph nodes. Plasmalemma vesicle-associated protein (PLVAP) is expressed by blood vessels lymphatic endothelial cells in the lymphatic sinus in lymph nodes. PLVAP-positive lymphatic endothelial cells contribute to the sieving of lymphocytes and high-molecular-weight antigens entering the lymph nodes [165]. Since TLS contain a conduit system [166], it is reasonable to question whether LVs in TLS and lymph nodes function similarly. Antigen transport may be less critical than in SLOs because the antigen is an actual component of TLS. However, since antigen-presenting cells are usually present in TLS, this is debatable.

As noted above, LVs in lymph nodes present self-antigens [167–169] either directly through the expression of major histocompatibility complex (MHC) molecules or through antigen on 'classical' antigen-presenting cells. Presentation of self-antigen by LVs [167] may facilitate induction of either tolerance or T-cell activation in lymph nodes or TLS. Studies investigating the ability of TLS LVs to present antigen and induce either of these outcomes have not been conducted.

HEVs are specialized peripheral-node addressin (PNAd)-positive blood vessels with a distinct structure. HEVs appear to have a role in the transport of blood-borne lymphocytes into TLS. This phenomenon is a kind of specialized infiltration that mainly memory T cells with a low expression of L-selectin (possibly due to the expression of PNAd) can enter to the kidney [139]. An experimental study in mice deficient in either LTα or LTβLN found that the development of PNAd-expressing HEVs is stunted, leading to a reduction in the size and cellularity of lymphoid infiltrates [139]. Thus, LTβR signaling may be required for the organized lymphoid aggregation and HEV formation.

4. Conclusions and open questions

Although immune responses generated in SLOs can be generate protection against pathogens, auto-immune responses in TLS may be destructive. The germinal centers in TLS have similar characteristic of germinal center in SLOs and provide a ground for immune call clonal expansion and somatic hypermutation [41]. Although the presence of immune complexes has been considered important in the formation of TLS, more recent evidence suggests that under the influence of cytokines tubular epithelial cells can produce cytokines able to attract T cells [7].

B cells present in TLS have been shown to have undergone somatic hypermutation [41] and therefore the local production of autoantibodies and the possible formation of *in situ* ICs is certain. Th17 cells have been shown to be present in the kidneys of people and mice with lupus indicating the direct contribution of these cells in the inflammatory response and kidney damage [101,170–172]. The fact that the TCR repertoire of kidney infiltrating cells in mice and people with lupus is restricted [93] indicates that kidney-specific antigens, still at large, are being recognized. Th17 cells are vital in TLS formation in the propagation of inflammation in the central nervous system and the neonatal lungs [173–175]. A similar role can be projected for the cells in the establishment and maintenance of inflammation in LN.

The presence of Treg cells in the kidney TLS and their possible function is unknown. It is possible that they are excluded through unknown mechanisms or if present they become bereft of their expected function. It is known that Treg cells in the presence of an inflammatory environment lose their regulatory function [78].

Although it has been claimed that the intensity of the interstitial inflammation represents an ominous sign of renal function it is still unknown how TLS contributes to kidney damage. It is possible that T cells destroy kidney resident cells, like it has been shown for podocytes [21], through direct cytotoxicity or my compromising the function of kidney cells though the action of cytokines as it was shown for IL-23 [7] and BAFF [123].

Completely unchartered is the field of the contribution of TLS to the development of kidney fibrosis which is irreversible and defines the end of function. Cytokines produced by the infiltrating cells along with the contribution of other factors produced by kidney resident cells may promote collagen production by fibroblasts.

Upcoming technologies including single cell RNA sequencing [176] and spatial transcriptomics will enable the characterization of the interactions between cells comprising the TLS and kidney resident cells. They may also allow the characterization of subsets among patients with LN, as it is certain that LN is clinically and pathogenetically heterogeneous. The efforts to reverse kidney pathology by delivering drugs to kidney resident cells (podocytes [22], tubular epithelial cells [7]) should allow more effective restoration of the function of kidney cells while side effects resulting from the systemic administration are obviated.

Acknowledgements

Supported by Northern Norway Regional Health Authority research grant HNF 1427-18.

References

[1] Tsokos GC. Systemic lupus erythematosus. N Engl J Med 2011;365(22):2110–21.
[2] Tsokos GC, et al. New insights into the immunopathogenesis of systemic lupus erythematosus. Nat Rev Rheumatol 2016;12(12):716–30.
[3] Lisnevskaia L, Murphy G, Isenberg D. Systemic lupus erythematosus. Lancet 2014;384(9957):1878–88.
[4] Urbonaviciute V, et al. Induction of inflammatory and immune responses by HMGB1-nucleosome complexes: implications for the pathogenesis of SLE. J Exp Med 2008;205(13):3007–18.
[5] Suurmond J, Diamond B. Autoantibodies in systemic autoimmune diseases: specificity and pathogenicity. J Clin Invest 2015;125(6):2194–202.
[6] Vlahakos DV, et al. Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. Kidney Int 1992;41(6):1690–700.
[7] Li H, et al. Interleukin-23 reshapes kidney resident cell metabolism and promotes local kidney inflammation. J Clin Invest 2021;131(12):e142428.
[8] van Bavel CC, et al. Glomerular targets of nephritogenic autoantibodies in systemic lupus erythematosus. Arthritis Rheum 2008;58(7):1892–9.
[9] Schwartz N, Goilav B, Putterman C. The pathogenesis, diagnosis and treatment of lupus nephritis. Curr Opin Rheumatol 2014;26(5):502–9.
[10] Perico L, et al. Podocyte-actin dynamics in health and disease. Nat Rev Nephrol 2016;12(11):692–710.
[11] Satyam A, et al. Cell-derived extracellular matrix-rich biomimetic substrate supports Podocyte proliferation, differentiation, and maintenance of native phenotype. Adv Funct Mater 2020;30(44):1908752.
[12] He B, et al. Podocin-green fluorescence protein allows visualization and functional analysis of podocytes. J Am Soc Nephrol 2011;22(6):1019–23.
[13] Wagner N, et al. The major podocyte protein nephrin is transcriptionally activated by the Wilms' tumor suppressor WT1. J Am Soc Nephrol 2004;15(12):3044–51.
[14] Kravets I, Mallipattu SK. The role of podocytes and podocyte-associated biomarkers in diagnosis and treatment of diabetic kidney disease. J Endocr Soc 2020;4(4). bvaa029.
[15] Reiser J, Altintas MM. Podocytes. F1000Res 2016;5.
[16] Fu R, et al. Podocyte activation of NLRP3 Inflammasomes contributes to the development of proteinuria in lupus nephritis. Arthritis Rheumatol 2017;69(8):1636–46.
[17] Wang Y, et al. Podocyte involvement in lupus nephritis based on the 2003 ISN/RPS system: a large cohort study from a single Centre. Rheumatology (Oxford) 2014;53(7):1235–44.
[18] Ferretti AP, et al. Calcium/Calmodulin kinase IV controls the function of both T cells and kidney resident cells. Front Immunol 2018;9:2113.
[19] Zhang C, et al. Activation of nod-like receptor protein 3 inflammasomes turns on podocyte injury and glomerular sclerosis in hyperhomocysteinemia. Hypertension 2012;60(1):154–62.
[20] Conley SM, et al. Inflammasome activation in chronic glomerular diseases. Curr Drug Targets 2017;18(9):1019–29.
[21] Chen A, et al. Bowman's capsule provides a protective niche for podocytes from cytotoxic CD8+ T cells. J Clin Invest 2018;128(8):3413–24.
[22] Maeda K, et al. CaMK4 compromises podocyte function in autoimmune and nonautoimmune kidney disease. J Clin Invest 2018;128(8):3445–59.
[23] Bhargava R, et al. N-glycosylated IgG in patients with kidney transplants increases calcium/calmodulin kinase IV in podocytes and causes injury. Am J Transplant 2021;21(1):148–60.
[24] Otomo K, et al. Cutting edge: Nanogel-based delivery of an inhibitor of CaMK4 to CD4+ T cells suppresses experimental autoimmune encephalomyelitis and lupus-like disease in mice. J Immunol 2015;195(12):5533–7.
[25] Flur K, et al. Viral RNA induces type I interferon-dependent cytokine release and cell death in mesangial cells via melanoma-differentiation-associated gene-5: implications for viral infection-associated glomerulonephritis. Am J Pathol 2009;175(5):2014–22.
[26] Patole PS, et al. Expression and regulation of toll-like receptors in lupus-like immune complex glomerulonephritis of MRL-Fas(lpr) mice. Nephrol Dial Transplant 2006;21(11):3062–73.
[27] Yung S, et al. Anti-dsDNA antibodies bind to mesangial annexin II in lupus nephritis. J Am Soc Nephrol 2010;21(11):1912–27.
[28] Yung S, Chan TM. Anti-dsDNA antibodies and resident renal cells - their putative roles in pathogenesis of renal lesions in lupus nephritis. Clin Immunol 2017;185:40–50.
[29] Kanapathippillai P, et al. Nucleosomes contribute to increase mesangial cell chemokine expression during the development of lupus nephritis. Cytokine 2013;62(2):244–52.
[30] Ichinose K, et al. Cutting edge: calcium/Calmodulin-dependent protein kinase type IV is essential for mesangial cell proliferation and lupus nephritis. J Immunol 2011;187(11):5500–4.
[31] Castellano G, et al. Local synthesis of interferon-alpha in lupus nephritis is associated with type I interferons signature and LMP7 induction in renal tubular epithelial cells. Arthritis Res Ther 2015;17:72.
[32] Schwarting A, et al. Renal tubular epithelial cell-derived BAFF expression mediates kidney damage and correlates with activity of proliferative lupus nephritis in mouse and men. Lupus 2018;27(2):243–56.
[33] Yung S, et al. Effect of human anti-DNA antibodies on proximal renal tubular epithelial cell cytokine expression: implications on tubulointerstitial inflammation in lupus nephritis. J Am Soc Nephrol 2005;16(11):3281–94.
[34] Chen Y, et al. Expression of the novel co-stimulatory molecule B7-H4 by renal tubular epithelial cells. Kidney Int 2006;70(12):2092–9.
[35] Basnakian AG, Singh AB, Shah SV. Identification and expression of deoxyribonuclease (DNase) I alternative transcripts in the rat. Gene 2002;289(1–2):87–96.
[36] Hengartner MO. Apoptosis. DNA destroyers. Nature 2001;412(6842):27 [29].
[37] Mackay F, et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. J Exp Med 1999;190(11):1697–710.
[38] Cancro MP, Smith SH. Peripheral B cell selection and homeostasis. Immunol Res 2003;27(2–3):141–8.
[39] Shin W, et al. BAFF-neutralizing interaction of belimumab related to its therapeutic efficacy for treating systemic lupus erythematosus. Nat Commun 2018;9(1):1200.
[40] van Vollenhoven RF, et al. Long-term safety and limited organ damage in patients with systemic lupus erythematosus treated with belimumab: a phase III study extension. Rheumatology (Oxford) 2020;59(2):281–91.
[41] Chang A, et al. In situ B cell-mediated immune responses and tubulointerstitial inflammation in human lupus nephritis. J Immunol 2011;186(3):1849–60.
[42] Kang S, et al. BAFF induces tertiary lymphoid structures and positions T cells within the glomeruli during lupus nephritis. J Immunol 2017;198(7):2602–11.
[43] Frieri M, Heuser W, Bliss J. Efficacy of novel monoclonal antibody belimumab in the treatment of lupus nephritis. J Pharmacol Pharmacother 2015;6(2):71–6.
[44] Bianco P. "Mesenchymal" stem cells. Annu Rev Cell Dev Biol 2014;30:677–704.
[45] Li W, et al. Mesenchymal stem cells: a double-edged sword in regulating immune responses. Cell Death Differ 2012;19(9):1505–13.
[46] Kaplan JM, Youd ME, Lodie TA. Immunomodulatory activity of mesenchymal stem cells. Curr Stem Cell Res Ther 2011;6(4):297–316.
[47] Dorraji SE, et al. Mesenchymal stem cells and T cells in the formation of tertiary lymphoid structures in lupus nephritis. Sci Rep 2018;8(1):7861.
[48] Girard JP, Moussion C, Forster R. HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. Nat Rev Immunol 2012;12(11):762–73.
[49] Schiffer L, et al. Activated renal macrophages are markers of disease onset and disease remission in lupus nephritis. J Immunol 2008;180(3):1938–47.
[50] Sahu R, et al. Structure and function of renal macrophages and dendritic cells from lupus-prone mice. Arthritis Rheumatol 2014;66(6):1596–607.
[51] Mantovani A, et al. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 2004;25(12):677–86.
[52] Davidson A. Renal mononuclear phagocytes in lupus nephritis. ACR Open Rheumatol 2021;3(7):442–50.
[53] Tabas I, Bornfeldt KE. Macrophage phenotype and function in different stages of atherosclerosis. Circ Res 2016;118(4):653–67.
[54] Lin SL, et al. Bone marrow Ly6Chigh monocytes are selectively recruited to injured kidney and differentiate into functionally distinct populations. J Immunol 2009;183(10):6733–43.
[55] Li L, et al. The chemokine receptors CCR2 and CX3CR1 mediate monocyte/macrophage trafficking in kidney ischemia-reperfusion injury. Kidney Int 2008;74(12):1526–37.
[56] Lee S, et al. Distinct macrophage phenotypes contribute to kidney injury and repair. J Am Soc Nephrol 2011;22(2):317–26.
[57] Filardy AA, et al. Proinflammatory clearance of apoptotic neutrophils induces an IL-12(low)IL-10(high) regulatory phenotype in macrophages. J Immunol 2010;185(4):2044–50.
[58] Picker LJ, Butcher EC. Physiological and molecular mechanisms of lymphocyte homing. Annu Rev Immunol 1992;10:561–91.
[59] Luo S, et al. Chronic inflammation: a common promoter in tertiary lymphoid organ Neogenesis. Front Immunol 2019;10:2938.
[60] Cruz-Migoni S, Caamano J. Fat-associated lymphoid clusters in inflammation and immunity. Front Immunol 2016;7:612.
[61] Jones GW, et al. Interleukin-27 inhibits ectopic lymphoid-like structure development in early inflammatory arthritis. J Exp Med 2015;212(11):1793–802.

[62] Stahl FR, et al. Nodular inflammatory foci are sites of T cell priming and control of murine cytomegalovirus infection in the neonatal lung. PLoS Pathog 2013;9 (12):e1003828.
[63] Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. Nat Rev Immunol 2006;6(3):205–17.
[64] Shipman WD, Dasoveanu DC, Lu TT. Tertiary lymphoid organs in systemic autoimmune diseases: pathogenic or protective? F1000Res 2017;6:196.
[65] Ruddle NH. High endothelial Venules and lymphatic vessels in tertiary lymphoid organs: characteristics, functions, and regulation. Front Immunol 2016;7:491.
[66] Pei G, et al. Renal interstitial infiltration and tertiary lymphoid organ neogenesis in IgA nephropathy. Clin J Am Soc Nephrol 2014;9(2):255–64.
[67] Miyanaga T, et al. Tertiary lymphoid tissue in early-stage IgG4-related tubulointerstitial nephritis incidentally detected with a tumor lesion of the ureteropelvic junction: a case report. BMC Nephrol 2021;22(1):34.
[68] Sato Y, et al. Heterogeneous fibroblasts underlie age-dependent tertiary lymphoid tissues in the kidney. JCI Insight 2016;1(11):e87680.
[69] Sato Y, Takahashi M, Yanagita M. Pathophysiology of AKI to CKD progression. Semin Nephrol 2020;40(2):206–15.
[70] Pitzalis C, et al. Ectopic lymphoid-like structures in infection, cancer and autoimmunity. Nat Rev Immunol 2014;14(7):447–62.
[71] Sato Y, et al. Developmental stages of tertiary lymphoid tissue reflect local injury and inflammation in mouse and human kidneys. Kidney Int 2020;98(2):448–63.
[72] He N, et al. Association of Serum CXCL13 with Intrarenal ectopic lymphoid tissue formation in lupus nephritis. J Immunol Res 2016;2016:4832543.
[73] Masum MA, et al. Vasculature-associated lymphoid tissue: a unique tertiary lymphoid tissue correlates with renal lesions in lupus nephritis mouse model. Front Immunol 2020;11:595672.
[74] Dorraji SE, et al. Kidney tertiary lymphoid structures in lupus nephritis develop into large interconnected networks and resemble lymph nodes in gene signature. Am J Pathol 2020;190(11):2203–25.
[75] Lucchesi D, Bombardieri M. The role of viruses in autoreactive B cell activation within tertiary lymphoid structures in autoimmune diseases. J Leukoc Biol 2013; 94(6):1191–9.
[76] Dieu-Nosjean MC, et al. Tertiary lymphoid structures in cancer and beyond. Trends Immunol 2014;35(11):571–80.
[77] Suarez-Fueyo A, et al. T cells and autoimmune kidney disease. Nat Rev Nephrol 2017;13(6):329–43.
[78] Sharabi A, Tsokos GC. T cell metabolism: new insights in systemic lupus erythematosus pathogenesis and therapy. Nat Rev Rheumatol 2020;16(2): 100–12.
[79] Li Y, et al. Phosphorylated ERM is responsible for increased T cell polarization, adhesion, and migration in patients with systemic lupus erythematosus. J Immunol 2007;178(3):1938–47.
[80] Suarez-Fueyo A, et al. Hyaluronic acid synthesis contributes to tissue damage in systemic lupus Erythematosus. Front Immunol 2019;10:2172.
[81] Biswas PS, et al. Phosphorylation of IRF4 by ROCK2 regulates IL-17 and IL-21 production and the development of autoimmunity in mice. J Clin Invest 2010;120 (9):3280–95.
[82] Crispin JC, et al. Expression of CD44 variant isoforms CD44v3 and CD44v6 is increased on T cells from patients with systemic lupus erythematosus and is correlated with disease activity. Arthritis Rheum 2010;62(5):1431–7.
[83] Kroeger DR, Milne K, Nelson BH. Tumor-infiltrating plasma cells are associated with tertiary lymphoid structures, Cytolytic T-cell responses, and superior prognosis in ovarian Cancer. Clin Cancer Res 2016;22(12):3005–15.
[84] Goc J, et al. Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8+ T cells. Cancer Res 2014;74(3):705–15.
[85] de Chaisemartin L, et al. Characterization of chemokines and adhesion molecules associated with T cell presence in tertiary lymphoid structures in human lung cancer. Cancer Res 2011;71(20):6391–9.
[86] Gobert M, et al. Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. Cancer Res 2009;69(5):2000–9.
[87] Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature 2011;480(7378):480–9.
[88] Montfort A, et al. A strong B-cell response is part of the immune landscape in human high-grade serous ovarian metastases. Clin Cancer Res 2017;23(1): 250–62.
[89] Shivakumar S, Tsokos GC, Datta SK. T cell receptor alpha/beta expressing double-negative (CD4-/CD8-) and CD4+ T helper cells in humans augment the production of pathogenic anti-DNA autoantibodies associated with lupus nephritis. J Immunol 1989;143(1):103–12.
[90] Rodriguez-Rodriguez N, et al. Pro-inflammatory self-reactive T cells are found within murine TCR-alphabeta(+) CD4(−) CD8(−) PD-1(+) cells. Eur J Immunol 2016;46(6):1383–91.
[91] Thomson CW, Lee BP, Zhang L. Double-negative regulatory T cells: non-conventional regulators. Immunol Res 2006;35(1–2):163–78.
[92] Rodriguez-Rodriguez N, et al. Programmed cell death 1 and Helios distinguish TCR-alphabeta+ double-negative (CD4-CD8-) T cells that derive from self-reactive CD8 T cells. J Immunol 2015;194(9):4207–14.
[93] Li H, et al. Systemic lupus erythematosus favors the generation of IL-17 producing double negative T cells. Nat Commun 2020;11(1):2859.
[94] Suzuki N, et al. Preferential adsorption of cationic anti-DNA antibodies with immobilized polyanionic compounds, dextran sulfate. Autoimmunity 1994;19(2): 105–12.
[95] Yamagata T, Skepner J, Yang J. Targeting Th17 effector cytokines for the treatment of autoimmune diseases. Arch Immunol Ther Exp (Warsz) 2015;63(6): 405–14.
[96] Kanaya M, et al. Simple determination of acid mucopolyssacharides in the ascitic fluid. Rinsho Byori 1974;22(10 Suppl):160.
[97] Turner JE, et al. The Th17 immune response in renal inflammation. Kidney Int 2010;77(12):1070–5.
[98] Deteix C, et al. Intragraft Th17 infiltrate promotes lymphoid neogenesis and hastens clinical chronic rejection. J Immunol 2010;184(9):5344–51.
[99] Mitsdoerffer M, et al. Proinflammatory T helper type 17 cells are effective B-cell helpers. Proc Natl Acad Sci U S A 2010;107(32):14292–7.
[100] Schaffert H, et al. IL-17-producing CD4(+) T cells contribute to the loss of B-cell tolerance in experimental autoimmune myasthenia gravis. Eur J Immunol 2015; 45(5):1339–47.
[101] Crispin JC, et al. Expanded double negative T cells in patients with systemic lupus erythematosus produce IL-17 and infiltrate the kidneys. J Immunol 2008;181(12): 8761–6.
[102] Ueno H. T follicular helper cells in human autoimmunity. Curr Opin Immunol 2016;43:24–31.
[103] Lahdes K, et al. Systemic absorption of topically applied ocular atropine. Clin Pharmacol Ther 1988;44(3):310–4.
[104] Bacon BR, Bailey-Newton RS, Connors Jr AF. Pleural effusions after endoscopic variceal sclerotherapy. Gastroenterology 1985;88(6):1910–4.
[105] Peterson RA. Regulatory T-cells: diverse phenotypes integral to immune homeostasis and suppression. Toxicol Pathol 2012;40(2):186–204.
[106] Chandran S, Feng S. Current status of tolerance in kidney transplantation. Curr Opin Nephrol Hypertens 2016;25(6):591–601.
[107] Kasper IR, et al. Empowering regulatory T cells in autoimmunity. Trends Mol Med 2016;22(9):784–97.
[108] Park CO, Kupper TS. The emerging role of resident memory T cells in protective immunity and inflammatory disease. Nat Med 2015;21(7):688–97.
[109] Zhou G, et al. Identification of systemically expanded activated T cell clones in MRL/lpr and NZB/W F1 lupus model mice. Clin Exp Immunol 2004;136(3): 448–55.
[110] Kato T, et al. Analysis of accumulated T cell clonotypes in patients with systemic lupus erythematosus. Arthritis Rheum 2000;43(12):2712–21.
[111] Jing F, Choi EY. Potential of cells and cytokines/chemokines to regulate tertiary lymphoid structures in human diseases. Immune Netw 2016;16(5):271–80.
[112] Bombardieri M, et al. Activation-induced cytidine deaminase expression in follicular dendritic cell networks and interfollicular large B cells supports functionality of ectopic lymphoid neogenesis in autoimmune sialoadenitis and MALT lymphoma in Sjogren's syndrome. J Immunol 2007;179(7):4929–38.
[113] Croia C, et al. Implication of Epstein-Barr virus infection in disease-specific autoreactive B cell activation in ectopic lymphoid structures of Sjogren's syndrome. Arthritis Rheumatol 2014;66(9):2545–57.
[114] Nacionales DC, et al. B cell proliferation, somatic hypermutation, class switch recombination, and autoantibody production in ectopic lymphoid tissue in murine lupus. J Immunol 2009;182(7):4226–36.
[115] Weinstein JS, et al. Maintenance of anti-Sm/RNP autoantibody production by plasma cells residing in ectopic lymphoid tissue and bone marrow memory B cells. J Immunol 2013;190(8):3916–27.
[116] Qin D, et al. Fc gamma receptor IIB on follicular dendritic cells regulates the B cell recall response. J Immunol 2000;164(12):6268–75.
[117] Suzuki K, et al. Visualizing B cell capture of cognate antigen from follicular dendritic cells. J Exp Med 2009;206(7):1485–93.
[118] Tumanov AV, et al. Lymphotoxin and TNF produced by B cells are dispensable for maintenance of the follicle-associated epithelium but are required for development of lymphoid follicles in the Peyer's patches. J Immunol 2004;173(1): 86–91.
[119] Shen P, Fillatreau S. Antibody-independent functions of B cells: a focus on cytokines. Nat Rev Immunol 2015;15(7):441–51.
[120] Badr G, et al. BAFF enhances chemotaxis of primary human B cells: a particular synergy between BAFF and CXCL13 on memory B cells. Blood 2008;111(5): 2744–54.
[121] Ng LG, et al. B cell-activating factor belonging to the TNF family (BAFF)-R is the principal BAFF receptor facilitating BAFF costimulation of circulating T and B cells. J Immunol 2004;173(2):807–17.
[122] Liarski VM, et al. Cell distance mapping identifies functional T follicular helper cells in inflamed human renal tissue. Sci Transl Med 2014;6(230). 230ra46.
[123] Hu H, et al. Noncanonical NF-kappaB regulates inducible costimulator (ICOS) ligand expression and T follicular helper cell development. Proc Natl Acad Sci U S A 2011;108(31):12827–32.
[124] Coquery CM, et al. BAFF regulates follicular helper t cells and affects their accumulation and interferon-gamma production in autoimmunity. Arthritis Rheumatol 2015;67(3):773–84.
[125] Kang S, et al. IgG-immune complexes promote B cell memory by inducing BAFF. J Immunol 2016;196(1):196–206.
[126] Luther SA, Ansel KM, Cyster JG. Overlapping roles of CXCL13, interleukin 7 receptor alpha, and CCR7 ligands in lymph node development. J Exp Med 2003; 197(9):1191–8.
[127] Drayton DL, et al. Lymphoid organ development: from ontogeny to neogenesis. Nat Immunol 2006;7(4):344–53.
[128] Luther SA, et al. BLC expression in pancreatic islets causes B cell recruitment and lymphotoxin-dependent lymphoid neogenesis. Immunity 2000;12(5):471–81.
[129] Greenbaum A, et al. CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. Nature 2013;495(7440):227–30.

[130] Okada T, et al. Chemokine requirements for B cell entry to lymph nodes and Peyer's patches. J Exp Med 2002;196(1):65–75.
[131] Luther SA, et al. Differing activities of homeostatic chemokines CCL19, CCL21, and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis. J Immunol 2002;169(1):424–33.
[132] Chen SC, et al. Ectopic expression of the murine chemokines CCL21a and CCL21b induces the formation of lymph node-like structures in pancreas, but not skin, of transgenic mice. J Immunol 2002;168(3):1001–8.
[133] Sundstrom P, et al. Human IgA-secreting cells induced by intestinal, but not systemic, immunization respond to CCL25 (TECK) and CCL28 (MEC). Eur J Immunol 2008;38(12):3327–38.
[134] Castelletti E, et al. The mucosae-associated epithelial chemokine (MEC/CCL28) modulates immunity in HIV infection. PLoS One 2007;2(10):e969.
[135] Rainone V, et al. CCL28 induces mucosal homing of HIV-1-specific IgA-secreting plasma cells in mice immunized with HIV-1 virus-like particles. PLoS One 2011;6(10):e26979.
[136] Jiang P, et al. Enhancing CCL28 expression through the gene transfer to salivary glands for controlling cariogenic microbe. Cytokine 2012;59(1):94–9.
[137] Eksteen B, et al. Epithelial inflammation is associated with CCL28 production and the recruitment of regulatory T cells expressing CCR10. J Immunol 2006;177(1):593–603.
[138] Ren L, et al. Hypoxia-induced CCL28 promotes recruitment of regulatory T cells and tumor growth in liver cancer. Oncotarget 2016;7(46):75763–73.
[139] Drayton DL, et al. Ectopic LT alpha beta directs lymphoid organ neogenesis with concomitant expression of peripheral node addressin and a HEV-restricted sulfotransferase. J Exp Med 2003;197(9):1153–63.
[140] Kratz A, et al. Chronic inflammation caused by lymphotoxin is lymphoid neogenesis. J Exp Med 1996;183(4):1461–72.
[141] Matsumoto M, et al. Affinity maturation without germinal centres in lymphotoxin-alpha-deficient mice. Nature 1996;382(6590):462–6.
[142] Sacca R, et al. Lymphotoxin: from chronic inflammation to lymphoid organs. J Inflamm 1995;47(1–2):81–4.
[143] Grabner R, et al. Lymphotoxin beta receptor signaling promotes tertiary lymphoid organogenesis in the aorta adventitia of aged ApoE−/− mice. J Exp Med 2009;206(1):233–48.
[144] Lotzer K, et al. Mouse aorta smooth muscle cells differentiate into lymphoid tissue organizer-like cells on combined tumor necrosis factor receptor-1/lymphotoxin beta-receptor NF-kappaB signaling. Arterioscler Thromb Vasc Biol 2010;30(3):395–402.
[145] Jacob CO, et al. Prevention of diabetes in nonobese diabetic mice by tumor necrosis factor (TNF): similarities between TNF-alpha and interleukin 1. Proc Natl Acad Sci U S A 1990;87(3):968–72.
[146] Goya S, et al. Sustained interleukin-6 signalling leads to the development of lymphoid organ-like structures in the lung. J Pathol 2003;200(1):82–7.
[147] Rangel-Moreno J, et al. Inducible bronchus-associated lymphoid tissue (iBALT) in patients with pulmonary complications of rheumatoid arthritis. J Clin Invest 2006;116(12):3183–94.
[148] Schiffer L, et al. Short term administration of costimulatory blockade and cyclophosphamide induces remission of systemic lupus erythematosus nephritis in NZB/W F1 mice by a mechanism downstream of renal immune complex deposition. J Immunol 2003;171(1):489–97.
[149] Peng SL, Moslehi J, Craft J. Roles of interferon-gamma and interleukin-4 in murine lupus. J Clin Invest 1997;99(8):1936–46.
[150] Haas C, Ryffel B, Le Hir M. IFN-gamma is essential for the development of autoimmune glomerulonephritis in MRL/Ipr mice. J Immunol 1997;158(11):5484–91.
[151] Winchester R, et al. Immunologic characteristics of intrarenal T cells: trafficking of expanded CD8+ T cell beta-chain clonotypes in progressive lupus nephritis. Arthritis Rheum 2012;64(5):1589–600.
[152] Seeger H, Bonani M, Segerer S. The role of lymphatics in renal inflammation. Nephrol Dial Transplant 2012;27(7):2634–41.
[153] Baluk P, et al. Functionally specialized junctions between endothelial cells of lymphatic vessels. J Exp Med 2007;204(10):2349–62.
[154] Ruddle NH. Lymphatic vessels and tertiary lymphoid organs. J Clin Invest 2014;124(3):953–9.
[155] Kerjaschki D, et al. Lymphatic endothelial progenitor cells contribute to de novo lymphangiogenesis in human renal transplants. Nat Med 2006;12(2):230–4.
[156] Kerjaschki D, et al. Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. J Am Soc Nephrol 2004;15(3):603–12.
[157] Motallebzadeh R, et al. Blocking lymphotoxin signaling abrogates the development of ectopic lymphoid tissue within cardiac allografts and inhibits effector antibody responses. FASEB J 2012;26(1):51–62.
[158] Mounzer RH, et al. Lymphotoxin-alpha contributes to lymphangiogenesis. Blood 2010;116(12):2173–82.
[159] Liao S, et al. Transgenic LacZ under control of Hec-6st regulatory sequences recapitulates endogenous gene expression on high endothelial venules. Proc Natl Acad Sci U S A 2007;104(11):4577–82.
[160] Huang Y, et al. Identification of novel genes associated with renal tertiary lymphoid organ formation in aging mice. PLoS One 2014;9(3):e91850.
[161] Mandala S, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science 2002;296(5566):346–9.
[162] Maki T, et al. Prevention and cure of autoimmune diabetes in nonobese diabetic mice by continuous administration of FTY720. Transplantation 2005;79(9):1051–5.
[163] Tsuji T, et al. Therapeutic approach for type 1 diabetes mellitus using the novel immunomodulator FTY720 (fingolimod) in combination with once-daily injection of insulin glargine in non-obese diabetic mice. J Diabetes Investig 2012;3(2):132–7.
[164] Penaranda C, et al. Prevention of diabetes by FTY720-mediated stabilization of peri-islet tertiary lymphoid organs. Diabetes 2010;59(6):1461–8.
[165] Rantakari P, et al. The endothelial protein PLVAP in lymphatics controls the entry of lymphocytes and antigens into lymph nodes. Nat Immunol 2015;16(4):386–96.
[166] Stranford S, Ruddle NH. Follicular dendritic cells, conduits, lymphatic vessels, and high endothelial venules in tertiary lymphoid organs: parallels with lymph node stroma. Front Immunol 2012;3:350.
[167] Cohen JN, et al. Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. J Exp Med 2010;207(4):681–8.
[168] Rouhani SJ, et al. Roles of lymphatic endothelial cells expressing peripheral tissue antigens in CD4 T-cell tolerance induction. Nat Commun 2015;6:6771.
[169] Tewalt EF, et al. Lymphatic endothelial cells induce tolerance via PD-L1 and lack of costimulation leading to high-level PD-1 expression on CD8 T cells. Blood 2012;120(24):4772–82.
[170] Pisitkun P, et al. Interleukin-17 cytokines are critical in development of fatal lupus glomerulonephritis. Immunity 2012;37(6):1104–15.
[171] Zhang Z, Kyttaris VC, Tsokos GC. The role of IL-23/IL-17 axis in lupus nephritis. J Immunol 2009;183(5):3160–9.
[172] Genovese MC, et al. One-year efficacy and safety results of secukinumab in patients with rheumatoid arthritis: phase II, dose-finding, double-blind, randomized, placebo-controlled study. J Rheumatol 2014;41(3):414–21.
[173] Peters A, et al. Th17 cells induce ectopic lymphoid follicles in central nervous system tissue inflammation. Immunity 2011;35(6):986–96.
[174] Pikor NB, et al. Integration of Th17- and Lymphotoxin-derived signals initiates meningeal-resident stromal cell remodeling to propagate Neuroinflammation. Immunity 2015;43(6):1160–73.
[175] Rangel-Moreno J, et al. The development of inducible bronchus-associated lymphoid tissue depends on IL-17. Nat Immunol 2011;12(7):639–46.
[176] Chen H, Ye F, Guo G. Revolutionizing immunology with single-cell RNA sequencing. Cell Mol Immunol 2019;16(3):242–9.