# T Cells and B Cells in Lupus Nephritis

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***Summary:*** T and B lymphocytes play diverse roles at multiple stages in the development and progression of lupus nephritis. Disruption of T- and B-cell regulatory functions by environmental and genetic influences permits pathogenic effectors to emerge in disease. New insights into the biology of these multifunctional cells offer novel targets for intervention in lupus nephritis and systemic autoimmunity.
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The immune system plays a central role in the pathogenesis of systemic lupus erythematosus. This review focuses on B and T lymphocytes, 2 pivotal cellular components of adaptive immunity and key culprits in mediation of glomerulonephritis, a common and serious complication of lupus. Glomerular immunoglobulin (Ig) deposition and renal lymphocyte and macrophage infiltration lead to clinical renal involvement at some point in the clinical course of up to 75% of lupus patients. Lymphocytes and their soluble mediators participate at all levels in disease pathogenesis: initiation, perpetuation, amplification, regulation, tissue and organ destruction, and disease relapse. They thus pose prime targets for novel interventions. Intense laboratory and clinical investigation over the past decade has brought important insights into cellular subsets, signaling, mediators and regulatory pathways, and introduced promising new immunotherapies.

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## INITIATION OF NEPHRITOGENIC AUTOIMMUNE RESPONSES

### Tolerance and Autoimmune Susceptibility

Lupus is a disease of failed immune tolerance to self. The repertoire of Ig and T-cell receptors is vast and self-recognition is common, generated by gene recombinatorial and somatic events in the thymus and bone marrow and, in the case of mature B cells, by somatic mutation in the peripheral lymphoid organs. It is estimated that up to 75% of newly formed, and up to 40% of mature germinal center, B cells recognize self-antigens.[1,2] This autoreactivity normally is held in check by regulatory mechanisms that operate on developing lymphocytes in the central lymphoid organs and on mature cells in the spleen and lymph nodes (Table 1).[3]

Specific tolerance is induced by interaction of the lymphocyte antigen receptor with self-antigen, either in native conformation for recognition by B cells or as peptide antigen presented by cell-bound major histocompatibility complex (MHC) molecules for recognition by T-cell receptor (TCR)-$\alpha/\beta$–expressing T cells (Fig. 1). The strength of this signal may influence the type of tolerance induced (deletion or anergy). Induction of tolerance by antigen contact requires an appropriate microenvironment and noninflammatory milieu that limits lymphocyte signaling to that initiated by antigen alone, also referred to as *signal 1*. Delivery of signal 2 to an autoreactive lymphocyte converts a

**Table 1. Mechanisms Regulating Autoimmunity**

| |
|---|
| Deletion |
| Anergy |
| Receptor editing |
| Dual-receptor expression |
| Clonal ignorance |
| Peritoneal homing |
| Follicular exclusion |
| Competition for survival factors or cognate cell help |
| Regulatory B- and T-cell subsets* |
| Th1, Th2, and Th17 skewing |
| Immune-suppressive cytokines (TGF-$\beta$, IL-10) |
| Receptor and coreceptor modulation |
| Idiotypic networks |
| Antigen sequestration |

*IL-10– or TGF-$\beta$–producing B cells, prototypic CD4+CD25+ Treg, T-helper-3 (Th3) T cells, T regulatory type 1 (Tr1) T cells, and inhibitory CD8+, TCR-$\gamma/\delta$, or NK T cells.

tolerogenic signal into an activating one, triggering an autoimmune response. Signal 2 typically is delivered by costimulatory molecules expressed on activated antigen-presenting cells (APCs) and lymphocytes. Major costimulators involved in T- and B-cell collaboration are CD40/CD40L and members of the B7/CD28 family (B7/CD28, inducible costimulator ligand [ICOSL]/inducible costimulator [ICOS]).[4] In addition, potent mitogens or microbial products can trigger danger signals that permit bypass of tolerance by binding Toll-like receptors (TLRs) on B cells and APCs.[5]

Failure or bypass of one or more regulatory mechanisms permits autoreactive lymphocytes to survive and expand (Table 2, Fig. 2). Dysregulation of sufficient magnitude or at a sufficient number of checkpoints in combination with lymphocyte activation, antigen exposure, and a permissive end-organ microenvironment ultimately leads to tissue destruction and disease. Dysregulation results from environmental exposures and inherited autoimmune susceptibility, often acting in concert.[6,7] Considerable data suggest that different influences alter different pathways, and that different regulatory mechanisms are breached in different individuals. It is evident from animal models that a wide variety of immune abnormalities can lead to a similar lupus-like disease.

Exogenous agents can provoke acquired defects in immune tolerance leading to a lupus phenotype. Procainamide and hydralazine induce systemic autoimmunity in human beings and rodents. In vitro they inhibit DNA methylation and upregulate T-cell lymphocyte function-associated antigen-1 (LFA-1) costimulator expression to promote T-cell autoreactivity. Adoptive transfer of T cells overexpressing LFA-1 induces lupus-like disease including glomerulonephritis in naive recipient mice.[8] Procainamide also has been shown to alter T-cell thymic selection and signaling thresholds.[9] Mercuric chloride and gold induce Th2-type autoreactive CD4+ T cells, interleukin (IL) 4 production, and an interferon (IFN)-$\gamma$–dependent lupus nephritis in rodents.[10,11] A similar phenotype is reported in allogeneic reactions. Heavy metals, hydrocarbon oil (pristane), and microbial products are potent B-cell mitogens, with the potential to activate nontolerant (ignorant) autoreactive cells or reverse anergy.

Autoimmune susceptibility arises from allelic variants or mutations in genes encoding a variety of immune-relevant proteins of diverse function.[7] Transgenic overexpression or targeted disruption of molecules involved in B- and T-cell signaling, lymphocyte selection, adhesion, activation, and survival can lead to spontaneous lupus-like disease and nephritis. Genetic mapping in murine lupus strains (MRL/lpr, [NZBxNZW]F1, BXSB, NZM2410) and their congenic variants has identified loci that modify tolerance. Autoreactive B- and T-cell–receptor transgenes established on lupus-prone backgrounds reveal defects in B-cell tolerance, induction of T-cell anergy, and defects in central T-cell deletion.[12,13] Studies in human lupus reveal associations with MHC class II alleles, molecules critical to antigen presentation, complement components that regulate B-cell activation, tolerance and effector functions, and antibody constant region fragment (Fc)-$\gamma$ receptors that regulate APC activation.[7]

## T- and B-Cell Abnormalities and Interactions in Induction of Lupus

Abnormalities in B and T cells characterize murine and human lupus, reflecting both lympho-

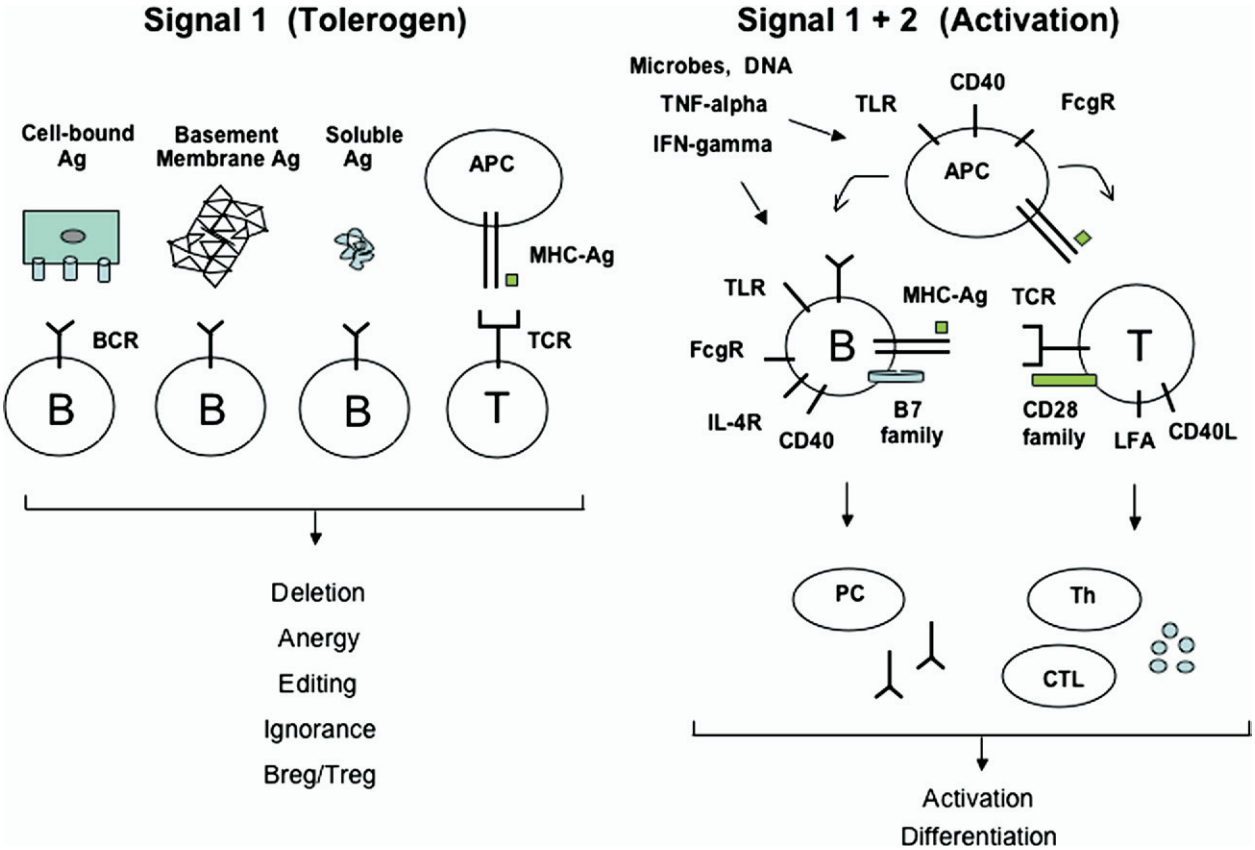


**Figure 1.** Antigen regulation of autoimmunity. Self-antigen contact with the T or B cell receptor can induce specific tolerance or activation in the responding lymphocyte. The outcome of the interaction depends on the microenvironment and whether an inflammatory milieu upregulates costimulatory signals. Ag, antigen; Breg, regulatory B cell; Treg, regulatory T cell; PC, plasma cell; CTL, cytotoxic T lymphocyte.

cyte-intrinsic defects and extrinsic influences.[14-17] Lupus T cells manifest decreased DNA methylation; defective TCR signaling; heightened calcium responses; decreased activation threshold; upregulated expression of costimulatory molecules CD40L, ICOS, $\beta 2$ integrins CD11a/CD18 (LFA-1), and CD11c/CD18 (Mac-1); upregulated focal adhesion kinase pp125FAK; decreased expression of TCR-$\zeta$ chain; increased intracellular phosphorylation; upregulated Fc-$\gamma$ receptor chain expression; mutations in type I protein kinase A regulatory subunit $\alpha$; deficient activation-induced death; deficient IL-2 production; upregulated cyclooxygenase-2; mitochondrial dysfunction; overexpression of perforin; and regulatory defects in cbl and extracellular signal-related kinase pathways. Paradoxic concurrent spontaneous T-cell hyperresponsiveness and defective in vivo and ex vivo T-cell proliferative responses are reported. Lupus B cells are hyperactive and show enhanced phosphorylation of mitogen-activated protein kinase pathways, altered Lyn expression, upregulated bcl2, upregulated CD40L, and defective B-cell receptor (BCR)-induced apoptosis.[18-20] Circulating B-cell–activating factor (BAFF, or BLyS), a B-cell survival factor, is increased in a significant proportion of lupus patients, and mice overexpressing BAFF develop a lupus-like disease.[21] BAFF rescues autoreactive B cells from peripheral deletion and initiates CD40L-independent IgG isotype class switch.[22] Circulating plasmablasts and cells bearing germinal center markers are reported in lupus patients, and plasmablasts and ectopic germinal centers and follicles populate murine lupus kidneys.[18,23]

## Local Regulation of Immune Responses

Renal parenchymal cells, and proximal tubular epithelial cells (TECs) in particular, may play a

**Table 2. Initiation and Amplification of Nephritogenic Autoimmune Responses in Lupus**

| |
|---|
| Altered DNA methylation or histone acetylation* |
| Dendritic cell activation† |
| Antigenic mimicry caused by foreign/self-homology |
| Exposure of cryptic or neo- (modified) self-antigen |
| Expression of dormant self-antigen (heat shock protein) |
| Imbalance or defect in T- or B-cell regulatory subsets |
| Bystander activation |
| Polyclonal activation (mitogens, superantigens, lectins) |
| Biased T-helper subset expansion (Th1/Th2/Th17 shift) |
| Defective, abnormal, or excessive apoptosis‡ |
| Altered antigen receptor signaling (BCR, TCR) |
| Decreased inhibitory receptor signaling (FcgRIIB, CD21, CD22, CD5) |
| Enhanced accessory receptor signaling (TLR, costimulators, activating FcgR, adhesion molecules) |
| Enhanced lymphocyte survival (increased BlyS/BAFF, mutated Fas) |
| Epitope spreading |

*A given type of environmental challenge (infectious agents, chemicals, drugs, pharmacologic agents, ultraviolet light) may induce acquired defects by multiple mechanisms shown here.

†Activation of IFN-$\alpha/\beta$–producing dendritic cells by viruses, nucleic acid–IgG immune complexes, or microbial products may be a major cause of loss of peripheral tolerance.

‡Apoptosis defects may contribute to autoimmunity at multiple levels. Apoptosis is crucial for autoreactive B- and T-cell deletion, and apoptotic cells expose a variety of lupus self-antigens that can either tolerize or immunize, depending on the context.

key role in modulating nephritogenic immune responses in lupus.[24,25] IFN-$\gamma$–stimulated TECs downregulate proliferation in autoreactive T-cell clones derived from MRL/lpr kidneys. Murine lupus TEC express MHC molecules and present antigens ex vivo for recognition by CD4+ T cells. This interaction can lead to either anergy or activation of responding T cells, depending on whether antigen-presenting TECs concomitantly upregulate costimulatory or inhibitory molecules. The reported tissue distribution of the costimulatory family molecules suggest that the pathways most likely engaged in parenchymal cell regulation of T lymphocytes are ICOS/ICOS-L and inhibitory programmed death (PD)-1/PD-L interactions. Collectively, these findings suggest that TEC–T-cell interactions in the kidney are primarily regulatory and maintain tolerance to self-antigen. A similar role may apply to other parenchymal cells, such as mesangial cells, glomerular podocytes, and endothelium, which have inducible antigen-presenting functions.

## T CELLS AND CELLULAR IMMUNITY IN LUPUS NEPHRITIS

### Regulation by T-Cell Subsets

T cells play central and multiple roles in the pathogenesis of lupus nephritis (Fig. 2). They provide help for B-cell production of nephritogenic autoantibodies, regulate B-cell responses, and modulate T-helper (Th) and effector functions and expansion. T cells infiltrate the kidney to injure renal parenchymal cells directly via cytotoxicity or indirectly through activation and recruitment of macrophages and natural killer (NK) cells.

The importance of T cells in lupus is well documented.[15,26] Oligoclonally expanded IFN-$\gamma$–producing T cells autoreactive to classic lupus-associated autoantigens can be isolated from peripheral blood of lupus patients and from lupus mice. Most lines or clones isolated to date are CD4+ TCR-$\alpha/\beta$ phenotype, although recovery of CD8+ and TCR-$\gamma/\delta$ T cells has been reported. An imbalance in the number and function of T-cell subsets has been reported, including an increase in CD4+ relative to CD8+ T cells, increased frequency of circulating double-negative CD4-CD8- T cells, and altered Th1 and Th2 cytokine production. Patient and murine lupus $\alpha/\beta$, $\gamma/\delta$ and CD4-CD8- T cells facilitate autoantibody production by B cells. Chromatin-reactive CD4+ T cells isolated from lupus patients and mice augment production of anti-DNA and antichromatin antibodies.[27] Evidence of T-cell help is found in the lupus autoantibodies themselves; IgG isotype switch and accumulation of replacement mutations sug-

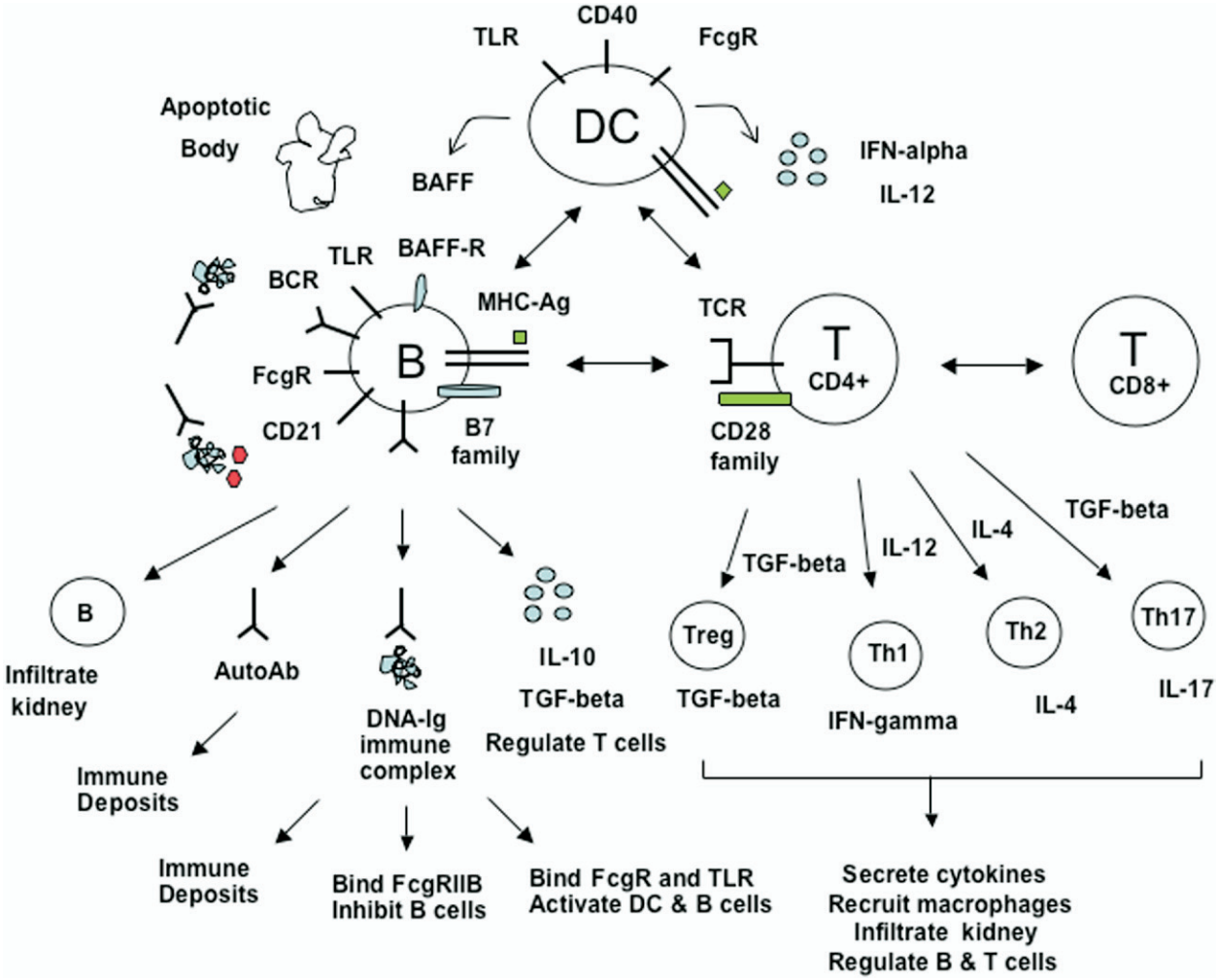


**Figure 2.** Diverse roles for B cells, T cells and dendritic cells (DC) in the control of autoimmunity and pathogenesis of lupus nephritis.

gest that they are products of an antigen-driven T-cell–dependent immune response.

Definitive evidence of the requirement for T cells comes from rodent lupus models, in which T-cell helper and effector subsets have been depleted and costimulatory pathways interrupted using monoclonal antibodies, fusion proteins, and gene-targeted deletion.[28] In general, murine lupus nephritis is delayed and ameliorated by depletion of CD4+ and $\alpha/\beta$ T-cell subsets and antagonism of CD28/B7, CD40/CD40L, and intercellular adhesion molecule-1/LFA-1 costimulatory interactions. Each strategy can delay nephritis and variably decrease proteinuria, improve urea and creatinine clearances, decrease renal histopathologic injury and lymphocytic infiltration, and decrease autoantibody production.[29-31] Recent results from interruption of the ICOS/ICOSL pathway vary by strain and experimental strategy; anti-ICOS monoclonal antibody (mAb) improved nephritis in the BWF1 mouse whereas targeted deletion of ICOS had no effect on MRL/lpr nephritis.[32,33] Efficacy of T depletion and costimulatory blockade in prolonging survival and ameliorating disease in nonrenal organ systems is variable, and the persistence of some lupus manifestations in mice lacking $\alpha/\beta$ T cells suggests $\alpha/\beta$-independent mechanisms.[34]

Notably, each major T-cell subset has regulatory and pathogenic effector roles such that the net effect of a given intervention appears to be context dependent and may be unpredictable. In this regard, deletion of class

I MHC and CD8+ T cells has yielded conflicting observations. Targeted deletion of β2-microglobulin, which eliminates both classic and nonclassic (CD1) MHC class I expression and thus eliminates CD8+ and NK1 T cells, ameliorates kidney injury but accelerates skin disease in MRL/lpr mice.[35,36] The effect is attributed to CD8+ T-cell depletion, because isolated CD1 deletion does not alter MRL/lpr nephritis. Conversely, β2-microglobulin deletion in BWF1 mice accelerates disease,[37] and prolonged anti-CD8 mAb treatment fails to alter disease in MRL/lpr and (NZWxBXSB)F1 mice, in contrast to a favorable response induced with anti-CD4 mAb.[38,39]

TCR-γ/δ T cells and NKT cells play an equally complex role in lupus.[40] MRL/lpr mice lacking TCR-γ/δ T cells develop accelerated nephritis and mortality accompanied by polyclonal expansion of CD4+ T cells, suggesting a dominant-negative regulatory function for this T-cell subset.[41] Conversely, TCR-γ/δ effectors appear to be responsible for the residual mild autoimmunity and nephritis observed in TCR-α/β-deficient MRL/lpr mice because autoantibody production and disease are absent in TCR-α/β TCR-γ/δ double-deficient MRL/lpr mice.[41] NKT cells express an invariant TCR and NK cell receptor NK1.1 and recognize glycolipid presented by CD1d. Activated NKT cells produce immunomodulatory cytokines and mediate cytotoxicity. As noted earlier, CD1 deficiency does not alter MRL/lpr nephritis, whereas in vivo activation of NKT cells has variably been associated with exacerbation and amelioration of murine lupus.[42]

Collectively these observations support an important role for regulatory T-cell subsets in lupus. This is consistent with numerous early reports of abnormalities of T-cell suppression in lupus, including decreased numbers of CD4+ CD25+ T cells in the peripheral blood of SLE patients with active disease.[15] The suppression of autoantibody production and nephritis in lupus mice using tolerogenic peptides to expand CD4+ CD25+ regulatory T cells and transforming growth factors (TGF)-β–producing inhibitory CD8+ T-cell subsets has been reported.[43]

## T-Cell Effector Mechanisms in Lupus

Differentiated T-cell effectors exert their functions by interactions with other cells or through overproduction of immunomodulatory cytokines, such as IFN-γ, tumor necrosis factor (TNF)-α, TGF-β, IL-4, and IL-17. Activated CD4+ and CD8+ T cells, and, in MRL/lpr mice, the unique population of B220+ class I–restricted double-negative CD4-CD8- TCR-α/β T cells are present in glomerular lesions in murine and human lupus, recruited by chemokines to exert actions locally.[44,45] In addition to promoting autoantibody responses, T-cell–generated proinflammatory cytokines amplify disease by upregulating adhesion and MHC class II molecules, recruit macrophages, promote fibrosis, and induce intrarenal nephritogenic cytokines colony-stimulating factor-1, granulocyte-macrophage colony-stimulating factor, and TNF-α.[46,47] Autoreactive double-negative T-cell clones propagated from nephritic lupus mouse kidneys induce MHC class II and intercellular adhesion molecule-1 expression and proliferate in response to renal parenchymal cells.[48] Autoreactive cytotoxic CD4+ TCR-α/β T cells can be isolated from nephritic kidneys of mice with chronic graft-versus-host disease, a lupus-like syndrome with glomerulopathy.[49] Graft-versus-host disease–derived T-cell effectors induce glomerular crescents and a focal mononuclear infiltration when transferred to naive syngeneic recipients by renal subcapsular transfer. In nonlupus model systems, nephritogenic CD4+ and CD8+ T-cell clones have been shown to mediate MHC-restricted, perforin and granzyme-mediated cytotoxicity toward cultured renal proximal tubular cells.[50] It is notable therefore that T-cell cytotoxicity in lupus may be predominantly regulatory, directed toward controlling pathogenic lymphocytes. Cytotoxic responses are mediated primarily through Fas, perforin, and TNF. Deficiency in each of these pathways introduced in lupus-prone mice by gene-targeted deletion of Fas, Fas ligand, perforin, or TNF-receptor leads to acceleration, not improvement, of autoimmunity and nephritis.[51] This may explain in part the association of drug-induced lupus with anti–TNF-α therapy.

Cytokines involved in CD4+ Th1/Th2 T-helper cell differentiation received early attention because of their proinflammatory (IFN-γ, IL-12, TNF-α/β) or anti-inflammatory (IL-4, IL-10) actions and potential therapeutic efficacy. However, in vivo biologic or genetic modulation of cytokine production or function has shown inconsistent results in modulating lupus nephritis.[52] In vivo administration of either IL-10 or IFN-γ accelerates nephritis in lupus-prone BWF1 mice, whereas antibody blockade of either cytokine delays disease. Nephritis in MRL/lpr mice is ameliorated by targeted disruption of either IFN-γ or IL-4. Administration of IL-12 accelerates nephritis and promotes intrarenal accumulation of IFN-γ–secreting CD4+, CD8+, and double-negative T cells in lupus mice. These results fail to implicate disease dependence on a single CD4+ T-helper subset. Notably, levels of both Th1 and Th2 cytokines are increased in murine lupus, and proteinuria and glomerulonephritis in MRL/lpr lupus is ameliorated by histone deacetylase inhibitors that downregulate transcription of both subsets (IL-12, IFN-γ, IL-6, and IL-10).[53]

Recognition that IL-23 and IL-27, additional members of the IL-6/IL-12 family, influence Th1 responses and identification of a third lineage of CD4+ T-helper cell, termed *Th17 cells*, explain some of the previous conflicting findings. Th17 cells develop under the influence of IL-6, TGF-β, and IL-23 and produce the proinflammatory cytokine IL-17.[54] Notably, deficiency for WSX-1, a component of the IL-27 receptor, alters the cytokine profile and phenotype of lupus glomerulonephritis in MRL/lpr lupus mice.[55] A significant decrease in IFN-γ production by CD4+ T cells accompanies a shift from diffuse proliferative glomerulonephritis (GN) to a clinical and pathologic picture resembling human membranous nephropathy. The importance and nature of local cytokines that mediate renal immune injury was elucidated using an innovative strategy of implanting genetically modified cytokine-secreting renal tubular cells under the kidney capsule. IL-12, macrophage growth factors, and the chemokine RANTES, but not IL-6, elicit autoimmune injury in lupus mouse kidneys.[56]

**Table 3. Diverse Roles for B Cells in Immunity and Lupus**

| |
|---|
| Produce pathogenic autoantibodies |
| Produce regulatory antibodies (anti-idiotypic, FcgRIIB-binding, mediator-neutralizing) |
| Produce immunomodulatory cytokines (IL-10, IL-6, TGF-β, IFN-γ, IL-12) |
| Differentiate into polarized B-cell effectors |
| Process and present antigen to T cells for activation or tolerance induction |
| Coordinate T-cell migration and differentiation |
| Directly cross-regulate Th1 and Th2 cell differentiation |
| Inhibit or delete T-cell effectors via direct contact or TGF-β |
| Recruit CD8+ and NKT regulatory T cells |
| Promote the development of follicular dendritic cells |
| Regulate dendritic-cell cytokine production |
| Maintain secondary lymphoid architecture |

## B CELLS AND HUMORAL IMMUNITY IN LUPUS NEPHRITIS

### Multifunctional B Cells

B cells serve diverse regulatory functions in immune biology and lupus (Fig. 2 and Table 3).[57,58] Although best appreciated for antibody release, B-cell production of regulatory cytokines and direct interactions with T cells and dendritic cells have a profound impact in shaping cellular immune responses. B cells are highly efficient antigen-presenting cells, capable of capturing antigens via cell-surface Ig receptors for subsequent internalization, processing, and presentation via class I or II MHC molecules. B-cell presentation of autoantigen peptides directly activates autoreactive T cells. B cells modulate T-cell memory and regulate dendritic cell development and activation. Distinct subsets of differentiated effector and regulatory B cells produce significant amounts of immune modulatory cytokines, such as IL-10 and TGF-β.[59] IL-10 suppresses macrophage activation and cytokine production and skews T-helper differentiation toward a Th2 phenotype. Notably, generation of IL-10–producing regulatory B cells occurs under inflammatory conditions, appears

to involve a marginal zone B cell precursor, occurs in human beings, and can be induced by TLR signaling. This latter observation is consistent with recent reports of apparent paradoxic immunosuppressive effects of TLR agonists. TGF-$\beta$ suppresses inflammation, induces apoptosis in effector T cells, and skews T-helper differentiation toward a Th17 phenotype. Experimental manipulations suggest multiple additional mechanisms, many of which are listed in Table 3, by which B cells modulate T-cell expansion and function, induce T-cell tolerance, and dampen inflammation. Secreted antibodies also function in regulation, via protective self-antigen specificities, IgG constant region engagement of inhibitory FcgRIIB receptors on APCs, or within regulatory idiotypic networks.[60] There has been a recent resurgence of interest in the regulatory role of B cells and autoantibodies with the discovery of B-cell dependence in autoimmune disease models previously thought to be primarily T-cell mediated and, conversely, exacerbation of several murine autoimmune and inflammatory diseases in B-cell–deficient mice as compared with their B-cell–sufficient counterparts. These diverse roles raise some concern regarding the predictability of outcomes with B-cell targeted therapies. Nonetheless, B-cell ablation through gene deletion or anti-CD20 monoclonal antibody administration eliminates early mortality, immune infiltrates, and organ injury in murine lupus, and early reports suggest similar therapeutic efficacy of anti-CD20 therapy in human lupus, suggesting a dominant pathogenic role for B cells in this disease.[19,58]

B cells serve multiple functions in lupus. Best characterized is their production of antinuclear and polyreactive autoantibodies, the cornerstone of diagnosis and characteristic feature of lupus glomerulonephritis. Autoantibodies deposit in the kidney and induce injury by a variety of mechanisms. It is clear, however, that the B-cell contribution to pathogenicity extends beyond antibody production.[58] Nephritis and vasculitis do not develop in B-cell–deficient MRL/lpr mice, whereas glomerulonephritis and early death are unaffected in MRL/lpr mice bearing genetically manipulated B cells incapable of secreting antibody.[61] B-cell–deficient MRL/lpr mice fail to develop the activated CD8 and CD4 T cells found in B-cell–sufficient mice, a finding attributed to loss of B-cell–CD4 T-cell interactions because activated CD8 T cells persist in MRL/lpr mice lacking MHC class I antigens on B cells. Tissue infiltrating antibody-secreting B cells and plasma cells are abundant in nephritic BWF1 kidneys, and B cells secreting nephropathic anti-DNA autoantibodies can be isolated from nephritic MRL/lpr kidneys.[23] BWF1 mice ectopically express a B-cell chemoattractant that promotes renal and other organ trafficking of B-1 B cells.[62] IL-10–producing regulatory B cells also are present in murine lupus where they may play a role in limiting disease. This may in part explain the conflicting results obtained from ablation of the marginal zone B-cell population, a proposed precursor of regulatory B cells, in BWF1 and BXSB lupus-prone mice.[57]

## Nephritogenic Autoantibodies and Antibody-Mediated Injury

Glomerular antibody deposition is a hallmark of lupus glomerulonephritis. Renal bound antibodies engage cell-bound Fc receptors, activate complement, mediate thrombosis, and directly alter cell functions. Mechanisms of immune deposition and injury have been reviewed extensively elsewhere,[63,64] and are summarized briefly here, with emphasis on recent new insights. Extensive investigation has revealed multiple IgG subclasses and diverse antigen specificities, avidity, and charge among Ig eluted from nephritic lupus kidneys or isolated from lupus serum or monoclonal antibodies. IgG is the predominant isotype deposited, but IgM and IgA also typically are present in human lupus nephritis, accompanied by C3, C4, and C1q. Specificity for DNA is important for pathogenesis for a subset of lupus Ig, but multiple experimental models indicate that anti-DNA activity is neither necessary nor sufficient to induce lupus nephritis, and many Ig eluted from lupus kidneys do not bind to DNA.

These nephritogenic antibodies localize to the kidney by multiple mechanisms. In situ formation of immune complexes may be a dominant mechanism of renal Ig deposition. Direct binding of cross-reactive anti-DNA Ig to purified glomerular antigens, including $\alpha$-actinin and

**Table 4. Targeted Immunotherapy in Lupus Nephritis**

| |
|---|
| B- and T-cell signaling and activation thresholds |
| Upregulation or stimulation of B-cell inhibitory Fc-γ RIIB |
| B-cell tolerance induction (dsDNA oligomers)* |
| Inhibition of DNA methylation or histone deacetylation† |
| B- and T-cell survival, proliferation, differentiation, collaboration, and costimulation |
| Anti–B-cell therapy (anti-CD20 and anti-CD19 mAbs) |
| Blockade of BAFF/BAFF-receptor pathway (anti-BAFF mAb, TACI–Ig fusion protein) |
| Modulate activating or inhibitory surface receptors (anti-CD22 mAb) |
| Anti–T-cell subset therapy |
| Blockade of B7/CD28 pathway (CTLA4-Ig, single-chain Fv CD28 inhibitor) |
| Blockade of CD40/CD40L (anti-CD40L mAb) |
| Blockade of ICOS/ICOSL pathway (anti-ICOSL mAb) |
| Blockade of CD137 (4-1BB)/4-1BBL pathway |
| Stimulation of T-cell inhibitory pathways PD-1/PD1-L, CTLA4, BTLA |
| Modulation of TLR signaling (inhibitory GpG oligodeoxynucleotides) |
| Modified antigen-presenting cells (dendritic cell therapy) |
| Regulatory cell function |
| In vivo or ex vivo induction of regulatory T or B cells |
| Administration of tolerizing self-antigen–expressing dendritic or stem cells |
| B- and T-effector cell function and inflammation |
| Blockade of Ig production |
| Inhibition of memory or plasma cell differentiation |
| Blockade or adsorption of autoantibody reactivity |
| Complement inhibition (anti-C5 mAb, soluble Crry or Crry-Fc fusion protein, C5a antagonist) |
| Blockade of activatory FcgRI, FcgRIII, or FcgRIV |
| Stimulation of inhibitory FcgRIIB |
| Inhibition of secondary mediators (cytokines, chemokines, nitric oxide, reactive oxygen species, lipid mediators, and so forth) |
| Antimacrophage or neutrophil therapy |
| T-helper subset skewing |

Abbreviations: dsDNA, double-stranded deoxyribonucleic acid; Crry, complement receptor-1–related gene/protein Y; CTLA-4, cytotoxic T lymphocyte antigen-4; BTLA, B- and T-lymphocyte attenuator.
*Selected examples of interventions currently in preclinical or clinical trials are indicated in parentheses.
†Some interventions modulate multiple checkpoints; for simplicity, only one category is shown.

α-enolase, and to cultured parenchymal cells, can be shown in vitro and in experimental models. Nuclear antigens with intrinsic affinity for glomerular cell or basement membrane surfaces can serve as planted antigens for subsequent binding by anti-DNA and antinucleosome antibodies. Collagen, laminin, fibronectin, and some cells have binding sites for DNA, whereas charge interactions attract anionic DNA to cationic immune complexes, histones, and nucleosomes previously captured by anionic sites in glomerular basement membrane or on cell membranes. Rheumatoid factors and anti-C1q Ig can engage previously deposited IgG or C1q. Murine IgG3 has a unique capacity to form cryoglobulins and occlude glomerular capillaries. Additional mechanisms of renal injury have been proposed: disruption of cell functions by autoantibodies that gain intracellular access, disruption of glomerular permeability by masking anionic sites, thrombus formation caused by interference with phospholipid-dependent coagulation, and complement-mediated cytotoxicity of renal parenchymal cells.

The ultimate renal disease phenotype is dependent on several factors, including antibody specificity and isotype and effector functions determined by Ig constant region domains, each

subclass of which has a unique profile of interactions with complement and multifunctional Fc-γ receptors. Outcomes are regulated in turn by a complex system of regulatory proteins and cytokines. Igs engage activating Fc-γ receptors on macrophages, neutrophils, and renal parenchymal cells to promote leukocyte adhesion and trigger release of multiple inflammatory mediators.[65] However, nephritis is variably attenuated in lupus mice with targeted deletion of the common Fc receptor γ-chain gene.[66,67] Discordant outcomes reflect in part the importance of Fc receptors in immune complex clearance; loss of activating Fc-γ receptors in nonautoimmune mice markedly increases immune deposition.[65] Loss of the inhibitory IgG Fc receptor, FcgRIIB, induces lupus-like nephritis in a strain-specific manner in nonautoimmune mice and accelerates disease in lupus-prone strains.[68]

Notably, secreted IgG, autoantibodies, and antigen–IgG immune complexes also serve regulatory roles to modulate lupus phenotypes. Autoantibodies reactive with phospholipids are implicated in the clearance of apoptotic bodies, one source of lupus self-immunogen. Nucleic acid (DNA and RNA) and chromatin-containing IgG immune complexes are potent amplifiers of autoimmunity. Dendritic cells and B cells capture and internalize DNA/anti-DNA immune complexes by activating Fc-γ receptors, FcgRIIa and FcgRIII, or by IgG-binding (rheumatoid factor) B-cell receptors. This sets the stage for subsequent nucleic acid engagement of TLR9 or TLR7 in cytoplasmic compartments, promoting cell maturation and activation, and, in autoreactive B cells, switch to pathogenic IgG isotypes.[65,69] Conversely, inhibitory Fc-γ RIIB receptors on B cells downmodulate B-cell–receptor signaling and plasma cell expansion.[65] Immune complexes also inhibit B-cell signaling by engaging complement-receptor CD21/CD35; deficiency of this inhibitory receptor interrupts induction of B-cell anergy and, in permissive backgrounds, promotes lupus nephritis.[70]

## SUMMARY AND CONCLUSIONS

T and B lymphocytes play diverse roles at multiple stages in the development and progression of lupus nephritis. Disease phenotype depends on the balance of pathogenic effector and immune regulatory cell subsets and their secreted products. New insights into their biology has brought the promise of new therapies in lupus nephritis (Table 4). Major recent advances include appreciation of a key modulatory function for innate immune elements, novel regulatory roles for B cells and antibodies, a newly discovered T cell subset (Th17), elucidation of additional immune regulatory checkpoints, an expanding list of genetic or acquired lymphocyte abnormalities that predispose to autoreactivity, and recognition of new functions of effector molecule cascades, including components that potently impact both the inductive and effector limbs of disease, sometimes with opposite effects on outcomes. Support for ongoing research in this effort is crucial, to broaden the arsenal of nontoxic targeted interventions for lupus nephritis and systemic autoimmunity.

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