Recent Advances in the Pathogenesis of Lupus Nephritis: Autoantibodies and B Cells

By Wanfang Su and Michael P. Madaio

Although many factors contribute to the clinical presentation and subsequent course of individuals with lupus nephritis, the formation of glomerular immune deposits is typically one of the initial events. In general, breakdown in immunologic tolerance leads to the production of autoreactive B and T cells that, either through direct infiltration and/or their secretory products, initiate inflammation. Immune deposition within glomeruli results in complement activation and recruitment of inflammatory cells, along with activation of endogenous renal cells. This inflammatory cascade leads to secretion of cytokines and chemokines, which in turn attract more infiltrating cells. Up-regulation of lymphoid-derived chemokines further enhance the cellular influx, augmenting inflammation and resulting in further tissue damage. The degree of inflammation is determined by the extent of this invasion along with both the systemic and local responses to the assault. This review focuses mainly on the contributions of pathogenic autoantibodies, autoreactive B cells to lupus nephritis, and potential immunologic therapies for lupus nephritis. Manipulation of both the cells and soluble mediators that initiate and perpetuate the disease are essential to suppressing autoreactivity and inflammation and preventing disease progression.
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INDIVIDUALS WITH SYSTEMIC lupus erythematosus (SLE) spontaneously produce autoantibodies that react with a variety of cellular and extracellular constituents, including DNA and other nucleic acids, nucleoproteins, cytoplasm components, cell surface antigens, and matrix components.[1-5] A large body of work suggests that anti-DNA antibodies play a crucial role in the pathogenesis of lupus nephritis, although autoantibodies with other specificities also participate.[4] Anti-DNA antibodies can be eluted from SLE kidneys; nephritis can be induced either by administration of anti-DNA antibodies to normal mice, or by overexpression of anti-DNA antibodies using a transgenic approach in nonautoimmune strains.[6,7] However, clinical and experimental evidence also suggests that only some anti-DNA antibodies are pathogenic.[8,9] In this regard, not all patients with high levels of circulating autoantibodies develop nephritis. Furthermore, after administration, not all monoclonal anti-DNA antibodies deposit in the kidneys or induce inflammation in nonautoimmune mice.[10] Other factors also contribute. Studies mapping genetic loci to SLE pathogenesis clearly have distinguished genetic loci linked to anti-dsDNA and antinucleosome antibody production from nephritis.[11] Furthermore, lupus-prone mice engineered so they lack either T cells or specific cytokines have limited disease despite abundant autoantibody deposition.[12-14] Nevertheless, autoantibodies form immune deposits and, along with cells and cytokines, participate in the initiation of glomerulonephritis. This discussion focuses on the factors that distinguish nephritogenic lupus antibodies, with emphasis on their mechanisms of deposition. The role of autoreactive B cells, per se, also are considered because strategies to either modulate their activity or eliminate B cells have potential therapeutic benefits.

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Supported by a PHS award (DK 33694) and a renal training grant (DK-07006).

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0270-9295/03/2306-0007$30.00/0
doi:10.1053/S0270-9295(03)00135-9

MECHANISMS OF IMMUNE DEPOSIT FORMATION IN LUPUS NEPHRITIS

How pathogenic autoantibodies form deposits has been the subject of debate. Over the years, 3 theories have emerged regarding how they form deposits: (1) a deposition of preformed, circulating immune complexes; (2) a direct binding of autoantibodies to intrinsic glomerular antigens; and (3) a binding of autoantibodies to autoantigens previously complexed to glomerular antigens. All 3 mechanisms likely participate in lupus nephritis, although their respective contributions differ.

Earlier studies that observed correlation of circulating immune complex levels and nephritis led to the conclusion that the deposition of these complexes was the proximate cause of disease.[15] However, it has been difficult to show that deposition of preformed circulating immune complexes initiates

nephritis.[3,16] After administration to normal animals, there is transient mesangial localization of complexes, however, inflammation is atypical. Furthermore, treatment of lupus-prone mice with DNA-anti-DNA complexes actually prolongs, rather than accentuates, disease by reducing the production of autoantibodies.[17] Nevertheless, immune complexes activate pro-inflammatory programs in renal cells and therefore this process likely contributes to ongoing inflammation in vivo.

Most evidence supports the initiation of immune deposits by in situ mechanisms, through the direct binding of autoantibodies to either intrinsic glomerular antigens or to circulating autoantigens previously localized within glomeruli. Antibodies eluted from lupus kidneys have been observed to bind directly to extracellular glomerular constituents.[1] Furthermore, a distinguishing feature of nephritogenic, murine, monoclonal autoantibodies is their capacity to bind to glomerular antigens. In this regard, many groups have shown that some but not all anti-DNA antibodies formed immune deposits after transfer to normal mice.[16] Of particular relevance, the location of deposition varied among nephritogenic antibodies, and this was related to the location of glomerular antigen bound to form deposits. Both intrinsic glomerular antigens, including cell surface and matrix proteins, and circulating autoantigens with an affinity for glomerular antigens, serve as the nidus for deposition. For example, we have shown that some nephritogenic lupus autoantibodies bind directly to glomerular endothelial or mesangial cells to initiate nephritis,[16] whereas other investigators have observed that some anti-DNA, antihistone, and antinucleosome antibodies bind to nucleosomes previously localized within glomeruli.[18] In the latter situation, charge-charge interactions between nucleosomes and glomeruli facilitate the initial interaction. Differences in autoantibody specificity (eg, which glomerular antigen the antibodies interact with) contribute to the differences in the location of immune deposition commonly observed among individuals. Quantitative differences in circulating autoantibodies and autoantigens also influence deposition, and concentration of immune reactants during filtration facilitates lower affinity interactions and deposit formation. Variation in the location of deposition influences recruitment and access of inflammatory mediators, which together determine the pathologic and clinical disease profile.

AUTOREACTIVE B CELLS IN LUPUS NEPHRITIS

Although the roles for autoantibodies in lupus and nephritis are well accepted, the precise roles of B cells are less appreciated. In addition to the generation of antibody-forming cells, B cells are important antigen-presenting cells for $CD4^+$ T cells.[19,20] They also can secrete a variety of cytokines and chemokines.[21-23]

A clear demonstration for the role of B cells in murine lupus nephritis, independent from autoantibody secretion, was observed when the B-cell inactivating mutation *JhD* was bred onto the MRL/lpr background.[24,25] There was a marked reduction in the number of activated memory T cells and a 10-fold reduction in the number of $CD4^+$ T cells in B-cell deficient mice, compared with B-cell intact controls. Similar effects were observed for $CD8^+$ T cells.[24] This work shows that B cells promote T-cell activation and expansion. Consequently, mice homozygous for the mutation lacked B cells and did not develop any manifestation of nephritis, including T-cell mediated interstitial nephritis and vasculitis. Furthermore, when the B-cell deficient MRL/lpr mice were engineered so they expressed a single surface anti-DNA immunoglobulin (Ig)M, but did not produce soluble Ig, the animals developed nephritis. Although the mice did not develop the full-blown nephritic phenotype,[25] the results established a role for B cells independent of circulating or deposited Ig. Similar conclusions were derived from studies of NZB/WF1 mice with a restricted B-cell repertoire.[26]

Thus, B cells not only promote and expand normal T cells, but they also serve as antigen-presenting cells for activation of autoreactive T cells. These effects are mediated via cell-cell contact through major histocompatibility complex class II, costimulatory (eg, CD80 and CD86), and accessory molecules. There is ample direct evidence that B cells can play critical antigen-presenting cell functions for T cells in normal immune responses.[19,20,27,28] This is particularly important when the antigen is a protein rather than a peptide,[28] and when the B-cell receptor is specific for the antigen.[29] Thus, although B-cell deficient MRL/lpr mice with limited disease activity possess intact dendritic cells and macrophages, they have reduced numbers of spontaneously activated and memory $CD4^+$ T cells.[25] Evidently, the T cells

Table 1. Targeting B Cells, Potential Therapies for Lupus Nephritis

Agents	Mechanisms	Study
Anti-CD20 mAB (Rituximab)	Inhibition of B-cell activation, proliferation, and differentiation	Anolik et al 2003[30]
Anti-CD40L	Blockade of CD40/CD40L interaction	Daikh et al 1997[31] Kalled et al 1998[32] Huang et al 2002[33]
CTLA4/Ig	Blockade of B7/CD28 interaction	Finck et al 1994[34] Daikh et al 2001[35]
CTLA4/Ig+Anti-CD40L	Coblockade of T- and B-cell interactions	Daikh et al 1997[31] Wang et al 2002[36]
Anti-BLys	Inhibition of B-cell proliferation and differentiation	Gross et al 2000[38] Sekut et al 2001[39]
LJP-394	Induction of tolerance of anti-dsDNA producing B cells and reduction of anti-dsDNA antibody	Jones et al 1995[40] Weisman et al 1997[41] Furie et al 2001[42]
Anti–IL-10	Blockade of the effect of B-cell mediator	Ishida et al 1994[43] Llorente et al 2000[44]
Anti–IFN-γ	Inhibition of B cells and reduction of antoantibody production	Haas et al 1998[14]
Anti-C5b mAB	Blockade of complement complex attack	Strand 2001[45] Wang et al 1996[46]
Anti-C3 (Crry-Ig)	Regulation of C3 activity	Bao et al 2003[47]

Abbreviations: mAB, monoclonal antibody; Crry, complement receptor 1–related protein Y.

activated in the absence of B cells are either too few or lack key autoreactivities to promote sustained disease (ie, because autoreactive B cells are not present). In support of this conclusion, there is a reduction in the number of activated T cells in the JHD/lpr mice.[24]

POTENTIAL THERAPIES DIRECTED AT B CELLS IN LUPUS NEPHRITIS

B cells most likely exert pathogenic effects through both direct cell contact and secretion of soluble mediators (Table 1). Based on these observations, new therapeutic targets and strategies have emerged, and a few of them are reviewed briefly. One approach is to deplete B cells (at least transiently) using antibodies versus CD20, a cell surface molecule specific to B cells. CD20 plays an important role in B-cell activation, proliferation, and differentiation, and Rituximab (Maria, Leandre, London, UK), a humanized monoclonal anti-C20 antibody, currently is under evaluation for the treatment of SLE.[30] An alternative strategy is to inhibit T- and B-cell interactions by interfering with CD40L (T cell)-CD40 (B cell) interactions and limiting autoantibody production.[31-33] A similar approach is being considered by inhibiting the interaction between T and B cells (activated B cells up-regulate B7 and B7 also is expressed on antigen-presenting cells) and CD28 (T cells).[31,34,35] Blockade of CD40L/CD40 and/or B7/CD28 interactions have showed a significant reduction in murine lupus nephritis.[31,35,36] More recently, attention has been devoted to BlyS, also known as BAFF, THANK, TALL-1, and zTNF4, and its receptors TACI, BMCA, and BAFF-R. These interactions stimulate B-cell proliferation and differentiation and increase immunoglobulin production.[37] Neutralization of BlyS activity or blockade of its receptors attenuates B-cell mediated inflammation.[38,39] Another strategy involves administration of LJP394, an immunomodulating B-cell agent that interacts with soluble and surface-bound (to B cells) autoantibodies against double-stranded DNA.[40] Animal and human studies showed a decrease in anti-dsDNA levels and proteinuria after LJP394 treatment, but in patients it appears to be most effective in individuals with high-affinity anti-DNA antibodies.[40-42]

Other future potential therapeutic targets include cytokines involved in either B-cell activation or produced by B cells, and complement components. For example, stimulated B cells secrete a variety of cytokines and chemokines that contribute to both autoreactivity and inflammation, including interleukin (IL)-2, IL-6, IL-10, transforming growth

factor β, interferon gamma;, tumor necrosis factor α, and lymphotoxin-β, among others.[21-23] IL-10 levels are increased in patients with active SLE and correlate with disease activity.[43,44] In murine SLE, continuous administration of IL-10 accelerated onset of renal disease, and treatment with an anti–IL-10 antibody delayed disease onset.[43] Interferon γ levels are increased in some forms of lupus, and depletion of interferon γ receptor prevented autoantibody production and glomerulonephritis in lupus-prone mice.[14] Complement proteins play important roles in both amplifying immune complex–initiated inflammatory reactions and in immunologic tolerance, and are good candidates for manipulation.[45] Monoclonal antibody to C5 (anti-C5b) blocks the membrane attacks of complement complex, delays the onset of proteinuria, improves renal histology, and prolongs survival in NZB/W mice.[45,46] Complement receptor 1–related protein Y is a potent regulator that inhibits complement C3 activation. Administration of complement receptor 1–related protein Y Ig, a recombinant protein, to MRL/lpr mice protects against renal disease owing to a significant reduction of C3d.[47]

Thus, therapeutic strategies aimed at either deletion of autoreactive B cells, limiting B-cell activation and/or interactions with other cells, or inhibition of the activity of its secreted products, has the potential to ameliorate lupus nephritis. Table 1 summarizes some of the novel strategies targeting B cells and its mediators. Further definition of the pathophysiologic events that leads to nephritis, perpetuates inflammation, and fosters fibrosis should provide the basis for more rationale therapy in the future.

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