Emerging Concepts Regarding B Cells and Autoantibodies in Murine Lupus Nephritis

B Cells Have Multiple Roles; All Autoantibodies Are Not Equal.

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ABSTRACT

Despite observations linking the severity of lupus nephritis to the quantity and location of glomerular immune deposits, it had been difficult to decipher the primary role of B cells and autoantibodies in this process. Newer technologies have provided the means to evaluate the roles of whole B cell populations and individual immunoglobulins in lupus lesions. In this review, recent advances in this area are summarized, with particular emphasis on work from the authors' laboratories. The results implicate a primary role for B cells and immunoglobulins in lupus nephritis, including glomerular, interstitial, and vascular lesions. Multiple antibody-ligand interactions participate in glomerular immune deposit formation in individuals with lupus nephritis. Recent evidence suggests that in situ formation of immune deposits by either cross-reactivity of autoantibodies with intrinsic glomerular antigens (i.e., anti-DNA antibodies with laminin) or direct interaction of autoantibodies with circulating autoantigens lodged within glomeruli (i.e., anti-DNA antibodies with histone/DNA). The predominant autoantibody-glomerular antigen interaction(s) in a given individual influences the principal location of immune deposition, which in turn influences the pathologic and clinical expression of disease. It is believed that these phenomena contribute to the phenotypic diversity commonly observed among individuals with lupus nephritis. Furthermore, these consequences are dependent on properties unique to both subsets of lupus autoantibodies and to their target antigen ligands within the glomerulus. Thus, the autoantibody variable or antigen binding region, along with the nature and location of the target glomerular antigen (or site of circulating antigen deposition), are influential in initiating these perturbations.

Key Words: Lupus, nephritis, autoantibodies

Lupus nephritis is a complex disorder that involves many cell types and immune reactants. Over the years, there has been considerable debate about the importance of the individual contributors. In this regard, it has been difficult to distinguish the relative contributions of deposited immunoglobulins (Ig), B cells, T cells, other infiltrating cells, and endogenous cells, to both the initiation of the autoimmune response and the induction of specific pathologic lesions. The interdependence of various cell types further complicates identification of the early events in what is undoubtedly a cascade.

Overall, emphasis on the relevance of each of these components to lupus lesions has paralleled recent discoveries and advances in immunology. Early studies emphasized the importance of B cells and autoantibodies, largely because immunoglobulins were invariably present within glomerular lesions, and the morphologic and clinical expression of disease closely paralleled the location, quantity and pro-inflammatory properties of these immune deposits(1,2). Furthermore, the formation of immune deposits was presumed to be the initial event in the inflammatory process. In recent years, however, with rapid advances in the areas of cellular immunology, genetics, and molecular biology, important contributions of T cells, various cytokines, and macrophages, along with resident glomerular and tubular cells, to the pathologic process have been demonstrated(3-51). Additionally, a number of background genes that influence disease expression have been identified (22,50,51). This body of work has greatly enhanced our understanding of lupus nephritis in general, and it is now evident that multiple cell types and other factors play active roles in the pathogenesis of lupus lesions.

B CELLS HAVE MULTIPLE ROLES

In the evolution of thought about lupus nephritis, B cells and autoantibodies have received less attention. At least two technical problems have limited progress in this area: (1) the inability to completely eliminate B cells/immunoglobulins from autoimmune mice (i.e., as was accomplished with thymectomy to eliminate T
cells); and (2) difficulties in the identification of autoantibodies with pathologic properties. With regard to the former, there have been attempts to eliminate B cells in lupus-prone mice by treatment with anti-IgM antibodies from birth (52). The anti-IgM-treated lupus mice did not develop severe glomerulonephritis; however, they had interstitial nephritis and vasculitis. Although these animals had reduced autoantibody levels, low levels of circulating immunoglobulins and B cells complicated interpretation. The use of heterologous antisera to deplete B cells in these experiments was also confounding.

With regard to the latter, understanding of the cellular and molecular events that lead to the production and glomerular localization of pathogenic autoantibodies has been impeded by characterization of nephritogenic immunoglobulins. In this regard, although the presence of anti-DNA, anti-SmRNP, and other autoantibodies are useful diagnostic markers in patients with lupus, serum autoantibody levels frequently do not correlate with disease activity (53-56). Furthermore, serum autoantibody levels in patients without clinical evidence of nephritis can be markedly elevated, and, conversely, are occasionally undetectable in patients with fulminant disease. Of particular relevance, there are multiple inbred strains of mice that spontaneously develop diseases that closely resemble various aspects of human lupus (57,58). Notably, some strains do not develop immune deposit-mediated nephritis despite elevated serum autoantibody levels (58,59). Collectively, these observations suggest that there are distinguishing properties of pathogenic B cells and nephritogenic Ig that enable them to initiate disease. Thus, although lupus has been established as a polygenic disorder with pathologic properties. With regard to the latter, understanding of the cellular and molecular events that lead to the production and glomerular localization of pathogenic autoantibodies has been impeded by characterization of nephritogenic immunoglobulins. In this regard, although the presence of anti-DNA, anti-SmRNP, and other autoantibodies are useful diagnostic markers in patients with lupus, serum autoantibody levels frequently do not correlate with disease activity (53-56). Furthermore, serum autoantibody levels in patients without clinical evidence of nephritis can be markedly elevated, and, conversely, are occasionally undetectable in patients with fulminant disease. Of particular relevance, there are multiple inbred strains of mice that spontaneously develop diseases that closely resemble various aspects of human lupus (57,58). Notably, some strains do not develop immune deposit-mediated nephritis despite elevated serum autoantibody levels (58,59). Collectively, these observations suggest that there are distinguishing properties of pathogenic B cells and nephritogenic Ig that enable them to initiate disease. Thus, although lupus has been established as a polygenic disorder with multiple factors that influence disease expression, there had not been general agreement on the specific contributions of pathogenic B cells and nephritogenic immunoglobulins to the process.

In recent years, by using novel experimental strategies and methodologies, it has become possible to more closely scrutinize the contribution of whole B cell populations, as well as individual B cells and antibodies, to the development of disease. In this discussion, we will focus on this recent information, with particular emphasis on the results of our own investigations. To approach this problem, we and others have used inbred strains of mice that spontaneously develop lupus. In the majority of our work, MRL- lpr/lpr mice were utilized. This strain spontaneously develops lupus with severe nephritis, and the mice die from renal failure at 5 to 6 months of age (58). The pathologic and clinical features of the glomerulonephritis closely resemble diffuse proliferative lupus nephritis in man: immune deposits are abundant at mesangial, subendothelial, and subepithelial locations; hypercellularity, as a result of both cellular infiltration and proliferation of endogenous cells, is associated with heavy proteinuria at 2 to 3 months; and progressive inflammation and fibrosis lead to glomerular obsolescence, interstitial scarring, and renal failure. Vasculitis and interstitial nephritis are prominent features of this disease. In our experience, the vasculitis can usually be detected at the time of onset of glomerulonephritis; the interstitial nephritis is usually present at this time or may appear shortly thereafter. The recessive lpr gene has been recently localized to a mutation in the Fas gene (60), and homozygous lpr mice have massive T-cell expansion, leading to lymphadenopathy and cellular infiltration of multiple organs, including the kidney. The T cells in the spleen and lymph nodes are predominantly CD4—CD8—, however, both CD4+ and CD8+ T cells are also expanded in these animals, and organ infiltration involves primarily CD4+ and CD8+ T cells. The lymphoproliferation accelerates disease, however, it has been dissociated from nephritis (61), and congenic MRL+/+ mice develop a more insidious form of disease in later life (58).

To address the question of whether B cells and/or immunoglobulins are necessary for the development of lupus nephritis, we took advantage of the availability of Jh “knockout” mice ( termed “JhD”), which have a targeted deletion of the Jh immunoglobulin variable gene locus (62). These mice cannot make functional immunoglobulin gene rearrangements, and therefore have neither circulating immunoglobulins nor B cells that express surface immunoglobulin. Initially, mice with the Jh knockout mutation were crossed with MRL-lpr/lpr mice (F1). The F1 mice were then intercrossed, and the F2 mice were examined (63). Adult F2 mice were typed for the lpr gene with Southern blot analysis by using a Fas cDNA as a probe to reveal a restriction fragment length polymorphism (RFLP) in the mutant gene. Polymerase chain reaction analysis of tail DNA, using primers to identify the region of the heavy chain locus deleted by gene targeting, was used to type mice for the JhD mutation. The lpr/lpr mice with (i.e., JhD+/+ or +/+ ) or without (i.e., JhD/JhD) B cells were thus identified and subjected to more thorough examination. This included measurement of circulating immunoglobulins and levels of serum autoantibodies, FACS analysis of lymphoid tissues, and pathologic analysis of kidneys. The lpr+/+ mice with and without B cells were used as additional controls. One cohort of lpr/lpr mice (with and without B cells) was analyzed at 4½ months, and another cohort was evaluated at 6 months for evidence of nephritis.

Both groups of lpr/lpr mice, including those with and without B cells, developed massive lymphadenopathy with expansion of CD4+, CD8+, and double-negative T cells, although in the older cohort, the absence of B cells reduced the lymphadenopathy. As expected, lpr/lpr mice with B cells had markedly elevated autoantibody and total Ig levels, whereas mice without B cells had neither.

Comparative histologic analysis of the kidneys were striking. All of the mice with B cells developed severe nephritis. The type and extent of disease was indistinguishable among JhD++, lpr/lpr, and ++/, lpr/lpr.
animals. (The morphology and severity of nephritis was also indistinguishable from MRL-lpr/lpr mice.) The nephritis included: severe proliferative glomerulonephritis with crescents; intense vasculitis with extensive perivascular lymphocytic infiltration; and severe interstitial nephritis with tubular necrosis. By contrast, none of the B cell-deficient (JhD/JhD) lpr/lpr littermates from either cohort developed clinical or histologic evidence of nephritis. What is particularly noteworthy is that neither glomerulonephritis, nor vasculitis, nor tubulointerstitial nephritis was ever observed in any of the lpr/lpr mice without B cells.

The results indicate that B cells and/or Ig are necessary for the development of murine lupus nephritis, including the glomerular, vasculitic, and interstitial lesions. We suspect that the relative role of B cells and Ig may vary for the different disease components. For example, Ig deposition may be required for the expression of glomerular disease, whereas B cells may serve as antigen-presenting cells for T cells in the initiation of interstitial nephritis. This, of course, is only speculation, and studies are currently planned that will address these issues. The JhD/JhD lpr/lpr mice should provide an ideal milieu for this purpose.

CROSS-REACTIVE ANTIGEN BINDING PROPERTIES DISTINGUISH NEPHRITOGENDIC AUTOANTIBODIES

In parallel studies, we and others have examined the role of individual autoantibodies in the development of lupus nephritis. From clinical observations of patients and other murine strains, it is evident that not all autoantibodies are pathogenic, as many lupus patients and autoimmune strains with high circulating levels of serum autoantibodies levels do not develop disease (57–59). Conversely, there are individuals with relatively low serum autoantibody levels and severe disease. Clearly, multiple factors contribute to the development of nephritis, however, because the lesions appear to be antibody and/or B cell dependent, we postulated that there must be physical properties unique to these Ig that facilitate their deposition and distinguish them from their non-nephritogenic counterparts.

To approach the issue of pathogenicity, we reasoned that it would be useful to isolate large amounts of individual autoantibodies; the nephritogenic capacity of these Ig could then be evaluated. In theory, hybridomas that produce large quantities of monoclonal autoantibodies would be ideal for these purposes. However, in light of the previous discussion of uncertainties regarding the distinguishing properties of pathogenic lupus autoantibodies, the selection criteria was not straightforward. How would nephritogenic Ig be distinguished from non-nephritogenic Ig? To approach these initial and crucial questions, the following experimental strategy was adopted. We reasoned that identification of the physical and immunochromal properties of autoantibodies, deposited within nephritic lesions that distinguished them from nonpathogenic autoantibodies, should provide clues. Once the crucial “nephritogenic properties” of pathogenic Ig were determined, monoclonal antibodies with similar properties could be selected. The capacity of these Ig to produce disease would then be determined. As monoclonal antibodies with nephritogenic properties were identified, then second-order questions relevant to the production and deposition of these Ig could be addressed, including: Are there unique genes or sets of genes that encode these antibodies? How do these antibodies form immune deposits? Can these interactions be interrupted to prevent immune deposit formation? Can B cells that produce these Ig selectively eliminated?

The identification and characterization of these antibodies has been particularly informative, and the results have provided both unique reagents and novel insights into the pathogenesis of lupus lesions. In initial studies, Ig were eluted from the kidneys of 3-month-old mice with active nephritis (64). At this age, nephritis is well established, the kidneys yield sufficient quantities of Ig for investigation, however, chronic disease is not yet present. The latter enhances the probability that Ig that initiated disease will be recovered.

By comparison with lupus serum Ig, the nephritogenic Ig were predominantly IgG, and they were enriched for autoantibody activity. These results confirm previous studies in both murine and human lupus; nephritogenic lupus autoantibodies in man had also been found to be enriched for complement-fixing capacity (59, 65–70). However, what distinguished the nephritogenic antibodies from their serum counterparts were properties unique to the antigen binding region; that is, they were enriched for autoreactivity for both intracellular and extracellular autoantigens (64,69,71). Of particular relevance, extensive cross-reactivities among individual Ig within the eluates were observed, including reactivity of eluted anti-DNA antibodies with extracellular matrix components, such as heparan sulfate and laminin, and with cell-surface proteins. (Cross-reactivity is defined as the capacity of an individual antibody to react with more than one antigen. In some cases this is the result of the presence of shared epitopes on the divergent antigen, as observed with DNA and cardiolipin; in other circumstances, the molecular basis for the interactions has not yet been defined.) As indicated in the MRL-lpr/lpr kidney eluates, individual Ig within the nephritogenic population were highly cross-reactive, interacting with multiple autoantigens, including DNA, phospholipids, proteins (i.e., SmRNP), and basement-membrane constituents (i.e., laminin, heparan sulfate) (64,71). Antibodies that reacted with basement membrane constituents but not intracellular antigens were also present in the eluates. By contrast, the cross-reactive antigen-binding properties of serum autoantibodies derived from the same lupus mice were more limited. For example, serum anti-DNA antibodies reacted with multiple polynucleotides, how-
ever, they did not react with either phospholipids or protein antigens. Furthermore, so-called natural autoantibodies (IgM), derived from normal mice, were the least cross-reactive; they bound only to individual polynucleotides. We made similar observations of autoantibodies isolated from the serum and kidneys of lupus patients, as compared with normals (69).

These findings suggested that the antigen-binding region of individual lupus autoantibodies was influential in immune-deposit formation. Additional support for this hypothesis was derived from idiootypic analysis of the same antibody populations. In MRL-lpr/lpr mice, a high-frequency idiootypic marker, termed IdH130, was present in higher quantities among kidney eluates than among their serum counterparts (64). Of additional relevance among nephritogenic Ig, IdH130 was present among both anti-DNA antibody and Ig that did not bind to DNA, indicating that the two populations shared structural properties and raising the possibility that they have common genetic origins. Similar observations have been made by other investigators in other murine lupus strains (72–74). Collectively, these results implicated the antigen-binding region in the formation of immune deposits, and raised questions about both the mechanisms of immune-deposit formation and the structure of pathogenic Ig.

We initially focused on the following questions: Are there specific genes that encode autoantibodies with nephritogenic properties?, and Do autoantibodies (cross-react) with glomerular antigens to initiate immune deposit formation? To approach these questions, we produced a large panel of monoclonal antibodies from MRL-lpr/lpr mice, and the Ig products were evaluated for their isotype, charge, and antigen-binding properties (75). Monoclonal antibodies that shared properties with Ig eluted from mice with active nephritis (i.e., monoclonal IgG that cross-reacted with multiple autoantigens) were identified. These individual monoclonal antibodies were then administered to normal mice. Initial experiments involved investigation of monoclonal anti-DNA antibodies with these properties.

**DIFFERENT NEPHRITIC PHENOTYPES ARE PRODUCED BY INDIVIDUAL AUTOANTIBODIES**

The results were extremely interesting: a subset of antibodies produced glomerular immune deposits and transferred nephritis to normal mice (76,77) (Table 1). However, this property was confined to only a subset, as not all anti-DNA antibodies formed deposits or produced disease. We interpreted this observation to be consistent with the notion that not all lupus autoantibodies are pathogenic. Additional observations among the pathogenic subset were of particular interest. The location, or pattern, of immune deposition varied with the administered antibody, and these phenomena were associated with clearly distinctive histologic lesions and variations in proteinuria.

For examples, the monoclonal anti-DNA antibody, termed H238, produced capillary wall and mesangial deposits in a granular pattern, and this was associated with proliferative changes and proteinuria. By contrast, the deposits induced by another anti-DNA antibody, termed H221, were predominantly intraluminal and subendothelial, and the H221 animals developed heavy proteinuria in the absence of significant glomerular hypercellularity. Subendothelial deposits and linear basement-membrane deposits were produced by a third anti-DNA antibody, termed H147; this was associated with proliferative glomerulonephritis involving both polynuclear and mononuclear cells. Another clearly defined pattern, observed with three other autoantibodies, was intranuclear localization of Ig in the absence of extracellular deposits. In some cases, this phenomenon was associated with glomerular hypercellularity and proteinuria. The unique properties of this latter subset of autoantibodies will be considered in greater detail near the end of this discussion.

Overall, these observations are consistent with those of other investigators, who reported variable

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**TABLE 1. In vitro and in vivo properties of selected immune-deposit forming MRL-lpr/lpr-derived anti-DNA antibodies reported from a single laboratory**

<table>
<thead>
<tr>
<th>mAb (iso)</th>
<th>ssDNA</th>
<th>dsDNA</th>
<th>smRNP</th>
<th>Laminin</th>
<th>IF</th>
<th>LM</th>
</tr>
</thead>
<tbody>
<tr>
<td>H147 (IgG2a)</td>
<td>3+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
<td>Linear GBM/TBM mes/cap wall</td>
<td>Proliferative glomerulonephritis</td>
</tr>
<tr>
<td>H221 (IgG2a)</td>
<td>3+</td>
<td>3+</td>
<td>2+</td>
<td>NT</td>
<td>Intraluminal mes/cap wall</td>
<td>Intraluminal deposits</td>
</tr>
<tr>
<td>H238 (IgM)</td>
<td>1+</td>
<td>-</td>
<td>2+</td>
<td>1+</td>
<td>Mes/cap wall small vessels</td>
<td>Hypercellularity</td>
</tr>
<tr>
<td>H161 (IgG3)</td>
<td>1+</td>
<td>-</td>
<td>3+</td>
<td>-</td>
<td>Mes/cap wall small vessels</td>
<td>Vessel wall thickening</td>
</tr>
<tr>
<td>H8 (IgG2a)</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>-</td>
<td>Mes/cap wall</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>H241 (IgG2a)</td>
<td>3+</td>
<td>4+</td>
<td>2+</td>
<td>3+</td>
<td>Mes/cap wall</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>H7 (IgG1)</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>NT</td>
<td>Intranuclear</td>
<td>Normal</td>
</tr>
<tr>
<td>H9 (IgG2a)</td>
<td>1+</td>
<td>1+</td>
<td>-</td>
<td>NT</td>
<td>Intranuclear</td>
<td>Hypercellularity</td>
</tr>
<tr>
<td>H72 (IgG1)</td>
<td>1+</td>
<td>1+</td>
<td>-</td>
<td>NT</td>
<td>Intranuclear</td>
<td>Hypercellularity</td>
</tr>
</tbody>
</table>

a IF, immunofluorescence; LM, light microscopy; mes, mesangial; cap wall, capillary wall; NT, not tested; -, nonreactive.
b,c See References, 76–78, 83, and 93 for details of in vitro and in vivo assessment.
c Animals developed proteinuria associated with glomerular epithelial-cell foot-process effacement.

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clonal anti-DNA antibody can transfer nephritis to normal mice. However, not all autoantibodies are nephritogenic. Furthermore, no single autoantibody completely reproduced the full-blown nephritic MRL-lpr/lpr phenotype, although together the autoantibodies recapitulate the spectrum of immune deposits typically observed in individuals with this disease. Moreover, the variable phenotypic expression of disease is reminiscent of the variable histologic and clinical presentation of patients with lupus nephritis. This variability also raises the possibility that the dominant nephritogenic autoantibody response in a given individual may influence the nature of disease expression.

These results led us to modify our thinking about the pathogenic relevance of the antigen binding region, and to hypothesize that this region influences both the capacity of individual autoantibodies to form immune deposits and the location of immune deposits in the kidney. This notion has important implications for the investigation of both the structural properties of nephritogenic antibodies and their antigen targets, because it suggests that different subsets of antibodies, each with unique structural properties, interact with different glomerular ligands to produce immune deposits at distinct locations within the kidney.

**IDENTICAL V GENES ENCODE ANTIBODIES THAT PRODUCE SIMILAR GLOMERULAR AND VASCULAR IMMUNE DEPOSITS**

To determine whether autoantibodies have similar structural motifs that facilitate immune-deposit formation, autoantibodies that produced immune deposits in similar locations were selected for V-region nucleotide sequence analysis. Our initial investigation involved three anti-DNA antibodies (78). Each produced subendothelial and mesangial immune deposits in a similar distribution; two of the three antibodies also produced deposits within small vessels. The antibodies were derived from three different MRL-lpr/lpr mice, ensuring their clonal independence.

The remarkable finding was that the VH genes of these mice were nearly identical, with over 99% homology, and CDR1 and CDR2 hypervariable regions were identical. As expected, their CDR3 and Framework 4 regions are encoded by different D and J genes, and were homologous. Furthermore, they were encoded by very different light-chain genes (<75% homology from three different light-chain gene families, including Vk1, Vk4, and Vk8).

The pathogenic relevance of this particular VH gene is further emphasized by independent observations from two laboratories. Raz et al. and Eliat and coworkers (Jerusalem) identified a nephritogenic anti-DNA antibody that produced similar a type of glomerular immune deposits, termed A52 and derived from an NZB/NZW mouse, that was encoded by a nearly identical VH gene sequence (79,80). In separate studies, Gangemi, Singh and Barrett (Boston) reported that this particular V gene appears to be present primarily in autoimmune strains, further emphasizing its importance (81). In addition, they also found that the VH gene sequences of two other anti-DNA antibodies that we had previously examined in vivo, were highly related to those encoding the trio just described (81,82).

Of particular interest, H241 formed morphologically similar immune deposits to other members of the group, whereas the other antibody, termed H102, did not form immune deposits (83). These results confirm the pathogenic relevance of this particular VH gene. They also indicate that in the latter instance (H102), either the CDR3 region or the light chain may actually impede immune-deposit formation, presumably by altering the antigen-binding properties of the Ig. The role of the VH CDR3 and the VL regions is further emphasized by the extraglomerular deposits produced by these nephritogenic Ig: the two antibodies that produced vascular deposits in addition to glomerular deposits in our original trio had the most homologous CDR3 regions (77), raising the possibility that this region influenced the formation of extraglomerular immune deposits. We are currently investigating the structural basis for these findings.

The contribution of individual residues to immune-deposit formation was recently demonstrated in elegant studies reported by Katz and coworkers in Diamond’s laboratory, where they observed that single amino-acid substitutions, produced by site-directed mutagenesis within either CDR or framework regions, could alter both autoantigen-binding activity and pathogenicity (glomerular immune-deposit formation) of a murine monoclonal anti-DNA antibody (84).

**DIRECT INTERACTION OF AUTOANTIBODIES WITH GLOMERULAR ANTIGENS**

These results further emphasize the relevance of the antigen-binding region of nephritogenic lupus autoantibodies, and they are consistent with the hypothesis that specific autoantibody-gglomerular lupus interactions initiate immune deposition. On the basis of these observations and the variability in the location of immune deposits after the administration of individual autoantibodies, we postulated that: (1) the direct interaction of autoantibodies and glomerular antigens was a major mechanism of immune-deposit formation in lupus nephritis; and (2) the observed variability in site immune deposition among individual autoantibodies was influenced by the location of the target glomerular antigen(s) (Figure 1). In support of these hypotheses, we and others observed that nephritogenic lupus autoantibodies cross-reacted with glomerular basement membrane and cell-surface constituents, including heparan sulfate, laminin, and other glomerular proteins (71,79,83,85–93). More re-
cently, we have evaluated the capacity of nephritogenic antibodies to interact directly with glomerular cell-surface antigens. Of particular relevance to the present discussion, specific autoantibody-cell surface protein interactions have been identified. Furthermore, these interactive cell surface proteins vary with the individual autoantibody and the type of lesion produced \textit{in vivo}. For example, we have identified nephritogenic antibodies with different \textit{in vivo} properties (forming deposits in different locations) that bind selectively to either endothelial or mesangial cell-surface proteins (94). We anticipate that more precise identification of these unique antigens should provide novel and potentially disease-relevant information.

\textbf{IN SITU IMMUNE DEPOSIT FORMATION OF AUTOANTIBODIES AND CIRCULATING AUTOANTIGENS DEPENDS ON PHYSICAL PROPERTIES OF AUTOANTIGENS}

Other mechanisms of immune-deposit formation may also contribute to the variable and diverse phenotypic expression of lupus nephritis. Most recently, interest has focused on \textit{in situ} formation of immune complexes, consisting of endogenous circulating antigens and autoantibodies. Nucleosomes, which consist of histone-DNA complexes, have received the most attention. They have been demonstrated to bind to the glomerulus and serve as a planted antigen for the subsequent binding of circulating autoantibodies (95–98). The nucleosome-glomerular interaction is facilitated by the relatively cationic charge of histones and the negative charge of glomerular basement membrane, although low molecular-weight DNA may also be capable of localizing within the glomerulus (99). A similar mechanism may also amplify disease in individuals with circulating rheumatoid factors, with deposited Ig serving as the planted antigen. Rheumatoid factor activity is particularly elevated in MRL-\textit{lpr}/\textit{lpr} mice and in some lupus patients. It has also been demonstrated that cryoglobulins (consisting in part of rheumatoid factors) can produce vasculitis and glomerulonephritis, although, in at least in one case, the rheumatoid factor activity was dissociated from pathogenicity of the Ig (100). In this regard, Dolcher and coworkers demonstrated that IgG within cryoglobulins derived from the serum of nephritic patients bound directly to normal human glomeruli, and this feature distinguished them from cryoglobulins derived from patients without clinical evidence of renal involvement (101).

\textbf{LOCALIZATION OF AUTOANTIBODIES WITHIN LIVING CELLS}

Cell-surface binding also appears to an important initial step for cellular entry of anti-DNA antibodies that negotiate both cell and nuclear membranes to localize within the nucleus. As we have previously alluded, we have identified a subset of anti-DNA antibodies that localize within the nuclei of cells of multi-

\textbf{Figure 1.} Mechanisms of immune deposit formation in lupus nephritis. Examples of each of the following are illustrated: (1) Direct binding of autoantibodies with cell-surface antigens (i.e., mesangial, endothelial, epithelial cells); (2) Direct binding of autoantibodies to matrix constituents (i.e., lammin, heparan sulfate, Type IV collagen); (3) \textit{In situ} complex formation of autoantibodies and circulating autoantigens that deposit within the glomeruli—the site of complex formation is largely determined by properties of circulating autoantigen (i.e., nucleosomes); (4) Nuclear localization of autoantibodies. Direct glomerular binding activities for both anti-nuclear antibodies (cross-reactivity) and antibodies that do not display antibody activity against intracellular antigens have been detected among Ig eluted from lupus kidneys. This antigen-binding property is associated with unique and distinguishing properties of antigen-binding regions of individual autoantibodies (i.e., all autoantibodies are not equal). Either antibody deposition per se (i.e., through receptor cross-linking), circulating immune complex uptake by glomerular cells, and/or inflammation, may lead to expression of neoantigens, which serve as targets for subsequent deposition of circulating autoantibodies. These local interactions may also directly influence local cellular responses that modulate inflammation and fibrosis. The cationic histone component of nucleosomes initiates the deposition of the complexes through charge-charge interactions with anionic sites within the glomerulus. Low molecular-weight DNA may also localize within glomeruli and serve as a target for subsequent antibody binding and deposit formation. Only a subset of autoantibodies enter cells and localize within nuclei; preliminary information suggests that antibody cell surface interactions initiate internalization. (Figure of glomerulus (without immune deposits) adapted from The Ciba Collection of Medical Illustrations, Volume 6, Kidneys Ureters and Urinary Bladder. F.H. Netter, Ciba 1973, Summit NJ.)
ple organs after administration to normal mice (76). In the kidney, nuclear localization was associated with glomerular hypercellularity, as well as epithelial foot-process fusion and proteinuria (76). The same anti-DNA antibodies that localized within nuclei in whole animals penetrated cells to localize within nuclei in cultured cells (102). These and subsequent cell-culture experiments were carried out by Dr. Kumico Yanase in collaboration with Dr. Leonard Jarett and Robert Smith of the Department of Pathology and Laboratory Medicine, and other colleagues. This phenomenon was clearly demonstrated by using labeled antibody and confocal microscopy, which show that the antibody traverses through the cytoplasm and into the nucleus in a time and temperature-dependent manner. Mild trypsinization of cells before antibody exposure significantly reduced both cellular entry and nuclear localization, consistent with the notion that a specific cell-surface protein may mediate cellular entry; in this regard, a candidate protein was recently identified (103).

Once these antibodies gain entry into the cell, an important question is how they gain access to the nucleus. Sequence analysis of these Ig provides clues. Our collaborator, Dr. Mary Foster, and her colleagues, made the rather astute observation that the CDR3 regions of these antibodies have significant homology with nuclear-localization sequences (NLS) of other proteins known to localize within nuclei (104). Of particular interest, although NLS-like positively charged amino-acid motifs were present in the CDR regions of the three different autoantibodies, the primary sequence of these regions were not highly related. However, simulated three-dimensional structures of two of the antibodies (created by Dr. Thomas Kleber-Emmons at the Wistar Institute; the third antibody could not be aligned to a known template) demonstrated that the positively charged amino acids were exposed. Therefore, they have the potential to mediate nuclear entry. Of additional interest and potential relevance, CDR1 and CDR2, along with frameworks 1, 2, and 3 of the two antibodies, closely align. Furthermore, although the primary amino-acid sequence of these antibodies in the CDR3 regions differed, there was a conformational relationship between the arginine and tyrosine residues in this region. Although they are reversed, the topological relationships between these potentially important residues are the same. These types of relationships have been described for other antibodies to account for shared antigen-binding properties, despite differences in primary amino-acid sequences. Recent preliminary observations by Dr. Yanase et al. (103) support the notion that this motif may be relevant to nuclear localization. When cells were permeabilized with digitonin before coculture with H7, so that intracellular contents leak out but the nuclear membrane remains intact, antibody was detected around the nuclear membrane but not in the nucleus. However, when the experiment was performed under identical conditions, except that ATP was added to the permeabilized cells, the antibodies were detected in the nucleus, confirming that nuclear localization is energy dependent, and suggesting that the NLS described by Dr. Foster may be sufficient to mediate nuclear localization. Experiments designed to further evaluate the relevance of these sequence motifs are planned.

CONCLUSIONS

B cells and autoantibodies are important for the expression of lupus nephritis. They are necessary for what appears to be antibody-mediated disease (i.e., glomerulonephritis and perhaps vasculitis) as well as for the expression of disease that is not linked to antibody deposition (i.e., interstitial nephritis). This conclusion implies a dual role for B cells: as the producers of pathogenic autoantibodies, as well as a highly specialized antigen-presenting cell for the activation of specific subsets of autoreactive T cells. Nevertheless, multiple antibody-ligand interactions appear to participate in glomerular immune-deposit formation among individuals with lupus nephritis. However, the predominant autoantibody-gglomerular antigen interaction(s) in a given individual determines the principal location of immune-deposit formation, which in turn influences the pathologic and clinical expression of disease. We believe that this contributes to the phenotypic diversity commonly observed among individuals with this disease. With regard to the expression of glomerulonephritis, these consequences appear to be dependent on properties unique to subsets of lupus autoantibodies and to their target antigen ligands within the glomerulus. Thus, the autoantibody variable, or antigen-binding region, along with the nature and location of the target glomerular antigen, are influential in initiating these perturbations. These observations provide the rationale for design and evaluation of specific therapies aimed at the interruption of either the events that lead to pathogenic autoantibody production or the most disease-relevant antigen-antibody interaction(s) in individuals with lupus nephritis.

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