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Autoimmunity to vimentin and lupus nephritis

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Lupus nephritis affects up to 60% of adults and 80% of children with lupus. Despite vast improvements in the survival and well being of patients with this disease our current understanding of its pathogenesis is incomplete and the risk of end stage renal disease is still unacceptably high.

The glomerular lesions of lupus nephritis have been intensively studied over the last 5 decades, with the establishment and refinement of the ISN/RPS system for classifying the glomerular lesions as well as the development of composite indices of activity and chronicity. Classification of glomerular lesions in SLE was initially based on 5 year outcomes in patients who were untreated except for corticosteroids. Using patient survival as the outcome it was clear that patients with focal glomerular disease or membranous disease progressed slowly and had a much better outcome at 5 years than those with diffuse disease most of whom succumbed within 2 years. The validation of the current classification criteria and of activity and chronicity indices for determining treatment and predicting long-term (>5 year) outcomes in the current era of optimized immunologic and medical interventions is still a work in progress. This is in part because outcome is linked to demographic factors including age, gender and ethnicity as well as compliance, responsiveness to therapy and number of relapses; these currently cannot be predicted from an initial renal biopsy.

Compared with the emphasis on glomerular lesions in lupus biopsies, less attention has historically been paid to lesions of the renal tubulointerstitial compartment that include infiltrates with mononuclear cells, tubular atrophy, fibrosis and tubular immune complex deposition. Several previous studies found that tubulointerstitial lesions correlate with glomerular injury (1-2). A recent study from Clark's group did not however find a clear association between the magnitude of tubulointerstitial infiltrates and either activity index or glomerular histologic class but rather with the tubular components of the chronicity score that include tubular atrophy and fibrosis (3). In addition there is consensus that the presence of infiltrates does not correlate with the degree of interstitial immune complex deposition. It

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is currently not possible to predict clinically which patients will have tubulointerstitial infiltrates.

Interstitial fibrosis is a component of the chronicity score and is recognized as a poor prognostic indicator in lupus nephritis. There is also strong agreement in the literature that the presence of tubulointerstitial infiltrates independently correlates with a worse long-term outcome (1-2, 4). Early studies indicated that tubulointerstitial infiltrates were associated with poorer glomerular function at presentation and poorer long-term outcome and that glomerular function at follow up correlated with the numbers of monocytes/macrophages in the initial biopsy. These findings were confirmed recently by Hsieh et al who found that tubulointerstitial inflammation was associated with a decreased GFR and higher serum creatinine at the time of biopsy but that a predominance of B or T cells per se did not correlate with either of these variables (3). Strikingly, 37% of patients with severe tubulointerstitial inflammation at biopsy progressed to renal failure in 24 months. Histologic involvement of the tubulointerstitial compartment has been observed in repeat biopsies even from patients in clinical remission. Poor long term outcomes were particularly noted when interstitial infiltrates of mononuclear cells were still present at a second biopsy (5).

Considerable recent interest has focused on the distribution and composition of the tubulointerstitial infiltrates in lupus biopsies as well as their effector functions. Lymphocytic infiltrates in lupus nephritis kidneys are heterogeneous in their anatomic structure with ≈50% of biopsies manifesting scattered infiltrates of B cells and plasma cells and 50% manifesting T:B aggregates that often accumulate in the periglomerular areas (6-8). Studies of the cellular composition of these infiltrates have shown that T cells, both CD4 and CD8, are the dominant cell type although B cells, NK cells and plasma cells are also found. There is extensive heterogeneity among patients with some patients having predominantly CD8 and others CD4 infiltrates. An extensive analysis of T cell infiltrates from 17 human SLE renal biopsies found that T cells are present both in periglomerular regions and in the interstitium with a somewhat different anatomic distribution and potentially different function of renal CD4 and CD8 T cells (9). Both Th1 and TH17 T cells have been identified in biopsies of patients with proliferative lupus nephritis. B cells are present in 50-60% of biopsies (6, 9) but germinal center formation occurs rarely, being found in 0/192 biopsies studied by Shen (6) and only 4/68 (6%) of biopsies studied by Clark's group (8). Macrophages/monocytes accumulate widely throughout the interstitium and may be found in the glomeruli, particularly in individuals with proliferative disease. There is an increasing appreciation that several subsets of dendritic cells can also infiltrate inflamed kidneys.

One important question is whether tubulointerstitial infiltrates are induced by or directed to renal antigens and therefore constitute a secondary aspect of autoimmunity that could generate long-lived organ specific memory/effector cells. The T cell aspect of this question was recently addressed by Winchester et al. who found that both CD4 and CD8 T cell infiltrates from lupus biopsies are oligoclonal (9). Clonal CD4 populations were found in periglomerular aggregates that also contain B cells, raising the possibility that such clones could provide help for B cells. The identification of B cell and plasma cell clusters in close proximity to ICOS+ TFH like T cells lends credence to this hypothesis (7, 10). In addition, clonal expansions of CD8+/CD28^{null} memory effector cells were shared among multiple

renal compartments and/or the peripheral blood. Which antigens are driving this response has not yet been addressed.

In a manuscript published in this issue of Arthritis and Rheumatology, Kinloch et al have asked which antigens drive the expansion of tubulointerstitial B cells (insert reference here). Previously this group sampled single B cells from T:B aggregates of 9 human SLE biopsies and found both clonal relationships and somatic mutations, evidence that T cell driven clonal expansion of B cells is occurring in situ (8). They have now extended their studies by co-expressing the heavy and light chains of 25 single B cells harvested by laser capture microscopy or single cell sorting and testing them for antigenic specificity. These cells are representative of clonal expansions from 8 different lupus biopsies, 7 with T cell:B cell aggregates and one with germinal center structures. Many previous studies of antibodies eluted from lupus biopsies, both mouse and human, have shown that a large proportion do not bind classical lupus autoantigens. Indeed, the group found by screening on HEp-2 cells that <20% of the reconstituted antibodies reacted with nuclear autoantigens whereas ≈40% reacted with structures in the cytoplasm and the rest had no reactivity. They then used two separate approaches to identify the putative autoantigens; the first was to screen the antibodies on commercial arrays displaying >9000 human proteins and the second was to perform immunoprecipitation with HEp-2 lysates followed by mass spectrometry. Most of the anti-cytoplasmic antibodies recognized fewer than 8 of the 9000 proteins on the proteome arrays, indicating that the local production of antibodies is driven by a limited number of antigens. When the two assays were cross-referenced, vimentin was the only shared putative autoantigen. Using co-localization studies with an anti-vimentin antibody and direct binding to vimentin coated slides the group confirmed that 10/11 of the antibodies with cytoplasmic reactivity could bind to vimentin. In accord with this finding they showed an increase in vimentin expression in biopsy samples with localization to a subset of cells within the interstitial infiltrates and a correlation between high serum titers of anti-vimentin antibodies and severe tubulointerstitial infiltrates.

These painstaking experiments by both Winchester and Kinloch represent a major advance in our ability to garner mechanistic information from vanishingly small samples of archived tissue and represent only the beginning of new technologies that can extract vast quantities of data from single cells. Together, the studies suggest that clonal expansion of both B and T cells occurs in situ within the renal interstitium and may be driven by local autoantigens. Furthermore these immune responses are likely to contribute to disease pathogenesis. Experiments in a mouse model of inflammatory glomerulonephritis have illustrated how a T cell directed immune response to a model renal antigen expressed in the glomeruli amplifies renal inflammation in a manner dependent on both peripheral and local dendritic cells; the ongoing immune response helps to recruit and maintain periglomerular infiltrates, promulgates an increase in infiltrating renal macrophages and dendritic cells and causes chronic tubulointerstitial damage (11). These experiments suggest that the infiltrates are not just biomarkers of more severe disease but that they play a causative role in disease progression.

It should not come as a surprise that many of the B cells in inflamed lupus nephritis kidneys are making antibodies directed at vimentin, a major component of the intermediate filament

protein produced by cells of mesenchymal origin. Antibodies to vimentin have been found in a variety of inflammatory and autoimmune conditions including RA and mouse models of SLE as well as in allograft rejection. Several experimental approaches have suggested potential mechanisms by which tolerance to vimentin is broken during inflammation [reviewed (12)]. First, cleavage of vimentin is one of the first steps in dismantling the cytoskeleton during the early stages apoptosis. Thus sites of inflammation and tissue damage are sources of vimentin fragments. Post-translational modifications such as citrullination and shedding of soluble fragments could also contribute to the exposure of cryptic immunogenic epitopes. In rheumatoid arthritis for example, vimentin fragments are found in synovial fluid and antibodies to citrullinated vimentin are as common as antibodies to CCP and correlate with disease activity. Although intact intermediate filaments are intracellular, vimentin or its fragments are expressed on the cell surfaces of activated macrophages, platelets, and apoptotic T lymphocytes, and neutrophils, where they can act both as immunogens and as targets of circulating autoantibodies. Vimentin is also expressed by injured endothelium and tubular cells. Of renal allograft transplant recipients, approximately 50% develop IgM and 12-25% develop IgG anti-vimentin antibodies despite conventional immunosuppression (13). In humans with glomerular diseases, including SLE, vimentin is found in the renal interstitium and correlates with declining GFR (14), consistent with the findings of Kinloch et al. In addition, the quantity of vimentin mRNA in urine pellets from allograft recipients is highly associated with tubulointerstial fibrosis and tubular atrophy (15).

Abundant evidence suggests that antibodies to vimentin have pathogenic potential. Pre-immunization with vimentin accelerates allograft rejection and graft vasculopathy in murine models in an antibody dependent fashion. IgM anti-vimentin antibodies can fix complement and colocalization of vimentin and complement on apoptosing leukocytes and platelet-leukocyte conjugates has been observed in rejecting allografts. Notably, this does not occur in isografts, suggesting that tissue damage and vimentin exposure is necessary for antibody mediated damage to occur. The interaction of anti-vimentin antibodies with platelets accelerates intimal occlusion in cardiac allografts, a result of platelet activation by anti-vimentin antibodies [reviewed (12)].

The findings of Kinloch et al raise several important considerations with respect to lupus nephritis. First, antibodies to vimentin appear to belong to a group of antibodies arising in response to antigens within damaged tissue that have potential for amplification of tissue damage. Whether or not anti-vimentin T cells are also elicited and what other antigens may elicit similar responses, including others identified in allograft recipients, remain to be determined. Several additional potential renal auto antigens were identified by Kinloch et al and need to undergo further validation. Importantly, these types of immune responses to renal antigens could result in subsequent memory autoimmune responses that are reactivated following any renal insult independently of lupus specific anti-nuclear responses.

The progression from acute kidney injury to chronic renal disease is influenced by many intrarenal factors including glomerular hypertension, endothelial injury and vascular dropout, interstitial inflammation and fibrosis and epigenetic changes within renal cell subpopulations. These mechanisms amplify each other and can be exacerbated by repeated injury. How the anti-vimentin response contributes to this process can now be investigated

in well characterized cohorts of lupus nephritis patients. We need to understand which patients express this specificity, whether these antibodies increase over time and whether either they or urine vimentin mRNA predict outcome or are biomarkers for deteriorating renal function. Importantly, high titer anti-vimentin antibodies 1 year after renal transplant are associated with allograft associated coronary artery disease 4 years later (13) . The factors contributing to this outcome are not currently understood but the implication of this finding for lupus nephritis patients is clear and bears further examination.

Given the ubiquity of anti-vimentin antibodies in allograft recipients and their potential pathogenic role, several studies have examined whether the emergence of these antibodies can be prevented by immunosuppression. Notably, formation of these antibodies in human allograft recipients is not prevented by cyclosporine and is suppressed to a greater degree by MMF than by azathioprine. Mouse studies have indicated that costimulatory blockade also delays the production of IgG anti-vimentin antibodies in allograft recipients [reviewed (13)]. Should these autoantibodies arise during or after the onset of lupus nephritis further studies to address how they can be prevented and whether this has an effect on long-term outcomes would then be warranted.

REFERENCES

1. Yu F, Wu LH, Tan Y, Li LH, Wang CL, Wang WK, et al. Tubulointerstitial lesions of patients with lupus nephritis classified by the 2003 International Society of Nephrology and Renal Pathology Society system. Kidney Int. 2010; 77(9):820–9. [PubMed: 20182417]
2. Park MH, D'Agati V, Appel GB, Pirani CL. Tubulointerstitial disease in lupus nephritis: relationship to immune deposits, interstitial inflammation, glomerular changes, renal function, and prognosis. Nephron. 1986; 44(4):309–19. [PubMed: 3540691]
3. Hsieh C, Chang A, Brandt D, Guttikonda R, Utset TO, Clark MR. Predicting outcomes of lupus nephritis with tubulointerstitial inflammation and scarring. Arthritis Care Res (Hoboken). 2011; 63(6):865–74. [PubMed: 21309006]
4. Ortega LM, Schultz DR, Lenz O, Pardo V, Contreras GN. Review: Lupus nephritis: pathologic features, epidemiology and a guide to therapeutic decisions. Lupus. 2010; 19(5):557–74. [PubMed: 20089610]
5. Hill GS, Delahousse M, Nochy D, Remy P, Mignon F, Mery JP, et al. Predictive power of the second renal biopsy in lupus nephritis: significance of macrophages. Kidney Int. 2001; 59(1):304–16. [PubMed: 11135084]
6. Shen Y, Sun CY, Wu FX, Chen Y, Dai M, Yan YC, et al. Association of intrarenal B-cell infiltrates with clinical outcome in lupus nephritis: a study of 192 cases. Clin Dev Immunol. 2012; 2012:967584. [PubMed: 22792121]
7. Hutloff A, Buchner K, Reiter K, Baelde HJ, Odendahl M, Jacobi A, et al. Involvement of inducible costimulator in the exaggerated memory B cell and plasma cell generation in systemic lupus erythematosus. Arthritis Rheum. 2004; 50(10):3211–20. [PubMed: 15476242]
8. Chang A, Henderson SG, Brandt D, Liu N, Guttikonda R, Hsieh C, et al. In situ B cell-mediated immune responses and tubulointerstitial inflammation in human lupus nephritis. J Immunol. 2011; 186(3):1849–60. [PubMed: 21187439]
9. Winchester R, Wiesendanger M, Zhang HZ, Steshenko V, Peterson K, Geraldino-Pardilla L, 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. [PubMed: 22130908]
10. Liarski VM, Kaverina N, Chang A, Brandt D, Yanez D, Talasnik L, et al. Cell distance mapping identifies functional T follicular helper cells in inflamed human renal tissue. Sci Transl Med. 2014; 6(230):230ra46.

11. Heymann F, Meyer-Schwesinger C, Hamilton-Williams EE, Hammerich L, Panzer U, Kaden S, et al. Kidney dendritic cell activation is required for progression of renal disease in a mouse model of glomerular injury. J Clin Invest. 2009; 119(5):1286–97. [PubMed: 19381017]
12. Rose ML. Role of anti-vimentin antibodies in allograft rejection. Hum Immunol. 2013; 74(11): 1459–62. [PubMed: 23777935]
13. Besarani D, Cerundolo L, Smith JD, Procter J, Barnardo MC, Roberts IS, et al. Role of anti-vimentin antibodies in renal transplantation. Transplantation. 2014; 98(1):72–8. [PubMed: 24978037]
14. Bob FR, Gluhovschi G, Herman D, Potencz E, Gluhovschi C, Trandafirescu V, et al. Histological, immunohistochemical and biological data in assessing interstitial fibrosis in patients with chronic glomerulonephritis. Acta Histochem. 2008; 110(3):196–203. [PubMed: 18155753]
15. Lee JR, Muthukumar T, Dadhania D, Ding R, Sharma VK, Schwartz JE, et al. Urinary cell mRNA profiles predictive of human kidney allograft status. Immunol Rev. 2014; 258(1):218–40. [PubMed: 24517436]