Emerging Paradigms in the Renal Pathology of Viral Diseases

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This review considers recent information that illuminates pathogenetic mechanisms that involve three of the major viral infections that cause renal injury in the form of HIV-associated nephropathy, polyoma virus nephropathy, and hepatitis C virus–associated glomerulonephritis.

HIV-Associated Nephropathy

A nephropathy that is associated with HIV infection was identified soon after the epidemic of HIV/AIDS first became recognized in the early 1980s. HIV-associated nephropathy (HIVAN) is a combined glomerular and tubular injury that is characterized by a collapsing glomerulopathy (CG) with collapse of glomerular capillary structures and a striking hyperplasia of podocytes, microcystic transformation of renal tubules, and concomitant and likely nonspecific interstitial inflammation and fibrosis consequent to the glomerular and tubular injuries (Figure 1). From a pathology standpoint, three major areas of investigation have led to a deeper understanding of this lesion.

The first of these addresses the issue of whether HIVAN is a direct consequence of viral infection of the renal parenchyma or is a secondary consequence of systemic infection. Cohen et al. (1) first reported the presence of HIV RNA in podocytes as revealed by in situ hybridization (ISH) in 1989, a finding that was supported by microdissection studies of Kimmel et al. (2). These studies were difficult to validate because of the failure of others to replicate these findings using conventional techniques of ISH, the inability to demonstrate the presence of viral peptides in renal parenchymal cells by immunohistochemistry, and the inability to demonstrate appropriate receptors for viral entry into cells, namely CD4 and the chemokine receptors CXCR4 or CCR5, on renal parenchymal cells (3). This created a conundrum in that a mechanism for viral entry into renal cells could not be defined. Some but not all of the difficulties were resolved by a more sophisticated form of ISH, which is based on a technique that detects forms of viral DNA that are characteristic of replicating virus (4–6). With these techniques, it was possible to substantiate the findings of Cohen et al. and convincingly demonstrate HIV infection of podocytes and tubular epithelial cells.

A second key development concomitant with the demonstration of HIV infection of human renal epithelial cells was the development of murine models of HIVAN. The best studied of these involves the Tg26 mouse: Mice are made transgenic for a nearly whole-length HIV virus but with a deletion of HIV gag and pol genes to render the virus nonreplicative (7). Studies of these mice, largely by the group of Klotman and their collaborators, have established that expression of HIV genes, presumably modeling the events of renal HIV infection, is sufficient to produce a renal injury in the mouse that is similar to HIVAN in humans. Studies in which kidneys from transgenic mice were transplanted into wild-type controls demonstrated that renal expression of HIV gene products was sufficient to initiate disease (8) and that systemic viral infection or other systemic conditions, such as the immunodeficiency that occurs in humans with HIVAN, were not necessary for the development of nephropathy. The murine models have been further refined to study the effects of selective transgenic insertion of specific HIV genes to determine their relative pathogenicity for the development of HIVAN. These studies have indicated that the product of the HIV gene nef, a gene that is involved in viral replication, is a particularly important contributor to the development of nephropathy, although it is not alone sufficient to produce this injury (9–11). Murine models in which transgenic expression of HIV is limited to podocytes (resulting from the use of a nephrin promoter construct) have been created recently and show that HIV infection of podocytes is sufficient to produce the CG phenotype of HIVAN (12).

The third major development that has led to the current pathology paradigm of HIVAN came largely from studies of renal biopsies of patients with CG and HIVAN. It had long been recognized that the proliferating hyperplastic podocytes of CG were morphologically different from the mature podocytes of normal kidneys and from the injured podocytes that are present in cases of minimal-change disease and other morphologic types of FSGS. A series of studies by D'Agati and colleagues (13) revealed that morphologic markers that characterize podocyte specification and maturation in developing kidneys (e.g., synaptopodin, WT-1, GLEPP1, cyclin kinase inhibitor p27) were lost in the podocytes of CG and HIVAN. Furthermore, it was shown that the podocytes in CG and HIVAN were actively proliferating and had a profile of cell-cycle regulatory molecule expression that recapitulated patterns of immature, proliferating podocytes in glomerular de-
Table 1. HIVAN paradigm: Renal injury is a direct consequence of viral infection of renal cells

| Viral infection of podocytes leads to perturbation of cell machinery, cell-cycle regulation, and dysregulated/differentiated phenotype. Inflammation has a minor role, at best, in pathogenesis. Mechanisms of viral entry into renal cells remain obscure (e.g., the best characterized receptors for virus CXCR4 and CCR5 are normally not found in glomerular cells). Subsequent role of perturbed cytokine networks is relatively unexplored and remains conjectural. |

aHIVAN, HIV-associated nephropathy.
that characterize affected patients versus patients with PVN but without such deposits (18). Levels of viremia or specific immunosuppression regimens also do not distinguish those groups of patients. There is some evidence that patients with tubular basement membrane immune deposits have a more severe form of PVN, most notably characterized by a lower degree of polyoma virus clearance after treatment with antiviral therapy. Table 2 indicates a current pathology paradigm for PVN.

**Hepatitis C Virus–Associated Glomerulonephritis**

Although a variety of glomerulonephritis have been associated with hepatitis C virus (HCV) infection, including membranous glomerulopathy and FSGS among others, by far the strongest association is that of membranoproliferative glomerulonephritis (MPGN) (19–23). MPGN typically presents many years, often decades, after initial infection with HCV. The majority of cases, but not all, have concomitant clinical features of mixed cryoglobulinemia, and the biopsy appearance of glomerular injury may reflect this noteworthy association (23,24). In the MPGN that is associated with cryoglobulinemia, termed cryoglobulinemic glomerulonephritis by some, there may be a marked infiltration of glomerular capillaries by leukocytes, typically with a large component of monocytes. Glomeruli show accentuated lobulation of the tuft architecture and may show some combination of increased mesangial cellularity, mesangiolysis, capillary endothelial swelling, duplication of capillary basement membranes, and sometimes intracapillary globular accumulations of eosinophilic material representing precipitated immune complexes/cryoglobulins (Figure 3) (20,25,26). On ultrastructural examination, the visualized immune complexes, typically subendothelial in location, may have a finely fibrillar or tactoid pattern of organization, a distinctive feature of some cryoglobulin deposits.

In noncryoglobulinemic MPGN, the features are similar to
that described in the previous paragraph except that the features of mesangiolysis, marked leukocyte influx, and intracapillary accumulations are less likely to be apparent. Both subendothelial and subepithelial immune complexes can be identified by electron microscopy, typically without distinctive substructure. In both forms of HCV-associated MPGN, mesangial and capillary wall deposition of IgM, IgG, and C3 are frequently but not invariably demonstrated (19,22,24).

Other forms of glomerular injury have been documented as well (21,22,24–27). Both individual case reports and small series have reported membranous glomerulonephritis (MGN) in HCV-infected patients (21,27,28). The clinical features and pathology in these cases do not distinguish those patients from those with idiopathic or other secondary forms of MGN. Other forms of immune complex–mediated glomerulonephritis, such as IgA nephropathy, may occur in these patients. We believe that there also may be a noteworthy population of HCV-infected patients who develop glomerular injury corresponding to FSGS (21,29). Many of these patients have a history of intravenous drug abuse as a probable route of infection with HCV. Cases demonstrating an association of HCV infection with fibrillary glomerulonephritis and immunotactoid glomerulopathy also have been reported (30).

Several recent studies point to the potential magnitude of the problem of HCV infection and kidney disease. A recent analysis of the current National Health and Nutrition Examination Survey (NHANES), involving more than 15,000 patients, indicates a stable prevalence of HCV seropositivity in the United States (1.6%; estimated 4.1 million infected people) and HCV viremia (1.3%; 3.2 million actively infected people) (31). Information from this survey and other sources indicates that the population of infected individuals is growing older and that the sustained high prevalence of HCV infection in the face of a declining rate of acute infections in the US population is due to a reservoir of infections that were acquired decades earlier (31,32). Because kidney disease and cryoglobulinemia are late manifestations of HCV infection, it can be anticipated that an increase in HCV-associated renal disease will be encountered in the near term.

Furthermore, a recent study of patients who underwent protocol renal biopsy at the time of liver transplantation for HCV-associated liver failure found that most of these patients (25 of 30 patients) had an immune complex–mediated glomerulonephritis, which was often clinically unsuspected (33). This points to a potential major contributing cause of renal failure that may occur after transplant in these patients and suggests that the patient group with clinically evident HCV-associated glomerular injury may be akin to the tip of an iceberg.

The precise pathogenetic sequence of injury that results in glomerulonephritis is not known. It is thought that glomerular injury in this setting results from the deposition of circulating immune complexes that contain HCV and anti-HCV antibody (HCV Paradigm 1, Table 3). These complexes may contain rheumatoid factors of IgM antibody, likely directed with some specificity toward the anti-HCV IgG antibodies. This scenario would be highly tenable if HCV virions or antigens would be demonstrated in the characteristic glomerular lesions. However, although isolated reports of successful immunohistochemical localization of HCV antigens in renal tissues have been published, it remains the consensus opinion of investigators in this area that reliable demonstration of HCV antigens in kidney tissues in general and nephritic lesions particularly has yet to be accomplished. Purported ultrastructural demonstrations of characteristic virions in renal lesions to date also are intriguing but not yet widely considered persuasive. The extent to which virus or viral products are present in the deposited immune complexes in glomeruli therefore remains a somewhat unsettled issue.

The development of MPGN subsequent to deposition of immune complexes requires amplification of the injury process: Recruitment of leukocytes, release of inflammatory mediators, and release of growth factors that produce the cellular proliferation and basement membrane alterations that are characteristic of this MPGN. There are two leading paradigms for the

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<th>Table 3. HCV-associated glomerulonephritis: Paradigm 1—HCV causes type II cryoglobulinemia by direct activation of B cells</th>
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<td>HCV infects B cells and can induce clonal proliferation of these cells.</td>
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<td>Cryoglobulinemia is a late manifestation (years to decades) after HCV infection.</td>
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<td>The cryoglobulins consist of monoclonal IgM (rheumatoid factor) possibly directed against epitopes of the HCV envelope that are cross-reactive with IgG.</td>
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*aHCV, hepatitis C virus.*
amplification phase of glomerulonephritis. The first of these involves complement activation by either the classic or alternate pathways by the deposited immune complexes, with subsequent release of anaphylotoxins C3a and C5a to recruit leukocytes, and local formation of membrane attack complexes that are composed of terminal portions of the complement pathway that may be injurious to tissues where they are deposited (34–36). The second paradigm is based on engagement and activation of Fc receptors on leukocytes by the Fc portion of Ig that are present in the deposited immune complexes (35). These paradigms are not mutually exclusive; nor do they exclude other contributing amplification pathways such as those of innate immunity, which may be initiated by engagement of Toll-like receptors by viruses or viral products. These paradigms are shown in Figure 4.

It has been difficult to establish which of these paradigms may be primary in the setting of HCV-associated MPGN because HCV remains an elusive virus. It remains resistant to culture, so mechanisms of its infectivity remain difficult to characterize. The virus infects only human and nonhuman primates, so useful rodent models of HCV infection are not available to study key pathogenetic mechanisms.

Despite the obstacles to studying HCV-related renal injury directly, we are fortunate in that several murine models of MPGN of nonviral etiology have been recently characterized and are suitable for dissecting downstream pathways that result in amplification of the glomerular injury (35). One of these, the thymic stromal lymphopoietin (TSLP) transgenic mouse, develops cryoglobulinemia and a cryoglobulinemic MPGN that closely resembles that of human HCV-associated MPGN (37). Studies from this model have strongly implicated the engagement of Fc receptors as a major regulator of glomerulonephritis. This comes from the observation in this model that deletion of inhibitory of Fc receptor Fcγ/H9253R11b, which normally dampens the inflammatory response, results in marked exacerbation of the MPGN in this model (38). Complementary studies of the effect of deletion of activating Fc receptors in this model are not yet complete. However, studies of these activating receptors in other models of glomerulonephritis, including murine models of lupus nephritis and anti–glomerular basement membrane antibody–mediated glomerulonephritis (39,40), indicate that the balance of activating and dampening Fc receptor engagement is a major and perhaps the major determinant of the severity of glomerulonephritis consequent to immune complex and/or antibody deposition.

The relative importance of complement activation in this type of injury remains uncertain. Efforts to interrupt the activation of complement have thus far not been successful in ameliorating the MPGN of TSLP transgenic mice (41), although such measures have been successful in other models of glomerulonephritis (34,36). A very recent and potentially important finding is the demonstration of upregulated expression of the Toll-like receptor 3 in human HCV-associated MPGN (42). This suggests a pathway whereby innate immune recognition of pathogens, perhaps independent of or in conjunction with immune complex deposition, may also initiate the events of HCV-associated glomerulonephritis. Information on the role of innate immunity in human glomerulonephritis is scanty, but this is expected to change dramatically in the near future.

Acknowledgments
Some of the work reported was supported by grant DK68802 from the National Institutes of Health.

Disclosures
None.

References

**Figure 4.** Overlapping paradigms for amplification of injury in HCV-associated membranoproliferative glomerulonephritis.
12. Zhong J, Zuo Y, Ma J, Fogo AB, Jolicoeur P, Ichikawa I,
18. Leca NB, Davis LD, Alpers CE, Kowalewska J: Clinical
17. Bracamonte E, Leca N, Smith KD, Nicosia RF, Nickeleit V,
19. Johnson RJ, Gretch DR, Yamabe H, Hart J, Bacchi CE,
20. D’Amico G, Fornasieri A: Cryoglobulinemic glomerulone-
16. Randhawa P, Brennan DC: BK virus infection in transplant
7. Dickie P, Felser J, Eckhaus M, Bryant J, Silver J, Marinios N,
8. Bruggeman LA, Dikman S, Meng C, Quagggin SE, Coffman
TM, Klotman PE: Nephropathy in human immunodefici-
virus-1 transgenic mice is due to renal transgene expression. *J Clin Invest* 100: 84–92, 1997
10. Kajiyama W, Koppl JB, Marinios NJ, Klotman PE, Dickie P:
Glomerulosclerosis and viral gene expression in HIV-
11. Husain M, D’Agati VD, He JC, Klotman ME, Klotman PE:
12. Zhong J, Zuo Y, Ma J, Fogo AB, Jolicoeur P, Ichikawa I,
14. Shankland SJ, Eitner F, Hudkins KL, Goodpaster T,
D’Agati V, Alpers CE: Differential expression of cyclin-
15. Peraldi MN, Maslo C, Akpososo K, Mougenot B, Rondeau E,
17. Bracamonte E, Leca N, Smith KD, Nicosia RF, Nickeleit V,
19. Johnson RJ, Gretch DR, Yamabe H, Hart J, Bacchi CE,
20. D’Amico G, Fornasieri A: Cryoglobulinemic glomerulone-
23. Roccatello D, Fornasieri A, Giachino O, Rossi D, Beltrame
24. Johnson RJ, Willson R, Yamabe H, Couser W, Alpers CE,
25. Beddu S, Bastacky S, Johnson JP: The clinical and mor-
26. D’Amico G, Colasanti G, Ferrario F, Sinico RA: Renal in-
27. Poutel-Noble C, Maiza H, DiJoud F, MacGregor B: Glo-
erular disease associated with hepatitis C virus infection in native kidneys. *Nephrol Dial Transplant* 15[Suppl 1]: 28–33, 2000
28. Stehman-Breen C, Alpers CE, Couser WG, Willson R, John-
son RJ: Hepatitis C virus associated membranous glome-
30. Markowitz GS, Cheng JT, Colvin RB, Trebbin WM, D’Agati
33. McGuire BM, Julian BA, Bynon JS Jr, Cook WJ, King SJ,
35. Smith KD, Alpers CE: Pathogenic mechanisms in mem-
branoproliferative glomerulonephritis. *Curr Opin Nephrol Hypertens* 14: 396–403, 2005
36. Trendelenburg M, Fossati-Jimack L, Cortes-Hernandez J,
37. Tandera S, Segerer S, Hudkins KL, Cui Y, Wen M, Segerer
M, Wener MH, Khairallah CG, Farr AG, Alpers CE: Cryo-
38. Muhlfield AS, Segerer S, Hudkins K, Carling MD, Wen M,
Farr AG, Raveth JV, Alpers CE: Deletion of the fcgamma


