Recent observations regarding intrinsic glomerular cell biology, particularly in the podocyte, have provided exciting new insights into potential pathogenic mechanisms of human glomerular disease. Although both immune and nonimmune mechanisms of glomerular injury have been studied previously, experimental models of disease and recent techniques that provide tools for molecular profiling show great promise for identifying glomerular disease biomarkers. Despite these recent advances, additional work in both basic and clinical studies of glomerular disease is needed to advance the field. Standardization of animal models of distinct forms of glomerular disease would likely facilitate the search for biomarkers. Several factors limit current efforts to implement clinical trials of glomerular disease. Identification of disease biomarkers, development of disease-specific end points, and organization of collaborative clinical groups are critical for ultimately designing and implementing appropriately powered trials of glomerular disease.


Although individual morphologic forms of glomerular disease occur uncommonly, glomerulonephritis (GN) as a disease category is the third leading cause of ESRD cases in the United States after diabetes and hypertension. Recent US Renal Data System (USRDS) data indicate that patients with primary GN compose 8.2% of incident and 15.6% of prevalent dialysis patients in the United States, but as many as 55.7% of incident and 51.3% of prevalent dialysis patients have a primary or secondary form of glomerular disease (including diabetic nephropathy) (1). To discuss recent noteworthy developments in glomerular disease research and potential opportunities for improving diagnosis and therapeutic intervention for human glomerular disease, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) convened a workshop on glomerular disease in January 2005. This 2-d workshop assembled both basic scientists and clinical investigators from the international research community to focus on glomerular cell biology and opportunities for translation into clinical studies. The workshop included scientific presentations and breakout sessions that discussed specific key topics for glomerular disease research.

Pathobiology of Glomerular Disease

Intrinsic Glomerular Cell Biology

Podocyte-Signaling at Slit Diaphragm (Thomas Benzing, University of Freiburg). Dr. Benzing provided an overview of the slit diaphragm and its role in maintaining the glomerular filtration barrier (2). A number of slit diaphragm proteins that seem to play a major role in podocyte cell signaling events have been identified (2–7). Dr. Benzing proposed a model by which alterations in signaling pathways might lead to significant functional changes in podocyte biology, such as changes associated with proteinuria or hypertension (2). The development of additional experimental models will allow for a better understanding of how defective signaling events at the slit diaphragm affect the induction or progression of glomerular disease.

Podocyte-Endothelial Interactions (Susan Quaggin, University of Toronto). Dr. Quaggin discussed the critical signaling impact of vascular endothelial growth factor A (VEGF-A) in endothelial cell biology, noting that dysregulated VEGF-A is associated with many glomerular diseases (8). Moreover, recent observations with conditional VEGF-A gene-targeting approaches suggest that VEGF-A not only plays a major role in glomerular development but also is likely requisite for maintaining normal glomerular function in the adult kidney (9,10). The potential role of podocyte hypoxia and the control of VEGF-A expression by the oxygen-sensitive von Hippel-Lindau tumor suppressor protein (pVHL) and hypoxia-inducible factors (HIF) transcription factors were also discussed.

Podocyte Depletion and Focal Segmental Glomerular Sclerosis (Roger Wiggins, University of Michigan). Dr. Wiggins discussed the hypothesis that podocyte damage and depletion are necessary and sufficient for progression of glomerular injury in focal segmental glomerular sclerosis (FSGS) and other forms of glomerular disease. Investigating this hypothesis, he described a transgenic rodent model with a transgene encoding the diphtheria toxin and a podocyte-specific promoter (11). Preliminary studies in this model suggest that diphtheria toxin administration produces a predictable loss of podocytes and proteinuria (12). Such experimental models provide important...
tools for further investigating mechanisms of glomerulosclerosis.

**Gene/Protein Discovery**

**Glomerular Transcriptome (Karl Tryggvason, Karolinska Institute).** Dr. Tryggvason described a comprehensive program, entitled Functional Genomics of the Renal Glomerulus, whose purpose is to identify glomerulus-specific gene transcripts using glomerulus-specific microarrays and other tools. The function of newly identified glomerular proteins will be approached systematically with gene knockout and knockdown techniques in mice and zebrafish, respectively.

**Inflammation and Autoimmunity**

**Complement, Therapeutics, and Glomerular Disease (V. Michael Holers, University of Colorado).** In view of the marked glomerular inflammatory response mediated by complement (C) proteins, Dr. Holers reviewed strategies for alternate C pathway inhibition in models of autoimmune glomerular disease. Reports of MRL/lpr (lupus-prone) mice deficient for either factor B or factor D reveal a marked disease-protective effect in deficient rodents (13,14). Newer strategies are focusing on the use of fusion protein technology to develop antibodies that target components of the C pathways that will localize to sites of inflammation, such as the glomerulus.

**Chemokines and Their Receptors in Glomerulonephritis** (Detlef Schlondorff, Ludwig Maximilians University). Dr. Schlondorff provided an overview of the role of chemokines and their receptors in renal disease noting that chemokines are inducible in all subsets of intrinsic renal cells, as well as infiltrating cells in settings of glomerular inflammation (15). Targeting distinct chemokines or receptors with depleting antibody studies or animal knockout technology has revealed both pro- and anti-inflammatory responses in various models of GN (16–18). Further studies with conditional targeting of chemokines and cognate receptors in models of GN would facilitate a better understanding of chemokine pathways in glomerular inflammation.

**Adaptive Nephritogenic Immunity (Steven Holdsworth, Monash University).** Dr. Holdsworth reviewed a model for T cell–mediated events in experimental models of crescentic GN (19,20). Preliminary findings in a murine model of crescentic GN induced by anti-myeloperoxidase were also presented, which suggest that T cell immunity plays a prominent role in glomerular injury in this model (unpublished observations).

**Autoantigen Complementarity and Autoimmunity** (Ronald Falk, University of North Carolina). Dr. Falk discussed a novel theory that inciting antigens for autoimmune diseases may be peptides that are complementary to relevant autoantigens. An antibody response that targets such a complementary peptide could also result in an anti-idiotypic response that targets the autoantigen. Evidence supporting this theory has been gleaned from patients with antineutrophil cytoplasmic antibody (ANCA) disease, in that several samples from patients with ANCA and predominant anti–proteinase-3 (PR-3) activity have antibodies directed against a protein product complementary to the middle fragment of PR-3 (21). Isolated anti-idiotypic antibodies also bind to PR-3 antibodies (21). Searches to identify complementary proteins in this setting suggest homology to previously identified proteins from various microorganisms that have been associated with ANCA vasculitis (22,23). Further studies are needed to assess better whether such complementarity is a critical factor in autoimmune disease induction.

**Toward the Identification of Glomerular Disease Biomarkers**

**Proteomic and Array Approaches in Cancer (Samir Hanash, Fred Hutchinson Cancer Research Center).** In view of the diversity of proteins and dynamic range of protein abundance, a wide range of proteomic techniques are needed to develop molecular profiles of disease (23–27). Dr. Hanash discussed molecular profiling approaches used for malignancy and then outlined a new effort that is currently under way, the Plasma Proteome Initiative, which will assess available technologies for characterizing serum proteins. The generation and availability of mAb to glomerular proteins would greatly facilitate molecular profiling of glomerular disease.

**Microarray Technology for Glomerular Disease (Erwin Böttinger, Mount Sinai School of Medicine).** Dr. Böttinger discussed recent observations that successfully exploited microarray technology in profiling glomerular disease associated with experimental models of diabetes (28,29). Further validation of glomerular disease marker genes in other experimental models will provide a basis for applying such microarray technology to human glomerular diseases. Optimizing methods for glomerular preparation and RNA handling may enhance use in clinical settings.

**Molecular Genetics and Glomerular Disease (Friedhelm Hildebrandt, University of Michigan).** Dr. Hildebrandt provided an overview of positional cloning and its impact on gene discovery in nephrotic syndrome and FSGS. These included discoveries related to mutations in nephrin, podocin, α-actinin-4, and WT1 and their associations with distinct forms of nephrotic syndrome/FSGS (30–33). Mutations identified by positional cloning might ultimately serve as disease biomarkers that assist in the diagnosis or treatment of glomerular diseases.

**Resources from Existing Sample Banks and Cooperative Studies**

**Glomerular Disease Networks (Matthias Kretzler, University of Munich).** Dr. Kretzler outlined the scope of the European Renal cDNA Bank, which includes >20 research centers that collaborate in collecting renal biopsies and clinical data from patients with multiple glomerular diseases. The goal of this cooperative effort is to generate glomerular gene expression maps and identify disease markers. Initial studies suggest that expression profiles of podocin/synaptopodin in biopsy material may reliably distinguish minimal-change disease from FSGS and may predict steroid responsiveness of disease (34). Further expansion of such efforts may suggest additional biomarkers of glomerular disease activity (35).

**Urine Protein Profiling with Mass Spectrometry (Peter Nickerson, University of Manitoba).** Dr. Nickerson reviewed recent findings regarding the utility of urine protein
profiling in the setting of kidney transplantation (36). The proteomic approach used in recent studies is a high-throughput mass spectroscopy platform (37). Identification of unique proteins from diseased urine revealed that levels of cleaved β-2 microglobulin were specific for patients with allograft rejection (38). Biomarker discovery in this setting, however, was critically dependent on correct handling of patient samples and strict adherence to definitions of clinical disease subsets (38).

How Can We Implement Clinical Studies More Effectively?
Dr. Howard Kaufman (Columbia University), a member of the Southwest Oncology Group (SWOG), discussed the organization and structure of the 283 cancer research institutions in this cooperative clinical studies network. In view of the high morbidity and mortality of cancer in the United States, SWOG was founded by the NIH/National Cancer Institute in 1956 and focuses on prevention, diagnostics, and therapeutics of a broad spectrum of oncologic diseases. In addition to clinical trial efforts, SWOG has developed a tissue bank for patient samples.

Recognizing budgetary constraints that typically are involved in clinical trial efforts, Dr. Denise Simons-Morton (NIH/National Heart, Lung, and Blood Institute) discussed mechanisms of costing a clinical trial. Early identification of the research question provides the best pathway for estimating trial costs and evaluating whether specific trials are actually feasible. Most trials are based on one of three general financial infrastructures: (1) Funding through a single coordinating center with capitated patient payments, (2) funding through a coordinating center and each clinical site, and (3) funding through a coordinating center and “network hubs” that pay clinical sites for patient enrollment and visits. Working through clinical trial costs is clearly a complex process, with the primary goal of determining whether a trial can effectively answer the specific research question.

Panel Discussion on How to Implement Clinical Studies of Glomerular Disease
To provide an overview of ongoing studies, investigators who are involved in current clinical trials in glomerular disease presented details of their work. Dr. Norman Siegel (Yale University) described the FSGS Clinical Trial, a multicenter, randomized trial to compare the effectiveness of two treatment regimens in children and young adults with steroid-resistant idiopathic FSGS. The trial, sponsored by the NIDDK (www.fsgstrial.org), will also collect patient data and samples that will be available to the greater research community to conduct ancillary studies. Dr. Daniel Catran (Toronto General Hospital) discussed the Toronto Glomerular Nephritis Registry, which has collected thousands of renal biopsy reports and initial and follow-up data points from patients in Canada and the United States. Dr. Susan Hogan (University of North Carolina) described the Glomerular Disease Collaborative Network, assembled at the University of North Carolina, which has collected patient data and samples from a large group of nephrologists across the southeastern United States. Dr. Michael Mauer outlined the Renin Angiotensin System Blockage Study (RASS) study, an NIDDK-funded primary prevention trial of nephropathy in type 1 diabetes. This randomized, placebo-controlled trial has been very successful in enrolling and following 285 patients over 5 yr at three clinical sites.

Breakout Sessions: Discussion and Recommendations
After completing the scientific program, meeting participants assembled in small groups to discuss four specific questions about several important aspects regarding future needs of glomerular disease research. The following text summarizes participant discussion from the final session of the Workshop.

Which Experimental Models Are Used Widely Enough that Standardized Protocol and Reagents Would Be Useful?
Standardization of methods and reagents for well-established models (MRL/lpr and NZB/W lupus-prone mice, nephrotic nephritis, anti–glomerular basement membrane disease, passive and active Heyman nephritis, anti-Thy1 nephritis, factor H–deficient mice, and toxic nephropathy models) would be useful to the research community. Additional recently studied models, such as anti-myeloperoxidase mice, CD2AP null mice, and podocyte depletion models, might also be of interest. Providing centralized open access to relevant reagents for basic studies with these models would also be useful. In addition, a more standardized approach to glomerular disease study end points would be of particular use to the community.

Which Issues Regarding Studies of Glomerular Diseases Are of Highest Priority?
Identification and characterization of glomerular disease biomarkers should be a high priority for future research as they would allow better disease classification, detection, and prognostic information and facilitate tracking response to therapy. This should be a high priority for future research.

Establishment of a cooperative network, with clinical trial infrastructure, disease registry capabilities, and tissue repository, would greatly facilitate glomerular disease clinical research. Widespread cooperation among clinicians, pathologists, and investigators will be needed, and a central mechanism must be established for collecting and storing samples and clinical information using well-defined protocols.

Although recent progress has been made in understanding the biologic basis of some forms of glomerulopathy, participants urged stimulation of further research in this area. IgA nephropathy, FSGS, membranous glomerulopathy, small vessel vasculitis, and lupus nephritis were identified as diseases that might receive initial research priority.

Participants voiced concern that renal biopsy safety should be clearly established for the renal community to encourage both appropriate balance between the legitimate safety concerns and the potential scientific benefits of this critically important reagent for future studies of glomerular disease. Development and phenotypic analysis of new genetic models (e.g., mouse ENU mutagenesis) and innovative glomerular imaging methods should be encouraged.
What Are the Major Barriers to Translational Studies?

A number of barriers were identified by participants, including a paucity of glomerular disease biomarkers, lack of infrastructure for collecting and distributing patient samples and data, absence of standardized end points, insufficient funding for clinical studies of glomerular disease, and insufficient public awareness of glomerular disease burden in the United States. A lack of standardized experimental models for preclinical glomerular disease testing was discussed.

Meaningful clinical investigations of glomerular diseases are scarce as a result of the relative rarity of individual glomerular disorders, limited infrastructure, and limited funding. In addition, except for measures such as serum creatinine, glomerular disease–specific end points have not been identified for the various morphologic forms of glomerular disease. It therefore would be useful for investigators to perform studies that would evaluate new methods of assessing clinical outcomes in glomerular disease. New outcome measures could significantly reduce both patient sample size and follow-up time for interventional trials. Ancillary studies to ongoing clinical studies of patients with glomerular disease should be encouraged to make use of patient data and samples for such purposes. Additional administrative barriers were also discussed, such as lack of uniformity of oversight with various institutional review boards and implementation of federal guidelines regarding access to patient data.

Which Novel Therapies Show Enough Promise to Move to Human Studies?

The results of small-scale trials may be sufficiently promising that large multicenter trials of well-selected cases of glomerular diseases should be considered. Reagents with therapeutic promise include anti-CD20 (rituximab) and anti-C5 (eculizumab) in active cases of severe membranous nephropathy, lupus nephritis, membranoproliferative GN, and perhaps IgA nephropathy. There is also extensive pharmaceutical interest in the development of effective antifibrotic agents. If there were agreement within the glomerular disease community that all types of chronic progressive glomerular diseases could be combined in a national cooperative trial, then it is likely that sufficient subjects would be available for such a trial using agents such as pirfenidone, TGF-blocking reagents, rapamycin, aldososterone blocking agents, IFN-β, and IFN-γ or other antifibrotic agents under development. Participants also recognized a number of administrative concerns regarding trial design issues, patient selection and recruitment, and intellectual property concerns.

Conclusion

Glomerular disease research should be encouraged to progress in the distinct areas of basic discovery, applied translational, and clinical efforts. Identifying glomerular disease biomarkers by a variety of approaches has great potential to advance both basic research and clinical research of human glomerular disease. Technical advances likely make the identification of biomarkers feasible. Rapid characterization of emerging biomarkers and their application to clinical studies of glomerular disease will require a coordinated national or international effort. Meaningful clinical investigations of glomerular diseases are currently scarce as a result of limited infrastructure and lack of disease-specific clinical end points. Discernment of disease-specific end points and development of collaborative clinical groups would provide adequate information and expertise to facilitate translational and clinical efforts for studies of human glomerular disease.

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