NIDDK GLOMERULAR DISEASE WORKSHOP

24-25 JANUARY 2005

SUMMARY REPORT

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NIDDK GLOMERULAR DISEASE WORKSHOP

Executive Summary

NIDDK convened this two-day Workshop to gather together basic scientists and clinical investigators from the international research community to discuss recent observations and potential opportunities for improving diagnosis and therapeutic intervention for human glomerular disease.

The first session of the Workshop focused on Intrinsic Glomerular Cell Biology. Highlights of this session included the observation by Dr. Thomas Benzing and colleagues that a serine to proline substitution in murine podocin, the protein mutated in steroid-resistant nephrotic syndrome in humans, augments nephrin signal transduction and leads to elevated blood pressure and podocyte foot process abnormalities in mice. These findings identify a potentially new paradigm for blood pressure regulation at the level of the glomerular podocyte, perhaps by influencing glomerular filtration surface area. Based on her work in genetically modified mice, Dr. Susan Quaggin emphasized the importance of tightly controlled podocyte-derived VEGF-A signaling via paracrine interaction with glomerular endothelial cells on glomerular development and maintenance. The relevance of this system in human preeclampsia has already been established, and the effects of VEGF-A up or down regulation bear striking resemblance to human collapsing glomerulopathy and endotheliosis, respectively. Moreover, regulation of VEGF-A transcription by intracellular oxygen levels opens a possible role for VEGF-A in glomerular diseases. Dr. Roger Wiggins described a model for inducing graded depletion of rat podocytes, the effect of which is to produce various degrees of proteinuria, glomerular sclerosis and loss of kidney function. These findings support the ‘podocyte depletion hypothesis” as a mechanism underlying the development of FSGS, and provide a potential tool for investigating diagnostic and therapeutic interventions.

In a session devoted to Glomerular Gene Discovery, Dr. Karl Tryggvason described a comprehensive program to identify glomerulus-specific gene transcripts and develop reagents, including glomerulus-specific microarray chips and antibodies to expressed proteins to establish their biological role and expression pathways in mouse and human glomerular diseases.

In the session on Inflammation and Autoimmunity, Dr. Michael Holers described strategies to counteract complement activation at sites of immune injury, focusing particularly on the potential to regulate the alternate pathway by inhibiting factor B. Of particular interest, are new strategies to target complement inhibitors to the inflamed glomerulus with the use of fusion proteins of complement receptors and regulators. Dr. Detleff Schlondorff reviewed the role of chemokines and chemokine receptors in animal models of glomerulonephritis (GN), including leukocyte recruitment to sites of inflammation, as well as their role in the development of a peripheral immune response. Turning from the innate to the adaptive immune response in experimental crescentic GN, Dr. Stephen Holdsworth described studies showing that Th1 lymphocytes and macrophages are able to induce GN in cooperation with intrinsic glomerular cells expressing CD40 in the absence of circulating antibodies. In the final presentation of this session, Dr. Ronald Falk reviewed the interesting and surprising finding that anti-proteinase-3 (PR-3) from patients with ANCA-associated GN is actually an anti-idiotypic antibody to a complementary peptide produced by certain bacteria. These interesting findings raise the possibility of a more general paradigm in which microbes with potential to encode “reverse peptides” may lead to autoantibody production.

In the session devoted towards the Identification of Biomarkers of Glomerular Disease, Dr. Samir Hanash described various proteomic techniques for identifying disease-related proteins by mass spectroscopy. He envisaged an effort such as the Human Antibody Initiative to develop monoclonal antibodies to 1000 proteins relevant to glomerular diseases that would be available to glomerular disease investigators to define biomarker profiles much as has been done to characterize hematological and soft-tissue malignancies. In a complementary strategy, Dr. Erwin Böttinger described work from his own group using microarray technology to identify biomarkers of glomerular disease. Using longitudinal phenotypic analysis and whole-genome array analyses of RNA

derived from isolated glomeruli from several murine models of glomerular disease, he and his colleagues have been able to sort animals with different phenotypes on the basis their gene expression profiles. Dr. Friedhelm Hildebrandt concluded this session by describing how modern molecular genetic techniques combined with a positional cloning approach are able to identify potential candidate genes that might underlie the development of common glomerular diseases such as focal and segmental glomerulosclerosis (FSGS). He pointed out that a mutated gene, such as podocin, associated with a particular form of glomerular disease provides an unequivocal biomarker.

On the second day, Dr. Matthias Kretzler opened the session on "the use of resources from existing sample banks and cooperative studies" by describing the efforts of the European Renal cDNA Bank. This initiative involves the cooperation of 24 research centers in the collection of renal biopsy material and clinical data from patients with a wide range of glomerular diseases. Dr. Peter Nickerson then described the use of a proteomic approach to identify non-invasive biomarkers in the urine for the diagnosis of renal allograft rejection. His presentation emphasized the importance of standardizing the technique for sample collection, preservation, and preparation for mass spectroscopy. If these issues are carefully attended to, he showed that it is possible to identify physiologically and pathologically relevant profiles in normal subjects and in patients undergoing transplant rejection. It was clear from this session that widespread cooperation between clinicians, pathologists and investigators would be required, and a central mechanism established for collecting and storing samples and clinical information, and that well-defined protocols for the collection, storage, and transport of samples would be required.

Dr. Howard Kaufman expanded on this theme in the session on how to implement clinical studies more effectively by recounting the history of the Southwest Oncology Group (SWOG), a cooperative network of 283 research institutions aimed at promoting cancer research. Dr. Kaufman also outlined the ground rules for participation in SWOG and described how the growth of the organization has enabled the development of sub-committees whose activities facilitate the translation of laboratory discoveries to the clinic. Dr. Denise Simons-Morton went on to describe, in detail, the blueprint for costing

a clinical trial based on her experience at NHLBI. She described three possible financial infrastructures for the distribution of awarded funds, and detailed the entities that must be funded in a trial.

The morning session concluded with a panel discussion on how to implement clinical studies of glomerular disease in which Dr. Norman Siegel described the FSGS Clinical Trial, a multi-center randomized trial to compare the effectiveness of two treatment regiments in children and young adults with steroid-resistant idiopathic FSGS sponsored by NIDDK (www.fsgstrial.org). Dr. Siegel emphasized that a goal of this study is to assemble clinical data and patient samples to conduct ancillary research, and he outlined the guidelines for gaining access this material. Dr. Daniel Cattran followed this presentation with a description of the Toronto Glomerular Nephritis Registry. This registry has developed over the past three decades and has collected over 10,000 renal biopsy reports and over 35,000 initial and follow-up data points based on the recruitment of patients in Canada and the U.S. into randomized clinical trials. Dr. Ronald Falk then described Glomerular Disease Collaborative Network based at the University of North Carolina that involves a large group of practicing nephrologists across the southeast U.S. This network has facilitated clinical trials and also provided clinical data and biological samples for observational and epidemiological studies, as well as fundamental research. Finally, Dr. Michael Mauer provided an update of the RASS study, a primary prevention trial in type 1 diabetic patients. This trial has been remarkably successful in patient recruitment and retention and Dr. Mauer expressed confidence that similar trials can be successful in other glomerular diseases.

The workshop concluded with a plenary session at which the chairs of four Breakout groups presented their responses to the following four questions.

A. Which experimental models are used widely enough that standardized protocol and reagents would be useful?
B. Which issues regarding studies of glomerular diseases are of highest priority?
C. What are the major barriers to translational studies?
D. What novel therapies show enough promise to move to human studies?

Conclusions and Recommendations of the Workshop and Breakout Sessions

Research must be encouraged to progress concomitantly on several levels: basic discovery, applied translational, and purely clinical.

1. Identification of biomarkers of glomerular disease by a variety of approaches has great potential to advance both basic and clinical research in glomerular diseases. Advances in technology make the identification of biomarkers feasible. Rapid characterization of emerging biomarkers such that these will be clinically useful will require a coordinated and unselfish national or international effort. Widespread cooperation between clinicians, pathologists and investigators will be required, and a central mechanism established for collecting and storing samples and clinical information, as well as well-defined protocols for the collection, storage, and transport of samples.

2. Meaningful clinical investigations of glomerular diseases are scarce due to the relative rarity of individual glomerular disorders, limited infrastructure and limited funding. In addition, glomerular disease-specific outcome measures have not been identified for the various morphologic forms of glomerular disease, other than measures such as serum creatinine. It would therefore be useful for investigators to perform studies that would evaluate new methods of assessing clinical outcomes in glomerular disease. Such new outcome measures could significantly reduce both patient sample size and follow-up time for interventional trials of glomerular disease. 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. With clear definition of standardized clinical outcomes in glomerular disease, the development of a clinical trial network within the US, similar to SWOG, would provide necessary infrastructure and expertise for performing clinical studies of various glomerular diseases.

3. The results of small-scale trials are sufficiently promising that large multicenter trials of well selected cases of glomerular diseases should be considered. These reagents include anti-CD20 (rituximab) and anti-C5 (eculizumab) in active cases of severe membranous nephropathy, lupus nephritis, MPGN and perhaps IgA nephropathy. There is also extensive pharmaceutical interest in the development of effective anti-fibrotic agents, mostly focused on interstitial lung disease and other more common chronic progressive diseases. 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, it is likely that sufficient subjects would be available for such a trial and that there might be sufficient interest of pharmaceutical companies with anti-fibrotic agents currently under development.
4. The following items are recommended to advance basic research into the etiology and pathogenesis of glomerular diseases, and provide suitable models for preclinical studies.
    a. Selected, well-established models should be standardized. This could be accomplished by developing a central supply of reagents and uniform protocols and endpoints.
    b. Support the development and phenotypic analyses of genetic models of glomerular diseases through targeted as well as ENU mutagenesis, and create a public database of available models.
    c. Encourage the development of an animal model of IgA nephropathy.
    d. Create repositories for tissues and pathology blocks/slides from disease models, as well as relevant monoclonal antibodies and glomerular cell specific DNA arrays and libraries.
    e. Require that transgenic and knockout mice be made available through existing public or private repositories.
    f. Develop core facilities for MS/MS, stereology and other specialized imaging techniques.

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Scientific Presentation Summaries

PATHOBIOLOGY OF GLOMERULAR DISEASE

Intrinsic Glomerular Cell Biology

The Podocyte -The Dynamic Slit Diaphragm Protein Complex: Signaling and Beyond

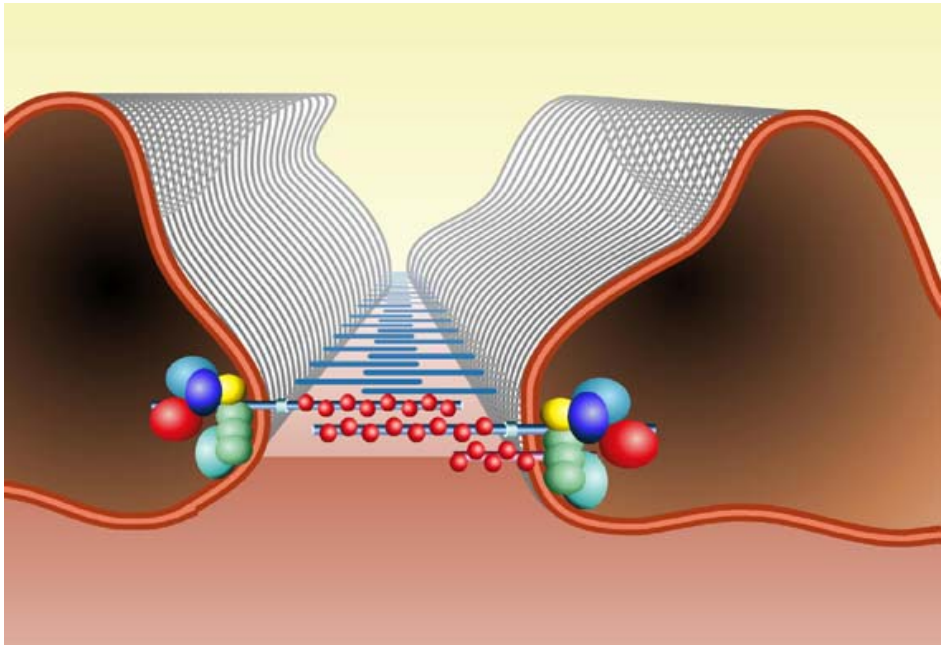
Thomas Benzing, MD, University of Freiburg

Once through the capillary endothelium and its basement membrane, fluid filtered by the kidney passes through the slit diaphragm before entering Bowman's space. In presenting a model from Karl Tryggvason depicting how interdigitating nephrin molecules compose the slit diaphragm between adjacent podocyte foot processes, Dr. Benzing underscored the complexity of the slit diaphragm and the fact that it is composed of a number other proteins, some yet to be identified.

Dr. Benzing and coworkers demonstrated that slit diaphragm proteins participate in complex signaling pathways in the podocyte. The cytoplasmic portion of nephrin is tyrosine phosphorylated by a *src* family kinase and along with an adaptor protein, CD2AP, can reversibly bind phosphatidylinositol (PI) 3 kinase; this complex, with the participation of podocin, activates downstream signaling cascades such as the PI 3

kinase/Akt pathway. Consequently, in mouse strains deleted for the gene encoding CD2AP, apoptosis of podocytes and glomerulosclerosis was observed.

Podocin binds the cytoplasmic portion of nephrin, and its deletion causes a steroid-resistant nephrotic syndrome in humans. Podocin-nephrin association and functional signaling occurs in lipid microdomains in the bilayer of the podocyte membrane; podocin itself is palmitoylated and appears to bind cholesterol to organize the lipid microenvironment of the slit diaphragm protein complex. In mice, a serine to proline substitution in podocin appears to be associated with elevated blood pressure. Interestingly, this mutant podocin is well-expressed at the slit diaphragm. However, the high blood pressure variant shows an increased ability to augment nephrin signal transduction and appears to have higher affinity for nephrin binding than the wild type protein. The proline/serine change, while not associated with proteinuria, also causes alteration in the ultrastructure of foot processes and perhaps reduced overall glomerular filtration area. Dr. Benzing suggested that signal transduction at the slit diaphragm could actively regulate the structure of podocyte foot processes and the area of the slit diaphragm, thereby playing a previously unrecognized role in controlling kidney function and blood pressure.



Nephrin, a sensor in the slit diaphram

The Glomerular Endothelial Cell

Susan Quaggin, MD, Mount Sinai School of Medicine

Dr. Quaggin discussed recent findings on the role of vascular endothelial growth factor (VEGF) in the endothelial cells, which interact with the podocytes to constitute the glomerular filtration barrier. VEGF is of interest to the kidney research community because changes in its expression are associated with diabetes and glomerulonephritis (GN). In mouse models of diabetic nephropathy, agents that interfere with VEGF activity appear to slow disease progression. VEGF also plays an important role in angiogenesis, and since VEGF antagonists are now used in clinical trials to treat certain cancers, a better understanding its role in normal and diseased kidneys is needed.

The protein is present in both developing and mature podocytes. Electron microscopy studies suggest other locations, including the glomerular basement membrane and the luminal surface of the endothelium. To better understand the mechanism and role of VEGF signaling in the glomerulus and overcome embryonic lethality seen in *vegfA* mouse knockouts, Dr. Quaggin used a transgenic construct in which Cre/Lox-mediated recombination activity (specifically expressed in podocytes using the nephrin promoter) would cause podocyte-specific deletion of *vegfA*. The loss of both *vegf* alleles in podocytes caused perinatal lethality, while loss of one allele caused endotheliosis a common complication of pre-eclampsia. Over-expression of VEGF-A also caused a variety of defects, with collapsing glomerulopathy observed at the highest levels of *vegf-a* construct, confirming that tight regulation of VEGF expression is critical in the developing kidney. The role of VEGF in the adult mouse was examined using constructs permitting inducible expression; over-expression caused pronounced proteinuria and complex changes in glomeruli. In transgenic mice permitting inducible Cre/Lox

mediated deletion of *vegfA*, post-natal deletion led to end stage renal disease (ESRD) and glomerulosclerosis

The result demonstrated an important role of tightly-controlled VEGF-A signaling in glomerular development and maintenance. Dr. Quaggin presented a model of paracrine signaling by podocyte-derived VEGF across the basement membrane to capillary endothelial cells expressing FLT1 and FLK1 receptors, while pointing out that autocrine signaling in the podocyte was also important. *VegfA* transcription is sensitized to intracellular oxygen levels through the concerted effect of VHL and oxygen-sensitive proline hydroxylases on HIF-1 stability, and this could be relevant to glomerular disease. Dr. Quaggin concluded by proposing that better understanding of the role of VEGF in the developing and mature kidney could lead to new therapeutic approaches to glomerular disease.

Podocyte Depletion and Focal Segmental Glomerulosclerosis

Roger C. Wiggins, MD, University of Michigan

The podocyte depletion hypothesis suggests that podocyte damage and depletion is necessary and sufficient for development of focal segmental glomerulosclerosis (FSGS), as well as other forms of proteinuric kidney disease. Dr. Wiggins listed relevant criteria for models of FSGS in which this hypothesis could be tested, including glomerular pathology closely resembling human disease, and the ability of investigators to deplete podocytes at a precise stage in the life of the animal and in a carefully controlled manner.

Using the human podocin promoter to drive expression of a receptor for diphtheria toxin, Dr. Wiggins was able to achieve podocyte-specific expression of the human receptor in rats. When proteinuria was measured shortly after toxin injection, transgenic mice expressing the receptor showed a consistent dose response over a broad range. Examination of the glomeruli of the rats subject to different toxin concentrations indicated that a range of percent podocyte depletion could be achieved. Other quantitative measures of glomerular pathology plotted against podocyte loss, including

level of matrix expansion, number of capsular adhesions and level of proteinuria, were all proportional to podocyte depletion.

Mild depletion of podocytes (<20%) resulted in mesangial expansion and transient proteinuria with normal renal function. Podocyte depletion in the range of 20-40% caused adhesions and FSGS with sustained and low level proteinuria; renal function remained normal. Loss of more that 40% of podocytes was associated with global sclerosis, severe proteinuria and a decline in renal function. Given that the histologic features in this model system were very similar to those observed in humans; Dr. Wiggins concluded that the results were compatible with the idea that podocyte loss was an important mechanism leading to ESRD in humans. He suggested that since proteinuria and decline in kidney function are similarly correlated in diabetes, podocyte depletion might explain diabetic glomerulosclerosis, as has been suggested by other investigators.

Podocytes can be lost due to detachment, cell death by apoptosis and necrosis, HIV infection, and the presence of crescentic GN. Dr. Wiggins indicated that the phenomenon of cell loss initiating deleterious consequences was a characteristic of tissues containing non-dividing, highly-differentiated cells. Other examples include loss of neurons in neurodegenerative diseases, rod cells in the eye followed by retinal degeneration and loss of hair cells and deafness. With general acceptance of the notion that podocyte depletion is a central causative factor in renal disease, Dr. Wiggins concluded that there would be increased focus on understanding podocyte injury and preventing loss from the glomerulus. Podocyte depletion might become a useful surrogate for progression in many conditions, and urinary podocyte markers might be validated as biomarkers. Special high-risk podocyte losing patients groups might also be identified as candidates for preventive treatments.

Gene/Protein Discovery

The Glomerular Transcriptome

Karl Tryggvason, MD, PhD, Karolinska Institute

Discussing the scientific rationale for his systematic approach to glomerular biology and disease processes, Dr. Tryggvason noted that the pathological changes resulting from glomerular injury of diverse origins are quite similar, making differential diagnosis and treatment difficult or impossible. Detailed information about glomerular diseases processes at the molecular level is therefore essential. The main objectives of the project (Functional Genomics of the Renal Glomerulus) are to: (1) Characterize the glomerular transcriptome; (2) Generate glomerulus-specific microarray chips; (3) Identify gene transcripts that are highly specific for glomeruli in the kidney, and therefore likely to be important for glomerular development and function; (4) Examine glomerular expression profiles in different glomerular disease processes; (5) Generate specific antibodies against novel "glomerulus-specific" proteins; (6) Study the biological role of "glomerulus-specific" proteins by using gene knockout and knockdown techniques in mice and zebrafish, respectively; (7) Elucidate glomerular expression pathways in mouse and human glomerular disorders to facilitate development of better diagnostic methods.

Isolation of glomeruli for analysis of gene expression is by a novel method in which animals are perfused with magnetic beads that become trapped in the glomeruli; the procedure is rapid, and RNA quality is high. Dr. Tryggvason addressed issues of reproducibility in expression profiles obtained across different platforms, and he compared his transcription profile with earlier results obtained by serial analysis of gene expression. Thus far, he and his colleagues have found about 7000 transcripts in the glomerular transcriptome, 200 of which encode novel proteins of unknown function that are, in the kidney, and appear specific to the glomerulus. Studies have been initiated to characterize biological function of genes in mice, where knockout strains for novel genes are being created, and in zebrafish, where morpholino-based inhibition of gene expression is used for rapid functional screening of the 200 glomerular genes. In addition, antibodies against all these novel proteins are being generated. Findings from this project will be reported in due course and may provide a wealth of knowledge about the development and biology of the glomerulus, identify novel genes with roles in glomerular disease, and create disease-specific expression profiles. This information will potentially be the basis of new diagnostic methods and the identification of novel drug targets.

Inflammation and Autoimmunity

Complement, Therapeutics and Glomerular Disease

Michael Holers, MD, University of Colorado Health Sciences Center

The complexity of the complement system offers multiple opportunities for intervention in inflammatory kidney disease. Dr. Holers summarized some current strategies under investigation to attenuate complement activation based on antibody targeting of complement components, and other approaches, some pathway specific and others acting downstream of C3 as pathway non-specific.

In studies of the MRL-*FAS* ^{lpr} model conducted by Dr. Gary Gilkeson of the Medical University of South Carolina, deletion of the gene encoding factor B in the alternative pathway ameliorated renal disease by reducing glomerular inflammation and level of proteinuria. The positive effects of factor B deficiency, including increased lifespan, were observed even though there was no change in circulating antibodies relative to controls. Dr. Holers discussed an additional approach to alternative pathway inhibition through continual injection of monoclonal antibody to Factor B generated in his laboratory. Blocking Factor B, either before or after appearance of disease symptoms decreased proteinuria, glomerular inflammation and urinary nitrate/ nitrate production. Blockade had no effect on anti-dsDNA antibody production or on IgG deposition in the kidney. Dr. Holers discussed briefly some underlying factors that might contribute to the inappropriate activation of the alternative pathway, such as alterations of complement regulatory proteins at the site of tissue injury, disruption of normal regulation by import of pathway components by inflammatory cells, local synthesis of pathway components, and action of the activation products of Factor B on as yet poorly characterized receptors.

Dr. Holers indicated that prior to the clinical use of therapeutics targeting the complement pathway the potential implications of complement inhibition on tissue repair and pharmacokinetic parameters need to be addressed. With the goal of developing safer and more effective therapeutics, he discussed several new strategies on the horizon for

specific renal targeting of complement inhibitors, particularly those of fusion proteins of complement receptors and regulators.

Chemokines: Mediators of Glomerular Disease

Detleff Schlondorff, MD, University of Munich

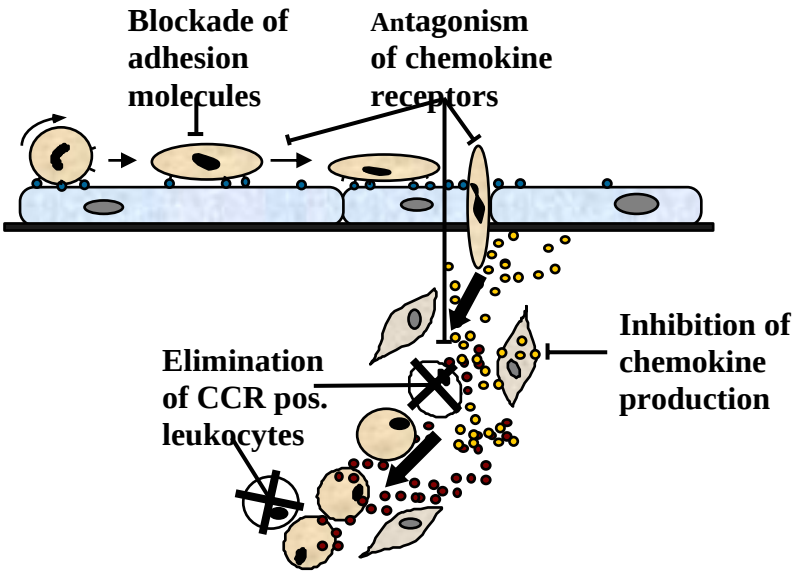
Chemokines are cytokines that act as chemoattractants, mediating the directional migration and activation of a variety of immune cell types. They are classified into four families based on the arrangement of cysteine residues (internal disulfide bonds are a characteristic of these peptides). Considerable progress has been made in identifying the cell types in which individual chemokines are expressed and the cells upon which they act via cognate receptors. Dr. Schlondorff briefly illustrated how chemokines are secreted by various tissues at sites of injury, establishing a concentration gradient on surfaces around the lesion, and then attracting and activating leukocytes.

Focusing on the kidney, he indicated that all types of renal cells can produce inflammatory chemokines upon stimulation. Prosecretory signals include reactive oxygen species, cytokines like IFN-gamma, angiogenic factors (angiotensin II), and LPS. Increased expression of particular chemokines is generally restricted to sites of renal injury and is accompanied by infiltration of receptor-positive lymphocytes, macrophages, and other immune cells.

Dr. Schlondorff showed data confirming the role of chemokines in a model of lupus-like symptoms and GN, the MRL/*lpr* mouse. Treatment of these mice with an antagonist to CCR-1, a receptor for the chemokines RANTES and MIP-1 (these are typically expressed in response to renal injury), reduced glomerular infiltration of cytotoxic lymphocytes, and fibrosis was markedly decreased. In spite of pronounced reduction in tubulointerstitial inflammation, proteinuria levels and serum levels of DNA autoantibodies were relatively unchanged, suggesting that the underlying disease process was perhaps unaltered. In a rat model of crescentic GN induced by antibodies to glomerular basement membrane, administration of antibody to CCL2, a chemokine highly expressed in nephritic glomeruli, markedly decreased infiltration of monocytes

and macrophages and reduced proteinuria and glomerular crescent formation in later stages of disease.

Dr. Schlondorff presented a table summarizing results of chemokine blockade in a variety of models of inflammatory kidney disease. He indicated that the role of chemokines is much broader than leukocyte recruitment and includes roles in uptake of apoptotic cells, dendritic cell maturation, and in regulatory T cell trafficking. The complex effects of pharmacological inhibition are therefore not surprising. In interpreting results in disease models, species differences in chemokine signaling must also be considered. New mouse strains permitting conditional targeting of chemokines and their receptors are greatly needed.



Targeting the chemokine network in renal inflammation

Mechanisms of Adaptive Immunity Inducing Crescentic Glomerulonephritis

Steven Holdsworth, MD, PhD, Monash University

Dr. Holdsworth began by pointing out that crescent formation associated with severe, rapidly progressing GN can be caused by several distinct disease entities. To understand the immunological mechanisms underlying crescentic nephritis, he has employed a model in which immunized mice are challenged by the glomerular planting of sheep globulin (targeted to glomeruli as sheep anti-mouse glomerular basement membrane globulin). Interference with the immune response of the mice by immunoneutralization had suggested that T cells and macrophages were critical mediators of GN in this system. Based on his experiments with specific cytokine deficient mice, Dr. Holdsworth presented a model of how critical Th1 lymphocyte-mediated responses cause GN.

To better understand the role of non-immune cells in the development of GN, Dr. Holdsworth used a series of knockout mice from which the bone marrow was depleted and replaced with marrow from wild type mice; the chimeras would have wild type immune cells, while resident renal cells would lack certain genes critical to immune function. These experiments indicated an important role for renal cell and immune effector cell cooperation in the immune responses leading to crescent formation after glomerular antigen challenge; immune response depended on renal cells expressing CD40 and local production of IL-12 to induce chemokine production.

Further insights into the etiology of crescentic GN in human disease were provided by a model in which mice were injected with human myeloperoxidase (MPO), a common antigenic specificity of ANCA (anti-neutrophil cytoplasmic antibody) isolated from affected humans. In these mice, subsequent injection of antibodies to glomerular basement membrane causes crescentic GN. Mice lacking the gene encoding the immunoglobulin gamma chain were, as expected, unable to make circulating ANCA, but even in the absence of humoral immune response, crescentic GN was observed. Depletion of CD4 T cells attenuated crescent formation. Therefore in this GN model, cellular immune responses to MPO is sufficient to cause glomerular damage independent of circulating ANCA. Dr. Holdsworth concluded by describing experiments which demonstrated that anti-MPO-bound neutrophils localized to the glomerulus leading to diffuse glomerular deposition of MPO; an observation that might explain the associated glomerular damage noted in MPO-ANCA patients. He suggested that continued progress

in understanding the complex immune mechanisms in crescentic GN could reveal many potential therapeutic targets.

Autoantigen Complementarity and Autoimmunity

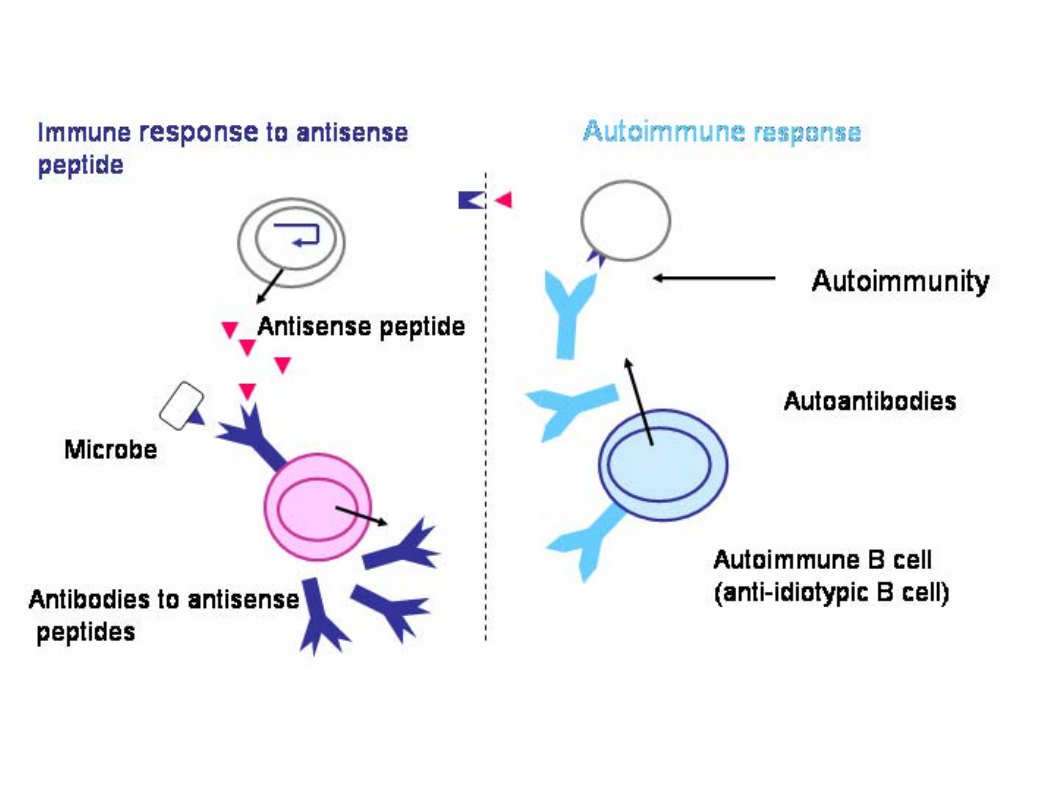
Ronald J. Falk, MD, University of North Carolina at Chapel Hill

Dr. Falk presented findings which offered new insights into the possible origins of autoimmunity. He and coworkers made the surprising observation that the sera of ANCA patients reacted with bacterial clones in which the gene encoding proteinase-3 (PR-3), a common ANCA antigenic specificity, was inserted in the reverse orientation, thereby expressing an antisense RNA encoding the "antisense peptide". Dr. Falk suggested that this antisense peptide might be the true initiator of immune response. In this scenario, the antisense peptide initiates the production of antibodies, which elicits a second immune response to produce anti-antibodies. These anti-idiotypic antibodies can be self-reactive if the self protein binds in complementary fashion to the "antisense peptide".

Sera from 7 of 34 patients with antibodies to PR-3 tested positive for a complementary peptide. Purified antibodies to PR-3 and antibodies to the complementary peptide bound each other, and the association was inhibited by complementary peptide, suggesting that the purified antibodies constituted an idiotypic pair. Consistent with this, mice immunized with the complementary peptide to PR-3 developed antibodies to PR-3 as well.

The model requires that PR-3 and its complement derived from reverse transcription (or a portion of the complementary peptide chain) interact. In support of this prediction, Dr. Falk found that the protease activity of PR-3 was inhibited in a concentration-dependent fashion by complementary peptide. Dr. Falk also referred to previous work which proposed that peptides encoded by sense and antisense sequences would tend to interact based on the tendency of codons to encode an amino acid of different hydrophilicity when read as the reverse complement. RT-PCR of extracts of circulating leukocytes using gene-specific primers confirmed the presence of a reverse transcript with the potential to encode complementary peptide in about half of PR3-ANCA patients (some MPO-3 ANCA patients also were positive). Interestingly,

pathogens thought to be associated with ANCA vasculitis, such as S*taphyloccus aureus* and *Entamoeba histolytica*, have DNA sequence with the potential to encode "reverse peptides" to ANCA antigens.



TOWARD THE IDENTIFICATION OF BIOMARKERS OF GLOMERULAR DISEASE

Proteomic/Genomic/Tissue Microarray Approaches in Cancer
Contribution of Proteomics to the Global Molecular Profiling of Disease
Samir Hanash, MD, PhD, Fred Hutchinson Cancer Research Center

Dr. Hanash presented a general overview of problems and considerations in applying proteomic methodologies to human disease. The potential of proteomics in the diagnosis and classification of disease and the identification of therapeutic targets is tremendous. However, the chemical diversity of proteins and their wide range of

concentrations in the body represent major challenges to those applying systematic approaches. Dr. Hanash recommended a "divide and conquer" strategy in which manageable subproteomes (phosphoproteins, glycoproteins, cell surface proteins etc.) are analyzed. As an example, he described a method for the biotinylation and affinity purification of diverse cell surface proteins and their separation by 2D gel electrophoresis prior to sequencing by mass spectroscopy (MS). A primary application of his approach has been in identifying tumor-specific cell surface markers.

The goal of the Plasma Proteome Initiative is to profile protein constituents of serum/plasma and identify protein markers that predict onset of common diseases. Dr. Hanash pointed out that the concentration of individual proteins in plasma can range over 12 orders of magnitude. Given this and other technical obstacles, the aims of the project in its pilot phase are to assess a range of technologies for serum protein characterization in terms of their resolving power, sensitivity, cost, and practicality, and to identify important factors in specimen collection, handling and storage. Thus far a total of 3000 plasma constituents have been identified by MS-based sequencing.

Dr. Hanash also discussed a new approach to identifying diagnostic biomarkers. In the Intact Protein Analysis System, sera from two individuals or time points are labeled with different fluorophores and then pooled for a series of chromatographic separations. Fluorescence scanning of gels of the fractions can identify those proteins which are increased, decreased, or remain unchanged in the two samples. Dr. Hanash referred to a recent application of this technique for patients with acute graft-versus-host disease, which indicated that these individuals had elevated serum levels of HGF.

Central to proteomic applications is the availability of capture agents; these are critical in assays of protein abundance, systematic mapping protein, localization and constructing networks of protein interactions. The Human Antibody Initiative, working from 2 centers in Europe and a third in China, will begin by producing antibodies to 3000 proteins. As more antibodies become available, Dr. Hanash recommended that monoclonal antibodies to 1000 proteins relevant to glomerular kidney disease be made available as a standard resource to the investigator community through an ATCC-like mechanism.

Dr. Hanash concluded by pointing out that no single technology can fit all applications in proteomics, and that multiple strategies must be pursued simultaneously if the potential of the systematic approach is to be realized.

Microarray Technology for Identification of Biomarkers of Glomerular Disease
Erwin Böttinger, MD, Mount Sinai School of Medicine

Dr. Böttinger discussed the optimal use of the wealth of gene expression data obtained by new microarray technologies as a means to improved understanding and treatment of kidney disease. He has focused in part on standardized longitudinal phenotype analysis and whole-genome array studies in several mouse models of progressive diabetic nephropathy, immune-mediated GN and FSGS. In spite of differences relative to human disease, the models overcome some obvious obstacles, such as genetic heterogeneity and lack of environmental control, encountered in human studies. Working across several mouse models and performing microarray analysis on samples from kidney biopsies, he was able to identify genes expressed in podocytes, whose decreased expression was closely associated with the degree of mesangial matrix expansion and other phenotypic outcomes. The validity of the statistical inferences was confirmed by successful sorting of animals into groups with or without markers of glomerulopathy based on the newly discovered biomarkers.

Dr. Böttinger briefly discussed plans to extend these methodologies to the problem of finding prognostic and diagnostic markers for diabetic nephropathy and non-diabetic glomerulopathies. He has optimized methods for separating glomeruli and tubules from human kidney biopsies and methods to ensure that RNA of sufficient quantity and quality can be obtained from as few as five glomeruli. Adequate biomedical informatics support is critical for large-scale marker discovery based on expression analysis, and he described data management infrastructure developed in his group. The paucity of human samples and the general reluctance to perform renal biopsies remain major obstacles.

There are now new technologies for the quantification of RNA transcripts based on fluorescent labeling and a proprietary microfluidic system (U.S. Genomics), which

permit direct detection of individual RNA molecules by laser excitation. This method may make amplification of RNA unnecessary and could make quantification of RNA of molecular markers more reproducible in clinical settings.

Molecular Genetics Approach to Nephrotic Syndrome and Focal Segmental Glomerulosclerosis (The Podocin Mutation)
Friedhelm Hildebrandt, MD, University of Michigan

Positional cloning approaches have identified a number of autosomal recessive and dominant mutations causing FSGS. This has focused the pathogenesis of FSGS at the glomerular basement slit membrane. By definition, positional cloning provides unequivocal "biomarkers" (mutations) that are useful to clinicians. For example, the cloning of multiple disease alleles of *NPHS2* encoding podocin has enabled physicians realize that patients with 2 mutations in *NPHS2* are resistant to standard steroid therapy but are also at reduced risk for recurrence of FSGS in the renal transplant. Dr. Hildebrandt underscored the point that FSGS can be caused by a number of different genes, and some remain completely uncharacterized. He added that using homozygosity mapping, his group has identified a new FSGS locus on human chromosome 14.

Critical to mapping additional FSGS disease genes will be the availability of large consanguineous pedigrees. Dr. Hildebrandt also described DNA microarray technology for rapid identification of multiple single nucleotide polymorphisms (SNP). He noted that these technologies and efforts toward construction of a comprehensive haplotype map in humans have greatly increased the power and speed of mapping on the basis of SNPs. Candidate genes from these mapping methodologies can now be quickly assessed using new mutation analysis methods. Systems biology and genomics approaches will provide a wealth of data (e.g. information on protein-protein interactions, and conservation across species) for evaluating candidate genes and understanding their role in disease. Showing a graph illustrating the steady rise in the last 15 years of the number newly identified renal disease genes, Dr. Hildebrandt predicted that the approach of positional cloning would continue to be a powerful one.

RESOURCES FROM EXISTING SAMPLE BANKS AND COOPERATIVE STUDIES

Glomerular disease tissue bank network: Analyzing the Transcriptome of Human Renal Disease

Matthias Kretzler, MD, University of Munich

Dr. Kretzler outlined strategies for applying modern methods of gene expression profiling to kidney disease. Central to the work is the European Renal cDNA Bank (ERCB) in which 24 research centers cooperate in the collection of renal biopsies from patients with the range of nephropathies encountered in clinical practice. Standard protocols for clinical description, biopsies, storage of tissue, dissection, RNA/cDNA preparation, gene expression array methods, and data analysis have been developed.

He presented recent data on the profiling of FSGS and minimal change disease, glomerular diseases difficult to distinguish in their early phases, yet having different treatments and prognoses. His group was able to extract a disease profile from the expression data: the ratio of genes encoding synaptopodin and podocin effectively distinguished the two patient populations and accurately predicted response to steroid therapy. Similar efforts in which gene expression analysis is integrated into diagnostic practice are underway for the differential diagnosis of tubular damage by proteinuria, the prediction of progression in tubulointerstitial inflammation, diagnosis of BK virus nephropathy, and other conditions.

Because gene expression arrays furnish an overwhelming amount of data, Dr. Kretzler advocated the use of focusing on subcategories of genes in identifying transcriptional programs relevant to disease. In diabetic nephropathy he showed that the developmental Wnt pathway is dysregulated, as are genes involved in inflammation under the control of NFkB. Another promising strategy involves promoter modelling, by analyzing promoter sequence across the entire genome, in which common mechanisms of transcriptional activation (binding of a common set of transcription factors) can be inferred and thereby predict expression of genes in particular cell types or disease

processes. As a proof of concept, Dr. Kretzler compared the nephrin gene promoter across several species, identified a common promoter element, and among candidate genes noticed conservation in another slit membrane associated gene, ZO1. Subsequent gene expression profiling showed that as predicted, the nephrin and ZO1 genes were the most similar genes in terms of expression profile in several nephropathies.

Finally, Dr. Kretzler discussed future strategies for the storage and organization of gene expression data for the investigative community to permit disease-specific data mining for the widest possible audience.

Urine Protein Profiling with Mass Spectroscopy

Peter Nickerson, MD, FRCPC, Canadian Blood Services

Dr. Nickerson's discussed his attempts to develop a non-invasive biomarker in urine for renal allograft rejection. He first investigated whether Seldi-TOF-MS would be a useful tool to profile protein composition in a complex mixture. The technique was reproducible if careful procedures and controls were followed. Along these lines, midstream urine profiling is preferred, freeze-thaw cycles of samples should be minimized, and centrifugation should be performed to address blood contamination. The method was reliable for urine volumes up to 3 liters/day.

Dr. Nickerson compared urine among normal controls and those experiencing acute transplant rejections and saw clearly distinguishable differences in the protein profile. Prominent peaks were observed in the rejection group (17/18 cases) that were wholly absent in the normals. Urine from stable (as defined by biopsy) transplant patients showed the characteristic rejection pattern in only a few cases. The rejection pattern was not characteristic of acute tubular nephrosis, glomeropathy, or post-transplant CMV viremia.

By fractionation of the samples prior to laser excitation and MS, Dr Nickerson was able to identify some of rejection-specific proteins as cleavage products of beta 2 microglobulin - the protease activity is probably released into the urine by damaged tubular epithelial cells. Cleaved beta 2 microglobulin may therefore be a sensitive biomarker for tubular cell stress injury. With these promising results, Dr. Nickerson

discussed plans to move to a low through-put, high resolution platform to compare the urine proteome in greater detail, looking for novel process-specific biomarkers.

HOW CAN WE IMPLEMENT CLINICAL STUDIES MORE EFFECTIVELY?

The Southwest Oncology Group Experience

Howard L. Kaufman, MD, Columbia University

The Southwest Oncology Group (SWOG) is a cooperative network of 283 research institutions and nearly 4000 physicians whose aim is to promote cancer research. Its inception dates to 1955 when a Clinical Studies Panel at the National Cancer Institute saw the need for physicians to work together on clinical trials along the lines by which physicians at Veterans Administration hospitals had cooperated in studying tuberculosis. One year later the Southwest Cancer Chemotherapy Study Group was formed in Houston with an initial focus on pediatric oncology. Under direction of the NCI, the group was directed to include the study of adult cancers; eventually the study of pediatric cancers devolved to a second organization, the Pediatric Oncology Group. Dr. Kaufmann presented major milestones in the development of SWOG into one of the leading clinical trials organizations in the world with an annual operating budget of approximately $28M.

Overseeing the group today is a Board of Governors consisting of Principal Investigators with cooperative agreements with NCI, representatives of SWOG Disease Committees (focusing on particular cancers) and SWOG Discipline Committees (mandates of the Discipline Committees include Cancer Control Research, Special Populations, and Correlative Sciences). The Board advises a chairman, Dr. Charles Coltman, who is located at the Cancer Research and Therapy Center in San Antonio Texas.

Dr. Kaufmann discussed three committees whose activities would be of special interest to the glomerular disease investigators: The Immunomolecular Therapeutics Committee was established in 1998 to rapidly translate laboratory discoveries to the

clinic—through protocol and product development, fostering communication between bench researchers and clinicians, establishing interactions with industry, and promoting clinical trials. The Early Therapeutics Committee addresses organ dysfunction in cancer and questions concerning pharmacokinetics. The Correlative Sciences Committee reviews and prioritizes laboratory correlates for clinical trials.

Dr. Kaufmann outlined standing procedures to assess institutional compliance and performance in terms of submission of samples, regulatory material and discussed procedures for the reporting of clinical trials, including adverse event reporting. Tissue and serum banking from patients in SWOG trials is now performed in a number of disease areas with confidentiality safeguards and standard handling protocols in place. Services provided by SWOG include the storage of a wide variety of biological samples, separation of plasma constituents, preparation of immunostained slides for microdissections, laser capture microscopy, DNA extraction and other applications. SWOG maintains its own statistical center, now in Seattle Washington.

Costing a Clinical Trial

Denise Simons-Morton, MD, PhD, NIH, National Heart, Lung, and Blood Institute

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Dr. Simons-Morton emphasized the importance of estimating trial costs to allow fiscal planning by participating institutes and investigators and to ensure that a given trial can answer the scientific questions it was designed to address.

She broke the estimation process down into discrete steps:

Identifying

- the research question
- types of participants
- intervention/treatment to be tested
- primary outcome

Estimating sample size

Mapping out the study timeline

Deciding on trial infrastructure

Estimating costs for all aspects of the trial

Clinical trials can have three possible financial infrastructures:

1. **An award can be made to a coordinating center with capitation payments for patient enrollment and visits.** One advantage of this mechanism is that central control can insure payment only for work performed and it allows redistribution of funds based on performance. Because this mechanism tends to limit the number of experts involved, it may require the addition of additional expert consultants. Dr. Simons-Morton indicated that its best use was in a large relatively simple trial.

2. **Awards are to a coordinating center and clinical sites based on the estimated number of personnel.** In this mechanism there are a number of principal investigators bringing scientific expertise to the trial. There is less central control of funds allocation and waste could be incurred because it is difficult to change initial funding distribution. This mechanism is best used in smaller multi-center studies.

3. **Awards are to "network" hubs that pay clinical sites.** This decentralizes some oversight activity, and individual networks can be negotiated independently. PIs of each network can constitute a steering committee. The decentralization inherent in this mechanism requires a great deal of coordination at many levels. This mechanism is best used in large multicenter trial requiring complex interventions.

Dr. Simons-Morton listed the following entities that must be funded in a trial.

- Drug distribution center
- Central reading center, e.g., for imaging studies (ultrasounds, x-rays) or other studies (ECG, pathology)
- Central laboratory
- Blood/DNA repository
- Genetics analysis core

- Educational materials development center

And she listed some standard categories of costs:

- Personnel (investigators, coordinators, interventionists, measurers, data entry staff,
- statisticians, programmers, consultants)
- Equipment (computers, measurement devices)
- Travel
- Recruitment costs (e.g., advertising)
- Intervention materials (e.g., drugs, videotapes)
- Office supplies
- Laboratory tests
- Other costs needed for the particular trial

If estimates indicate costs are too high an investigator will need to revise his plans. Interventions may need to be reduced or low priority measurements may need to be eliminated. A surrogate outcome could possibly be adopted as a primary endpoint. In some cases, it may be possible to include participants with a higher event rate to insure the study is powered adequately. Costing a trial is a complex activity forcing the investigator to readdress all the scientific assumptions underpinning the effort. Dr. Simons-Morton said it is an essential effort if the trial is to succeed in answering a scientific question.

PANELISTS DISCUSSION OF EXISTING CLINICAL STUDIES OF GLOMERULAR DISEASE

The Focal Segmental Glomerulosclerosis Clinical Trial (FSGS-CT)

Norman J. Siegel, MD, Yale University

The Focal Segmental Glomerulosclerosis Clinical Trial (FSGS-CT) is a multi-center, prospective, randomized trial comparing the effectiveness of a treatment regimen including cyclosporine A to a regimen including Mycophenalate Mofetil and oral pulse steroids in inducing remission of proteinuria in patients with steroid- resistant FSGS. The study is now actively recruiting 500 patients (www.fsgstrial.org). Dr. Siegal who chairs the steering committee for the trial, discussed the steps that were taken to encourage productive use of resources generated by the trial: Ancillary research was actively encouraged during the trial's planning phase, and the study protocol was made available to potential investigators. Also, the establishment of a sample repository, including patient DNA, serum, plasma and unprocessed urine, was incorporated into the primary protocol.

Dr. Siegal also discussed some guidelines for evaluating ancillary studies: a peer-review process is used to evaluate proposals, and applicants are required to include a request for funds for the release of repository samples and data. A number of ancillary studies have been approved. Patient data will be used to identify risk factors, and biopsy, urine, and DNA samples will support investigations of pathogenic mechanisms, disease classification, and the identification of novel biomarkers.

The Toronto Glomerulonephritis Registry
Daniel Cattran, MD, FRCP, Toronto General Hospital

Dr. Cattran outlined the structure of the Toronto Glomerulonephritis Registry, a regional organization in place for the past three decades, designed for research into both basic and clinical aspects of glomerular diseases. He emphasized that the organization includes all of the regional nephrologists, who contribute their initial as well as their ongoing clinical and laboratory data on a standardized form to the Registry, and that this data is aligned with the biopsy reports of all cases of GN forwarded directly to the Registry from the renal pathologists. The central registry takes advantage of the latest information technology and statistical support to maintain this data in an up-to-date and accessible format. The Registry currently contains over 10,000 renal biopsy reports and over 35,000 initial and follow-up time points. The functionality of the database is best

exemplified by the large number of publications, including studies of basic mechanisms of disease, natural history reports, algorithms developed for predicting prognosis and numerous randomized controlled trials focused on determining the effectiveness as well as the risks of new forms of therapy in the treatment of various types of primary GN. The Toronto Registry has used this format not only in local regional studies but also has successfully incorporated multiple centers from across Canada and the United States in a number of their randomized clinical trials. Utilization of this standardized format has made this type of Registry-based trial center very cost-effective as the need for redesigning all of the clinical and laboratory forms for each subsequent study has been largely eliminated. The need for expanding and using the Registry as a template for the development of other regional centers and the linking of these registries together to form an effective platform for GN research was emphasized.

The Glomerular Disease Collaborative Network

Ronald Falk, MD, University of North Carolina, Chapel Hill

The Glomerular Disease Collaborative Network (GDCN) is a network of 275 nephrologists with private practices located across the Southeast US that was established to explore causes and therapeutic options for patients with glomerular disease. Current activities of the GDCN include the maintenance of patient registries, epidemiologic studies, collection of biological samples, and clinical intervention trials. The patient registries support a variety of research activities:

- Long term follow-up of patients
- Establishment of demographics, clinical characteristics & outcomes within the GDCN
- Evaluation of predictors of outcome (dialysis, death, remission, relapse)
- Observation of current treatment trends
- Design and recruitment for clinical trials
- Recruitment for other studies - acquisition of samples, questionnaires, interviews

RASS, a Diabetic Nephropathy Primary Prevention Trial

Michael Mauer, MD, University of Minnesota

The RASS study is a primary prevention trial for diabetic nephropathy (DN). Its basic hypothesis is that reduction of renin-angiotensin system activity with enalapril or losartan will prevent or retard early histologic changes in the kidney associated with DN. The recruitment goal for the trial, 285 type 1 diabetic patients with normal blood pressure and kidney function, has been met. Two percutaneous renal biopsies will be performed, one as a condition for entry, and one after 5-year participation. Patients receiving enalapril, losartan or placebo have been stratified by center and gender. The primary endpoint is mesangial fractional volume change over 5 years. Secondary structural endpoints will include a change in a nephropathy index score and other individual structural variables, e.g., GBM width. Secondary functional endpoints will include albumin excretion rate. glomerular filtration rate, and post-drug washout blood pressure. The study is powered to detect a 50% reduction in the mesangial fractional volume increase. Dr. Mauer indicated that the study is now more than 2/3 completed, and expressed confidence in the feasibility of this kind clinical trial for this and other chronic renal diseases.

BREAKOUT SESSIONS

After listening to the scientific program, meeting participants met in small groups to discuss four specific questions about several important aspects pertaining to future needs of glomerular disease research. The following text summarizes participant discussion from the final session of the Workshop.

A. Which experimental models are used widely enough that standardized protocol and reagents would be useful?

Existing well established models

1. **Murine models of SLE**, especially MRL/*lpr* and NZB/W, are valuable for studying the pathogenesis of SLE, lupus nephritis and for pre-clinical therapeutic trials.
2. **Nephrotoxic nephritis (NTN, anti-GBM nephritis)** in rats or mice can be studied as a predominantly proteinuric self-limited heterologous phase model or as an aggressive inflammatory model by preimmunization as described by Dr. Holdsworth. Although their pathogenesis bears only superficial resemblance to human GN, they are the most widely used induced models in mice for studying disease susceptibility, immune modulation, and preclinical therapeutic studies of proliferative GN.
3. The **passive and active Heymann nephritis** models of experimental membranous nephropathy (MN) in rats closely resemble the clinical and pathological features of human MN. They have been valuable in defining the mechanisms of immune deposition and the role of complement and complement regulatory proteins, but the target antigen is not the same as in human MN, mice are not susceptible, and they are induced rather than spontaneous models of autoimmunity. They can be modified by uni-nephrectomy to produce a model of chronic proteinuria-associated tubulointerstitial disease. They are useful for studying the pathogenesis of MN and for preclinical trials of proteinuria modifying drugs and complement regulatory agents.
4. **Anti-Thy1 nephritis** in rats is an acute model of mesangial proliferative GN. It can be modified to produce a chronic model of glomerular matrix expansion. It has been useful to define the role of growth factors in glomerular disease and the therapeutic effects of growth factor inhibition.
5. Norway pigs with **membranoproliferative GN type 2** (MPGN2) were found to be genetically deficient in factor H, a control protein of the alternate complement

pathway. The pig strain has been destroyed but **factor H-deficient mice** with a similar phenotype have been produced at Imperial College in London. The phenotype is quite representative of human MPGN2, which is most often associated with C3NEF, an autoantibody that promotes alternate pathway activity. These mice will be valuable to evaluate agents that can regulate the alternate complement pathway.

6. **Non-immune models** in rats, including purine aminonucleoside (PAN) and adriamycin nephrosis are simple and reliable methods of inducing podocyte injury and proteinuria. The 5/6 nephrectomy model, though well-established, is labor intensive.

Promising models

1. **Anti-myeloperoxidase** (anti-MPO) in mice resembles pauci-immune GN and may prove more relevant than NTN if it can be standardized for widespread use.
2. **CD2AP null and other null or transgenic models** promise to define the pathogenesis of non-immune forms of glomerular disease (see presentations by Drs. Benzing and Quaggin for examples).
3. **Podocyte depletion models** to induce FSGS (as described by Dr. Wiggins or by Matsusaka and Ichikawa).

Models of uncertain value

Murine IgA nephropathy and apoferritin-induced immune complex GN.

Recommendations:

Standardization

1. Establish a central supply of antibodies and antigens for well-established models and uniform protocols for generating and studying the models.
2. Develop and publish uniform protocols for inducing non-immune models of proteinuria and progressive disease.
3. Standardize endpoints.

Development

1. Genetic models – Identify (e.g. from mouse ENU mutagenesis banks) and generate genetically modified mice and define their phenotype over time. Create a public database of available models.
2. Offer input from the nephrology community to ENU mutagenesis programs to ensure appropriate screening for kidney phenotypes.
3. Encourage development of a mouse model of IgA nephropathy.

Resources

A. Establish repositories of:

1. Tissues and pathology slides/blocks from disease models.
2. Transgenic and knockout mice (e.g. Jackson Labs).
3. Cell lines from normal and genetically modified animals (e.g. ATCC).
4. Monoclonal antibodies (and distribute information on existing lines).
5. Glomerular cell specific DNA arrays and libraries.

B. Develop core facilities for MS/MS, stereology and other specialized imaging techniques.

B. Which issues regarding studies of glomerular diseases are of highest priority?

1) **Biomarkers of glomerular disease**: There was broad consensus among participants that identification and characterization of disease biomarkers should be a high priority for future research. Participants suggested that both researchers studying and clinicians treating various glomerulopathies were hampered by an inability to precisely classify these disease processes. It was recognized that with appropriate characterization, new biomarkers might:

a) Allow sub-classification of glomerular disease beyond that obtained by histology alone for defining subject inclusion and exclusion criteria for clinical studies; disease sub-classification might overcome the effects of study population heterogeneity that have complicated interpretation of past studies of glomerular disease
b) Provide prognosis of disease natural history
c) Allow prediction of response to therapeutic intervention
d) Allow early detection of disease
e) Provide indicators of disease activity

Recent advances in technology, progress in other organ systems, and emerging work in nephrology presented earlier in the workshop has demonstrated both the feasibility and the potential value of the recommended research effort.

2) **Establishment of a national cooperative network for clinical investigation in glomerular disease**: Participants were concerned about the absence of a concerted national effort to organize and facilitate clinical studies in glomerular disease. Several breakout groups suggested that establishing a cooperative network among nephrology groups at academic medical centers (and nephrologists in the community) might provide a cost effective means of fostering glomerular disease clinical research. In particular, participants envisioned a program that could serve several purposes including:
   a) Creating a permanent infrastructure for performing prospective multi-center clinical trials that would include for example, core centers for administration, statistical analysis, histopathological analysis, and appropriate laboratory analysis (e.g., genetic, biochemical);
   b) Creating a patient clinical data registry that could be used for epidemiological and cost analysis studies;
   c) Creating a biological tissue repository that might include biopsy material, genetic material, blood and urine that should be linked to the clinical data registry.

Regarding the creation of a cooperative network, it was suggested that:

a) Such a program might be particularly useful in the short term to facilitate identification and particularly characterization of new relevant biomarkers of disease;
b) A national program could be built upon existing regional infrastructures;
c) A glomerular disease cooperative network might be attractive to the private sector as well. As such, it was envisioned that new or existing compounds could be more efficiently tested and that funding from the private sector might be attracted to support the network in part.

3) **Focus on glomerular disease etiology**: While recent progress has been made in understanding the biological basis of some forms of glomerulopathy, participants urged stimulation of further research in this area. IgA nephropathy, focal and segmental glomerulosclerosis, membranous glomerulopathy, small vessel vasculitis, and lupus nephritis were identified as processes that might receive initial research priority. Several participants pointed to the importance of podocyte number/depletion in predicting or determining disease outcome and urged investigation of this issue.
4) **Focus on glomerular basic biology**: It was generally acknowledged that while important recent progress had been made in this area, much remains undiscovered about the biology of this structure that is likely to improve the likelihood of understanding disease pathogenesis.
5) **Establish safety of biopsies**: Several participants voiced concern that renal biopsy safety must be clearly established both to encourage biopsy use in present clinical practice and to facilitate access to this tissue for scientific use.
6) **Bio-imaging technique development**: Impressive advances in imaging technology were noted. As such, it was suggested that innovative imaging techniques might be developed for diagnosis or prognostication in the area of glomerular disease.

C. What are the major barriers to translational studies?

A number of barriers were identified by participants:

1. Paucity of specific biomarkers of glomerular disease: It was generally acknowledged that recognizable glomerular histopathological patterns may represent the common outcome of distinct pathogenic mechanisms. The perceived value of identifying and thoroughly characterizing new biomarkers of glomerular disease is described above.
2. Poor investigative infrastructure to facilitate collection, cultivation, and access to human biological material and associated clinical data
3. Absence of standardized clinical investigative approaches and end points
4. Funding: While it was acknowledged that human studies are expensive, it was generally perceived that there exists insufficient public and private funding for clinical investigation of glomerular disease
5. Insufficient public awareness: it was suggested that funding for investigation of glomerular biology and human glomerular disease could be improved by increasing public and congressional awareness of the human consequences and societal cost of these diseases
6. IRB/HIPAA barriers that complicate collection and dissemination of human data and materials
7. Intellectual property barriers that complicate establishing cooperative investigations particularly between private and public sector investigators
8. Absence of standardized and validated animal models and standardized reagents useful for pre-clinical glomerular disease investigations: As described above, it was envisioned that cultivation of such reagents would facilitate independent basic science investigations and might be attractive to the private sector for pre-clinical animal studies.

D. What novel therapies show enough promise to move to human studies?

Before embarking on therapeutic trials in glomerular diseases it will be important to:

1. Get cooperation of the whole nephrology community beyond academic institutions.
2. Get cooperation of pharmaceutical industry perhaps through joint development with NIDDK of promising therapeutic agents.

3. In designing clinical trials, target those cases within each disease category that are most likely to derive benefit, e.g. anti-inflammatory agents should be targeted at active cases not those progressing because of chronic damage.
4. Ensure safety of intellectual property rights in collaborative studies with industry.

Promising therapies:

1. **Rituximab (anti-CD20)**
   a) Membranous nephropathy –idiopathic and lupus MN; high-risk cases with active disease (defined as C3 and or C5b-9 deposits on biopsy until better biomarkers are available).
   b) Proliferative lupus nephritis – Rituximab + MMF or Cytoxan vs. MMF or Cytoxan alone.
   c) IgA nephropathy – high risk progressive cases vs. prednisone
2. **Eculizumab (anti-C5)** – a better designed study to include active MN (as defined above), as well as other complement-mediated glomerulopathies such as MPGN, lupus nephritis and perhaps IgA nephropathy.
3. **Anti-fibrotic agents** in progressive disease. The results of clinical trials in other fibrotic diseases such as idiopathic pulmonary fibrosis may provide evidence of potential efficacy. Consideration should be given to the following agents. Anti-TGFβ or TGFβ receptor antagonists (e.g. Cystatin C); pirfenidone; aldosterone antagonist (epleronone, spironolactone); rapamycin (sirolimus); IFNβ (Avonex); IFNγ-1b (Actimmune); Gleevec. Solicit industry to submit drugs in their pipelines for consideration.

CONCLUSIONS

1. Glomerular disease research must be encouraged to progress concomitantly on several levels: basic discovery, applied translational, and purely clinical.

2. Identification of biomarkers of glomerular disease by a variety of approaches has great potential to advance both basic research and clinical research in glomerular disease. Advances in technology make the identification of biomarkers feasible. Rapid characterization of emerging biomarkers such that these will be clinically useful will require a coordinated and unselfish national or international effort.

3. Meaningful clinical investigations of glomerular diseases are scarce due to limited infrastructure and limited funding. Cost efficient cooperative networks should be built upon existing regional networks that might facilitate translational research.

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