Why Kidneys Fail: Report from an American Society of Nephrology Advances in Research Conference

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A recent American Society of Nephrology Conference, entitled “Why Kidneys Fail: Translating Basic Mechanisms of Disease Pathogenesis into Novel Therapies,” explored basic mechanisms and their potential translational implications. In this article, the conference organizers summarize the conference presentations.

The American Society of Nephrology Advances in Research Conference “Why Kidneys Fail: Translating Basic Mechanisms of Disease Pathogenesis into Novel Therapies” was held as part of Renal Week 2005. A total of 243 participants and 29 faculty heard presentations in four thematic areas. The first session considered models of fibrosis in various organs and their relevance to kidney fibrosis. The second reviewed molecular mechanisms of fibrosis. The third examined the cellular response to injury and its potential role in progressive renal disease. A final session addressed the pathway of drug development, from initial idea through preclinical testing to clinical testing and Food and Drug Administration (FDA) approval. This brief report is intended to summarize the major ideas and insights of the meeting. Further materials, including abstracts and slide presentations, are available on the American Society of Nephrology Renal Week web site (http://asn-online.org/education_and_meetings/Renal%20week/ARC-05.aspx). The interested reader can go to this URL and click on the “presentation” and “syllabus” links under each presenter’s name.

An organizing principle of the conference was a model of progressive renal injury involving twin pathways: Fibrogenesis and cellular injury (Figure 1). The process begins with triggering stimuli, including immune attack, viral infection, toxins, glomerular hyperfiltration and hyperperfusion, genetic mutations, and likely unidentified processes. These stimuli may activate fibrogenic pathways, leading to a net accumulation of fibrotic matrix proteins, or they may induce cell injury and death, including reversal of differentiated phenotype, transdifferentiation, loss as a result of de-adhesion, and apoptosis or necrosis. Importantly, fibrogenic pathways contribute to cell injury by distorting normal tissue architecture, reducing perfusion and diffusion of oxygen and nutrients, and altering cell-matrix interactions that regulate cell phenotype. Similarly, cellular injury contributes to fibrosis as dedifferentiated and transdifferentiated cells elaborate more matrix proteins. These two interlocking pathways threaten to initiate and maintain a vicious circle of injury. Under some circumstances, counter-regulatory factors can halt this injury cycle, and under favorable conditions, fibrosis and cell injury can even be reversed. Successful therapeutic approaches to progressive kidney injury likely will involve the use of various agents that suppress fibrogenesis and promote cytoprotection.

Models of Tissue Fibrosis: What Insight Can We Gain into Mechanisms?

Robert Diegelmann: Wound Healing and Fibrosis

Acute wounds normally heal in an orderly and efficient manner that is characterized by four distinct but overlapping phases: Hemostasis, inflammation, proliferation, and remodeling. In the pathologic responses to tissue injury, the normal balance between scar formation and remodeling is distorted, leading to replacement of normal tissue elements by scar. Mast cells have been associated with fibrosis in many tissues, including keloids and hypertrophic scars, nerve injury, liver cirrhosis, and fibrotic nonunion in bone; the provocative hypothesis was advanced that mast cells also may contribute to renal fibrosis.

Karl Weber: Hyperaldosteronism and the Proinflammatory/Vascular Phenotype

Congestive heart failure is characterized by chronic neurohormonal activation that is triggered by various stresses, including altered oxidative and nitrosative states. Hyperaldosteronism plays a central role in cardiac fibrosis. Recent evidence suggests that hyperaldosteronism also promotes excretion of calcium and magnesium, leading to secondary hyperparathyroidism, and that this in turn promotes perivascular cardiac fibrosis. Spironolactone and parathyroidectomy each reduce or prevent cardiac fibrosis.
Marcos Rojkind: Role of Acetaldehyde in Hepatic Stellate Cell Activation

Hepatic stellate cells resemble mesangial cells in a number of ways. Both cell types are normally quiescent, producing a modest amount of matrix protein. After liver injury, stellate cells differentiate into myofibroblasts (identified by the expression of α-smooth muscle actin); enter the cell cycle; migrate; and produce scar collagen, including collagens I and III, and regulators of extracellular matrix (ECM) turnover such as tissue inhibitor of metalloproteinases (TIMP-1) and matrix metalloproteinase-2 (MMP-2). Ethanol is metabolized to acetaldehyde, a process that induces oxidant stress and alters the hepatic redox state. Acetaldehyde also directly contributes to shifting the stellate cell toward a fibrogenic phenotype, via acetaldehyde-response elements within collagen I genes and by induction of Smad2 phosphorylation.

Shawn Cowper: Nephrogenic Systemic Dermopathy

Nephrogenic systemic dermopathy first was recognized in 1997 and was formerly called nephrogenic fibrosing dermopathy; the new name emphasizes its potential to affect many organs. The primary lesion consists of papules or nodules that coalesce to form plaques, affecting the limbs and trunk. More than 200 cases have been reported, and the only common feature is renal insufficiency, including chronic kidney disease (CKD), dialysis, and transplantation. The cause remains an enigma, but several fascinating clues have emerged, including the presence of CD31+ fibrocytes in blood and the frequent historical feature of trauma, suggesting a possible role for tissue injury and vascular injury. Despite extensive epidemiologic investigation, we do not know why the disease appeared only in the past decade, and we do not understand the role of kidney disease in promoting a systemic profibrotic state.

Julie Ingelfinger: Perinatal Programming and Nephron Number

The Barker hypothesis proposes that certain adult diseases may have their origins in developmental processes such as in utero growth retardation, nutritional deficiency, and exposure to medication or maternal hyperglycemia. Perinatal programming is proposed to affect nephron number or structure, leading to suboptimal nephrogenesis and, in later life, hypertension and CKD.

Wilhelm Kriz: Relationship between Glomerular and Interstitial Disease: Insights from Animal Models

Most progressive renal diseases that originate in the glomerulus are transferred to the tubulointerstitium, resulting in a combined glomerular and tubulointerstitial process. How does glomerular disease affect the tubulointerstitium? There are two opposed views. On the one hand, Dr. Kriz argues that the two distinct classes of glomerular disease (sclerotic and inflammatory) damage the tubule by particular anatomic pathways. In sclerotic diseases, misdirected glomerular filtrate is conducted along the outer aspect of the tubule, leading directly to obstruction and degeneration or alternatively leading to atrophy and thus to degeneration. In inflammatory diseases, proliferative crescents may encroach upon the glomerulotubular junction, also inflicting tubular injury. On the other hand, others have argued that filtered proteins damage the tubule as a result of excessive reabsorption (e.g., albumin or albumin-bound proteins), direct toxicity (e.g., complement), or activation (e.g., cytokines and chemokines). Dr. Kriz argued that although the latter process may damage the tubule, there is no evidence that they irreversibly injure the tubule. Meticulous histologic analysis of murine crescentic glomerulonephritis demonstrated that only tubules that are connected to glomeruli with crescents that...
extend to the tubular junction showed atrophy. Furthermore, review of multiple models suggests that tubular injury is invariably associated with injury to the attached glomerulus. Therefore, injury does not propagate from tubule to tubule or from interstitium to tubule.

Matrix and Remodeling: What Are the Molecular Mechanisms of Fibrosis?

Dale Abrahamson: Matrix Proteins in Renal Development and Fibrosis

Developmental changes in ECM expression are essential for establishing glomerular structure, because the ECM molecules direct glomerular development. Both the endothelium and the epithelium contribute to the glomerular basement membrane. The bidirectional signaling and mutual regulation between the cells and the ECM during development demonstrate the concept of “dynamic reciprocity” in tissue homeostasis, a theme that also was sounded by several subsequent speakers.

Francesco Ramirez: Transcriptional Control of ECM Protein Expression

The activation of COL1A2, encoding the α2 chain of collagen I, provides a useful model of ECM gene transcription. Activation mechanisms show tissue and species specificity. Specificity is determined by the relative expression and activity of different molecules in the transcription complex and on chromatin structure.

Lynn Sakai: Interaction between ECM Aggregates and Signaling Molecules in Development and Disease

The aggregation of ECM molecules into higher ordered structures is critically dependent on particular large, multidomain proteins such as fibrillin. Fibrillin is a 350-kD molecule that is mutated in patients with Marfan syndrome. It participates in the formation of complex microfibrilar networks. Because these structures focus and concentrate intercellular signaling molecules to direct cellular responses, defects in their structure could play a role in determining cell function.

Joanne Murphy-Ullrich: Matricellular Proteins

Whereas many ECM proteins serve both structural and functional purposes, the matricellular proteins only modulate cell function. They include thrombospondin, SPARC/osteonectin, osteopontin, and connective tissue growth factor. Accumulation of these proteins has been implicated in a number of fibrotic diseases. For example, a peptide antagonist of thrombospondin-1 decreases TGF-β activation and fibrosis in hypertensive mice, demonstrating the potential pathogenic role of this matricellular protein.

Duk-Hee Kang: Vascular Injury and Regeneration in CKD

The close association between the degree of hypoxia and the extent of interstitial injury in progressive renal disease suggests the possibility that vascular compromise promotes progression. Hypoxia simulates the expression of several angiogenic factors, including basic fibroblast growth factor, vascular endothelial growth factor (VEGF), and erythropoietin. It is interesting that there may be two sequential phases of angiogenesis in the injured kidney. The first is deleterious, because blocking VEGF prevents glomerular hypertrophy. In the second phase, angiogenesis may be beneficial, because erythropoietin and VEGF promote revascularization that may enhance recovery of normal function.

David Basile: Acute Tubular Necrosis and Progressive Renal Disease

Acute ischemia/reperfusion injury in rats is followed by progressive fibrosis. Progression is characterized by decreased renal vascular density and salt-sensitive hypertension. TGF-β plays an important role, but it does so by promoting blood vessel regression rather than directly stimulating fibrosis.

Allison Eddy: Lipids, Proteinuria, and Interstitial Fibrosis

The renal interstitium becomes involved in a pathologic vortex that involves inflammation, myofibroblast activation, and tubular cell injury. A critical outcome is loss of differentiated tubular epithelial cells and interstitial capillaries. Because proteinuria correlates with progression, transtubular transport of filtered proteins by megalin and cubulin likely plays a role in tubular cell injury. The critical filtered protein probably is not albumin but rather cytokines and lipoproteins that are present in the pathogenic glomerular filtrate.

Cell Injury and Recovery: How Do the Cell and Its Milieu Mediate Progressive Renal Disease?

Mina Bissell: Regulation of Cell Function by the ECM

Maintenance of a differentiated and functioning tissue is an active process, in many cases defined by the presence of a specific ECM components. When that ECM is altered, cells may undergo phenotypic changes. Dynamic reciprocity describes the bidirectional pathways of influence: Cells make ECM, and ECM modulates cell behavior. The principles of dynamic reciprocity help us understand both normal processes (tissue morphogenesis, cell differentiation, and wound repair) and certain disease processes (neoplasia and fibrogenesis).

Allen Cowley: Impact of Hypertension on Fibrosis

A hypertensive rat model has been developed using a servo-controlled aortic balloon that exposes the right kidney to high perfusion pressure while maintaining normal perfusion pressure to the left kidney. This allows the teasing apart of local and systemic factors in hypertensive tissue injury. The high-pressure right kidney shows much greater fibrosis than does the control left kidney. TGF-β and NF-κB activity is increased in the hypertensive kidney but not the control kidney. Analysis of these results suggests that systemic hormonal effects account for no more than 20% of the fibrogenic stimulus.

William Schnaper: The Cytoskeleton Mediates Multiple Fibrogenic Signals

Glomerular hypertension may have an impact on a network of signal transduction pathways that modulate TGF-β–stimulated mesangial cell type I collagen expression. In one of these
pathways, tension that is generated in the cell enhances TGF-β signal transduction. Experimental maneuvers that decrease stress fiber formation do not decrease the collagen-producing response, suggesting that the tension acts at the site of cell–matrix interaction, rather than by deforming the cytoskeleton itself. These data describe a mechanism by which physical forces could enhance fibrogenic biochemical signals.

Eric Neilson: Epithelial-Mesenchymal Transition

Experiments with genetically engineered mice have demonstrated that renal fibroblasts derive primarily from intrarenal precursors, with a minority of cells coming from bone marrow precursors (10% of fibroblasts in normal kidney, 15% of fibroblasts in fibrotic kidneys). Expression of fibroblast-specific protein-1 is associated with a genomic program that is characteristic of epithelial-mesenchymal transition (EMT). EMT first was described in development and contributes to cancer and to renal injury. The role of EMT was the subject of much discussion, with conference participants noting the lack of ultrastructural data and the absence of EMT in other fibrotic organs, such as lung and liver.

Christine Abrass: Mechanisms of Renal Injury and Repair in the Aging Kidney

With aging, both the amount and the composition of ECM change in the glomerulus, and the tubulointerstitium shows thickening of the tubular basement membrane and interstitial fibrosis. A number of molecules have been implicated, including IGF-binding protein, histone deacetylase, and the Sox9 transcription factor.

John Savill: Apoptosis and the Progression of Kidney Disease

Regulation of apoptosis is increasingly recognized as critical to blocking or implementing the progressive injury program. Apoptotic bodies that are not cleared promptly by phagocytosis represent a potent immunologic stimulus. Apoptosis is a “two-edged sword” in renal disease: It can eliminate inflammatory cells or excessive resident glomerular cells, yet it also can reduce podocyte number.

Matthias Kretzler: Podocyte Injury

Integrin-linked kinase is an adapter molecule that contributes to both inside-out and outside-in integrin signaling. Integrin-linked kinase activates β-catenin and LEF-1 and leads to the activation of the protein kinase B/Akt pathway. It also serves as a regulator of focal contact formation in epithelial cells.

Developing Therapeutics for Progressive Renal Disease

George Martin: Collaboration between Academia and Industry

Prolyl-hydroxylase inhibitors first were developed by Fibrogen as antifibrotic agents, because this enzyme is required for the synthesis and secretion of collagens. Certain prolyl-hydroxylases act on hypoxia-inducible factor-1, a nuclear factor that stimulates erythropoietin, VEGF, and glucose transporter gene expression. Inhibition may be cytoprotective in ischemia-reperfusion injury or CKD. Clinical trials for liver fibrosis were abandoned because of difficulty in assessing disease progression. Although chemical library screening holds much promise and has a key role in the National Institutes of Health Clinical Research Roadmap initiative, it is worth keeping in mind the words of James Black, Nobel Laureate in Chemistry for 1988: “The most fruitful basis of the discovery of a new drug is to start with an old drug.”

Agnes Fogo: Preclinical Models: Which Are Most Faithful to Human Disease?

Diabetic nephropathy has not been reproduced closely with available rodent models, which show at best mild mesangial expansion. National Institutes of Health–funded projects, including the Animal Models of Diabetic Models Consortium, may introduce new approaches. Some animal models are relevant to focal segmental glomerulosclerosis (FSGS); these include the remnant nephron model (which resembles postadaptive FSGS), podocyte toxin models such as puromycin aminonucleoside and Adriamycin (presumably similar to idiopathic FSGS), and genetically manipulated podocyte models (which resemble genetic FSGS or HIV-associated glomerulopathy, among others). Therefore, some animal models are suitable for preclinical drug development, but important gaps remain.

Michael Mauer: Morphologic Analysis of Renal Tissue: What Are Suitable Trial End Points?

CKD, in particular diabetic nephropathy, is characterized by a long silent period in which substantial morphologic changes occur and then a shorter period of renal functional decline. Therapies that slow progression of the later stages of renal disease can be tested using clinical end points such as loss of GFR or ESRD. If the goal is to prevent ESRD, however, then we must treat patients in early stages of kidney disease. Various therapies may be required, as various pathogenic mechanisms are likely operative, and various end points must be developed. Proteinuria is considered by the FDA to be too imprecise, as variance in proteinuria among subjects in trials accounts for only 50% of the variance in clinical end points. Renal structural measures, to serve as adequate surrogates, will have to be shown to be closely related to clinical end points. Clinical trials using multiple renal biopsies can be done and offer a potential solution for developing therapies for early kidney disease.

Erwin Böttinger: Molecular Analysis of Renal Tissue: What Are Suitable Trial End Points?

Dr. Böttinger presented evidence that expression of a set of seven RNA species predicts likelihood of progression of kidney disease in diverse mouse models and proposed that this panel of biomarkers should be applied to human renal biopsy tissue.

Jon Klein: Biomarkers in Plasma and Urine: What Type and Quality of Markers Are Available for Fibrotic Disease?

Some biomarkers for progressive kidney disease, such as macrophage chemoattractant protein-1 and the procollagen III N-terminal propeptide, have been pursued in hypothesis-
Driven experimentation. For biomarker discovery, several platforms are available, including two-dimensional PAGE, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry and liquid chromatography–mass spectrometry/mass spectrometry, and antibody arrays. Newer techniques include comparative proteomic profiling by two-dimensional liquid chromatography–mass spectrometry and comparative peptidomic profiling. Each approach has unique advantages and disadvantages.

**Douglas Throckmorton: FDA Perspectives on Approval of New Therapeutics: Kidney Disease, Biomarker Development, and the Critical Path**

New research tools, including pharmacogenomics, proteomics, metabolomics, molecular imaging, and nanotechnology, offer new opportunities to identify new disease targets and new therapeutic approaches. The FDA has defined a “Critical Path,” a concerted effort to bring attention to the need for targeted scientific efforts to modernize the techniques and methods that are used to evaluate the efficacy, safety, and quality of medical products at each stage of development (www.fda.gov/oc/initiatives/criticalpath/). Stages along the critical path include basic research, prototype design or discovery, preclinical development, clinical development, approval, and postapproval assessment. The FDA is particularly interested in assisting in biomarker development to aid in efficient evaluation of early signals of efficacy and safety. Biomarkers hold promise for dose selection, patient selection, and population selection and therefore offer much more than simply increased efficiency. One biomarker, proteinuria, has been accepted as an end point for a pivotal clinical trial, with the requirement under accelerated approval that the results of phase IV studies using clinical end points are supportive (FDA regulation 21 CFR 314.510, subpart H). A major policy concern is that although many groups have an interest in biomarker development, including academia, the pharmaceutical and biotechnology industries, and the FDA, no one sector is charged with ensuring rigorous validation of biomarkers so that they are suitable for use in clinical trials. In general, the next steps in speeding drug development include the use of biomarkers, gap analysis (what is missing?), and trial analysis (what trials are needed to fill these gaps?).

**Thomas Hostetter: Trial Designs for Progressive Renal Disease: Phase III Trials**

Thomas Addis said, “The problem with studying kidney disease is that no man lives long enough.” Advances in treatment for progressive CKD have increased the required size and duration of new clinical trials. Rates of GFR decline are slow (1 to 10 ml/min per yr) compared with the variability in GFR measurement (coefficient of variation 0.1 to 0.15). There are several approaches to increasing trial efficiency. First, we can select patients who are most susceptible to progression, using risk factors such as proteinuria, race, and gender. In the future, susceptibility genes may be identified. These approaches increase efficiency at the cost of reducing generalizability. Second, we can consider new approaches to assessing GFR. Third, structural end points may be developed using biopsy tissue or innovative imaging techniques.

**Jeffrey Kopp: Trial Designs for Phase II Trials**

An expanding number of molecules and pathways have been implicated in the processes of renal fibrosis and cellular injury. Furthermore, the list of possible therapeutic agents, many with proven efficacy in experimental kidney disease, has expanded exponentially. Clinical nephrology has transitioned from a target-poor, drug-poor environment to a target-rich, drug-rich environment. A major task of phase II trials is to choose efficiently the most promising agents from among many and to choose the optimal dose of each agent with regard to efficacy and safety in diverse populations. Several phase II trial designs are worthy of consideration, including traditional hypothesis testing, selection design, two-stage design, and screening design. Selection design is particularly attractive and aims to select efficiently the most promising agent (or dose) from among three, four, or more treatments.

**Conclusion**

We have made considerable progress in defining the microenvironment that promotes cell injury and scarring. It is clear that many different stimuli contribute to these pathogenic processes. Many challenges remain. These include integrating the progress made regarding molecular and cellular observations into models that can be used to define the most significant pathogenic events. Such models and the resulting definition would be major steps in identifying the most promising targets for the next generation of therapeutic agents. Questions to be addressed include the following: What is the role in progression of EMT versus misdirected filtration? How can we modify intracellular signals in a way that specifically affects disease processes? Finally, translating these findings into useful therapies will require both animal models that are relevant to human disease and the determination of markers for disease activity that accurately predict progression to renal insufficiency. Much work remains to be accomplished.

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