

Summary of ISN Forefronts Symposium 2015: 'Immunomodulation of Cardio-Renal Function'



Xiong Z. Ruan^{1,2}, Youfei Guan³, Zhi-Hong Liu⁴, Kai-Uwe Eckardt⁵ and Robert Unwin^{1,6}

¹Centre for Nephrology, University College London, London, UK; ²AstraZeneca-Shenzhen University Joint Institute of Nephrology, Centre for Nephrology & Urology, Shenzhen University Health Science Center, Shenzhen, China; ³Advanced Institute of Medical Science, Dalian Medical University, Dalian, China; ⁴National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China; ⁵Department of Nephrology and Hypertension, University of Erlangen-Nürnberg, Erlangen, Germany; and ⁶AstraZeneca, Gothenburg, Sweden

The International Society of Nephrology Forefronts Symposium Immunomodulation of Cardio-Renal Function took place October 22 to 25, 2015, in Shenzhen, China. The program covered basic and clinical aspects of cardio-renal pathophysiology and immunity. Leading scientists from different and related disciplines of clinical and basic research described and reviewed recent discoveries, and discussed emerging topics under the headings "Immunity and Renal Pathophysiology"; "Autoimmunity and the Inflammasome"; "Immunity and the Gut Microbiome"; "Immuno-Metabolism"; "Immunogenetics, Transcriptomics and Epigenetics"; "Immunity and Hypertension"; and "Immunity, Fibrosis, and Kidney Disease."

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Hypertension and vascular disease are well-known associations and complications of chronic kidney disease (CKD), and both are major contributors to its continuing high morbidity and mortality. Although the association between inflammation in CKD and cardiovascular disease is broadly recognized, its pathophysiological basis is still unclear, as is the relationship of inflammation in CKD to innate and adaptive immunity. The pathophysiologies affecting cardiovascular function and immunity have, until now, been considered very different and separate fields of clinical interest and research; however, increasing evidence indicates that there is significant overlap and interplay between them. This interdisciplinary symposium focused on cardio-renal pathophysiology and its links to immunology and inflammation, and this meeting report summarizes the main highlights of the meeting and provides background to some of the related short review articles that also appear in this issue of *Kidney International Reports*. In addition to the keynote

speakers, 69 abstracts were presented orally or as posters. A selection of the presentations can be accessed at www.theisn.org/education. Figure 1 is a photograph the organizers and invited participants.

Immunity, Inflammation, and Renal Pathophysiology

Dr Jan-Eric Turner (Universitätsklinikum Hamburg-Eppendorf, Germany) introduced us to the function of innate lymphoid cells (ILCs) in tissue injury and repair. Although these recently described lymphoid cells can be found in the kidney and are part of the innate immune system, their activation is antigen-independent. They include conventional natural killer cells, now termed "killer" ILCs or "helper-like" ILCs, which can be subdivided according to their cytokine profile, transcription factors, and tissue localization. Important functions for ILCs in immune barrier protection against various pathogens in tissues such as gut, lung, and skin have been identified, as well as roles in autoimmune inflammation in skin and intestine, metabolic control of adipose tissue.¹ Interleukin (IL)-5- and IL-13-producing ILCs are crucial in the regulation of eosinophil number and differentiation of alternatively activated macrophages.² By producing mediators that directly enhance epithelial growth, ILCs can contribute to tissue remodeling and repair following acute injury. But they can also

Correspondence: Xiong Z. Ruan, AstraZeneca-Shenzhen University Joint Institute of Nephrology, Centre for Nephrology & Urology, Shenzhen University Health Science Center, University College London, Rowland Hill Street, London NW3 2PF, UK. E-mail: x.ruan@ucl.ac.uk

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Figure 1. Group photograph of the meeting organizers and invited speakers.

have deleterious effects on progression of organ fibrosis by employing similar cytokine pathways. Most ILCs are GATA-3 positive and long-lasting; however, the role of ILC subtypes in the kidney as a source of T helper-cell-associated cytokines and their function in renal inflammation and fibrosis requires more study.³

Dr Stephen Alexander (Children's Hospital at Westmead, Australia) provided an overview of the interaction of the innate and adaptive or cognate immune systems in glomerulonephritis (GN). Inflammation and repair in kidney disease are affected by both systems, with important mechanisms of repair involving regulatory T cells. These cells provide protection against innate immune-mediated (macrophage) kidney injury and renal impairment in children with cardiac disease, as well as playing a role in different macrophage and dendritic cell subsets in various models of GN.^{4,5}

Dr Jeremy Hughes (University of Edinburgh, UK) provided further insight into the diversity of function of macrophages beyond the conventional M1/M2 paradigm, suggesting a potentially wider role for these cells in the response to renal injury and repair. Macrophage depletion can limit renal injury and scarring, but also impair regeneration and repair. These cells are involved in GN, diabetic nephropathy, tubulointerstitial fibrosis, acute kidney injury (AKI), transplant rejection, cystic renal disease, and ischemic preconditioning, all of which emphasize their importance in renal pathology.⁶ Recent studies have also

highlighted the significance of the local tissue microenvironment: Exposure to chemokines and growth factors secreted by tubular epithelial cells such as colony stimulating factor-1, with upregulation of microRNA-21, can direct macrophage polarization and limit inflammation and facilitate repair. Although this can expedite resolution of acute injury, it may be less successful in chronic disease. A deeper understanding of the induction and control of macrophage activation and resulting phenotype, as well as the intracellular signaling pathways that regulate macrophage responses, and mediators involved in the cross-talk between macrophages and parenchymal cells, will lead to new therapeutic options in renal disease.

Dr Kunling Ma (Southeast University, China) discussed the contribution of increased circulating platelet microparticles, the most abundant microparticle subtype,⁷ to glomerular endothelial injury in diabetic nephropathy. The release of platelet microparticles is thought to be proinflammatory, eliciting a cytokine response. Platelet microparticles may be useful biomarkers of endothelial injury, as well as suitable targets for treatment in diabetes.

Autoimmunity and the Inflammasome

Dr Minghui Zhao (Peking University First Hospital, China) discussed small vessel antineutrophil cytoplasmic antibody-associated vasculitis and immunity. The majority of patients in China with antibody-associated

vasculitis have myeloperoxidase–antineutrophil cytoplasmic antibody positive microscopic polyangiitis, which is more common in elderly persons and typically affects the kidneys as pauci-immune crescentic GN. Activation of complement via the alternative pathway has been shown to be critical, particularly the interaction between C5a and its receptor CD88 (C5aR), producing an amplification loop for neutrophil recruitment and activation that involves sphingosine-1-phosphate signaling⁸; mice lacking C5aR are protected from antineutrophil cytoplasmic antibody-related GN, and C5a *in vitro* primes neutrophils for an antineutrophil cytoplasmic antibody-induced oxidative burst and degranulation. A small molecule antagonist of C5aR, CCX168, is currently in clinical trials.

Dr Felix Knauf (University of Erlangen-Nürnberg, Germany) provided a renal perspective on the inflammasome. Inflammasomes are part of the innate immune system and are signaling platforms assembled in response to microbe-specific, as well as nonmicrobial, antigens. On activation, proinflammatory cytokines such as IL-1 and IL-18 are released that engage immune defenses to trigger an inflammatory form of cell death termed pyroptosis.⁹ He reviewed current knowledge of the role of the inflammasome in the pathogenesis of some forms of kidney injury and disease.¹⁰ Crystalopathies are particular examples of NLRP3 inflammasome-mediated injury and crystal (urate or oxalate) deposition in the kidney can cause both acute and chronic kidney injury.¹¹

Dr Chuan Wu (Brigham and Women's Hospital and Harvard Medical School, USA) discussed the regulation of T helper 17 cells and a link with salt sensing and serum glucocorticoid kinase-1 signaling via IL-23 and its receptor (IL-23R). IL-23R is also associated with inflammatory autoimmune disease, particularly in the intestine. In a T cell-dependent model of colitis, serum glucocorticoid kinase-1 promotes inflammatory T helper 17 cells and inhibits Foxp3+ regulatory T cells.¹² Serum glucocorticoid kinase-1 regulates IL-23R expression, which in turn maintains T helper 17 cell function. This illustrates an important role for serum glucocorticoid kinase-1 in the interplay between T helper 17 effector cells and Foxp3+ regulatory T cells in inflammatory bowel disease.

Dr Dan Liu (Zhong Da Hospital, China) considered the role of albuminuria in the development and progression of CKD by promoting tubulointerstitial inflammation and fibrosis and discussed the part played by megalin or cubilin-mediated albumin uptake, its transfer into lysosomes, and the link to tubular cell activation of the NLRP3 inflammasome and tubulointerstitial inflammation. Silencing of megalin or cubilin expression was shown to inhibit albumin-induced NLRP3 activation as a

result of lysosomal leakage of hydrolases, such as cathepsin B, when tubular epithelial cells are exposed to excess albumin.¹³

Dr Rujun Gong (Nanjing Drum Tower Hospital, China) talked about glycogen synthase kinase 3 β (initially identified as an inactivator of glycogen synthase) inhibition, which can promote macrophage polarization and reduce inflammation and acute and chronic kidney damage following AKI. Gong's group has shown that inhibition of glycogen synthase kinase 3 β by TDZD-8, a selective inhibitor, reduces kidney inflammation and injury in AKI by regulating macrophage recruitment and polarization. Recent studies demonstrated that systemic administration of a small molecule glycogen synthase kinase 3 β inhibitor given before and 36 hours after AKI significantly attenuated renal inflammation. Kidney macrophage infiltration was also reduced, and switching from a proinflammatory M1 to an anti-inflammatory M2 phenotype was promoted. These changes were associated with less tissue injury and more rapid recovery of renal function, with fewer histologic features of AKI and less fibrosis.¹⁴

Dr Fan Yi (Shandong University, China) reported on a podocyte-specific knockout of Sirtuin-6 (SIRT6) (a deacetylase with anti-inflammatory properties) that leads to more renal injury in a rat model of diabetic nephropathy by heightening inflammation. Dr Yi has shown that high glucose selectively down-regulates SIRT6 expression in podocytes, rather than in glomerular endothelial or mesangial cells, or in tubular epithelial cells. *In vivo*, podocyte-specific knockout of SIRT6 aggravated podocyte dysfunction and enhanced the inflammatory response. *In vitro*, overexpression of SIRT6 restored reduced nephrin expression caused by high glucose. SIRT6 can also decrease NLRP3 inflammasome activation by selectively inhibiting Notch 1 signaling, indicating a role for SIRT6 in hyperglycemia-induced podocyte injury.

Immunity and the Gut Microbiome

Dr Liping Zhao (Shanghai Jiao Tong University, China) gave an overview of the relationship between changes in the gut microbiota and chronic inflammation due to bacterial endotoxins. Zhao reported that the endotoxin-producing bacterium *Enterobacter cloacae* B29 isolated from the gut of morbidly obese humans caused an obesity phenotype with increased adiposity, systemic inflammation, and insulin resistance in germ-free mice. Specific types of gut microbiota have the genetic potential to contribute to the development of metabolic disease.¹⁵ Profiling of urinary metabolites from bacteria by nuclear magnetic resonance and mass spectrometry can be correlated with the gut microbiome. Using this approach in the dietary treatment of patients with

Prader-Willi syndrome (with hyperphagia and obesity), Zhao has compiled and correlated 100 draft genomes of prevalent gut bacteria from a large metagenomic dataset, and identified bacteria with enzymes that convert choline to trimethylamine. Trimethylamine is metabolized in the liver to trimethylamine N-oxide and is a recognized uremic toxin linked to atherogenesis and increased cardiovascular morbidity and mortality.¹⁶ The significance and role of the gut microbiome in chronic metabolic diseases is just emerging.

Dr Gregory F. Sonnenberg (Weill Cornell Medicine, USA) discussed the immune system and its critical role in protecting against infection from pathogenic organisms. However, studies in patients indicate that abnormal host immune responses to the commensal gut bacteria that normally colonize the body's barrier surfaces are causally linked to the pathogenesis and progression of several chronic infectious, and inflammatory and metabolic diseases, including HIV, inflammatory bowel disease, and cancer. Using advanced immunologic and microbiologic approaches to interrogate the relationship between the mammalian immune system and intestinal commensal bacteria, Sonnenberg identified a critical role for ILCs in orchestrating the host relationship to defined subsets of commensal bacteria.^{17,18} Delineating these complex interactions will provide a better understanding of the pathogenesis of multiple chronic inflammatory diseases and help in developing novel therapeutic strategies that target commensal bacteria-dependent chronic inflammation.

Dr Frank J. Gonzalez (National Cancer Institute, USA) discussed the gut microbiome in the context of obesity and fatty liver disease (nonalcoholic steatohepatitis), describing effects of the gut microbiota on host bile acid composition and hepatic and intestinal farnesoid X receptor (FXR) signaling. Bile acid metabolism is tightly linked to intestinal and hepatic FXR signaling, and FXR plays an important role in the regulation of bile acid synthesis and transport, and hepatic *de novo* lipogenesis. Manipulation of the gut microbiota in mice to increase the *Lactobacillus* population, which metabolize the endogenous FXR antagonist tauro- β -muricholic acid, is correlated with inhibition or resolution of obesity, decreased insulin resistance, and reduced nonalcoholic steatohepatitis.¹⁹ Intestine-specific FXR-null mice showed that the gut microbiome acts by regulating the bile acid-intestinal FXR-ceramide axis to alter serum ceramide levels, which in turn affect adipose tissue and the liver to decrease weight, insulin resistance, and hepatic lipid levels.²⁰ These studies have revealed a new target and potential lead compounds for nonalcoholic steatohepatitis and the treatment of metabolic disease.

Immuno-Metabolism

Dr Hans Stauss (University College London, UK) addressed the importance of modulating activity of the key cell growth and metabolism mechanistic target of rapamycin pathway to control T cell function for immunotherapy in cancer. The design of translational strategies for generating heterogeneous T cell immunity against cancer allows for up-regulation, as well as inhibition of the mechanistic target of rapamycin complex 1 pathway, and can be highly selective for therapeutic T cells without affecting systemic mechanistic target of rapamycin complex 1 functions.²¹

Dr Youfei Guan (Dalian Medical University, China) gave an overview of prostaglandin E2's role in the regulation of immunity and inflammation. Prostaglandin E2 also has a specific influence on CD4+ T cells: It inhibits the production of the T helper 1 cytokine interferon- γ but increases T helper 2 cytokines such as IL-4, IL-5, and IL-13. Prostaglandin E2 shifts the balance away from T helper 1 toward T helper 2, favoring chronic infection and allergic diseases.²² A better understanding of the biological actions of prostaglandin E2 and its receptors, and signal pathways, in innate and adaptive immunity should facilitate the development of molecular inhibitors or activators for the treatment of infectious and inflammatory diseases, including renal inflammation.

Dr Morgan Fullerton (McMaster University, Canada) discussed the role of adenosine monophosphate-activated protein kinase (AMPK) in inflammation and metabolism in obesity. A model in which the β 1 subunit of AMPK is deleted reveals an anti-inflammatory, insulin-sensitizing role for AMPK β 1 in obesity, and that AMPK β 1 protects macrophages from lipid-induced inflammation.²³ Such studies have also shown that salicylate is an AMPK activator by binding to AMPK β 1²⁴ and that macrophage AMPK is anti-atherogenic. These findings highlight the therapeutic potential of targeting macrophage AMPK with new or existing drugs to restore lipid homeostasis and resolve inflammation during the early stages of obesity-induced metabolic dysfunction.

Immunogenetics, Transcriptomics, and Epigenetics

Dr Xueqing Yu (Sun Yat-sen University, China) talked about the genetic susceptibility to lupus nephritis, which is more common in Asian and African patients with systemic lupus erythematosus (51%–55% compared with 14% in whites), and has a strong association with a major histocompatibility complex class II gene cluster region on chromosome 6. Yu's group did a deep sequencing analysis of the major histocompatibility complex region and the protein

coding sequences of 693 known immunity genes for lupus nephritis. They discovered 5 independent risk variants within the major histocompatibility complex region, as well as 3 nonmajor histocompatibility complex risk loci with genome-wide significance; in addition, they also discovered another 2 novel loci with suggestive associations. Of the 6 single-nucleotide polymorphisms, 4 single-nucleotide polymorphisms are novel susceptibility loci for lupus nephritis, and the other 2 are within the reported systemic lupus erythematosus loci. The genes located at these newly associated loci implicate altered function in the modulation of sex hormones, the epidermal growth factor receptor network, and CD4+ T cell signaling. About 16.2% of the phenotypic variance can be explained by all 10 susceptibility variants discovered in these lupus nephritis samples.²⁵

Dr Jun Wang (Shenzhen Beijing Genome Institute, China) provided a general overview of the million genomes project at the Beijing Genome Institute. The original Human Genome Project and later 1000 Genome Project demonstrated the power of the context and variation catalog of genetics, and showed the importance of genetic variation in disease. However, much larger datasets are needed to resolve and extend this knowledge with a view to developing personalized or precision medicine.

Dr Joakim Lundeberg (Science for Life Laboratory, Sweden) discussed the innovative and novel technique that his group has developed of transcriptomic or spatial imaging. They have devised a simple strategy that makes it possible to carry out global gene expression analysis in histologic tissue sections, matching the tissue transcriptome with the underlying histologic structure and features at a current resolution of 100 μm , and aiming eventually for 30 μm . Monitoring of all cells in a tissue section individually by digital RNA sequencing will reshape our basic understanding of cell circuitry and will have profound consequences for our understanding of complex subjects such as tissue differentiation and proliferation, the causes of cancer, organogenesis, and the responses to tissue injury, and also provide new diagnostic insights. Striking (as yet unpublished) examples in prostate cancer and in cardiac development were presented.

Dr Peter Maxwell (Queen's University of Belfast, UK) discussed the importance of epigenetics in diabetic kidney disease, which is known to have an inherited susceptibility that probably underlies the familial clustering of nephropathy and the differences in prevalence of kidney disease in different ethnic populations. Epigenetics refers to heritable (transgenerational) or dynamic changes in gene expression resulting from factors other than a direct change in the DNA

sequence of the genome. These modifications may be inherited or occur in response to current or past environmental exposures such as diet, drugs, or illness. Epigenetic mechanisms may include chromatin remodeling, altered DNA methylation, or changes in the expression of specific microRNAs. Extreme environmental stress may result in long-term epigenetic modification of the genome with an increased lifetime risk of diabetes, cardiovascular disease, or CKD.²⁶ Altered metabolic states, such as hyperglycemia in diabetes, may lead to changes in gene expression mediated by epigenetic mechanisms and this has been referred to as hyperglycemic memory.²⁷ Epigenetic biomarkers could prove useful in stratifying individuals at higher risk of developing diabetic kidney disease. Epigenetic features are also attractive therapeutic targets, because they may be amenable to alteration or reversal, offering potential for drugs that can selectively target epigenetic changes in particular tissues and cell types.

Dr Assam El-Osta (Baker IDI Heart and Diabetes Institute, Australia) discussed DNA methylation and sequencing that identifies CTCF (an enhancer blocking protein) regulated genes implicated in diabetic complications. Genetic variability cannot explain why some individuals and some families seem programmed to have a high rate of complications.²⁸ El-Osta discussed the Finnish Diabetic Nephropathy (FinnDiane) Study result suggesting that specific epigenetic events contribute to programming for the development and progression of diabetes complications.

Immunity and Hypertension

Dr Bernard Rossier (University of Lausanne, Switzerland) delivered the opening lecture on evolutionary medicine and the hypertension pandemic. He emphasized the critical role of the renin-angiotensin-aldosterone-system (RAAS) in salt conservation during evolution in vertebrates, and in the control of blood pressure, and focused on the importance of genetic and environmental factors in explaining the variability of the blood pressure trait.²⁹ He also discussed the gene-culture-environment mismatch hypothesis as the basis of the hypertension pandemic, and why evolutionary medicine is an important and emerging field with potential to identify new diagnostic tests, drug targets, and therapies.³⁰

Dr Jens Titze (Vanderbilt-University, USA, and University of Erlangen-Nürnberg, Germany) discussed gut-kidney interactions, metabolism, and fluid and electrolyte homeostasis. Investigation of salt and water balance traditionally relies on short-term studies of bodily responses to extremes in salt intake. Ultra-long-term sodium balance studies suggest that steady-state

sodium balance in humans is characterized by extra-vascular storage and release of sodium from the body, particularly skin and muscle. Experimental work has revealed that sodium storage is dependent on lymphatic regulation, and recent data show that sodium storage can be imaged and quantified *in vivo* in humans using sodium MRI. A novel link toward a better understanding of the relationship between renal and cardiovascular disease could be that sodium storage in tissue leads to urea production in the liver and extra-renal tissues, which couples sodium metabolism to urea metabolism. Body sodium content in humans and animals is not constant, does not always readily equilibrate with water, and is not exclusively controlled by the kidney.³¹ This different view of sodium balance provides new research avenues for basic and clinical investigation.

Dr Matthew Bailey (University of Edinburgh, UK) discussed autonomic regulation of the immune system and its implications for blood pressure control and hypertension. Hypertension is still a major modifiable risk factor for cardiovascular disease and CKD, yet for many affected individuals, despite treatment compliance, blood pressure control is inadequate. Chronic activation of RAAS enhances sympathetic tone, which in turn directly enhances the immune response. Any single component of the neuro-immune-endocrine axis may initiate hypertension. When acting in concert, the progression of cardiovascular or renal disease is accelerated.³² He reviewed the major studies in this area to establish points of convergence between the autonomic nervous system, the immune system, and RAAS. He also illustrated a novel contribution from macrophages to blood pressure homeostasis. Unraveling the complex biology of the neuro-immune-endocrine axis is challenging, but it is likely to improve our understanding of hypertension and offer more mechanism-based therapies to control blood pressure.

Dr Tianxin Yang (Sun Yat-sen University, China) discussed the (Pro)renin receptor (PRR) and blood pressure control. PRR was cloned around 10 years ago and is an inactive precursor of prorenin, as well as an endocrine hormone and component of RAAS. PRR binding to prorenin activates nonproteolytically an angiotensin II-dependent pathway, but also a PRR-dependent (and angiotensin II-independent) signaling pathway. PRR may play an important role in blood pressure regulation and cardiovascular function through its effect on local RAAS activity in various tissues, including the brain, the vasculature, and the kidneys.³³ Central PRR has been shown to affect sympathetic outflow mediating the hypertension induced by angiotensin II and deoxycorticosterone acetate-salt. Mice with overexpression of human PRR

in the vasculature have an elevated blood pressure. Unpublished data support a direct role for the renal PRR in promoting sodium and water reabsorption.³⁴ PRR may play an important part in the pathogenesis of hypertension and might offer a new therapeutic target in hypertension.³⁵

Dr Yanfei Qi (University of Florida, USA) from Mohan Raizada's group provided a summary of neuro-immune communication in hypertension and obesity. Resistant hypertension is thought to be primarily neurogenic in origin and characterized by a dysfunctional autonomic nervous system with heightened inflammatory and neuro-inflammatory profiles. Raizada's group has proposed that brain-bone marrow communication is critical in the maintenance of vascular repair and the inflammatory status of the cardiovascular system. An autonomic-mediated increase in sympathetic nerve activity to the bone marrow impairs this balance, resulting in increased production of proinflammatory progenitor cells and a decrease in angiogenic progenitor cells. This increases peripheral inflammation and compromises vascular repair, both hallmarks of hypertension.³⁶ Furthermore, some of the proinflammatory progenitor cells migrate into autonomic brain regions, differentiate into activated microglia, and contribute to neuroinflammation. Neuroinflammation-induced release of cytokines, chemokines, and reactive oxygen species enhances autonomic neuronal activity. This perpetual cycle of increased sympathetic nerve activity, proinflammatory progenitors, and neuroinflammation is critical in driving resistant hypertension.³⁷ Centrally administered minocycline can dampen neuroinflammation and may be the basis for development of more selective drugs to treat resistant hypertension.³⁸

Dr Alicia McDonough (University of Southern California, USA) discussed the importance of proximal tubular sodium transport in the regulation of extracellular fluid volume and blood pressure. Excessive sodium input can acutely increase extracellular fluid volume and blood pressure, but the kidneys normally excrete enough sodium and water to normalize blood pressure, a response known as pressure natriuresis. The molecular mechanisms underlying the interdependence of blood pressure and extracellular fluid volume converge on the regulation of nephron sodium transporters. Angiotensin II-dependent hypertension is associated with cytotoxic T cell infiltration of the kidneys; these cells secrete IL-17A and interferon- γ that oppose the down-regulation of sodium hydrogen exchanger isoform 3 and sodium-dependent phosphate cotransporter.³⁹ Female rodents have a lower set point for pressure natriuresis that is also due to a relative redistribution and depression of sodium hydrogen exchanger isoform 3 and sodium-dependent phosphate

cotransporter. Understanding how pressure natriuresis is blunted during hypertension may provide new ways of affecting blood pressure control.⁴⁰

Dr Zhiming Zhu (Third Military Medical University, China) focused on the likely role of transient receptor potential (TRP) channels in intracellular calcium regulation and vascular tone in hypertension. Mammalian TRP channels can be divided into 6 subfamilies, including transient receptor potential cation channels, TRP vanilloid, and TRP melastatin among others. Increased transient receptor potential cation channels 3 (TRPC3) and transient receptor potential cation channels 5 (TRPC5) channel protein expression is found in circulating monocytes of patients with essential hypertension, and in vascular smooth muscle cells from spontaneously hypertensive rats, and is associated with increased cell calcium influx.⁴¹ In contrast, activation of TRPV1, present in the endothelium and linked to nitric oxide release, and TRPM8 cause vasodilatation and lower blood pressure.⁴² Agonist actions at these TRP channels may underlie the cardiovascular benefit of foods containing capsaicin (TRPV1) or menthol (TRPM8). An imbalance in TRP channel function may contribute to the pathogenesis of hypertension, and these channels might be suitable drug targets for cardiovascular disease and hypertension.⁴³

Dr Andrew Newby (University of Bristol, UK) considered macrophage activation and atherogenesis. Macrophages are found at all stages of human and experimental atherosclerosis and deleting them can reduce atheroma in animal models. Although smooth muscle cells can adopt a macrophage-like phenotype when exposed to oxidized low-density lipoprotein, monocyte-derived macrophages are the major players in foam cell formation and plaque progression, and this is supported by a positive correlation between the number of circulating monocytes and susceptibility to atherosclerosis. Although the so-called M1 macrophage phenotype seems to promote plaque rupture and myocardial infarction, the important mediators of macrophage activation remain unclear, which is a barrier to finding better and more selective treatments.⁴⁴ He presented new data on the importance of foam cell formation from lipid accumulation, and the role of acquired and innate immunity in atherogenesis, but emphasized the limitations in identifying precise mediators in human atherosclerosis and in animal models. Production of matrix degrading enzymes, and the importance of their transcriptomic and epigenetic regulation, is becoming evident from population studies, and a key feature linking macrophage activation to plaque instability. Targeting macrophage activation is a therapeutic goal in preventing stroke and myocardial infarction.

Dr Entai Hou (Xi'an Jiaotong University, China) described the role of fumarase, a mitochondrial and cytosolic enzyme involved in the tricarboxylic acid cycle and amino acid metabolism, respectively, and its role in salt-sensitive hypertension. High-salt loading limits arginine availability for nitric oxide synthase, and, thus, nitric oxide production (from arginine metabolism to citrulline) is reduced in the kidney because of its restricted capacity to regenerate endogenous arginine via the citrulline-nitric oxide pathway. Fumarase activity is significantly lower in the kidneys of Dahl salt-sensitive rats compared with (consomic) control SS-13^{BN} rats.⁴⁵ In studies, fumarase activity contributed to the reduced arginine synthesis for nitric oxide production and the blood pressure elevation in Dahl salt-sensitive rats challenged with high salt.

Immunity, Fibrosis, and Kidney Disease

Dr Richard Kitching (Monash University, Australia) discussed the role of dendritic cells in CKD and fibrosis. Although it is attractive to view fibrosis as the final common pathway leading to end-stage renal disease, the drivers of persistent and progressive renal disease are many and varied. Active inflammatory and metabolic factors accelerate fibrosis and determine progressive fibrotic disease, but when chronic injury is advanced, fibrosis still tends to progress even without an ongoing stimulus. Current evidence suggests that macrophages can promote progression in antigen-independent renal fibrosis, whereas the role of interstitial dendritic cells is less clear.⁴⁶

Dr Karl E. Kadler (University of Manchester, UK) presented the concept of the circadian clock in matrix synthesis in fibrosis and the illustrative example of tendon biology. Although fibrosis due to ectopic deposition of type I collagen is a recognized feature of many chronic diseases, the molecular mechanisms of collagen fibril formation are still unclear, in part because large and insoluble collagen fibrils are refractory to conventional biochemical or molecular analysis. The highly organized 3-dimensional matrix in tendon is an ideal model for studying all stages and timing of collagen fibrillogenesis, and findings from studying tendons may also be applicable to renal fibrosis. Kadler's group has used serial block face-scanning electron microscopy to investigate collagen fibrillogenesis *in vivo*.⁴⁷ This technique can be used to obtain high-resolution images of cells synthesizing collagen fibrils at sites on the plasma membrane, which has led to a 2-stage model of fibrous tissue expansion.⁴⁸ His group has also shown that the tendon is a peripheral clock tissue in which the expression of 750 genes is regulated on a 24-hour rhythm.⁴⁹ Disruption of the tendon clock, and some identified key regulatory

genes, leads to abnormal collagen fibril formation and fibrosis.

Dr Youhua Liu (Southern Medical University, China) discussed epithelial-mesenchymal communication in fibroblast activation and kidney fibrosis. Fibroblasts are the cells responsible for excessive production of extracellular matrix, and the tubular epithelium is often the main target of kidney injury. How an injured tubular epithelium communicates with interstitial fibroblasts and causes them to undergo phenotypic activation is a central question. Liu's group recently discovered a critical role for sonic hedgehog and Wnt signaling in mediating an epithelial-mesenchymal communication in renal fibrogenesis.⁵⁰ In fibrotic kidneys, sonic hedgehog is induced in tubular epithelium, but not in fibroblasts. However, when sonic hedgehog is induced in fibroblasts it causes their proliferation and activation.⁵¹ When sonic hedgehog is active in fibroblasts, these cells secrete a variety of Wnt ligands that lead to β -catenin activation in the tubular epithelium. Tubular Wnt/ β -catenin activation in turn induces a wide range of fibrogenic genes, including all components of the RAAS system.⁵² These results suggest that sonic hedgehog and Wnt/ β -catenin pathways can mediate bidirectional communication between the tubular epithelium and interstitial fibroblasts.

Dr Huiyao Lan (Chinese University of Hong Kong, China) talked about the TGF- β /Smad signaling pathway in kidney and cardiovascular disease. Lan's group has found that in hypertension, angiotensin II is able to activate the transforming growth factor- β /Smad signaling pathway via transforming growth factor- β -dependent and -independent mechanisms.⁵³ Transforming growth factor- β /Smad3 signaling may be a key pathway in cardio-renal fibrosis, and increasing Smad7 or microRNA-29 expression may be a valid therapeutic approach.⁵⁴

Dr Mark Okusa (University of Virginia, USA) described sphingolipid signaling in kidney disease. Sphingosine 1-phosphate is the product of sphingosine phosphorylation by 2 sphingosine kinase (SphK) isoforms (SphK1 and SphK2). Localization of SphK1 near the plasma membrane provides sphingosine 1-phosphate, which is exported out of the cell by ABC transporters and spinster homolog 2, where it can bind to its 5 different G-protein coupled receptors (sphingosine 1-phosphate 1–5). SphK1 promotes cell survival and proliferation, and regulates cell transformation. Less is known about SphK2, but several reports suggest that SphK2 may have proapoptotic functions.⁵⁵ Dr Okusa discussed the role of the sphingolipid pathway in AKI, repair, and fibrosis. Sphingosine 1-phosphate 1, sphingosine 1-phosphate 3, and SphK2 may be new therapeutic targets for AKI and progressive kidney disease.⁵⁶

Dr Jun Ni (Chinese University of Hong Kong, China) discussed the finding that angiotensin-converting enzyme 2 (ACE2)/angiotensin 1-7 (Ang 1-7)/Mas receptor (Mas) deficiency can promote angiotensin II-related renal fibrosis by enhancing ERK1/2-MAPK-Smad3 crosstalk. Ni provided evidence for the ACE2/Ang1-7/Mas axis having a protective role in CKD by countering the effects of angiotensin II through its AT1 receptor. They have found that deletion of ACE2 or Mas in mice causes high blood, which is even higher following angiotensin II infusion in double knockouts. Compared with single ACE2 or Mas knockouts, double knockouts develop more severe hypertensive kidney injury, including markers of renal fibrosis. Disruption of the ACE2/Ang1-7/Mas axis enhances AT1 receptor-dependent ERK/MAPK-Smad3 signaling.

Dr Yi Guan (Huashan Hospital, China) reported on nicotinamide mononucleotide, a key intermediate in nicotinamide adenine dinucleotide biosynthesis, which can rescue the age-related susceptibility to cisplatin-induced AKI. This effect involves SIRT1 activation, which is a nicotinamide adenine dinucleotide biosynthesis-dependent deacetylase, linking nicotinamide mononucleotide supplementation to its protective effect. Studies have shown that in the aging kidney nicotinamide adenine dinucleotide biosynthesis levels and SIRT1 expression are both reduced, which may underlie the more general age-associated increase in the risk of AKI. Giving nicotinamide mononucleotide, a metabolite of the lipid-lowering drug niacin, may have a preventative role in managing patients at high risk of cisplatin-induced AKI, but perhaps also more generally.

Dr Weidong Wang (Sun Yat-sen University, China) discussed dual activation of the insulin-sensitizing bile acid receptors, nuclear receptor FXR (see earlier) and G-protein-coupled receptor TGR5, which together can attenuate lithium-induced nephrogenic diabetes insipidus. Wang's group has used INT-767, a dual FXR and TGR5 agonist, and INT-747, an FXR agonist, in lithium-induced nephrogenic diabetes insipidus; INT-767, but not INT-747, prevented the lithium-induced decrease in urine osmolality and increase in urine output. INT-767 also attenuated lithium-induced down-regulation of the aquaporin-2 protein, and mRNA expression of aquaporin-2, V2R, FXR, and TGR5 in the kidney inner medulla. In primary cell cultures of rat inner medulla collecting duct, INT-767 increased aquaporin-2 protein expression in control and lithium-treated rats, whereas INT-747 increased aquaporin-2 expression in control rats only. Activation of both FXR and TGR5 in the kidney can affect water transport and prevent lithium-induced nephrogenic diabetes insipidus, the latter mainly via TGR5 activation.

Dr Jing Nie (Southern Medical University, China) described the effects of targeted deletion of *NUMB*, which encodes the ubiquitous adaptor protein Numb, involved in many cellular functions, including cell fate, adhesion, migration, and proliferation. It is expressed in the renal tubule and glomerulus and attenuates interstitial fibrosis by mitigating G2/M cell cycle arrest when deleted in proximal tubular cells. Studies in a knockout mouse model reveal that Numb may be yet another target in treating tubulointerstitial fibrosis.

Conclusion

International Society of Nephrology Forefronts Symposia are intended to educate and inform, and to broaden and advance basic and applied renal science by drawing on expertise from diverse fields of biology and clinical medicine. As can be seen from the breadth of subjects covered in the summaries listed above, we believe these goals were achieved. In addition, it was a unique opportunity to bring research scientists, clinical scientists, and nephrologists from Asia (specifically China), Australia, Europe, and North America together in such a closely interactive format (Figure 1). The 2015 International Society of Nephrology Forefronts Symposium in Shenzhen attracted more than 200 active scientists—senior and junior—and has, and will, lead to new exchanges and productive collaborations to innovate in nephrology research and improve clinical practice.

DISCLOSURE

All the authors declared no competing interests.

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