First Joint Meeting of the French Society of Nephrology, the UK Renal Association and the Nephrology Section of the Royal Society of Medicine

Royal Society of Medicine, London,
February 28–29, 2008

Abstracts

Guest Editors

Philip Mason, President of the Section of Nephrology of the
Royal Society of Medicine

Peter Mathieson, President of the UK Renal Association

Pierre Ronco, President of the French Society of Nephrology

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Nephrocystin-4 Loss of Function Induced Convergent Extension Defect and Pronephric Cysts in Zebrafish

Burcklé C¹, Vesque C², Delous M¹, Salomon R¹, Schneider-Maunoury S², Saunier S¹

¹INSERM U574, Hereditary Nephropathies and Kidney Development GH NECKER – Enfants Malades 149 Rue De Sevres Tour Lavoisier, 75743 Paris Cedex 15; ²CNRS UMR7622, Biologie du Développement, CNRS UMR7622, Université Pierre et Marie Curie, 9 Quai Saint-Bernard, 75252 Paris Cedex 05, France

Nephrocystin-4, mutated in nephronophthisis, is located at the primary cilia. To investigate the function of nephrocystin-4 *in vivo*, we performed loss of function experiments with specific antisens Morpholino[®] (MPO) in zebrafish. High doses of MPO inhibited convergent extension (CE), inducing shortened embryonic axis, elongated somites, and notochord imperfections in 75% of the morphants. Clusters of cells aggregated on the dorsal side of strongly affected morphants. With lower doses of MPO, at 3 days post fertilization, all morphants displayed ventral axis curvature, 25% of them had pronephric cysts.

CE is under the control of two major signaling pathways, Wnt-PCP and BMP. To test epistatic interaction of nephrocystin-4 with these pathways, MPO injections will be performed in heterozygous mutant embryos for the Wnt-PCP (VANGL2) and BMP (Swirl) pathways.

The observation of cell aggregates on the dorsal side of the embryos suggests a cellular adhesion defect, as described in some cadherin mutants. The role of nephrocystin-4 in the delivery of cell-cell adhesion proteins at the plasma membrane will be investigated.

We have demonstrated that nephrocystin-4 is required for CE in zebrafish suggesting that nephrocystin-4 mutations could affect planar cell polarity (PCP) resulting in tubular cysts formation in mammals.

2

Important Factors in Initiation of Haemodialysis as an Emergency in ESRD Patients Known to the Renal Services

Raza MN, Dudley CRK

Richard Bright Renal Unit, Southmead Hospital, Bristol, BS10 5NB

Background: Emergency dialysis for ESRD patients requires hospital admission and has clinical and economic consequences.

We investigated the frequency of its occurrence and associated factors in patients presenting in a controlled way to the renal services.

Methods: We investigated records of a cohort of 137 patients who started haemodialysis in the year 2005 and divided them in to inpatient or outpatient dialysis group.

Results: Of the 137 patients, 121 patients had been known to renal services for a variable length of time, 71 started dialysis as inpatients and 50 as outpatients.

At the time of first RRT, the inpatient group had a higher median urea (36 vs 31mmol/L $p=0.036$), a lower serum bicarbonate (19 vs. 22 mmol/L $p=0.005$), were more anemic (Hb 9.4 vs. 10.5 g/dl, $p=0.002$) and had higher mortality at 12 months (44% vs. 24% $p=0.026$). Most diabetics started dialysis as inpatients (37% vs. 18% $p=0.02$).

Subjects in the inpatient group has had less review by the pre-dialysis education team (60% vs. 96% $P=0.0002$); majority started dialysis with temporary access (81% vs. 19% $P=0.00001$).

Regression lines for stepwise rise in serum Cr 6 months in advance of the first RRT were linear and parallel for both groups.

Conclusions: Patients known to renal services who start RRT as inpatients have adverse biochemical and hematological parameters, are less likely to have permanent access and have a significantly higher mortality at one year.

3

Sodium Retention in Acromegaly Results from Direct Growth Hormone Action in the Late Distal Nephron

Kamenicky P¹, Viengchareun S¹, Blanchard A², Meduri G¹, Zizzari P³, Doucet A⁴, Chanson P¹, Lombès M¹

¹INSERM U693, Faculté de Médecine, Univ Paris-Sud 11, 94276 Le Kremlin Bicêtre; ²CIC, Hôpital Européen Georges Pompidou, 75908 Paris; ³INSERM U549, Centre Broca, 75013 Paris; ⁴CNRS/UPMC 7134, IFR 58, Institut des Cordeliers, 75006 Paris, France

Acromegalic patients present with volume expansion and arterial hypertension but the renal sites and molecular mechanisms of direct antinatriuretic action of growth hormone (GH) remain unclear.

Renal metabolic studies performed on acromegalic GC rats revealed a decreased natriuretic response to furosemide compared to controls, whereas exposure to amiloride induced increased natriuresis, which normalized after acromegaly treatment. Despite low plasma aldosterone concentrations, an enhanced cleavage of α -subunit of ENaC and an increased abundance of α ENaC were observed in GC rats, supporting ENaC activation in the late distal

nephron in acromegaly. In vitro experiments on KC3AC1 cells, a cortical collecting duct (CCD) cell model revealed the expression of functional GH receptors (GHR) coupled to activation of JAK2/STAT5 and ERK signaling pathways. That GH directly controls sodium reabsorption in CCD cells is supported by i) stimulation of transepithelial sodium transport inhibited by GHR antagonist pegvisomant, ii) induction of α ENaC mRNA expression, iii) identification of STAT5 binding to a response element located in the α ENaC promoter, indicative of the transcriptional regulation of α ENaC by GH.

Our findings provide first evidence that GH stimulates ENaC-mediated sodium transport in the late distal nephron, accounting for the pathogenesis of sodium retention in acromegaly.

4

Evidence for a Role for Extracellular Signal-Regulated Kinase-5 (ERK5) in EGF-Induced Renal Epithelial Cell Survival – A Possible Role for MEF2C?

Browne J¹, Paramasivam K¹, Baines D², Colville-Nash P¹, Dockrell MEC¹

¹SW Thames Institute for Renal Research, St Helier Hospital, Carshalton; ²Department of BMS, St George's Hospital Medical School, London, UK

The exact role of the atypical MAP kinase, ERK5 remains unknown however it is critical for cell survival as highlighted by the lethality of the ERK5 knock-out. ERK5 possess a transactivation domain and may act as a transcriptional co-activator. ERK5 can induce its translocation to the nucleus to activate the transcription factor, MEF2C. Hence ERK5 could regulate the functions of MEF2C. ERK5 is anti-apoptotic possibly via MEF2C activation.

Lysates of HKC-8 cells treated for 5 min with either EGF (10 ng/ml) or vehicle (0.1% BSA) were used for western blotting or subjected to immunoprecipitation prior to blotting using antibodies targeting proteins of interest. ERK5 siRNA (100nM) transfected cells were similarly treated, but for 24h and caspase-3/7 activity measured to quantify apoptosis.

We show that EGF-induced activation of ERK5 in PTECs is associated with increased association of MEK5 with not only ERK5 but also MEF2C. EGF inhibited staurosporine-induced apoptosis. siRNA knockdown of ERK5 significantly increased apoptosis and EGF did not alter apoptosis in the presence of ERK5 siRNA.

Our work provides evidence that ERK5 may mediate EGF-induced cell survival in PTEC. We present the first demonstration of MEF2C expression in PTEC and propose that it has a role in EGF-ERK5 cell survival.

5

Differential Effect of Cyclosporin A, Mycophenolic Acid, and Everolimus on the Treg/Th17 Cells Balance

Mariat C

University Jean Monnet, Nephrology Department, EA3065, Saint-Etienne, France; Groupe de Recherche Sur Les Glomérulonéphrites et La Transplantation Rénale (EA 3065), Université Jean Monnet, et Département de Néphrologie et de Transplantation Rénale, Hôpital Nord, Saint-Etienne, France

Beyond the usual TH1/TH2 polarisation, a reciprocal pathway between FoxP3+ regulatory T cells (Tregs) and IL17 secreting effector T cells (TH17) has recently been identified. We have investigated the effects of three immunosuppressive treatments, cyclosporin A (CsA), mycophenolic acid (MPA) and everolimus (RAD) on the human Treg/TH17 cells response development.

Using an in vitro proliferation assay in healthy volunteers, we compared the influence of CsA, MPA and RAD on (i) the proliferation profile of CFSE-stained CD4+ T cell, and (ii) the transcription level of FOXP3 and IL17 in PBMC.

CsA, MPA and RAD similarly inhibited CD4+ T cells proliferation. After activation, the Tregs -TH17 balance was naturally tipped toward TH17. CsA, MPA and RAD did not affect the level of transcription of FOXP3. Likewise, IL17 expression remained stable when PBMC were stimulated in the presence of CsA and RAD. Conversely, MPA was consistently found to extinguish IL17 transcription. This appeared to be a direct effect of MPA on T cells since the drug did not modify IL6 production by monocytes.

MPA might favourably influence the Treg/TH17 balance. Our results suggest that the immunosuppressive drugs used in the clinic may differentially influence lymphocyte polarisation, including the newly identified TH17 pathway.

6

Pronephric Tubule Dilatation in Two Zebrafish Models of Cystic Kidney Diseases Is Related to an Obstruction in the Distal Part of the Pronephric Duct

Burtey S, Gilmour D, Karsenti E

Cell Biology and Biophysic Unit, EMBL Heidelberg, Heidelberg, Germany

Introduction and Aims: The physiopathology of cystic kidney diseases is still mysterious, despite the identification of numerous genes involved. Recently, it had been suggested, in *pkd2* morphant, that dilatation is secondary to obstruction. Our aim is to confirm and understand the origin of the obstruction.

Methods: We used a transgenic fish expressing GFP in the pronephros under the control of the *claudin b* promotor. We injected two morpholinos to knock-down the expression of *neph-*

rocystin-6 and *pkd2*. The morphants were observed in live imaging with confocal microscopy.

Results: We determined the normal morphology of pronephros in live, non fixed normal zebrafish at different times. In *Nephrocystin-6* and *Pkd2* morphants we observed that tubular dilatation begins after 36 hpf. By three days, we observed in more than 70% of morphants a huge tubular dilatation. The pronephros duct dilatation is extended to the 18th somites. In this part we observed the destruction of the normal tubule architecture, with disappearance of the pronephric duct lumen.

Conclusions: We demonstrated using live imaging microscopy that pronephric tubular dilatation in two models of cystic kidney disease is secondary to an obstruction of the distal part of the pronephric duct. We propose a come back of the obstructive theory of cystic kidney diseases, at least in zebrafish.

7

Early Expression of Epithelial to Mesenchymal Transition Markers Is Predictive of Long-Term Renal Graft Function

Hertig A¹, Anglicheau D², Verine J³, Touzot M¹, Mesnard L¹, Brousse N⁴, Glotz D⁵, Legendre C², Rondeau E¹, Xu-Dubois Y-C¹

¹Urgences Néphrologiques et Transplantation Renale, APHP, Hôpital Tenon, Paris; ²Transplantation Renale, APHP, Hôpital Necker, Paris, France; ³Anatomo-Pathologie, APHP, Hôpital Saint Louis, Paris; ⁴Anatomo-Pathologie, APHP, Hôpital Necker, Paris and ⁵Transplantation Renale, APHP, Hôpital Saint Louis, Paris, France

Introduction: Epithelial to mesenchymal transition (EMT) is a potential mechanism of tissue fibrogenesis. Here, we report the long-term follow-up of a cohort of kidney transplanted patients with or without early expression of EMT markers in their grafts.

Patients and Methods: 83 patients in whom sequential protocol graft biopsies had been performed at 3 and 12 months, were included. The phenotype of epithelial cells was studied at three months and included two EMT markers: expression of vimentin and cellular translocation of β -catenin (if than 10% of tubular cells were positive, graft was considered as EMT+).

Results: Early phenotypic changes were associated with a progressive and sustained deterioration of the graft function: EMT+ patients had a statistically higher serum creatinine from twelve months after transplantation, and a significantly lower creatinine clearance from 18 months after transplantation (EMT+ 49.4 ± 4.5 ml/min vs. EMT- 61.1 ± 2.3 ml/min, $p=0.01$).

Conclusion: The expression of EMT markers by tubular epithelial cells at an early time point post-transplant is highly suggestive of an ongoing fibrogenic process, and has repercussions on the long-term graft function. Therefore, these epithelial phenotypic changes are relevant and promising biomarkers for an early detection of Interstitial Fibrosis and Tubular Atrophy of the grafts.

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Cyclosporin Induces Epithelial to Mesenchymal Transition in Renal Grafts

Hertig A¹, Hazzan M², Buob D³, Noel C², Copin M-C³, Rondeau E¹, Xu-Dubois Y-C¹

¹Urgences Néphrologiques et Transplantation Renale, APHP, Hôpital Tenon, Paris; ²Néphrologie, Hôpital de Lille, Lille; ³Pathologie, Hôpital de Lille, Lille, France

Introduction: The expression of epithelial to mesenchymal transition (EMT) markers is a reliable predictor of the progression towards interstitial fibrosis in renal grafts. *In vitro* experiments suggest that calcineurin inhibitors (CNI) can induce EMT of tubular epithelial cells. No evidence was ever provided *in vivo*.

Patients and Methods: We have previously reported the results of a prospective randomized trial comparing the elimination at month 3 of either cyclosporine or mycophenolate from a triple drug regimen in *de novo* renal transplant patients. All of them had 2 systematic graft biopsies at months 3 and 12. We detected in tubular cells and by immuno-histochemistry the expression of two markers of EMT: the *de novo* expression of vimentin (Vim) and the cytoplasmic translocation of β -catenin (Cat).

Results: 68 patients (34 in each group) were analyzed. Remarkably, the 3-month EMT scores were correlated with the 1-year eGFR and the Chronic Allograft Damage Index. In the CsA group, the Vim and Cat scores had progressed between 3 and 12 months from 1.53 ± 0.86 to 1.99 ± 0.97 ($p=0.004$) and 1.55 ± 0.79 to 1.88 ± 1.04 ($p=0.041$), respectively, while they remained stable in the MMF group.

Conclusion: Interruption of CsA alleviates epithelial stress in renal grafts.

9

The Soluble ST2 Protein Is a Marker of Idiopathic Nephrotic Syndrome Recurrence After Transplantation

Bruneau S, Le Berre L, Hervé C, Naulet J, Souillou J-P, Sahali D, Dantal J

University of Nantes, France INSERM U643

Idiopathic Nephrotic Syndrome (INS) appears in the context of immune response disturbances according to unclear mechanisms. After transplantation, corticosteroid-resistant INS recurs in 30–50% of the patients, suggesting the presence of a circulating factor endowed with glomerular pathogenic properties and probably produced by Th2 lymphocytes. Based on these observations, we were interested in the Th2 cells marker ST2, and especially in its soluble form (sST2), in the development of INS recurrence.

We analyzed the sST2 protein in the serum of 43 patients with INS recurrence, 49 without recurrence and 35 with a different glomerulonephritis disease in the transplanted kidney. We found that sST2 is highly produced after transplantation in patients undergoing INS recurrence unlike in the two control groups ($p<0.001$). Moreover, a bi-dimensional analysis

showed that recurrent patients display the normal sST2 isoform. Nevertheless, we could not demonstrate, either *in vitro* or *in vivo*, that this protein is capable of acting directly on the kidney to induce proteinuria or glomerular/podocytes damages.

Thus, our study shows that although sST2 does not seem to be directly implicated in INS development, it could constitute a new marker for INS recurrence after transplantation that may be of great interest for its diagnosis in ambiguous clinical situations.

10

Higher Doses of Cholecalciferol Than Usual Are Needed to Correct 25OH-Vitamin D Insufficiency in Renal Transplant Recipients

Courbebaisse M¹, Thervet E², Souberbielle JC², Zuber J², Martinez F², Eladari D¹, Mamzer MF², Legendre C², Friedlander G¹, Prié D¹

¹Explorations Fonctionnelles; ²Service de Transplantation, Necker Hospital, Paris, France

Usual cholecalciferol (Clc) treatment (ClcT) (800 IU/day) cannot correct 25OH-vitamin D (25OHD) insufficiency in renal transplant recipients (RTR). However, the risk of inducing hypercalcemia and/or hypercalciuria limits the prescription of effective vitamin D supplementation.

During 10 months, 49 RTR with 25OHD < 30 ng/ml and iCa < 1.35 mmol/l were included 3 months after transplantation (M3). Patients received 100.000 IU of Clc twice monthly, then 100.000 IU every 2 months. After randomization ClcT was initiated at M4 (group A, n = 23) or at M6 (group B, n = 26). Biological assessments were performed at M6, M8 and M12. Groups A and B had similar pre-transplant characteristics, initial post-transplant evolution and immunosuppressive therapy. All patients of group A normalized their 25OHD after ClcT (M4 to M6) vs. no patient in group B. Group B normalized 25OHD after treatment (M6 to M8). No case of hypercalcemia or hypercalciuria was induced by ClcT. Serum PTH concentration decreased between M3 and M12 (106 +/- 121 vs. 75 +/- 57 pg/ml, n = 49 p = 0.03), without GFR modification.

In conclusion: 25OHD does not improve spontaneously in RTR, whereas ClcT corrects both 25OHD insufficiency and secondary hyperparathyroidism, without side effects.

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Acute Hypercalciuria Induced by Cinacalcet in Renal Transplant Recipients

Courbebaisse M¹, Le Quintrec M², Martinez F², Eladari D¹, Zuber J², Legendre C², Friedlander G¹, Thervet E¹, Prié D¹

¹Explorations Fonctionnelles; ²Service de Transplantation, Necker Hospital, Paris, France

Calcimimetic (CNC) decreases serum PTH and calcium concentrations in renal transplant recipients (RTR) with autono-

mous hyperparathyroidism. However, because the calcium sensor is expressed all along the nephron, CNC can potentially induce severe hypercalciuria.

We analysed the kinetics of serum PTH, ionized calcium concentrations (Ca_i) and urinary Ca/creatinine excretion (Cau/Creatu) every hour during the 5 hours following CNC morning dose in 23 RTR with autonomous hyperparathyroidism.

Serum PTH levels fell within the first hour following CNC dose, reached a minimum at the third hour and then increased again. The Ca_i decrease began 3 hours after CNC ingestion and persisted beyond the 5th hour. Cau/Creatu increased from the first hour, reached a maximum of 800 +/- 1139 %, 4 hours after CNC intake and then remained above pretreatment values. Kinetics analysis suggests that the decrease in Ca_i is mainly due to the marked increase in Cau/Creatu. This is supported by the normalization of Ca_i in 10 patients while serum PTH remained elevated.

In conclusion, CNC treatment induces a transient and marked increase in Cau/Creatu that peaks 4 hours after treatment intake. Effect of CNC on graft calcification or kidney stone formation remains to be determined.

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Pharmacodynamic Evaluation of the Anticalciuric Effect of Hydrochlorothiazide in Association with 5 mg of Amiloride in Dent's Syndrome

Blanchard A¹, Vargas-Poussou R¹, Peyrard S¹, Mogenet A², Baudouin V³, Boudailliez B, Charbit M², Deschesnes G³, Loirat C³, Macher M-A³, Niaudet P², Azizi M¹

¹Assistance Publique des Hôpitaux de Paris, Hôpital Européen George Pompidou; ²Hôpital Necker enfant malade; ³Hôpital Robert Debré; ⁴Centre Hospitalier Amiens Sud

Thiazide diuretics have been proposed as treatment for the hypercalciuria of Dent's disease to prevent renal failure. Since high doses are known to cause adverse metabolic effects, we assessed the risk/benefit ratio of 3 doses of hydrochlorothiazide (HCTZ) in seven patients. After a one month run-in period, each patient received sequentially 6.25 mg/day, 12.5 mg/day, or 25 mg/day HCTZ given for 2 months. Amiloride 5 mg/day was also given at the start of the study to minimise HCTZ-induced hypokalaemia and continued throughout study period. HCTZ exerted a dose-dependent stimulatory effect on renin and aldosterone levels, and markedly increased plasma protein concentration. Compared with baseline values, fasting urinary calcium excretion was not significantly altered by amiloride alone, or with 6.25 mg HCTZ, but it was decreased by ~40% and ~60%, respectively, by 12.5 mg and 25 mg HCTZ, though without any change in daily calcium excretion. All measurements made, except for aldosterone, returned to baseline after 1 month of stopping treatment. Four patients had adverse reactions: muscle cramps, seizures (n = 2) and hypokalaemia (n = 4).

In conclusion, although HCTZ exerted a dose-dependent natriuretic effect, a decrease in calcium excretion occurred only at the higher doses, which were associated with significant adverse events.

IQGAP 1 and The Glomerular Slit Diaphragm: Molecular Interactions and Expression in Nephropathies

Rigother C¹, Auguste P¹, Saleem M², Ripoche J¹, Combe C^{1,3}

¹GREF-INERM U889, INSERM-Universite Bordeaux, Bordeaux, France; ²Children's Renal Unit And Academic Renal Unit, University Of Bristol, Bristol, United Kingdom; ³Service de Nephrologie, CHU Bordeaux, Bordeaux, France

IQGAP1 is a scaffold protein, interacting with cytoskeleton proteins and adhesion complex molecules. It has been implicated in the formation of the actin cytoskeleton and in the regulation of cell migration and adhesion. IQGAP1 has recently been identified associated to nephrin in the slit diaphragm, where it may connect cytoskeleton to cell membrane anchored proteins. Modification of its expression or localization could alter podocyte and glomeruli permeability.

The aim of our work was to determine IQGAP1 interactions with slit diaphragm proteins and to understand its role in this complex.

IQGAP1 was expressed in immortalized human podocytes (Western blot analysis) such as others proteins. Co-immunoprecipitation experiments showed interactions between IQGAP1 and nephrin, MAGI-1, α -actinin-4 or podocalyxin. These results suggest that IQGAP1 may be intermediate between the actin cytoskeleton and different proteins in podocytes. Immunocytolocalisation experiments showed colocalization of these molecules with IQGAP1, confirming the previous results.

Immunohistolabelling suggested a decrease in glomerular IQGAP1 expression on biopsy sections taken from patients suffering from minimal change disease.

IQGAP1's multiple interactions with podocyte proteins suggest that it has a pivotal role in maintaining the physiological properties of the glomerular barrier.

Adherence to Therapy in Sub-Saharan Patients with Chronic Kidney Diseases

Seck SM, Ka EF, Cisse MM, Niang A, Diouf B

Department of Nephrology and Dialysis, Aristide Ledantec Teaching Hospital, Dakar, Senegal

Background: Poor adherence to medication regimens accounts for substantial morbidity, mortality, and increased health care costs in developing countries.

Objective: To assess adherence to therapy in non-dialysed patients with chronic renal diseases and to identify the major barriers to adherence.

Patients and Method: We conducted prospective study during three months at the nephrology department of teaching hos-

pital in Dakar-Senegal. Data were collected using a questionnaire. Rate of adherence (ROA) was defined as the percentage of the prescribed doses of the medication actually taken by the patient within a four weeks period. Statistical analysis was done with SPSS 11.0.

Results: Mean ROA was 81 ± 12 % (46–100%) with a difference between male (75%) and female (84%). Three quarters of patients reported ROA more than 80%. Patients' adherence was inversely proportional to daily frequency of dose but not correlated to number of drugs ($r = -0.002$; $p = 0.25$). Major obstacles to adherence were: complexity of drug regimen (OR=3.33; $p < 0.001$), forgetfulness (OR=1.37; $p = 0.004$), healthcare system inaccessibility (OR=2.65; $p = 0.002$), lack of information (OR = 2.35; $p = 0.04$, side effects (OR = 1.654; $p = 0.002$), auto-medication with plants (OR=3.26; $p = 0.005$) and high cost of medications (OR=1.47; $p = 0.004$).

Conclusion: Most of barriers to adherence can be by improving communication between patients and health providers and accessibility of healthcare system.

Does the Endothelium Enhance Platelet Formation by Bone Marrow Derived Megakaryocytes in vitro?

Spencer NB, Savill J, Brown SB

MRC Centre for Inflammation Research, University of Edinburgh, The Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh

Introduction: Aberration in platelet numbers is a feature of renal disease. Thrombopoiesis is highly inefficient *in vitro* and has proved difficult to characterise at a molecular level. Oestradiol, nitric oxide (NO) and Fas ligand (FasL) are all known to modulate thrombopoiesis either *in vitro* or *in vivo* in which oestradiol is reported to upregulate FasL expression by endothelial cells in an NO-dependent manner.

Aims: This study aims to model the bone marrow environment by co-culturing megakaryocytes with endothelial cells.

Methods: Primary rat megakaryocytes were co-cultured with a rat microvascular cell line (GPNT) exposed to IL-1 β , oestradiol or GSNO (an NO donor). Proplatelet counts were measured after 24 hours using light microscopy. GPNT cells were assessed for FasL by RT-PCR.

Results: Fewer than 3% of megakaryocytes formed proplatelets when cultured in the absence of GPNT cells. This was not found to significantly alter when co-cultured with GPNTs regardless of their prior exposure to IL-1 β , oestradiol or GSNO. Furthermore, we found no evidence that GPNT constitutively or inducibly expressed Fas ligand.

Conclusion: Although megakaryocytes are in close proximity to endothelial cells in the bone marrow *in vivo*, we have so far found no evidence that endothelial cells enhance platelet production *in vitro*.

Long-Term Outcome of Idiopathic Steroid-Resistant Nephrotic Syndrome: A Multicenter Study

Mekahli D¹, Liutkus A¹, Ranchin B¹, Yu A², Bessenay L³, Girardin E⁴, Damme-Lombaerts Van R², Palcoux JB³, Cachat F⁵, Lavocat MP⁶, Bourdat-Michel G⁷, Nobili F⁸, Cochat P¹

¹Pediatrics, University Hospital Edouard-Herriot, Lyon, France; ²Pediatrics, University hospital Gasthuisberg, Leuven, Belgium; ³Pediatrics, University Hospital, Clermont-Ferrand, France; ⁴Pediatrics, University Hospital, Geneva, Switzerland; ⁵Pediatrics, University Hospital, Lausanne, Switzerland; ⁶Pediatrics, University Hospital, Saint-Etienne, France; ⁷Pediatrics, University Hospital, Grenoble, France; ⁸Pediatrics, University Hospital, Besançon, France

The long-term outcome of idiopathic steroid-resistant nephrotic syndrome (SRNS) was retrospectively studied from 78 children in 8 centers (Belgium, France and Switzerland) during the last 20 years.

The median age at onset of NS was 4.4 years [1.1–15.0] and the sex ratio was 1.4. The median follow-up was 7.7 years [1.0–19.7] and the median age at last examination was 13.8 years [2.8–32.3]. Forty five patients (58%) were initial steroid non-responders and 33 (42%) late non-responders. The first renal biopsy showed 35 (45%) MCD, 10 (13%) DMP and 33 (42%) FSGS.

The renal survival rate at 5, 10 and 15 years was 75, 58 and 53 %, respectively. The age at onset of NS was the only independent predictor of ESRD in a multivariate analysis using a Cox regression model ($P < 0.001$).

Twenty patients (26 %) were transplanted; 10 presented recurrence of the NS. Seven patients lost their graft, from NS recurrence in 4.

Due to better management, the renal survival of idiopathic SRNS has improved during the last 20 years. Further prospective controlled trials will delineate the potential benefit of new immunosuppressive treatment.

Patients with Wegener's Granulomatosis Demonstrate a Relative Deficiency and Functional Impairment of T Regulatory Cells

Day CJ¹, Piper KL², Khan N², Moss PA², Savage COS¹

¹Renal Immunobiology, Division of Immunity and Infection; ²CRUK Institute of Cancer Studies, Medical School, University of Birmingham, UK

CD4+CD25hi regulatory T cells have been implicated in the control of auto-immunity. Wegener's granulomatosis (WG) is an autoimmune disease causing rapidly progressive glomerulonephritis. As an increased proportion of CD4+CD25+ T cells has

been reported in WG, this study investigated the role of regulatory T cells in this disease.

Methods: The proportion of regulatory T cells was determined in patients ($n = 55$) and age-matched healthy controls ($n = 33$) by measurement of CD25, Foxp3 and CD127 expression on CD4+ T cells. Functional activity of T regulatory cells was determined by the ability of CD4+ CD25hi cells to suppress proliferation and cytokine production in response to auto-antigen proteinase-3.

Results: Although increased proportions of CD4+CD25hi T cells were seen in patients ($p = 0.0006$) the percentage of Foxp3 positive cells within this CD4+CD25hi population was decreased ($p < 0.0001$) with this pattern positively associated with rate of relapse. CD4+CD25hi T cells suppressed proliferation to proteinase-3 in healthy controls ($p = 0.002$) and ANCA negative patients ($p = 0.007$) but this effect was lost in ANCA positive patients.

Conclusions: These data demonstrate an upset in both the immune balance and regulatory T cell function in patients with WG and suggest that T regulatory cells may play an important role in the natural history of this disease.

Differential Impact of End-Stage Kidney Disease on Mortality in Type 2 Diabetics and Non-Diabetics

Villar E^{1,2}, Polkinghorne KR^{1,3}, Chang SH^{1,4}, Chadban SJ^{1,5}, McDonald SP^{1,4}

¹Australia and New Zealand Dialysis and Transplant Registry, Woodville, South Australia, Australia; ²Hospices Civils de Lyon, Department of Nephrology, Centre Hospitalier Lyon Sud, Pierre Benite, France; ³Department of Nephrology, Monash Medical Centre, Melbourne, Victoria, Australia; ⁴Department of Nephrology, The Queen Elizabeth Hospital, Woodville, South Australia, Australia; ⁵Renal Medicine and Transplantation, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia

Patients with end-stage kidney disease (ESKD) and patients with diabetes mellitus experience a higher rate of mortality than the general population. Whether ESKD imparts the same increment in mortality risk for those with diabetes as it does for non-diabetics (noDM) is not known. Using data from the ANZDATA Registry, we computed standardised mortality ratios (SMR) for incident Australian ESKD patients from April 1, 1991 to December 31, 2005 in the first eight years after first renal replacement therapy against age-, gender- and diabetes status-specific mortality rates provided by the Australian Diabetes, Obesity and Lifestyle (AusDiab) Study. SMR comparisons were performed between ESKD patients with type 2 diabetes (T2DM) and noDM patients, taking into account the age and gender structures of both groups. The study included 4141 T2DM and 13289 noDM ESKD patients. In the AusDiab population without ESKD, the mortality rates were 20.6 deaths per 1000 person-years in T2DM and 4.9 in noDM population ($p < 0.0001$). When ESKD patients were compared to their non-ESKD counterparts, SMR was 10.8 [95% CI 10.4–11.2] in T2DM and 14.2 [13.9–14.6] in noDM ESKD patients ($p < 0.01$).

ESKD was associated with a greater increment in mortality in noDM than in T2DM population.

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Targeting the Kinin B1-Receptor Attenuates Renal Interstitial Fibrosis in vivo

Klein J, Gonzalez J, Esposito L, Calise D, Neau E, Bascands J-L, Schanstra JP

INSERM U858/I2MR Equipe n°5, CHU Rangueil, Bâtiment L3, BP84225, 31432 Toulouse Cedex 4, France

The bradykinin B1-receptor (B1R) receptor is weakly expressed under physiological conditions but induced by inflammatory stimuli. Here we report a role for the B1R in renal fibrosis using two different animal models.

Using a model of accelerated tubulointerstitial fibrosis induced by unilateral ureteral obstruction (UUO) we showed that genetic and pharmacological blockade of the B1R, either before or after UUO, reduced the induction of several markers of renal fibrosis: macrophage infiltration, myofibroblast accumulation and collagen deposition.

Since the majority of chronic kidney diseases are initiated by glomerular injury we also used the model of nephrotoxic serum (NTS) induced-glomerulonephritis and investigated whether blocking the B1R was effective in slowing-down the fibrotic process in this model. B1R antagonist treatment, starting 2 weeks after NTS administration, improved significantly renal function and histology.

Mechanistic studies showed that B1R blockade is reducing macrophage infiltration most likely by acting on chemokine expression.

In conclusion we demonstrate that delayed treatment with a B1R antagonist is leading to a significant decrease in the progression of renal fibrosis in two different animal models. Our findings show that blocking the B1R is a promising antifibrotic therapy and that this is feasible as orally active and stable antagonists are available.

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Pre-Transplant Screening for Complement System Abnormalities and Risk Assessment of Hemolytic and Uremic Syndrome (HUS) after Kidney Transplantation

Lionet A¹, Provot F¹, Glowacki F¹, Buob D², Fremeaux-Bacchi V³, Hazzan M¹, Noel C¹

¹Lille University Hospital, Department of Medicine, Division of Nephrology; ²Lille University Hospital, Department of Anathomopathology; ³European Hospital Georges Pompidou, Immunology Department, Paris, France

Introduction: Several mutations of the complement regulatory genes predispose to HUS and are linked to a high risk of relapse after kidney transplantation.

Patients: Since 2005, we have screened 42 patients on the waiting list for complement system abnormalities (CSA). Levels of circulating complement components (CCC), detection of nephritic factor (C3NEF) and the presence of mutations of the factor H, I, and C3 genes were systematically assessed. These 42 patients were selected according to the following clinical criteria: history of atypical HUS, post partum HUS, membrano-proliferative glomerulonephritis without cryoglobulinemia or C3NEF, atypical hypertensive nephropathy, or a previous kidney graft failure related to thrombotic microangiopathy without acute rejection.

Results: Complete results are available for 28 patients. CSA were found in 17 (61%) patients (isolated mutation: n=7; both mutation and CCC abnormalities: n=5; CCC abnormalities without mutation: n=3; C3NEF: n=2).

Ten of these 28 patients were transplanted. Six recipients without CSA had a successful outcome. Strikingly, the 4 remaining patients had mutations and all of them lost their kidney because of HUS (n=3) or graft vein thrombosis (n=1).

Conclusion: In selected patients, detection of CSA seems to be useful to predict the relapse of HUS after renal transplantation.

21

Effect of Neutrophil Adhesion, in the Presence of Anti-PR3 Anti-Neutrophil Cytoplasmic ANCA Antibodies, on Membrane Expression of PR3 and Endothelium Injury

Camous L, Brachemi S, Mambole A, Lesavre P, Halbwachs-Mecarelli L

INSERM U 845 and Nephrology Department, Necker Hospital, Paris, France

Background: Although the role of ANCA in vasculitis is established, two puzzling questions remain: the accessibility of ANCA to their antigens in the blood and the localization of vascular lesions, restricted to small vessels in ANCA vasculitis.

Materials and Methods: Human PMN were allowed to adhere to gelatin-coated plates or TNF-activated HUVEC, in the presence of anti-PR3 mAb or isotype control. PR3 membrane expression (mPR3) was measured by flow cytometry. Endothelial injury was measured by horseradish peroxidase permeability through HUVEC on transwells and by thrombomodulin release.

Results: Neutrophil activation by TNF- α or fMLP, adhesion and adhesion with anti-PR3 mAb resulted, respectively, in 2-, 11- and 24-fold increase of mPR3 levels. Similar results were obtained with human anti-PR3 ANCA but not with anti-MPO ANCA, anti-CD43 or anti-HLA mAbs. This mPR3 up-regulation required activation by TNF- α or by TNF-preactivated HUVECs and occurred without significant exocytosis of azurophilic granules. It involved β 2 integrins (inhibited by blocking anti- β 2) and Fc γ -receptors (not observed with F(ab')₂ ANCA). While plasma inhibited the binding of anti-PR3 to mPR3, TNF-induced adhesion allowed this binding in undiluted plasma. PMN adhesion to

HUVECs in the presence of anti-PR3 enhanced the endothelial injury, as shown by increased permeability and thrombomodulin release.

Conclusion: Cytokine activation, cell adhesion and the presence of anti-PR3 antibodies are three conditions required to allow an efficient binding of anti-PR3 ANCA in plasma and maximal endothelial lesions. This could explain that ANCA-associated vasculitides are restricted to capillaries and venules, where adhesion is favored by the small vessel diameter and the low blood flow.

22

A Novel Approach for the Treatment of Idiopathic Nephrotic Syndrome in a Rat Model

Le Berre L, Bruneau S, Naulet J, Renaudin K, Souillou J-P, Dantal J

INSERM 643, Institut de Transplantation et Recherche en Transplantation (ITERT), Nantes, France

Buffalo/Mna rats spontaneously develop a nephrotic syndrome (NS) associated with focal segmental glomerulosclerosis (FSGS) lesions. We demonstrated that this disease recurs after renal transplantation and results from an immune disorder.

We tested the effect of different immunosuppressive treatments on the Buff/Mna disease and focused on the only one to induce a complete remission, the deoxyspergualin derivative, LF15-0195 (Fournier, France).

Buff/Mna proteinuria was poorly sensitive to corticosteroids, cyclosporin A and anti-TCR treatment and resistant to others. In contrast, treatment with LF15-0195 led to a rapid and complete normalization of proteinuria associated with a regression of renal lesions, in the initial disease as well as in the post-transplantation recurrence. This remission was associated with a highly significant increase in splenic and peripheral CD4⁺CD25⁺FoxP3⁺T lymphocytes, not observed in controls. We measured also an increase of CD25, CTLA4, IDO, iNOS and GITR transcripts in these rats. Moreover, the transfer of purified CD4⁺CD25⁺T cells to irradiated Buff/Mna rats reduced proteinuria close to the normalization threshold. These results highlight the induction of regulatory T cells by LF15-0195 treatment, able to induce a regression of rat nephropathy.

Thus, we have demonstrated that idiopathic NS/FSGS can be immunologically “regulated”, opening up a new field of investigation, especially how this regulation leads to podocyte recovery. These data support the implementation of clinical trials for cortico-resistant patients suffering from FSGS recurrence.

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Cation Channel Activity in Functional Anion Exchangers: A Novel Property of Distal Renal Tubular Acidosis (dRTA)-Associated Anion Exchanger 1 (AE1, Band 3) Mutants

Walsh, S^{1,2}, Guizouarn H¹, Borge F¹, Unwin, R²

¹Bâtiment de Sciences Naturelles, Université de Nice-Sophia Antipolis, Nice, France; ²Centre for Nephrology and Department of Physiology, Royal Free and University College Medical School, London, United Kingdom

Anion exchanger 1 (AE1) is a glycoprotein that mediates anion exchange across the cell membrane; it is present in the erythrocyte and the α -intercalated cell. Distinct mutations may cause erythrocyte disease (hereditary stomatocytosis) or renal disease (dRTA). Certain stomatocytosis-associated AE1 mutations can abolish anion exchange and produce a cation transport property. We determined whether such a property was present in the dRTA-associated AE1 mutants.

We examined anion transport (by chloride influx and intracellular pH measurements) and cation transport (by rubidium influx and intracellular cation measurements) in several dRTA-associated mutations, both autosomal dominant (AD) (R589H, G609R, S613F), and autosomal recessive (AR) mutants (G701D, S773P, Δ 850, A858D); all co-expressed with glycophorin A (GPA - to enhance membrane expression) in *Xenopus laevis* oocytes.

Anion exchange was normal in all the dRTA mutants. All had a ‘cation leak’, which was much greater in the AR mutants, notably G701D. When we coexpressed the AR mutants with wt AE1 in the absence of GPA, we observed a significant Rb⁺ flux, indicating the mutant was ‘rescued’ by the wt molecule to the cell surface.

Thus these mutants have a cation leak and intact anion exchange, and ‘cation leaky’ mutants are rescued to the cell surface by wtAE1.

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Investigating the Pathway from Mutant Complement Factor H to Endothelial Injury in Atypical Haemolytic Uraemic Syndrome

Johnson SA¹, Williams JM¹, Hakoyan S², Richards A³, Goodship THJ³, Morgan BP², Taylor CM⁴, Savage COS¹

¹Department of Renal Immunobiology, University of Birmingham; ²Department of Medical Biochemistry and Immunology, Cardiff University; ³Department of Medicine and Human Genetics, University of Newcastle;

⁴Department of Nephrology, Birmingham Children’s Hospital, Birmingham

Introduction: Mutations in proteins of the alternative complement pathway are found in over 50% of cases of atypical haemolytic uraemic syndrome (HUS). The pathway from complement dysregulation to glomerular thrombotic microangiopathy is not elucidated. This study addresses mechanisms of endothelial

damage in factor-H associated HUS using serum from a pedigree with HUS and low serum factor H.

Results: Mutation analysis revealed compound heterozygous HF-1 mutations in the index case (T2768G/Y899D and G3654A/G1194D). An ELISA measuring the 402His variant of FH revealed absence of Y899D in serum. G1194D was purified from serum using immunoaffinity and ion exchange chromatography. G1194D exhibited normal cofactor activity in the inactivation of C3b, and normal heparin affinity. FH binding to human umbilical vein endothelial cells was reduced when incubated with serum containing G1194D compared to normal serum. Sheep erythrocytes incubated with serum containing G1194D were more susceptible to lysis, implying defective surface complement regulation. Finally, glomerular endothelial cells incubated with serum containing G1194D showed increased deposition of membrane attack complex compared with those incubated with normal serum.

Conclusion: These data provide the first evidence of complement dysregulation on glomerular endothelium resulting from mutant FH, an important step in the elucidation of the pathway to thrombotic microangiopathy.

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Discovery of Herbal Compounds with in vitro Anti-Fibrotic Activities

Hu Q, Hendry B, Xu Q

¹Department of Renal Medicine, Kings College London School of Medicine; ²Weston Education Centre, 10 Cutcombe Road, London SE5 9RJ

We recently developed innovative cell-based models of renal fibrosis suitable for high-throughput discovery of inflammation-independent anti-fibrotic activities. Here, we report the application of this method to discover herbal anti-fibrotics. Twenty-two herbal compounds were identified as potential candidates based on literature. They were examined in transforming growth factor β 1 (TGF- β 1)-induced in vitro models of renal fibrosis established in rat kidney fibroblasts. TGF- β 1-induced total collagen accumulation was visualised and quantitated by picro-Sirius red staining and spectrophotometric analysis. Cell detachment scores and lactate dehydrogenase assay were performed to monitor cytotoxicity. Four flavanoids (quercetin, baicalein, baicalin, and silybin) and two non-flavonoids (salvianolic acid B and emodin) showed anti-fibrotic activities. The flavanoids and salvianolic acid B had dose-dependent anti-fibrotic effects and low cytotoxicity. Emodin had an anti-fibrotic activity with an ED50 close to its toxic dose. The remaining 16 compounds either had little anti-fibrotic activity or had high cytotoxicity. We conclude that the cell-based model of renal fibrosis can be successfully used to detect inflammation-independent anti-fibrotic activities in herbal compounds. Flavonoids and salvianolic acid B have the most promising in vitro anti-fibrotic activities. These data warrant further studies to explore the effects and mechanisms of these herbal compounds in preventing renal fibrosis.

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Primary Hyperoxaluria Type 1: Mutation Analysis and Outcome

Fargue S¹, Harambat J², Liutkus A¹, Ranchin B¹, Schott AM³, Rolland MO⁴, Cochat P¹ (on behalf of the French Society of Nephrology and Paediatric Nephrology)

¹Department of Paediatrics, Hôpital Edouard-Herriot, Lyon;

²Department of Paediatrics, Hôpital Pellegrin, Bordeaux;

³Department of Medical Information, Hôpital Edouard-Herriot, Lyon; ⁴Laboratory of Paediatric Biochemistry, East Biological Center, Lyon, France

Primary Hyperoxaluria type 1 is due to a deficiency in alanine-glyoxylate amino-transferase (AGT). We retrospectively studied the clinical history of 155 patients (129 families) whose biological diagnosis, based on enzymatic deficiency of AGT and/or specific mutations in AGXT, and was made in Lyon between 1993 and 2007.

Patients came from West Europe for 49%, family history was positive in 60%, consanguinity present in 56%. Urolithiasis occurred in 83% of patients, extra-renal involvement in 39%, while 11% were diagnosed as family screening and 5 as prenatal diagnosis. Median age at first symptom was 4 [0–58] years and 12 [0–67] years at diagnosis at which time 42% of patients had reached end-stage renal failure. Fifty-nine (43%) patients underwent 78 transplantations. Patient cumulative survival was 96%, 90%, 67% at 10, 20 and 60 years, respectively. Renal survival was 83%, 62%, 50% at 10, 20 and 30 years. Among the 138 patients with genetic analysis, 26% had the G170R mutation and a cumulative renal survival of 69% at 30 years (vs. 40% for others).

These results underscore the severe prognosis and the necessity for early diagnosis and treatment as well as confirm the better prognosis of the mistargeting mutation.

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V α 14j α 18 Cells (iNKT) Confer Resistance to the Development of Passive Experimental Anti-Glomerular Basement Glomerulonephritis by Increasing TGF- β Levels

Mesnard L^{1,3}, Keller AC^{2,4}, Vandermeersch S¹, Rafat C¹, Rondeau E^{1,3}, Leite-de-Moraes MC²

¹INSERM UMR S 702, Université Pierre et Marie Curie, Hôpital Tenon, Paris; ²Unité Mixte de Recherche 8147, Centre National de la Recherche Scientifique, Faculté de Médecine René Descartes, Paris V, Hôpital Necker, 75743 Paris, Cedex 15; ³APHP, Hôpital Tenon, Urgences Néphrologiques & Transplantation Rénale, et Université Pierre et Marie Curie (UPMC), Paris, France; ⁴Universidade Federal de São Paulo (UNIFESP), Nephrology Division, Brazil

Invariant natural killer T cells (iNKT) are a subset of T lymphocytes with ability to promptly secrete a large number of cytokines

after TCR stimulation, through which they can exert regulatory or effector function depending on the immunological context. In this study we show that iNKT play an important role in the resistance to the development of passive anti-glomerular basement membrane induced glomerulonephritis (anti-GBMGN). Injection of sheep anti-rat GBM serum induced a significant gain in the weight, blood urea nitrogen (BUN) and proteinuria in comparison to non-treated mice. These parameters were associated with renal tissue injury, an important influx of macrophages to the kidney and increased levels of TGF- β and CXCL16, an iNKT associated chemokine. The iNKT deficient C57BL/6J $\alpha 18^{-/-}$ gained weight more rapidly than WT mice consistently with higher histological injury, more pronounced BUN levels and proteinuria. These data demonstrate a major role for iNKT cells in the resistance to the development of experimental anti-GBMGN. Q-PCR analyses show lower renal levels of TGF- β in $\alpha 18^{-/-}$ animals compared to WT or non-injected mice. Furthermore, in vivo administration of the TGF- β neutralizing antibody, 11D1, reverted WT resistance, indicating that iNKT cells exert its protective effect by increasing TGF- β levels in renal tissue.

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IgA1-Triggered CD71 Activation Induces MAPK and PI3K/AKT/MTOR Pathways Implicated in Proliferation and Cytokine Secretion in Human Mesangial Cells: Role in IgA Nephropathy

Tamouza H^{1,2}, Tiwari M^{1,2}, Averty M^{1,2}, Demion M^{1,2}, Vrtovnik F^{1,2}, Launay P^{1,2}, Benhamou M^{1,2}, Monteiro RC^{1,2}, Moura IC^{1,2}

¹INSERM U699, Paris; ²University of Paris 7, Denis Diderot, Paris, France

IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide. The hallmark of the disease is glomerular deposition of circulating IgA-immune complexes accompanied by mesangial cell proliferation and matrix expansion. We previously identified the transferrin receptor (TfR/CD71) as the mesangial IgA receptor that is highly expressed in biopsies of IgAN patients and co-localizes with deposited IgA. We have shown that IgA/TfR interaction initiates a positive feedback loop inducing increased receptor expression, mesangial cell proliferation and release of inflammatory and fibrogenic cytokines. Understanding mesangial cell activation by IgA-bound TfR is therefore critical to design therapeutic strategies aiming at inhibiting mesangial cell activation in IgAN. Here we show that IgA but not apo or holo-transferrin leads to calcium mobilization in human mesangial cells. IgA/TfR triggering of mesangial cells involves the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and phosphatidylinositol 3-kinase (PI3K)/AKT pathways evidenced by the phosphorylation of these molecular effectors. Selective inhibition of these pathways with PD098059 (ERK inhibitor) and Wortmannin (PI3K inhibitor) demonstrated that they are involved in pro-inflammatory cytokine secretion and cell proliferation, respectively. Interestingly, PI3K/AKT led to the activa-

tion of the mammalian target of rapamycin (mTOR). Its specific inhibitor, rapamycin, confirmed that this pathway is critical for IgA-dependant mesangial cell proliferation. This study identifies several new therapeutic targets within the IgA/TfR-triggered signaling pathways in mesangial cells that could be relevant in IgAN therapy.

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Progesterone in Male: Production and Renal Effects Under Potassium Depletion

El Abida B, Bloch M, Doucet A, Crambert G

CNRS UPMC UMR7134, Institut des Cordeliers, 75006 Paris, France

In addition to their functions in reproduction, sexual hormones have an important role in the maintenance of the blood pressure and ionic homeostasis. Progesterone is an intermediary in gluco- and mineralocorticoid synthesis and, therefore, circulates also in male. Interestingly, we found expression of the progesterone nuclear receptor in male kidney. Therefore, the presence of the ligand and the receptor prompted us to search for renal effects of progesterone in male.

We have injected progesterone to male mice and analyzed their urine ionic contents. The main effect was a decrease of urinary potassium. Then, we made the hypothesis that progesterone could be involved in potassium retention. We therefore placed male and ovariectomized female mice under low potassium diet and observed an increase of their progesterone plasma concentration.

Then, we demonstrated that adrenals are the site of progesterone production in male and that mRNA and proteins of steroidogenic enzymes located upstream of the synthesis of progesterone are upregulated during potassium depletion. Conversely, the two last enzymes are downregulated. This specific regulation is in total agreement with an accumulation of progesterone.

In summary, during potassium restriction, progesterone is produced by adrenals, in males and females, and plays a role in potassium retention.

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Ultra-Structural Integrity of Carbothane Tunneled Dialysis Catheters after Ethanol Solution Exposure

Guenu S, Heng A, Lautrette A, Charbonné F, Blavignac C, Deteix P, Traoré O, Souweine B, Lartigue C

Université de Clermont1, Clermont-Ferrand, France

Background: Ethanol lock is a therapeutic option for preventing and controlling catheter-associated infection. This in vitro study investigates the effect of ethanol on carbothane catheter integrity.

Methods: The catheters were immersed at 37°C in saline, 60% and 95% ethanol solutions for 5 min, 30 min, 4 hours and 15 days. The topography of the inner surface of the catheters was observed at x100, x1000, x3000 by scanning electron microscopy. A reference non-immersed catheter was used as a control.

Results: By comparison with both the reference catheter and the catheter immersed for 15 days in saline, a 15-day 60% and 95% ethanol exposure induced a disappearance of embedded granulations and a major lifting of the surface with flakes. Such abnormalities were less pronounced after a 4-hour ethanol immersion. After a 30-min exposure, no alteration was found using 60% ethanol, and using 95% ethanol only slight flakes were found. Whatever the solution used, a 5-min immersion did not produce any structural alteration of the surfaces.

Conclusion: The ethanol lock technique using carbothane catheters is conceivable using a 60% ethanol solution for up to 30 minutes.

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Ethanol Rapidly Eradicates Organisms in Experimental Biofilm Formed on Silicone Catheters

Heng A, Guenu S, Lautrette A, Charbonnel N, Charbonné F, Deteix P, Lartigue C, Traoré O, Souweine B, Forestier C

Université de Clermont1, Clermont-Ferrand France

Background: The use of ethanol as dialysis catheter lock solution is debated. This in vitro study investigated its bactericidal effect on sessile bacteria.

Methods: Experimental 24-hour catheter-associated biofilms of *S. aureus* (SA), *S. epidermidis* (SE), *K. pneumoniae* (KP), and *P. aeruginosa* (PA) grown on catheters were exposed to 60% ethanol, 46.7% citrate and a saline solution for 5, 20, and 30 min, 1, 4, and 24 hrs. The log values of the viable bacterial counts were compared using the Mann and Whitney test.

Results: Whatever the exposure time to saline, the bacterial counts of SA, SE, KP and PA biofilms were >5 log CFU. Complete eradication (no detectable bacterial growth) was only observed with ethanol treatment, after 20 min of exposure for KP and PA, and 30 min for SA and SE. By comparison with saline, after a 5 min exposure, ethanol but not citrate induced a significant decrease of the bacterial counts ($P < 0.05$). Such an effect was also observed with citrate but required at 20 min of exposure for SA, KP and PA, and 1 hour for SE.

Conclusion: A 60% ethanol solution exhibits a rapid and dramatic bactericidal effect on catheter-associated biofilms of clinically relevant microorganisms.

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The Basolateral Na/H Exchanger NHE4 Participates in NH_4 Absorption in The Medullary Thick Ascending Limb (MTAL) and Is Required for Adaptation to Metabolic Acidosis

Bourgeois S, Van Meer L, Bloch M, Gawenis L, Houillier P

¹UMRS872, INSERM, Paris, France; ²University of Utah, Salt Lake City, Utah, USA

We have previously provided evidence that NHE4 may be involved in NH_4 efflux out of MTAL cells. Here we investigated the role of NHE4 in NH_4 handling under basal condition (BC) and during metabolic acidosis. Studies were performed in NHE4^{+/+} and ^{-/-} littermate mice. Under BC, NH_4 absorption by ex vivo microperfused MTAL from NHE4^{-/-} mice was 45% lower than that by MTAL from NHE4^{+/+} mice. Under BC, plasma acid-base status was similar in the two genotypes and urinary NH_4 excretion was not reduced in NHE4^{-/-} mice. After 2 days of acid load, plasma HCO_3^- concentration similarly decreased in NHE4^{-/-} and NHE4^{+/+} mice but urinary NH_4 excretion was lower in NHE4^{-/-} mice. This defect remained after 7 days of acid load.

In conclusion, NHE4 participates in NH_4 absorption by MTAL. NHE4 disruption does not impair the ability to excrete a moderate amount of NH_4 but alters the renal response to acid loading.

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Novel Amiloride-Resistant Thiazide-Sensitive Electroneutral NaCl Transport Pathway in Mouse CCD

Morla L², Leviel F¹, Pech V³, Wall SM³, Shull G⁴, Chambrey R¹, Eladari D¹

¹Centre de Recherche des Cordeliers (INSERM UMRS 872), Eq. 3, Paris; ²UMR 7134, CNRS, Paris; ³Renal Division, Emory University, Atlanta, GA; ⁴Department of Molecular Genetics, University of Cincinnati, Cincinnati, OH, USA

In a mouse model of Gitelman syndrome (NCC^{-/-} mice), NaCl loss should be compensated by increased Na^+ absorption, which normally promotes K^+ secretion. However, hypokalemia is not observed in NCC^{-/-} mice. Thus, Na^+ , Cl^- , and K^+ transports (J_{Na} , J_{K} , J_{Cl}) were examined in CCDs perfused in vitro from NCC^{-/-} and wild type mice (WT).

In CCDs from WT mice given a Na^+ -deplete diet to upregulate aldosterone, electrogenic Na^+ absorption and K^+ secretion were observed. Amiloride 10⁻⁵M abolished V_{Te} and J_{K} , but decreased J_{Na} and J_{Cl} only 65 %. Amiloride-insensitive NaCl absorption was abolished with 10⁻⁴M luminal hydrochlorothiazide (HCTZ). Significant net NaCl absorption was also observed, in CCDs from NCC^{-/-} mice, although V_{Te} and J_{K} were not distinguishable from zero. In NCC^{-/-} mice, NaCl transport was unaffected by luminal amiloride 10⁻⁵M, while luminal HCTZ 10⁻⁴M eliminated NaCl

absorption. In conclusion, in CCDs from WT mice, when aldosterone secretion is stimulated, two transport pathways mediate net NaCl absorption. The first, which is most likely ENaC-mediated, is electrogenic and amiloride-sensitive, which promotes K⁺ secretion. The second is electroneutral, amiloride resistant, but HCTZ-sensitive. By favoring this electroneutral pathway, NCC^{-/-} mice are able to maintain NaCl balance while minimizing K⁺ loss.

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Antibiotics' Removal During High Volume Continuous Venovenous Hemofiltration (HVCVVHF) in Septic Patients

Jaubert D¹, Coudore F², Gazuy N¹, Souweine B¹, Deteix P¹

¹Service de Néphrologie et de Réanimation Médicale et

²Laboratoire de Pharmacologie et Toxicologie, CHU Gabriel Montpied BP 69 63000 Clermont-Ferrand, France

Objectives: The pharmacokinetic of amikacin or gentamycin was described during high volume continuous venovenous hemofiltration (HVCVVHF).

Design: A 9-bed medical intensive care unit (ICU) at university teaching hospital.

Subjects: Five patients admitted to ICU for septic shock with acute renal failure, treated by amikacin or gentamycin and requiring predilutional HVCVVHF (ultrafiltration rate of 6000 ml/h) with Polyflux 14S.

Measurements: The aminoglycosides' concentrations were measured in the prefilter and postfilter plasma and in the ultrafiltrate at the beginning and 1h, 4h, 8h, 12h, 18h, 24h, 36h after setup of the HVCVVHF. The mass transfer (MT) and the sieving coefficient (SC) were determined using the calculation of the mass balance.

Main Results: During the 36 first hours, SC remained unchanged ranging from 0.68 to 0.78 for amikacin and from 0.62 to 0.78 for gentamycin. A total of 30 to 40% of the entry mass was ultrafiltered. Adsorption to the membrane appeared to be a minor clearance mechanism and was unrelated to the entry mass.

Conclusion: The results indicated that aminoglycoside removal was achieved by HVCVVHF due to high ultrafiltration clearance.

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Molecular Analysis of the Apoptosis-Induced PR3 Membrane Expression: A New Proinflammatory Pathway Leading to Impairment of Apoptotic Neutrophil Clearance in Vasculitis

Kantari C¹, Hajjar E², Pederzoli-Ribeil M, Moura IC³, Benhamou M³, Reuter N², Witko-Sarsat V¹

¹INSERM U845 Necker Research Center, Necker Hospital, Paris, France; ²Computational Biology Unit, BCCS, University of Bergen, Bergen, Norway; ³INSERM U699, Bichat Hospital, Paris, France

Proteinase 3 (PR3) is the main target of ANCA in Wegener granulomatosis. Because ANCA binding to their antigen triggers neutrophil activation, PR3 membrane expression is a pro-inflammatory factor in vasculitis. We recently described a novel mechanism leading to PR3 membrane expression during apoptosis which involves the association between PR3 and human phospholipid scramblase 1 (hPLSCR1), a membrane protein controlling phosphatidylserine externalization. Moreover, apoptosis-induced PR3 membrane expression decreased macrophage phagocytosis thus suggesting that PR3 was a «don't-eat-me signal». Our modeling analysis using dynamic simulation demonstrated that PR3 has a unique ability to associate with plasma membrane through the combined effect of hydrophobic and charged amino acids clusters. Using the model of Rat basophilic cell line (RBL) stably transfected with PR3 or its mutants, we observed that the mutation of two hydrophobic amino acids are sufficient to inhibit apoptosis-induced membrane expression. Thus, this hydrophobic pocket seems to be the site of a direct interaction with plasma membrane or with a partner that is required for PR3/membrane expression. Elucidation of the molecular basis of PR3 membrane expression will help us to investigate this new PR3 driven pathway of impairment of apoptotic neutrophil clearance which ultimately result in exacerbation of inflammation and vasculitis.

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H⁺-Atpase Deficient Mice Develop a Bartter-Like Syndrome

Gueutin V¹, Chambrey R¹, El-moghrabi S¹, Vallet M¹, Wagner C², Eladari D¹

¹INSERM UMRS872-Equipe 3 (Centre de Recherche des Cordeliers); Université Paris Descartes, 15 rue de l'Ecole de Médecine, F-75006 Paris, France; ²Institute of Physiology, University of Zurich, Winterthurerstrasse 190, CH-8057, Zurich, Switzerland

Human patients suffering from type 1 distal renal acidosis often exhibit NaCl, K and water loss. These features are generally believed to be the consequence of nephrocalcinosis. The B1-subunit deficient mice (*Atp6v1b1*^{-/-}), which display type 1 distal renal acidosis don't develop nephrocalcinosis. The purpose of

this study was to examine whether these mice have alterations in Na and water balance. Sodium-replete deficient mice exhibited a mild polyuria, hypokalemia, and hypercalciuria. When submitted to a sodium-depleted diet, knockout mice exhibited major polyuria, along with renal loss of NaCl leading to a marked secondary hyperaldosteronism. Abundance of Na and Cl transporters along the nephron was examined by semiquantitative immunoblotting in *Atp6v1b1*^{-/-} and control mice. NKCC2 and AQP2 protein abundances were dramatically decreased. In contrast, the abundance of the proximal NHE3 was increased, as well as the three subunits of the epithelial sodium channel ENaC in the medullary collecting duct. Abundance of NCC was unchanged. In conclusion, *Atp6v1b1*^{-/-} mice have a phenotype resembling to Bartter syndrome. NaCl and water losses are likely consecutive to the down-regulation of NKCC2 and AQP2. Increased ENaC abundance might drive higher K secretion through ROMK and thus might account for renal hypokalemia in *Atp6v1b1*^{-/-} mice.

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Antimicrobial Interventions for the Prevention of Haemodialysis Catheter-Related Infections: A Systematic Review of Randomised Controlled Trials Has Been Submitted and Recorded in Our Database

Rabindranath K¹, Bansal T², Adams J³, Das R⁴, Macleod A⁵, Moore C⁶, Besarab A⁶

¹Renal Unit, Churchill Hospital, Oxford; ²Renal Unit, Royal London Hospital, London; ³Renal Unit, Kings College Hospital, London; ⁴Renal Unit, Royal Berkshire Hospital, Reading; ⁵Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, UK; ⁶Department of Nephrology and Hypertension, Henry Ford Health System, Detroit, USA

Background: Haemodialysis (HD) catheter-related bacteraemia (CRB) is a major reason for catheter loss and has been associated with substantial morbidity.

Purpose: To evaluate the benefits and harms of antimicrobial interventions for the prevention of catheter-related infections (CRIs) in HD patients.

Methods: MEDLINE, EMBASE, and CENTRAL were searched for potential RCTs. Data concerning trial methodological quality and relevant clinical outcomes were extracted, and where appropriate entered into RevMan for meta-analysis. Estimates of relative risk (RR) for dichotomous data and mean difference (MD) for continuous outcomes with 95% confidence intervals (CI) were calculated.

Data Synthesis: 24 trials with 2314 patients and 2387 catheters were included. Antimicrobial catheter locks significantly reduced the rates of CRB (Rate Ratio, 0.38, 95% CI 0.28 to 0.51) and exit-site infections (ESI) (Rate Ratio 0.66, 95% CI 0.46 to 0.95). Exit-site antimicrobial application also significantly reduced the rates of CRB (Rate Ratio 0.21, 95% CI 0.12 to 0.36) and ESI (Rate Ratio 0.22, 95% CI 0.10 to 0.47). Antimicrobial coating of HD catheters and peri-operative systemic antimicrobials (intrave-

nous vancomycin) did not result in a significant reduction in rate or risk of CRIs.

Conclusion: The use of AMLs and ESAs are useful measures in the reduction of CRIs, whereas antimicrobial impregnated catheters and perioperative systemic antimicrobial administration have not been found to be beneficial.

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Differential Dose Dependent BMP-7 Signalling in Proximal Tubule Epithelial Cells and Implication in Fibrosis

Motazed R, Colville-Nash P, Kwan JTC, Dockrell MEC

SWT Institute for Renal Research, St Helier Hospital, Carshalton, Surrey UK

Bone Morphogenic protein-7 found in adult kidney at levels which activate Smad 1. Reduced BMP-7 levels occur in diabetic nephropathy models, with reduced Smad 1 activation correlating with pathology, and BMP-7 treatment activating Smad 1 ameliorating disease. Previously we described novel BMP-7-induced p38 signalling in (Proximal Tubule Epithelial Cells) PTECs at concentrations below those activating Smad's. Here we investigate p38 signalling events, PTEC survival, and modulation of TGF- β -induced fibrotic outcomes by BMP-7. We hypothesize that Smad and p38 activation may be mutually exclusive.

BMP-7 activated Smad 1/5/8 at 200ng/ml, but activated p38 at 2.5ng/ml. With 2.5ng/ml BMP-7, MKK3/6 was shown to be upstream of p38 and MAPKAPK-2 downstream. BMP-7 at 2.5ng/ml did not affect cell proliferation/apoptosis. 200ng/ml BMP-7 significantly reduced TGF- β -induced p38 activation and associated fibronectin secretion.

Here we further elucidate a novel BMP-7 pathway with low doses causing p38 activation via upstream MKK3/6 and downstream MAPKAPK-2, which may be important in modulation of inflammation/fibrosis. We also demonstrate high dose can modulate TGF- β -induced p38-dependent fibrotic outcomes. Our results suggest high dose BMP-7 activating Smad 1 to be PHYSIOLOGICAL, whilst low doses activating p38 may be PATHOLOGICAL. The full interaction of the above signalling pathways remains to be classified.

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BMP 7 Regulation of EMT and Its Implication in Renal Fibrosis

Veerasamy M, Colville-Nash PR, Kwan JTC, Dockrell MEC

Epsom and St Helier University Hospitals NHS Trust

Bone Morphogenic protein 7 has been shown to improve renal injury and fibrosis in animal models. However the cellular signalling mechanisms responsible for these effects are still unclear. We investigated whether BMP 7 would inhibit TGF β 1

induced epithelial mesenchymal transition in human renal proximal tubular epithelial cells (PTECs). The experiments were performed on HKC 8 cells, a virally transformed human PTECs. We have for the first time identified that BMP 7 itself induced features of EMT (loss of E-cadherin, induction of vimentin) which is a Smad1/5 dependent event. Indeed BMP 7 had an additive effect with TGF β 1 in E-cadherin down regulation. Interestingly we have identified that BMP 7 inhibits TGF β 1 mediated upregulation of α -Smooth muscle actin, a marker of activated mesenchymal phenotype. This effect was mediated by Inhibitor of DNA binding 2, a bHLH class transcriptional regulator. This is the first report suggesting that loss of epithelial phenotype does not necessarily lead to a functional mesenchymal phenotype. Rather it requires further transcriptional programming. We have also identified that BMP 7 upregulates another bHLH protein, Id1 which seems to regulate cell proliferation. The later may induce PTECs proliferation to facilitate recovery from injury.

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Renal Expression of Parvalbumin Is Critical for NaCl Handling and Response to Diuretics

Belge H¹, Gailly P¹, Schwaller B², Loffing J², Beauwens R³, Devogelaer JP¹, Hoenderop JG⁴, Bindels RJ⁴, Devuyst O¹

¹UCL Medical School; ²ULB Medical School, Belgium;

³University of Fribourg, Switzerland; ⁴Radboud University Nijmegen, The Netherlands

Parvalbumin (PV) is a Ca²⁺-binding protein that is selectively expressed in the distal convoluted tubule (DCT), where its role remains unknown. We investigated the renal phenotype of PV knockout mice (Pvalb^{-/-}) vs. wild-type (Pvalb^{+/+}) littermates. PV colocalized with the thiazide-sensitive Na⁺-Cl-cotransporter (NCC) in the early DCT. The Pvalb^{-/-} mice showed increased diuresis and kaliuresis at baseline with higher aldosterone levels and lower lithium clearance. Acute furosemide administration did not increase calciuria in Pvalb^{-/-} mice, unless after NaCl supplementation. The Pvalb^{-/-} mice showed no significant diuretic response to hydrochlorothiazide, but an accentuated hypocalciuria. A decreased expression of NCC was detected in the early DCT of Pvalb^{-/-} kidneys. The PV-deficient mice had also increased bone mineral density. Studies in mouse DCT cells showed that endogenous NCC expression is Ca²⁺-dependent and can be modulated by the levels of PV expression. These results suggest that PV regulates the expression of NCC. They also provide insights into the Ca²⁺-sparing action of thiazides and the pathophysiology of distal tubulopathies.

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A Novel Hypertensive Transgenic Mouse to Study Protein Expression in Chronic Renal Disease

Huby A-C¹, Rastaldi MP², Smithies O³, Dussaule J-C¹, Chatziantoniou C¹

¹INSERM U702, Université Paris VI, Hôpital Tenon, Paris 75020, France; ²Fondazione D'Amico per la Ricerca sulle Malattie Renali, Milano, Italy; ³Department of Molecular and Cell Physiology, University of North Carolina, Chapel Hill, USA

The objective of the present study was to investigate gene regulation during progression and reversal of hypertension-associated renal failure. We used a new model of transgenic mice, the RenTg strain, expressing a renin transgene at a constant high level.

RenTg started displaying high blood pressure and abnormal albuminuria after 3 months. At 12 months, kidneys of RenTg showed severe histological alterations of all renal compartments. These changes were associated to decreased expression of nephrin, podocin, E-cadherin and megalin, while profibrotic genes of the TGF β superfamily (particularly TGF β and GDF7) were activated.

Treatment of RenTg for 6 weeks with a low dose of an AT1 antagonist (irbesartan) did not reduce systolic pressure. However, albuminuria markedly dropped to normal levels. The normalization of albuminuria was accompanied by reversal of vascular lesions and reappearance of podocin, nephrin, E-cadherin and megalin. In addition, expression of TGF β and GDF7 was normalized.

Using a novel transgenic strain of mice, we showed that long lasting proteinuria and alterations of the expression of key proteins of the integrity and function of podocytes and tubules are reversible. RenTg is a useful tool adapted to provide clues for a better understanding of mechanisms of progression and reversal of renal disease.

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Does Sex Matter? Outcomes After Renal Transplant Differ between Males and Females Depending on Comorbidity

Villar E^{1,2}, Chang SH^{1,3,4}, McDonald SP^{1,3,4}

¹Australia and New Zealand Dialysis and Transplant Registry, Woodville, South Australia, Australia; ²Hospices Civils de Lyon, Department of Nephrology, Centre Hospitalier Lyon Sud, Pierre Benite, France; ³Department of Nephrology, The Queen Elizabeth Hospital, Woodville, South Australia, Australia

In the general population, female survival advantage compared to males is lost in chronic diseases. We examined the relationship of gender with renal transplant (RTx) outcomes by comorbidity status using data from the ANZDATA registry. We analysed RTx

outcomes among adult patients who started chronic renal replacement therapy and who received a first RTx in Australia and New Zealand from 1 April 1991 to 31 December 2006. Multivariate analyses were adjusted for donor and recipient characteristics and stratified by comorbid status using Cox regression. Of the 7166 patients, 5310 were without comorbidity and 1856 were with at least one comorbidity (type 1 or 2 diabetes, cardiovascular diseases and/or chronic lung disease). Among recipients without comorbidity, RTx restored female survival advantage: male gender had higher overall death and death with graft function rates (1.35, $p=0.0003$ and 1.29, $p=0.005$), with higher cardiovascular (1.59, $p=0.02$) and malignancy death rates (1.80, $p=0.01$). Among recipients with comorbidity, male gender had lower graft loss rate (0.72, $p=0.002$) and lower mortality risk (0.73, $p=0.01$ for overall death; 0.73, $p=0.04$ for death with graft function) due to lower non-cardiovascular death rate (0.57, $p=0.005$). In this cohort, RTx outcomes differ between genders by recipient comorbidity status.

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LPA and the LPA1 Receptor in Renal Interstitial Fibrosis

Gonzalez J, Pradère J-P, Klein J, Grès S, Guigné C, Nevoit C, Neau E, Valet P, Calise D, Bascands J-L, Saulnier-Blache J-S, Schanstra JP

INSERM U858/I2MR Equipe n° 3 and 5, CHU Rangueil, Bâtiment L3, BP84225, 31432 Toulouse Cedex 4; Animal facility, IFR31 Institut Louis Bugnard, BP 84225, 31432 Toulouse Cedex 4; Microsurgery Facility, IFR31 Institut Louis Bugnard, BP 84225, 31432 Toulouse Cedex 4, France

Lysophosphatidic acid (LPA) is a bioactive phospholipid known to be involved in a number of biological processes via the activation of G-protein coupled receptors (LPA1-4). The aim of the present study was to analyze the possible involvement of LPA and its receptors in the development of renal tubulo-interstitial fibrosis (TIF) induced by unilateral ureteral obstruction (UUO) in mice.

UUO induced a 5-fold increase in LPA1 receptor- and no change in LPA2 receptor- mRNA levels. LPA3 and LPA4 receptors were not detected. Furthermore, LPA1 null mice and wild

type mice treated with LPA1 receptor antagonist (Ki16425) display significantly lower UUO-induced TIF than non-treated wild type mice. This protection might involve inhibition of connective tissue growth factor (CTGF), since LPA treatment of proximal tubular cells (MCT) rapidly and significantly increased CTGF expression. Currently we explore the effects of delayed pharmacological blockade of the LPA1 receptor and co-treatment with an angiotensin converting enzyme inhibitor in the UUO model.

In conclusion these data should make it possible to determine if LPA1 receptor blockade might become an alternative therapeutic strategy to slow down the progression of renal tubulointerstitial fibrosis.

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T-Type Calcium Channel Inhibition Reduces Glomerular Injury in Thy1 Nephritis in Rats

Cove-Smith A

Department of Renal Medicine, King's College London

Background: T-type calcium channels are low-voltage activated Ca channels that are thought to regulate cell cycle progression in certain cell types. Three isoforms exist (α_1G , α_1H and α_1I) and we have previously demonstrated that human mesangial cells express mRNA for the α_1H isoform. Inhibition of these channels is antiproliferative in human and rat MCs in vitro. The aim of this study was to investigate the effect of T-type Ca channel inhibition on MC proliferation in vivo.

Methods: Thy1 nephritis was induced in rats by a single iv injection of Ox7 monoclonal antibody. One group received daily IP injections of the T-type Ca channel antagonist TTL1177, while the other received vehicle only. At days 7 and 10 animals were sacrificed and their kidneys harvested for analysis.

Results: Expression of mRNA for the α_1H T-type Ca channel isoform was 3-fold higher in Thy1 nephritis at day 10 than in normal kidneys ($p=0.0073$). Blinded histological analysis of the TTL1177 treated kidneys revealed a significant reduction in glomerular injury and average glomerular cell number compared to vehicle treated kidneys ($p=0.0141$).

Conclusion: These results support the hypothesis that T-type Ca channel inhibition may be a promising therapeutic strategy in mesangial proliferative renal disease.

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