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ABSTRACTS

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Prevention and treatment of progressive experimental glomerulonephritis with type IV phosphodiesterase inhibitor. *S. Agarwal, F.W.K. Tam, J. Smith, D. Morel, E.M. Thompson, and C.D. Pusey, Renal Unit and Department of Histopathology, Royal Postgraduate Medical School, London, England, United Kingdom.* TNF-α has an important role in acute glomerular inflammation. Rolipram, a type IV phosphodiesterase inhibitor, has multiple anti-inflammatory effects including inhibition of TNF-α synthesis. We investigated the effects of rolipram in a model of crescentic glomerulonephritis in WKY rats in two sets of experiments. Glomerulonephritis was induced by injection of nephrotoxic serum (NTS). In both experiments, rats were injected i.p. with either 6.25 mg/kg of rolipram or vehicle only twice daily. In the first experiment, the first injection of either rolipram or vehicle was given 2.5 hours before injection of NTS, and rats were killed 96 hours later. By 96 hours, vehicle treated rats ($N = 6$) developed glomerular injury which was significantly abrogated by rolipram treatment (albuminuria 14.9 ± 11.8 mg/17 hr vs. 0.04 ± 0.03 mg/17 hr, $P < 0.01$; focal segmental necrosis with fibrin deposition affecting 7.7 ± 2.4% of glomeruli vs. 0.3 ± 0.3% of glomeruli, $P < 0.01$). In the second experiment, treatment was started 96 hours after injection of NTS. By day 7 after injection of NTS, rolipram treated rats ($N = 6$) had significantly less albuminuria than vehicle treated rats ($N = 6$; 82.5 ± 10.7 mg/17 hr vs. 133.2 ± 14.9 mg/17 hr, $P < 0.05$). These rats were killed 7 days after injection of NTS. Glomeruli from the rolipram treated rats had less focal segmental damage (23.7 ± 4.4% vs. 52 ± 4.9%, $P < 0.01$) and fewer crescents than the vehicle treated rats. Renal production of TNF-α was assessed by measurement of serum and urinary TNF-α by ELISA. On day 7, urinary TNF-α was lower in the rolipram treated rats (139 ± 54 pg/17 hr vs. 372 ± 79 pg/17 hr, $P = 0.05$). TNF-α was not detected in the sera from either group of rats. We conclude that rolipram is effective in both prevention and treatment of experimental anti-glomerular basement membrane glomerulonephritis. This effect was associated with reduction of renal production of TNF-α.

Arginase activity in nephritic glomeruli is inhibited by hydroxyarginine (HOArg) and increased by IL-4. *S.N. Waddington, V. Cattell, and H.T. Cook, Department of Histopathology, St. Mary's Hospital Medical School, London, England, United Kingdom.* Arginase activity is present in mesangial cells and nephritic glomeruli. Arginase converts arginine into ornithine, a precursor of proline and polyamines, and urea and competes for arginine with nitric oxide synthase. There is recent evidence that HOarg, an intermediate in the conversion of arginine to nitric oxide, and IL-4 modulate arginase activity in macrophages. We therefore examined their effect in nephritic glomeruli. Glomeruli from rats with accelerated nephrotoxic nephritis (day 4), normal rat glomeruli, rat mesangial cells, and rat thioglycollate-elicited macrophages were assayed for arginase activity (^{14}C arginine to urea conversion) after a 48 hr incubation with or without HOArg (200 μM) and IL-4 (80 ng/ml). Arginase activity was increased in nephritic glomeruli, as reported previously (Jansen et al. *Kidney Int* 42:1107–1112, 1992). HOArg suppressed arginase activity in nephritic glomeruli, macrophages, cultured mesangial cells and normal glomeruli. IL-4 increased arginase activity in nephritic glomeruli and macrophages but not in mesangial cells or normal glomeruli (Table).

Arginase activity *(nmol urea/48 hr)* ± SE

	Normal glomeruli *per 2000 gloms.*	Day 4[a] Nephritic glomeruli *per 2000 gloms.*	Macrophages *per 10^6 cells*	Mesangial cells *per mg protein*
Basal	9.3 ± 1.7	20.5 ± 3.5[c]	7.1 ± 0.3	279 ± 8
+HOarg	2.7 ± 0.4[b]	9.1 ± 1.6[b]	4.1 ± 0.1[b]	125 ± 5[b]
+IL-4	7.7 ± 1.9	85.5 ± 21.9[b]	26.1 ± 1.4[b]	287 ± 8

[a] Day 7 glomeruli showed a similar response
[b] $P < 0.001$ vs. Basal
[c] $P < 0.05$ vs. Normal glomeruli

These results show that the response in nephritic glomeruli is similar to macrophages. In contrast normal glomeruli behave more like basal mesangial cells. This is the first demonstration that arginase activity can be modulated in glomeruli and cultured mesangial cells. Both HOArg and IL-4 may influence the progression of glomerulonephritis.

Hemin, an inducer of haem oxygenase, ameliorates experimental nephrotoxic nephritis. *T. Cook, K. Mosley, V. Cattell, Department of Experimental Pathology, Imperial College School of Medicine at St. Mary's, London, England, United Kingdom.* Haem oxygenase is the rate limiting enzyme in haem metabolism, catalyzing the degradation of haem to biliverdin, iron and carbon monoxide. Two isoforms exist, a constitutive form and an inducible form (HO-1) which can be induced in mesangial cells by cytokine stimulation. *In vivo* induction of HO-1 has anti-inflammatory and anti-oxidant effects. We have looked for HO-1 in accelerated nephrotoxic nephritis (NTN) and examined the effect of hemin, an inducer of HO-1. Male Lewis rats were preimmunized with rabbit IgG and Freund's complete adjuvant 7 days before intravenous rabbit nephrotoxic globulin (NTG). Hemin (30 μmol/kg; $N = 3$) or saline vehicle ($N = 3$) was administered subcutaneously 24 hours before and 48 hours after NTG. Proteinuria was measured in urine collected from day 0-1 and day 3-4. Renal biopsies were taken at 24 hours and rats were killed at day 4. Formalin fixed paraffin embedded sections were stained with H&E for assessment of glomerular thrombi and immunohistochemistry was performed for HO-1 and macrophages (EDI). HO-1 was not detected immunohistochemically in normal glomeruli, but was induced in glomeruli in NTN at days 1 and 4. Double labeling showed that at day 4 the majority of HO-1 expressing cells were macrophages. Proteinuria was reduced on day 0-1 (hemin 33 ± 15 mg/24 hr control 79 ± 5, $P < 0.05$) and day 3-4 (22 ± 9 mg/24 hr; control 72 ± 12, $P < 0.05$) as was glomerular macrophage infiltration on day 1 (14.3 ± 2.5 cells/glom; control 18.2 ± 1.1, $P = 0.1$) and day 4 (10.2 ± 1.2 cells/glom; control 15.6 ± 0.5, $P < 0.05$). Glomerular thrombi were counted as the mean number of glomerular quadrants involved and were reduced by hemin at day 1 (0.24 ± 0.13; control 1.53 ± 0.49, $P < 0.05$). We conclude that HO-1 is induced in NTN. Stimulation of its synthesis with hemin leads to a reduction in glomerular thrombosis, macrophage infiltration and proteinuria. The mechanism remains to be determined, but possibilities include anti-complement or anti-oxidant effects of biliverdin, and vasodilator and anti-platelet effects of CO.

Polarity of TGF-β1 stimulation by proximal tubular cells. *A.O. Phillips, K. Morrisey, R. Steadman, and J.D. Williams, Institute of Nephrology, Royal Infirmary, Cardiff, Wales, United Kingdom.* We have previously demonstrated that treatment of human proximal epithelial cells with 25 mM D-glucose induces the mRNA for TGF-β1, but protein synthesis is initiated only by the subsequent application of PDGF. To investigate whether there is a selective polarity to this stimulation and to determine the polarity of TGF-β1 synthesis we have grown LLC-PKI cells on porous tissue culture inserts. Once confluent (assessed by transepithelial resistance), cells were treated with 25 mM D-glucose on either their apical or basolateral aspect. TGF-β1 mRNA induction (RT-PCR) occurred only following basolateral application. TGF-β1 secretion was induced only by the subsequent application of PDGF to the basolateral aspect of the cells, and was detected equally in the apical and basolateral compartments. This effect was maximal after 48 hr PDGF stimulation and represented a 3-fold increase over controls for TGF-β1 in both the apical and basolateral compartments ($N = 3$, $P < 0.05$ vs. control). The glucose transporter inhibitors, phlorizin and phloretin, were used to investigate this effect on specific D-glucose transport. Application of either basolateral phlorizin or phloretin at the time of addition of 25 mM D-glucose to the same compartment inhibited PDGF stimulated TGF-β1 synthesis. Maximal inhibition was achieved at 0.5 mM of either inhibitor (Phlorizin % inhibition of apical TGF-β1, 75%, $P = 0.015$, basolateral TGF-β1, 78% $P = 0.015$; Phloretin % inhibition of apical TGF-β1, 68%, $P = 0.03$, basolateral TGF-β1, 79% $P = 0.001$, $N = 5$, P vs. control). No inhibition was seen with apical application of either inhibitor. These data demonstrate that priming of proximal tubular cells for TGF-β1 synthesis results from basolateral exposure of the cells to 25 mM D-glucose. Based on the known activity of phlorizin and phloretin, they further suggest that this mechanism is dependent on the activity of the basolateral D-glucose transporter GLUT-1.

Macrophages modulate the pro-sclerotic responses of mesangial cells. *I.Z.A. Pawluczyk and K.P.G. Harris, Department of Nephrology, Leicester General Hospital, Leicester, England, United Kingdom.* Glomerulosclerosis (GS) is the final outcome of a number of different causes of glomerular injury during which the structures of the glomerulus are obliterated by extracellular matrix. Accumulating evidence suggests that macrophages (mΦ) play a pivotal role in the pathogenesis of this process. We previously reported that macrophage conditioned medium (MPCM) stimulates mesangial cells to secrete fibronectin (Fn). We have now examined other pro-sclerotic effects of MPCM on mesangial cells (MC). The genes for the matrix proteins laminin and collagen IV, as well as fibronectin, were up-regulated (4.94 ± 0.17-, 3.03 ± 0.31- and 2.86 ± 0.24-fold, respectively, $P < 0.001$). mRNA for tissue inhibitor of metalloproteinases (TIMP-1) was also increased (15.2 ± 2.5, $P < 0.001$), suggesting that a decrease in degradation rate could contribute to Fn accumulation. Although the genes (and the proteins) for the pro-fibrogenic growth factors TGFβ1 and PDGF were also increased in response to MPCM (2.2 ± 0.4, $P < 0.001$, 5.7 ± 1.2, $P < 0.004$, respectively), the use of suranim (a growth factor: receptor binding antagonist) and neutralizing antibodies to these cytokines suggested that these growth factors only played a minor role in MPCM stimulated MC Fn production. MC expression of MCP-1 mRNA was augmented 5.8 ± 0.8 fold ($P < 0.001$) in response to MPCM suggesting that mΦ can stimulate their own recruitment and activation by establishing a positive autocrine feed back loop. TGFβ1 (25 ng/ml) pretreatment of macrophages prior to the generation of MPCM significantly reduced the ability (by 43.1 ± 2.6%, $P < 0.001$) of the MPCM to induce MC FN secretion, suggesting that TGFβ1, as well as inducing pro-fibrogenic effects, may provide a counter-regulatory mechanism whereby the matrix inducing factor(s) of mΦ are suppressed. In conclusion, these data demonstrate that infiltrating glomerular mΦ play a pivotal role in the regulation of the sclerotic process by MC.

Enhanced uptake of LDL and oxidized LDL (OxLDL) by endothelial cells in uremic plasma. *R.C. Thuraisingham, G.V.R. Born, M.M. Yaqoob, and L.E. Cardonna-Sanclemente, Anthony Raine Research Laboratory, Royal Hospitals Trust and Pathopharmacology Unit, William Harvey Research Institute, St. Bartholomew's Hospital, London.* Endothelial abnormalities and increased oxidant stress are recognized features of the uremic syndrome. These features are generally accepted as predictors of increased cardiovascular morbidity and mortality. Our hypothesis is that one such abnormality is altered LDL and Ox-LDL fluxes in endothelial cells under uremic conditions. In order to test this hypothesis, we examined the uptake of these lipoproteins by human microvascular endothelial cells (HMEC) under uremic and control conditions. Human LDL, prepared by sequential ultracentrifugation, was labelled with ^{125}I-tyramine cellobiose (^{125}I-TC). Following cellular uptake, the lipoprotein is degraded and removed; however, the labelled adduct remains, indicating the total LDL flux. OxLDL was obtained using the copper technique. Uremic plasma (Ur) was collected from male non-diabetic hemodialysis patients just prior to a dialysis session. Control plasma (Con) was obtained from healthy male volunteers. HMEC were grown on 24 well plates at 6×10^5/well at 37°C in 5% CO_2. Starved cells were then incubated with medium supplemented with 10% control or uremic plasma for 0, 4, 14, 24 hours in the presence of either ^{125}I-TC-LDL or ^{125}I-TC-Ox-LDL. At the end of the incubations, supernatants were collected and three PBS washes performed. The cells were then trypsinized, harvested and their radioactivity counted in a γ-counter. Results are expressed as mean ± SEM counts per well. Statistical analysis was by Student's t-test for unpaired data.

	LDL		
Time, *hours*	Con ($N = 6$)	Ur ($N = 6$)	P
0	7150 ± 1208	6693 ± 717	NS
4	59090 ± 5368	72603 ± 5820	NS
14	222485 ± 20696	280356 ± 20028	<0.04
24	279486 ± 23577	351003 ± 22912	<0.03

	OxLDL		
Time, *hours*	Con ($N = 6$)	Ur ($N = 6$)	P
0	7957 ± 1462	8415 ± 1462	NS
4	79760 ± 12729	110823 ± 13466	<0.01
14	264868 ± 32258	336524 ± 21673	<0.05
24	358450 ± 42088	447593 ± 33234	<0.02

These data indicate an increased flux of LDL and Ox-LDL into endothelial cells under uremic conditions. The reasons and consequences of these findings and the effect of the increased lipoprotein flux on nitric oxide metabolism will be the subject of future work.

Ligation of ICAM-1 on the surface of renal cortical fibroblasts stimulates *de novo* expression of ICAM-1 and VCAM-1. *A. Clayton, J.D. Williams, and R. Steadman, Institute of Nephrology, University of Wales College of Medicine, Royal Infirmary, Cardiff, Wales, United Kingdom.* Progression to end-stage renal failure is accompanied by renal interstitial inflammation and fibrosis in which the activity of resident fibroblasts is believed to be of central importance. Intracellular adhesion molecule (ICAM)-1 has been implicated as the principal adhesion molecule controlling leukocyte infiltration of the inflamed interstitium. We have reported previously the isolation of fibroblasts from the normal renal cortex and shown that, following treatment with inflammatory cytokines, they increased their expression of ICAM-1 and bound peripheral blood neutrophils and monocytes in a manner that was dose-dependently inhibited by anti-CD18 antibodies. This interaction of the fibroblasts with leukocytes triggers an influx of extracellular calcium ions and is mimicked by specifically cross-linking the ICAM-1 receptor with anti-ICAM-1 antibodies. The present study tested the hypothesis that one effect of cross-linking ICAM-1 on the surface of the cell is to stimulate the *de novo* synthesis and expression of cellular adhesion molecules. Following cross-linking there was a 2- to 3-fold increase in the steady-state levels of ICAM-1 mRNA. The expression of this mRNA peaked at 1–3 hours and was dose-dependent on the concentration of secondary cross-linking antibody. There was no response as a result of incubation with irrelevant antibody (anti-ELAM-1). The expression of ICAM-1 protein was also increased in response to cross-linking pre-formed ICAM-1. Peak protein expression was between 18–48 hours after cross-linking and was also dependent on the concentration of cross linker. Additional data demonstrated a similar increase in the expression of VCAM-1 following ICAM-1 cross-linking. Preliminary studies using BAPTA-AM to chelate intracellular calcium ions suggest that the up-regulation of adhesion molecules is partly triggered as a result of the associated flux of calcium ions. The present study demonstrates that interstitial fibroblasts can be stimulated

by the direct cell surface interaction of ICAM-1 with its ligands on inflammatory cells. One phenotypic change resulting from this interaction is the up-regulated synthesis and expression of more cellular adhesion molecules. This may have profound implications for the role of the fibroblasts in controlling the progression of inflammatory renal disease.

Golgi co-localisation of TNFR1 and signalling proteins in endothelial cells. *S.J. Jones, K. Wolfreys, J. Savidge, and J. Bradley, Department of Medicine, University of Cambridge, Cambridge, England, United Kingdom.* Tumor necrosis factor receptor 1 (TNFR1) can initiate intracellular signal transduction pathways which induce apoptosis or activate NF-κB. Insight into the molecular mechanisms involved has been provided by the identification of receptor associated signalling proteins containing death domain (TRADD) and TRAF domain (TRAF1 and TRAF2) motifs. We have previously demonstrated, by confocal immunofluorescence and electron microscopy, that the TNFR1 is Golgi and the TNFR2 is plasma membrane-associated. This disparate localization of the two receptors may have important implications with respect to endothelial cell activation. We aimed to determine whether signalling proteins, importantly TRADD and TRAF2, are also co-localized with the TNFR1 in the Golgi. Human umbilical vein (HUVEC) and a spontaneous human endothelial cell line (ECV304) were subjected to subcellular fractionation using differential and sucrose density gradient ultracentrifugation techniques. The interfaces were assayed for enrichment of the Golgi enzyme marker alpha mannosidase II and Western blotted using antibodies to the Golgi specific marker p58, TNFR1, TNFR2, TRAF2 and TRADD. Antibodies to TNFR1, TRAF2 and p58 proteins specifically immunoblotted proteins of approximately 60 kDa, 56 kDa, and 58 kDa, respectively from Golgi enriched fractions. However, TNFR2 and TRADD were not recognized in this fraction. Using confocal microscopy anti-TRAF2 antibodies exhibited cell surface, Golgi and nuclear staining, but no detectable staining was observed with anti-TRADD antibodies. This study confirms that in endothelial cells the TNFR1 is Golgi-associated and further demonstrates the presence of TRAF2 in the Golgi. The effect of TNF on localization and expression of TRADD, which is required to bridge TRAF2 to TNFR1, is under investigation.

mRNA estimation in single human glomeruli by competitive RT-PCR: Methods and initial applications. *L.L. Hall, G.R. Bicknell, J.A. Shaw, J.H. Pringle, and P.N. Furness, Department of Pathology, Leicester Royal Infirmary, Leicester, England, United Kingdom.* Measurement of a specific mRNA in a tissue is often the best available index of that tissue's rate of synthesis of the corresponding protein. Needle biopsies are too small for Northern blotting; *in situ* methods give localization but poor quantitation. The best quantitative method for small samples is reverse transcriptase-polymerase chain reaction (RT-PCR) with initial addition of a known amount of a competitor strand (competitive RT-PCR) to permit quantitation of specific mRNA sequences. Single glomeruli can be obtained either by plucking them by hand from the surface of fresh renal biopsies, or by washing the biopsy needle after the procedure. mRNA is extracted from a single glomerulus using oligo-dT-linked paramagnetic beads. Reverse transcription and PCR are carried out conventionally. Where appropriate, a competitor strand is added; we have developed a novel ligase-free PCR approach to the synthesis of these strands. Detection of the amplified product is by an ELISA-based plate assay with specific digoxygenin-labelled oligonucleotide probes. This facilitates rapid and specific measurement of native and competitor products, without electrophoresis. The bead-linked cDNA is stable, and cDNA from one glomerulus can be used for at least 20 separate mRNA estimations, or considerably more if bead-linked cDNA is re-used with different primers. The method is also applicable to sections of frozen tissue, including archival material. Extraction of mRNA from glomerular epithelial cells alone can be achieved, but this has been limited by unpredictable contamination by mesangial mRNA. We have applied these methods to "housekeeping genes" (GAP3DH and β-actin), cytokines, extracellular matrix molecules, degradative enzymes, and enzyme inhibitors. Initial results suggest that in glomeruli from stable renal transplants, levels of mRNA for collagen III and collagen IVα2 correlate with levels of TGFβ. Glomeruli from cases of IgA nephropathy show considerable variation in expression of collagen III and collagen IVα2, but expression of collagen IVα5 is comparatively stable, supporting recent reports that α2, but not α5, is involved in glomerular sclerosis.

Growth of metanephric endothelia in a realistic experimental milieu. *S. Loughna, P. Hardman, K. Alitalo, L. Jussila, and A.S. Woolf, Developmental Biology Unit, Institute of Child Health, London, England, United Kingdom, and University of Helsinki, Helsinki, Finland.* Previous observations suggested that embryonic kidney vessels formed by in-growth, or angiogenesis, because explanted murine metanephroi were invaded by avian chorioallantoic membrane (CAM) vessels and also formed avascular glomeruli in organ culture. Our recent studies, however, found that avascular embryonic day 11 (E11) metanephroi and cell lines derived from E11 renal mesenchyme expressed vascular endothelial growth factor and its receptor tyrosine kinases (RTK) VEGFR 1/2. Thus, the molecules for vasculogenesis, or *in situ* vessel formation, are present at the onset of nephrogenesis. The orphan RTK, TIE-1, is expressed early in the endothelial lineage and we have detected TIE-1 mRNA at the inception of nephrogenesis. In *TIE-1 promoter/LacZ* transgenic mice, the expression of the reporter gene was detected *in vivo* in E12 metanephroi in sparse capillaries around the ureteric bud and also in isolated mesenchymal cells which we postulate are either endothelia differentiating *in situ* or angioblasts which have migrated into the organ. Next, we transplanted these metanephroi into the renal cortex of non-transgenic neonates, a site known to permit growth of implants into filtering nephrons. Chimeric kidneys were harvested at nine days and differentiated implants were detected which contained a rich vascular network with transgenic endothelia in clefts of S-shaped bodies and in glomerular arterioles and capillaries. In contrast, when E12 rudiments were grown in organ culture or in the chick CAM only a few transgenic vessels were detected despite glomerular differentiation. Hence, we conclude that the growth of embryonic kidney endothelia and their precursors is only favored when the experimental setting closely mimics the conditions which are normally found *in vivo*.

Hyperglycemia induces cytochrome P450 in proximal tubular cells. *M. Varagunam and M. Yaqoob, Anthony Raine Research Laboratories, St. Bartholomew's Hospital, London, England, United Kingdom.* The cytochrome P450 (CYP450) family has mainly been studied in the context of its ability to metabolize xenobiotics into excretable hydrophilic compounds which are known to cause cellular damage. Insulin deficiency has been shown to alter the CYP450 profile in the livers of streptozin-induced diabetic rats. The number of P450 isoforms varies between species. CYP2EI has been shown to produce reactive oxygen species such as superoxide radicals and hydrogen peroxide in high amounts relative to other P450 isoforms. Evidence of free radical generation and oxidant injury has been shown in patients, with diabetes mellitus and particularly in those with diabetic nephropathy. However, the source and cellular mechanism of free radical generation is not known. We therefore considered this study to assess the effects of hyperglycemia on the CYP450 profile in renal proximal tubular cells. Cells from a porcine proximal tubular cell line (LLC-PK1) were grown in DMEM containing either high (25 mM) or normal (5 mM) glucose and 10% FCS for one week, in a 5% CO_2, 37°C incubator. The cells were washed, harvested and sonicated in ice cold buffer A (0.25 M K_2PO_4, 0.15 M KCl, 1 mM EDTA, pH 7.25). The sonicate was spun for a further 60 minutes at 38,000 rpm. The resulting microsomal pellet was resuspended in buffer B (0.25 M K_2PO_4, pH 7.25, 30% glycerol) and the protein concentration determined by the Lowry assay. The microsomal fraction was subjected to Western blot analysis using a specific CYP2EI antibody and developed using ECL reagents. There was a threefold increase in CYP2EI in cells grown in hyperglycemic medium. These preliminary results suggest that hyperglycemia induces increased levels of CYP2EI protein in LLC-PK1 cells. The significance of this increase will be the subject of future work.

Apoptosis: A mechanism of cell deletion in an experimental model of chronic renal failure. *G.L. Thomas, B. Yang, and A.M. El Nahas, Sheffield Kidney Institute, Northern General Hospital Trust, Sheffield, England, United Kingdom.* The model of 5/6 subtotal nephrectomy (SNx) in age-matched male Wistar rats (University of Sheffield strain) has been used to study chronic renal failure and progressive fibrosis in the remnant kidney. Tissues were excised from experimental (SNx) and control (SNc) animals on 7, 30, 90 and 120 days post-SNx. These were examined for morphological signs of apoptosis by H&E staining, electron microscopy and acridine orange fluorescent staining of nuclear chromatin; and for signs of DNA cleavage by endonucleases via the principal of TUNEL staining (Apop Tag). Maximal apoptosis is seen at day 90 when the increase with

respect to controls being 21.5-fold ($P < 0.006$) for glomerular cells, 7.1-fold ($P < 0.001$) for interstitial cells and 14.2-fold (***$P < 0.0001$) for tubular cells. In conclusion, we have shown a time-dependent increase in apoptosis in the subtotal nephrectomy model of chronic renal failure. This may contribute to the progression of tubular atrophy and play a role in the pathogenesis of tubulointerstitial scarring.

The effect of acidosis and insulin upon protein degradation in cultured muscle cells. *R.G. Roberts, C.P.F. Redfern, and T.H.J. Goodship, Department of Medicine, University of Newcastle upon Tyne, Newcastle upon Tyne, England, United Kingdom.* That acidosis increases protein degradation in chronic renal failure is well-established. We believe that this is mainly due to increased activity of the ubiquitin-dependent proteolytic pathway. We have used the L6 rat myoblast line together with primary cultures of human muscle to investigate this further. Using ^{14}C-phenylalanine release to measure protein degradation (PD) we found an increase (expressed as log % of initial cellular ^{14}C/hour) from $-3.4 \pm 0.3 \times 10^{-3}$ at pH 7.40 to $-4.4 \pm 0.3 \times 10^{-3}$ at pH 6.95 in L6 cells. Addition of 100 nM insulin reduced PD under both conditions. In a separate experiment we found a dose-response relationship between PD and insulin with a just significant effect using 1 nM insulin. We obtained the same pattern of results with human myocytes. Using Northern blotting to investigate the ubiquitin-dependent proteolytic pathway, we found that in L6 cells insulin reduced the expression of the main components of the pathway, ubiquitin (UbA & UbB), activating enzyme E1, conjugating enzyme E2 and proteasome component C2. In the presence of insulin plus acidification, the expression of UbB, E2 and E1 was higher than with insulin alone, whereas the other genes were sensitive to insulin despite the acid pH. We did not find increased expression of any of the genes in the presence of acid alone. In the human cells we found that insulin reduced the expression of E2 but did not affect ubiquitin expression (Renal Association 1995). Regulation of this pathway by insulin supports the hypothesis that it is important in acidosis. That ubiquitin expression was not up-regulated by acidosis in L6 cells may be a consequence of the model, perhaps because the genes are already highly expressed in these cells.

Fatty acids on albumin cause injurious effects in protein overload proteinuria. *M.E. Thomas, N.J. Brunskill, S.J. Harper, K.P.G. Harris, P.N. Furness, and J. Walls, Department of Nephrology, Leicester General Hospital, Leicester, England, United Kingdom.* Interstitial inflammation is a characteristic finding in protein overload proteinuria. To investigate whether the fatty acids carried on injected albumin mediate these deleterious effects, two g per day of fatty acid bearing (FA-BSA, $N = 7$) or delipidated (DL-BSA, $N = 7$) bovine serum albumin were injected intraperitoneally into female Lewis rats for 7 days. Kidneys were perfused *in situ* with saline on day 7. Cortical macrophage (MΦ) infiltration was measured by immunoperoxidase staining with an ED-1 antibody. Percent area stained was measured blindly using the program *NIH Image* to analyze video images of the sections. S phase cells were identified by *in situ* hydridization for histone mRNA, and counted blindly per high power field. Results shown are means (SEM). Groups were compared by Mann-Whitney tests.

	Urine protein $(U)_p$ *mg/day*		
Day	1	3	6
FA-BSA	634	1078	986
	(179)	(60)	(93)
DL-BSA	688	561	704
	(72)	(129)	(102)
P	0.49	0.01	0.04

	MΦ	S-phase *cells/hpf*	
	%	Tubular	Interstitial
Day	7	7	7
FA-BSA	0.99	2.38	0.99
	(0.15)	(0.35)	(0.19)
DL-BSA	0.47	1.47	0.44
	(0.11)	(0.31)	(0.08)
P	0.02	0.10	0.04

Serum BSA levels were similar in both groups. FA-BSA provoked a significantly greater cortical MΦ influx and tubulointerstitial cell proliferation than DL-BSA. FA-BSA produced higher U_p levels at days 3 and 6 than DL-BSA, suggesting greater glomerular epithelial injury. The administration of DL-BSA, compared to FA-BSA, is associated with reduced renal injury. This is characterized by a marked reduction in the tubulointerstitial abnormalities seen in protein overload proteinuria. In conclusion, the fatty acids carried by BSA have deleterious effects in protein overload proteinuria.

Renal papillary interstitial fluid osmolality in experimental uremia. *R.M. Chamberlain, J. Skinner, and D.G. Shirley, Department of Physiology, Charing Cross & Westminster Medical School, London, England, United Kingdom.* The pathophysiology of the impaired urine concentrating ability of chronic renal failure is ill-understood. Concentration of urine is dependent on two factors: hypertonicity of the medullary interstitium and vasopressin-induced water transport in the collecting ducts. The latter may be impaired in renal failure, owing to down-regulation of the V_2 receptor. In the present study we have investigated the osmolality of the papillary interstitium in uremic rats. Rats were subjected to either two-stage 5/6 nephrectomy (5/6 NX) or sham operations (SO). In 5/6 NX rats, urine flow rate (V) increased 2–3 fold and urine osmolality (U_{osm}) was reduced. After 5 weeks, the rats were anesthetized (thiopental, 110 mg/kg, i.p.) and clearance measurements made. Relevant data (means ± SEM, $N = 8$ per group) are shown below.

	GFR *ml/min*	PUN *mmol/liter*	V *μl/min*
SO	3.1 ± 0.1	7.4 ± 0.7	8.5 ± 0.3
5/6 NX	0.8 ± 0.1[a]	15.0 ± 0.8[a]	22.8 ± 2.1[a]

	FE_{H2O} %	U_{osm} *mOsm/kg H_2O*
SO	0.3 ± 0.1	2018 ± 337
5/6 NX	3.2 ± 0.5[a]	779 ± 77[a]

Abbreviations are: GFR, glomerular filtration rate; PUN, plasma urea nitrogen; FE_{H2O}, fractional water excretion; *$P < 0.001$ compared with SO group.

Samples of papillary interstitial fluid were obtained by the method of sequential centrifugation and their osmolalities measured. Papillary osmolality in SO rats was 1791 ± 140 mOsm/kg H_2O ($N = 7$), but only 620 ± 37 mOsm/kg H_2O ($N = 7$; $P < 0.001$) in the 5/6 NX group. This diminished hypertonicity is likely to be a major factor in the reduced concentrating ability characteristic of experimental uremia.

HDL and protein farnesylation in a tubular cell monolayer model of the nephrotic syndrome. *C.P. Streather, J.S. Owen[1], B.M. Hendry, and J.E. Scoble, Renal Unit, Department of Medicine, King's College Hospital, and Department of Medicine, Royal Free Hospital, London, England, United Kingdom.* Urinary HDL is a marker of unselective proteinuria and therefore heralds a worse renal prognosis. HDL has been shown to stimulate renal tubular cells, and this effect is sensitive to Hydroxymethylglutaryl Coenzyme A (HMG CoA) reductase blockade with simvastatin (SIM). This effect may be related to the non-sterol products of this pathway, such as farnesol, since protein farnesylation is necessary for the biological activity of proteins, such as p21 ras, which are involved in cell proliferation. LLC-PK1 porcine tubular cell monolayers were exposed to apical, corresponding to urinary, HDL and monolayer resistance and the synthesis of sterols measured using 14C-acetate as a substrate. The effects of SIM, farnesol pyrophosphate (FPP), the substrate for farnesol protein transferase (FPT), and hydroxy farnesyl phosphonic acid (HFPA), a blocker of this enzyme on monolayer resistance (MR) and sterol synthesis, were investigated. Three hundred μg/ml HDL added atypically to confluent monolayers, increased MR (initial value = 100% to 341% ± 88), SIM totally abolished this effect (MR 49% ± 16, and 400 μg/ml) and FPP partially restored it (MR 154% ± 53). In further experiments 20 μM HFPA significantly reduced the effect of HDL on MR (96% ± 6 vs. 286% ± 38; $P < 0.002$) without reducing sterol synthesis (4.26 ± 0.61 pmol/μg

protein vs. 3.52 ± 0.97 pmol/μg protein). SIM significantly reduced sterol synthesis (0.22 ± 0.03 pmol/μg protein). Blockers of the protein kinase C (PKC) system and tyrosine phosphorylation (TK), chelerythrine and herbimycin A, respectively, had no effect on the resistance response to HDL. Apical HDL in this model, reproducing urinary HDL in nephrotic proteinuria, stimulates MR. This effect is sensitive to HMG CoA reductase and FPT blockade and appears to be independent of effects on cholesterol synthesis. HDL may therefore act on tubular cells via the stimulation of protein farnesylation. We speculate that statins might be used to interrupt this non-sterol pathway and prevent tubular damage in proteinuria independent of their action on cell cholesterol.

Functional evidence for up-regulation of H^+ ATPase during potassium depletion. *M.A. Bailey, R.J. Unwin, and S.J. Walter, Department of Physiology, Charing Cross and Westminster Medical School and Division of Nephrology, UCL Medical School, London, England, United Kingdom.* Potassium depletion is associated with metabolic alkalosis and enhanced bicarbonate reabsorption in the distal nephron. Although the underlying mechanisms are unclear, we have recently presented immunocytochemical data that suggest increased expression of H^+ ATPase in the apical membrane of distal nephron segments. To investigate this possibility *in vivo*, we have examined the effect of the specific H^+ ATPase inhibitor, bafilomycin A1 (BAF), on transepithelial potential difference (Vte) in the late distal tubule of control ($N = 5$) and potassium-depleted (low-K; $N = 5$) rats. Animals were anesthetized (Inactin; 110 mg/kg i.p.) and prepared for microperfusion experiments. Distal tubules with at least two accessible loops were perfused with two solutions designed to mimic early distal tubular fluid. In addition, amiloride (1 mM), barium (3 mM) and NPPB (10 μM) were added to both perfusates in order to abolish possible shunt pathways: BAF A1 (1 μM) was included in the second perfusate. Late distal tubule Vte was recorded during perfusion at 10 nl/min as previously described. Perfusion with BAF resulted in the Vte becoming more lumen-negative in both groups of animals. The absolute change in Vte was greater ($P < 0.05$) in the low-K rats (4.7 ± 0.8 mV) than in controls (2.5 ± 0.5 mV). The enhanced effect of BAF may indicate increased activity of electrogenic H^+ ATPase during potassium depletion.

Recognition of recombinant rat α3(IV)NCI by autoantibodies from WKY rats with EAG. *J.J. Ryan, J. Reynolds, P.J. Mason, A.N. Turner, and C.D. Pusey, Departments of Medicine and Haematology, RPMS London, London, England; Department of Medicine and Therapeutics, Aberdeen University, Aberdeen, Scotland, United Kingdom.* Goodpasture's disease is an autoimmune disorder that presents with rapidly progressive glomerulonephritis and lung hemorrhage. It is characterized by pathogenic autoantibodies to the glomerular basement membrane (GBM), which recognize the non-collagenous domain of the α chain of type IV collagen, α 3(IV)NCI. We and others have found evidence that α3(IV)NCI is also the target of autoimmunity in experimental autoimmune glomerulonephritis (EAG), a model of Goodpasture's disease in the rat which shares many characteristics with the human condition. In order to investigate immune mechanisms in this model, we set out to clone, sequence and express rat α3(IV)NCI. cDNA encoding part of the rat α3(IV)NCI domain was obtained by RT-PCR of RNA extracted from rat kidney. A 350 bp DNA fragment was radiolabelled and used to screen a rat kidney cDNA library. One positive clone of 1,350 bp was isolated from 2×10^6 pfu. Restriction enzyme digestion, Southern blot analysis and sequencing confirmed that it contained the α3(IV)NCI sequence. The clone encoded 210 bp of the 5′ collagenous triple helical region, 690 bp of the NCI domain and 450 bp of the 3′ non-coding region. The overall sequence homology with human α3(IV)NCI was 90%. Recombinant rat α3(IV) NCI was expressed in COS-7 cells, using a mini-collagen chain gene as previously described. Western blot analysis of COS supernantants showed that the recombinant molecule was recognized by sera from rats with EAG, and from patients with Goodpasture's disease, at the predicted molecular weight of 41 kDa. Rat and human control sera did not bind. Chimeric molecules, in which parts of the rat α3(IV)NCI domain are substituted by sequences from other rat or human α(IV) chains, are being used to localize B cell epitopes in EAG. This is the first report, to our knowledge, of the expression of recombinant rat α3(IV)NCI. This work confirms that α3(IV)NCI is an autoantigen in EAG, and the recombinant protein will be a valuable tool for further analysis of autoimmune responses in anti-GBM disease.

Macrophage time of residence in the experimentally inflamed peritoneum depends upon bacterial strain. *M.J. Andrews, D. Wakelin, R.P. Burden, A.G. Morgan, and J. Savill, City Hospital Renal Unit Nottingham and University of Nottingham, Nottingham, England, United Kingdom.* Inflammatory macrophages (MØ) have been implicated in both local and systemic complications of CAPD peritonitis, but little has been known of factors determining MØ life span in the inflamed peritoneum which, in turn, may regulate injurious potential of these cells. We investigated MØ kinetics in a simplified model of peritonitis induced in healthy Balb/c mice by i.p. administration of 100 million heat killed bacteria in 1.0 ml PBS, thereby excluding complicating effects of bacterial replication, uremia and dialysis fluid. Although *S. aureus* and *S. epidermidis* both induced pmn infiltration of similar magnitude, kinetics, and rate of pmn apoptosis, there were profound differences in the time course of MØ infiltration. Following i.p. *S. aureus* MØ number in a 4 ml PBS lavage peaked at 3 days, falling to baseline by 16 days, whereas with *S. epidermidis* MØ number remained maximally elevated at 16 days, suggesting increased time of residence. To address this possibility we developed a new technique for *in vivo* "pulse-labelling" of peritoneal MØ by i.p. administration of the fluorescent stable cell linker PKH. Cytospin preparations of labelled and unlabelled cells sorted by FACS confirmed 96% sensitivity and specificity of MØ labelling by PKH. By following the rate of disappearance of labelled MØ from the peritoneum we determined that in *S. aureus* peritonitis MØ half-life was 2.5 ± 0.4 days (mean ± SEM, $N = 6$), whereas with *S. epidermidis* half-life was significantly longer at 4.75 ± 0.3 days. We conclude that hitherto unrecognized bacterial factors may regulate the kinetics of the peritoneal inflammatory response by modulating MØ lifespan, suggesting a new target for studies of the pathogenesis of CAPD peritonitis.

Improvement of neutrophil function in pyruvate peritoneal dialysis fluid is related to intracellular pH acidification. *D. Kaur, J.D. Williams, T. Wilkinson, A. Mahiout, M. Hallett, and N. Topley, Institute of Nephrology, Dept of Surgery, UWCM, Cardiff, Wales, United Kingdom; Institute of Cell and Protein Engineering, Hannover, Germany.* Acidic lactate buffered peritoneal dialysis fluid (PDF) inhibits many cell functions related to peritoneal host defense. These effects appear to be related to intracellular acidification mediated by the combination of low pH and lactate concentration. In order to reduce the inhibitory effects associated with lactate buffered PDF, alternative fluid formulations have been developed. The present study compares the effects of lactate buffered PDF (Lac-PDF, 40 mM Lactate, pH 5.2) with a solution of identical composition buffered with pyruvate (Pyr-PDF, 40 mM pyruvate, pH 5.2) and examines their effects on PMN intacellular pH $[pH]_i$ and respiratory burst activation measured as luminol-dependent chemiluminescence (CL). Exposure of PMN to Lac-PDF for 15 minutes resulted in significant reduction in PMN CL (RLU). These were reduced from 2.96 ± 0.32 in control solutes (M199, pH 7.3) to 1.11 ± 0.36 and 0.83 ± 0.21 in solutions containing 1.36 and 3.86% glucose, respectively ($N = 7, P < 0.01$). In contrast, exposure to Pyr-PDF did not significantly reduce the PMN CL response (2.08 ± 0.26 and 1.97 ± 0.13 in 1.36 and 3.86% glucose). Exposure of PMN ($N = 3$) to Lac-PDF reduced $[pH]_i$ to a mean of 5.0 after 200 seconds exposure. In contrast, in Pyr-PDF $[pH]_i$ was only reduced to a mean of 5.7 under the same conditions. The addition of 5% pooled 4 hour spent dialysate to PMN did not inhibit the intracellular acidification induced by Lac-PDF but abolished that mediated by Pyr-PDF irrespective of glucose concentration. In order to further examine the effect of extracellular pH on PMN function, external pH was maintained at pH 5.2 (with 25 mM Hepes) in the presence of Pyr-PDF or Lac PDF (1.36% glucose) throughout a 15′ exposure period. Under these conditions both solutions showed substantial inhibition of PMN CL (Pyr-PDF: 0.39 ± 0.06, Lac-PDF 0.134 ± 0.04 vs. control 2.19 ± 0.22, $N = 3, P < 0.05$ vs. control for both). These data suggest that the improved function of PMN following exposure to pyruvate buffered PDF is related directly or indirectly to its ineffectiveness as a buffer at pH 5.2 and thus its reduced effect on PMN $[pH]_i$. These features suggest potential advantages of pyruvate as a buffer in PDF.

Pumped haemofiltration in small/pre-term infants. *M.A. Lewis, Renal Unit, Manchester Children's Hospital, Manchester, England, United Kingdom.* The management of renal failure in the newborn is difficult. When dialysis is instituted peritoneal dialysis (PD) is usually the technique of choice. This can be problematic and impossible in some patients with pre-existing intra-abdominal pathology. Continuous arteriovenous hemofiltrarion (CAVH) has been described in infants, but sick pre-term infants

are not able to support the circuit. I have devised a means of having pumped hemofiltration in small/preterm infants (PHIS/PI) and describe its use in ten patients ranging in size from 750 to 3000 g for periods of 1 to 7 days. Vascular access was achieved through 24 gauge up to 5 French gauge cannulae in either a peripheral artery or a central vein or through two central veins. Blood was pumped out using an IVAC 572 infusion pump and through a Gambro FH22 hemofilter. A second IVAC pump was used to remove hemofiltrate from the filter and a third to infuse replacement solution. Removal rate was set to give a clearance of 15 ml/min/1.73 sq.m (45 to 105 ml/hr for the above weight range) and blood flow rate set to between 5 and 10 times the removal rate (225 to 999 ml/hr). Heparin was infused into the circuit to prevent clotting of the filter. Biochemical and fluid balance control was achieved in all infants. Guaranteed fluid removal allowed the administration of full nutritional support. Five patients died when treatment was withdrawn because of an untreatable underlying problem. One recovered renal function but died some weeks later from unrelated problems, and four patients survived and recovered renal function. This system allows a secure means of achieving fluid and electrolyte control in the pre-term infant. The use of this technique may allow hemofiltration to become as applicable to pre-term infants as it is to older children and adults.

Skeletal muscle cell phosphate homeostasis following bicarbonate hemodialysis. *T.R. Ringrose, C.H. Thompson, S. Kumar, G.J. Kemp, D.J. Taylor, and G.K. Radda, MRC NMR Unit, and Churchill Hospital Renal Unit, Oxford; Department of Orthopaedics, Liverpool University, Liverpool, England, United Kingdom.* Intracellular transfer of extracellular phosphate (Pi) during dialysis is suggested as an explanation for inadequate Pi removal by hemodialysis (HD). Skeletal muscle, comprising 40% of lean body mass, can act as an important reservoir of Pi, and skeletal muscle free cytosolic Pi is elevated in CRF. The effect of bicarbonate hemodialysis on plasma and muscle pH and Pi was examined in six male patients. The right calf muscle was studied by ^{31}P magnetic resonance spectroscopy immediately before and after a 4 hour dialysis session (35 mM HCO_3^- dialysate). Plasma arterial pH, pCO_2, pO_2, HCO_3^-, Pi and K^+ were taken from the fistula within 10 minutes of the MRS study.

	Plasma pH	Muscle pH	Plasma Pi *mM*	Muscle Pi *mM*
Pre-dialysis	7.41	7.04	1.66	4.25
Post-dialysis	7.45[a]	7.03	1.06[a]	4.57

[a] $P < 0.05$ Wilcoxon signed rank test

In the group as a whole, there was no significant change in resting muscle phosphocreatine, pH or Pi, despite significant changes in plasma pH and Pi. However, paradoxical muscle acidification was seen in 3 patients and muscle pH changes during dialysis appeared dependent on changes in arterial HCO_3^- ($r = -0.865$; $P = 0.06$). Changes in muscle Pi during dialysis correlated with these intracellular pH changes ($r = 0.808$; $P < 0.04$). Conclusions: (i) skeletal muscle intracellular acidification can occur following bicarbonate dialysis and (ii) skeletal muscle intracellular Pi during dialysis is dependent upon intracellular rather than extracellular pH changes. This study suggests that other cell compartments, such as bone, may be more important than skeletal muscle in any sequestration of Pi that occurs during hemodialysis.

Reuse bicarbonate reservoir for hemodialysis. *C.P.R. Soper, M.K. Lam-Po-Tang, and A. Hodge, St. Helier Hospital, Carshalton, England, United Kingdom.* Bicarbonate buffer for intermittent hemodialysis requires composition shortly before administration. Contemporary systems for administering bicarbonate are suitable for single use only. We report 12 months' use of a reuse cylindrical reservoir for bicarbonate mixture to diluent, compatible with most dialysis systems. The cylinder is engineered from polycarbonate, with an inbuilt sprinkler, and is suitable for autoclaving. Between uses it was cleansed by soaking in 1000 ppm sodium hypochlorite for 60 minutes and then in a standard washing machine, with exposure to 60°C for 60 minutes. Each reservoir batch is discarded after 6 months. Dialysate was analyzed for Na, K, HCO_3 and ionized Ca. Dialysate samples and supply water were cultured in tryptone glucose extract agar weekly at 37°C for 7 days. Over a 2 week period crossover comparisons in serum bicarbonate were made in 7 patients, with a commercially available single-use cartridge. Predialysis HCO_3 values were 20.3 mmol/liter in the reservoir and 19.5 mmol/liter in the cartridge group ($N = 66$, $P = 0.816$). Dialysate HCO_3 was not different (1.86 mmol/liter lower in reservoirs, $P = 0.122$, $N = 5$) and showed no fall over dialyses up to 4 1/2 hours. Dialysate Na, K, Ca were not different and did not change significantly. Serum HCO_3 levels rose by 5.8 ± 3.0 mmol/liter (SD) and 7.1 ± 4.1 mmol/liter, respectively, (not significant by paired sample Student's *t*-testing, $N = 13$). Since switching patients over to the reservoir, over the last 12 months our supply water and dialysis water have met the AAMI and European Pharmacopoiea standards (although cultured at 37°C) in 100% of dialysate samples ($N = 31$). Reuse bicarbonate reservoirs have resulted in a fiscal saving of approximately £50,000, without compromising alkalization, purity from microbial contamination, or dialysate composition.

Preliminary experience of metacryloylphosphorylcholine laurylmethacrylate coated vascular access catheters. *F.E. Harris, S.R. Nelson, J.C. Russell, V. O'Byrne, and T. Sutton, Department of Renal Medicine, King's College Hospital, London, and Biocompatibles Ltd., Farnham, England, United Kingdom.* Prolonged central venous access is required for many patients requiring hemodialysis. Conventional catheters used for vascular access are prone to occlusion, poor flow rates, and biofilm deposition. Angiography catheters coated with the copolymer metacryloylphosphorylcholine laurylmethacrylate (MPC:LM), a biomembrane mimic, avoid these problems. We report our initial experience of MPC:LM coated catheters for prolonged intravascular placement in patients requiring hemodialysis. Ten consecutive patients, aged 18–75, presenting with acute or end-stage renal failure, requiring hemodialysis who were otherwise fit and well, were randomized to receive either coated or conventional catheters ($N = 5$ in each group). Standard 15 cm hemodialysis catheters were used, and 5 catheters were coated with MPC:LM and repacked to appear identical to uncoated catheters. Catheters remained *in situ* for between 4 and 9 days (median 5 days), and were electively removed. They were fixed in 2% glutaraldehyde, analyzed using scanning electron microscopy and the surface area coated with biofilm quantitatively assessed. Blood samples were taken at the time of insertion and removal of catheters and analyzed for routine biochemistry, acute phase response and hematology. Blood flow rates during each dialysis were recorded, as were any complications such as catheter blockage or infection. MPC:LM coated catheters were well-tolerated with no adverse features over conventional catheters. No significant difference was found between groups for any of the measured variables. However, the duration that the catheters remained *in situ* was short, and complications related to biofilm deposition may not be seen early. Larger patient numbers and longer duration of catheter placement should enable better evaluation of the advantages of coating hemodialysis catheters with MPC:LM.

Use of catheter brushes to diagnose internal luminal colonization of hemodialysis catheters. *I.D. Dittmer, D. Sharp, A.J. Williams, C.A.M. McNulty, and R.A. Banks, Renal Unit, Gloucestershire Royal Hospital, Gloucester, England, United Kingdom.* Central venous catheters are regularly used for vascular access in patients with renal failure requiring hemodialysis. We have previously shown that 95% of these catheters become colonized with bacteria on their internal luminal surfaces. A total of 75% are associated with peripheral bacteremia during dialysis that can result in clinically relevant sepsis, including life-threatening metastatic sepsis. The gold standard method for detecting colonization and catheter associated sepsis is differential quantitative blood cultures. This method involves culturing blood drawn from the catheter ports and peripheral venepuncture. The laboratory processing of these specimens is laborious and expensive. Long, narrow brushes (FAS Endoluminal Brush) have recently become available for obtaining "swabs" of the internal lumen of central venous catheters. This is the first evaluation of these brushes in the dialysis situation. Swabs and quantitative blood cultures were taken from both ports of the catheters of 6 patients. The blood cultures were processed in the standard manner. Swabs were rolled on agar plates for culture. Four samples had no growth by both blood and brush culture. Six samples had positive cultures by both techniques. The organisms identified by blood culture were identical to those isolated by brush culture. In one case a positive culture was obtained by brushing while no blood could be aspirated from the catheter port. In a further case the brushing was culture negative but an organism was isolated from the blood culture at a concentration of less than 5 colony forming units per ml of blood. There

were no complications associated with the brushings. This preliminary study shows that catheter brushes may be an accurate, reliable and rapid method of diagnosing central venous hemodialysis catheter colonization.

Temporary venous access for first dialysis is common, undesirable and usually avoidable. *A.M.S. Chesser and L.R.I. Baker, Department of Nephrology, St. Bartholomew's Hospital, London, England.* Late referral to a renal unit for patients with end-stage renal failure is associated with increased morbidity and mortality. An increased mortality has also been described when emergency first dialysis is required via temporary venous access, irrespective of whether the patient has received prior nephrological follow-up. Despite this, we have found that many patients are still referred to a renal unit too late to avoid emergency first dialysis (see table), and a significant number (19.7%) require emergency first dialysis despite having been under nephrological follow-up for more than three months. The mortality in the first 90 days following first dialysis is significantly greater in all those groups requiring emergency first dialysis.

Temporary access required for first dialysis			
Eventual preferred modality of dialysis treatment	Permanent access *in situ* at time of first dialysis	More than 3 months prior nephrology follow-up	Late referral to renal unit by referring doctor
Hemodialysis	8 (0)	17 (0)	27 (4)
CAPD	43 (1)	18 (4)	20 (1)
Total	51 (1)	35 (4)	47 (5)

Eventual preferred modality of dialysis treatment	Late presentation to medical profession	Patient decided late on modality of dialysis treatment
Hemodialysis	24 (13)	6 (1)
CAPD	13 (2)	2 (0)
Total	37 (15)	8 (1)

Numbers of patients requiring temporary venous access for first dialysis (90 day mortality figures are in brackets).

We conclude that nephrologists are often too slow to institute appropriate planning for first dialyses, and referring doctors are often still too late in referring their patients with chronic renal failure to a renal unit.

Transfer from pediatric to adult renal unit: Survey and strategy. *A.R. Watson and D. Phillips, Children and Young Peoples Kidney Unit, City Hospital, Nottingham, England, United Kingdom.* The transfer of young people with chronic illness from pediatric to adult units can be difficult for all concerned. There is little information and discussion on the factors involved in the timing and process of transfer. A questionnaire survey dealing with aspects of the process was completed by 43 pediatric nephrologists (23 from the USA) and 18 adult nephrologists (all from the UK). Seventy-five percent of the pediatric units were located in a separate children's hospital and only one third of the adult nephrologists were based in the same hospital as the pediatric unit. Respondents were asked to rank the importance of factors to be taken into account when considering the timing of transfer to the adult unit. Sixty-seven percent of pediatric nephrologists and 77% of adult nephrologists rated maturity as the top factor, followed by patient wishes and age. Few nephrologists would quote a specific age for transfer, with some patients being cared for in pediatric units until they are in their twenties. Factors mentioned for prolonged follow up in the pediatric unit included the need for other sub-specialty follow-up or the inability to offer the same treatment modality in the adult unit; for example, 5/43 pediatric units and 4/18 adult units mentioned the lack of automated peritoneal dialysis at home as being a factor. Five of 43 pediatric units also mentioned the lack of psychosocial support in the adult unit. Liaison visits by the young person to the adult unit seem to be encouraged but there is no set pattern. Many pediatric units favour a liaison visit by the patient accompanied by a nurse or social worker. Only 4/43 pediatric units held a joint clinic in conjunction with adult colleagues, whereas 2 adult nephrologists share joint clinics with pediatricians and 5 thought it was a good idea. It would appear that there are problems to be addressed on both sides. Young people in the pediatric unit need to be actively prepared for transfer to the adult unit and possibly weaned off the very close multi-disciplinary support many enjoy. Transfer to the adult unit needs to be more formalized and there may be problems with "level transfer,' especially with automated peritoneal dialysis. Better liaison and discussion between pediatric and adult units is necessary, and the experience of other sub-specialties transferring young people with chronic illness needs to be shared.

Vascular co-morbidity in patients with atheromatous renovascular disease (RVD). *P. MacDowall, P.A. Kalra, S. Cain, D.J. O'Donoghue, S. Waldek, I. Lang, and H. Mamtora, Department of Renal Medicine, Hope Hospital, University of Manchester, Manchester, England, United Kingdom.* It is well recognized that atheromatous renal artery stenosis (RAS) is strongly associated with more generalized vascular pathology, particularly peripheral vascular disease. Three hundred and seventy-two patients with suspected RVD underwent angiography in this unit between 1986 and 1993; 115 patients with RAS were identified. Data of clinical characteristics were available for analysis in 95 of these latter cases. The mean age of the population was 62.9 years (27–88 years), male: female ratio was 1:1.1 and 60% were smokers. Significant RVD (RAS > 50% or occlusion) were present in 80%. The mortality of this group was 36.8% to Jan. 1996. Comparison was made between those patients with unilateral RAS and those with bilateral disease for manifestations of generalized atheroma. The findings are shown below, and the numbers are % of patients in each group.

	Unilateral stenosis $N = 63$	Bilateral stenosis $N = 32$	Total number $N = 95$
Atheroma distal aorta	36.5%	56.3%	43.2%
Aortic aneurysm	12.6	21.9	15.8
CVA	7.9	9.4	8.4
TIA	9.6	6.3	8.4
Myocardial infarction	4.8*	18.8[a]	9.5
Angina	14.3	25.0	17.8
Cardiac failure	14.3	15.6	14.7
Claudication	27.0	31.3	28.4
Vascular bruits	35.4	31.3	34.7

[a] $P = < 0.05$

The occurrence of symptomatic peripheral vascular disease, renal or femoral vascular bruits and of cerebrovascular disease was similar in the two groups. There was a statistically higher incidence of MI in those patients with bilateral RVD, but this finding was not reflected by an increased mortality. Severe aortic atheroma was also more common in the group with bilateral RVD, and may be a marker of severity and extent of RAS.

Cardiovascular events on dialysis: A prospective analysis of lipoprotein (a) [Lp(a)] and other risk factors. *M. Misra, D.A. Reaveley, M. Seed, and E.A. Brown, Departments of Renal Medicine and Chemical Pathology, Charing Cross Hospital, London, England, United Kingdom.* Lp(a) is an independent cardiovascular risk factor in the general population. We have prospectively examined the association between Lp(a) levels and risk of future clinical cardiovascular events in patients with ESRF, who are about to start dialysis. Lp(a) was measured by ELISA and baseline cardiovascular risk assessment was performed for 95 consecutive patients with ESRF. The relation between future cardiovascular events (using defined criteria) and baseline Lp(a) was studied over a mean follow-up of 25.6 (1 to 38) months. Median Lp(a) was 27.3 mg/liter in patients and 10.3 mg/liter in controls ($P = <0.001$). Median Lp(a) levels on entry, though higher in patients with cardiovascular disease (414 mg/liter), were not significantly different from those without the disease (340 mg/liter, P = NS). Baseline median Lp(a) levels were significantly higher in those with cerebrovascular disease (698 mg/liter), than in those without the disease (334 mg/liter, $P = 0.003$). Baseline Lp(a) was only correlated to pre-existing cerebrovascular disease ($P = 0.005$), but not to pre-existing ischemic heart disease (IHD) or peripheral vascular disease (PVD). Median serum Lp(a) was higher in patients who had events (426 mg/liter)

than in those who did not have events (350 mg/liter), but this was not significant. Stepwise multiple logistic regression analysis revealed that serum Lp(a) at start was not an independent risk factor for future cardiovascular events. However, low albumin ($P = 0.013$), diabetes mellitus ($P = 0.012$) and hemoglobin ($P = 0.032$) were found to be independent risk factors. When data for prevalence of IHD ($N = 65$) and PVD ($N = 64$) was included, previous clinical events due to IHD became significant as a risk factor ($P = 0.015$), unlike pre-existing PVD ($P = 0.081$). These data suggest that elevated Lp(a) levels are not an independent risk factor for predicting future cardiovascular events in patients on dialysis.

Magnetic resonance angiography as a screening test for atheromatous renal artery stenosis. *P. Donohoe, T. Doyle, D. Goldsmith, A. King, J.C. Kingswood, M. Stewart, and P. Sharpstone, Renal Unit, and Department of Diagnostic Imaging, Royal Sussex County Hospital, Brighton, England, United Kingdom.* Magnetic resonance angiography (MRA) is now a first line screening test for atheromatous renal artery stenosis (ARAS), and has benefits over standard arteriography (SA) in that it is completely non-invasive. A prospective comparative study of MRA versus SA was performed in 21 patients with clinical suspicion of ARAS, over a 15 month period. MRA and SA were performed, in most cases, within the same 3 months. SA was performed as an aortic flush, with or without selective renal studies, via the femoral artery. Intravenous digital subtraction angiography was performed (instead of a flush procedure) in 2 patients with severe femoral atheroma. MRA was performed on a Phillips 0.5 Tesla T5 release III, using T1 gradient echo, and 3D phase contrast. One radiologist reported the MRA's, five reported the SA's; all were blinded to the results of other scans. MRA correctly identified the number of renal arteries in 20 of the 21 patients. Using SA as the "gold standard,' MRA had a sensitivity of 92% and a specificity of 62.5%, with regard to detection of ARAS. MRA had a positive predictive value of 62%, but a negative predictive value of only 9%. In 2 cases, MRA correctly identified ARAS lesions that had not been noted on reporting of SA (but evident on re-examining of the films). Statistical analysis showed a chi-squared value of 9.997, indicating excellent statistical agreement between the 2 tests. In 4 patients, MRA overestimated the severity of ARAS due to a tortuous renal artery. Only 3 patients were excluded from the trial, as a consequence of having standard MRA exclusion criteria. MRA was well tolerated by all patients. We conclude that MRA is a useful, non-invasive screening test for ARAS, demonstrating sensitivity and specificity which are in general comparable to that reported with captopril renography (85% and 72%, respectively) and Doppler ultrasound (31 to 91% and 73%), apart from a very low negative predictive value. We will continue to use MRA as a first line screening test for patients with possible ARAS.

Neutrophil FCγRIIA-polymorphism as heritable risk factors for ANCA positive vasculitis. *W.Y. Tse, K. Quibell, D.S. Kumararatne, C.O.S. Savage, and D. Adu, Department of Nephrology, Queen Elizabeth Medical Centre and Department of Immunology, University of Birmingham, Birmingham, England, United Kingdom.* TNFα-primed neutrophils can be activated by ANCA to produce reactive oxygen species (ROS) by cross-linking surface ANCA antigens with neutrophil FcγRIIa receptors. These FcγRIIa receptors exhibit a genetically determined polymorphism (H/H131, R/R131, H/R131) resulting in heterogeneity of affinity for human IgG subclasses. Neutrophils homozygous for the H/H131 allotype bind more efficiently to IgG3 than the R/R131 allotype and is the only human FcγR which binds IgG2. Although ANCA activity can be found in all IgG subclasses, there is a relative increase of IgG3 in acute disease, with an increase of IgG2 in remission. These observations lead us to hypothesize that FcγRIIa polymorphism may play a role in determining disease susceptibility or expression in ANCA-associated vasculitides due to differential IgG binding and activation. The FcγRIIa allelic frequencies were determined in 91 patients with ANCA-positive vasculitis (45 Wegener's granulomatosis, 41 microscopic polyangiitis, 4 polyarteritis nodosa, 1 Churg Strauss syndrome; 68 cANCA, 23 pANCA) and 100 healthy controls. FcγRIIa allotype was detected by Southern blotting using allele specific oligonucleotide probes end labeled with ^{32}P-γ-ATP, after PCR amplification of genomic FcγRIIa DNA; and also by quantitative flow cytometry. We found no overall difference in the allotypic frequencies between vasculitis patients and controls ($\chi^2 = 1.80$, $P = 0.43$). However, there was a significant increase in H/H131 allotype frequency in patients with pANCA vasculitis (odds ratio 3.28, 95% CI 1.20 to 8.99, $P = 0.02$) but not in patients with cANCA vasculitis, compared with controls. These preliminary data suggest that individuals with the homozygous FCγ RIIa-H/H131 allotype are at increased risk of developing pANCA-positive systemic vasculitis. It is possible that the increased reactivity of this receptor for IgG3/IgG2 could lead to a greater production of ROS after binding by ANCA. This preliminary data suggest that individuals with the homozygous FcγRIIa-H/H131 allotype are at increased risk of developing ANCA-positive systemic vasculitis. It is possible that the increased reactivity of this receptor for IgG3/IgG2 could lead to a greater production of ROS after binding by ANCA.

Genetic analysis of susceptibility to EAG in Wistar Kyoto rats. *J. Reynolds, V.O. Newman, T.J. Aitman, D.J. Evans, and C.D. Pusey, Renal Unit, Department of Medicine and Molecular Medicine Group, CSC, Royal Postgraduate Medical School and Department of Histopathology, ICSM St Mary's Hospital, London, England, United Kingdom.* We have previously shown that experimental autoimmune glomerulonephritis (EAG) can be induced in Wistar Kyoto (WKY) rats by an i.m. injection of collagenase-solubilized rat GBM in FCA. This resulted in circulating and deposited anti-GBM antibodies, accompanied by albuminuria, deposits of fibrin in the glomeruli and focal necrotizing glomerulonephritis with crescent formation. We have also shown that Lewis (LEW) rats immunized with the same antigen developed similar levels of circulating anti-GBM antibodies, but minimal glomerular deposits, and no albuminuria or histological evidence of nephritis. This work demonstrates that WKY rats are susceptible to EAG, while LEW rats are resistant. To investigate the genetic base of susceptibility to EAG, we examined the response of both F1(WKY × LEW) and backcross (BC1)(WKY × F1) rats to immunization with rat GBM in FCA. F1 animals developed circulating and deposited anti-GBM antibodies, but no albuminuria or glomerular abnormalities. However, BC1 animals showed a range of responses to the GBM antigen. Thirty-one percent of BC1 rats developed severe crescentic glomerulonephritis, 31% developed mild proliferative glomerulonephritis, and 38% showed no histological evidence of nephritis. The severity of the nephritis correlated with the amount of albuminuria ($r = 0.93$, $P < 0.001$). All BC1 animals developed circulating and deposited anti-GBM antibodies. The Table shows the number of rats from each generation with glomerulonephritis.

		Albuminuria		
Group	*N*	0–10 mg	10–100 mg	>100 mg
WKY	5	0	0	5
LEW	5	5	0	0
F1	12	12	0	0
BC1	13	5	4	4

	Abnormal glomeruli		
Group	0–10%	10–50%	50–100%
WKY	0	0	5
LEW	5	0	0
F1	12	0	0
BC1	5	4	4

These results indicate that susceptibility to EAG is inherited as a recessive trait in this strain combination, and demonstrate the feasibility of mapping the underlying genes by genetic linkage analysis. Mapping studies are now underway using PCR-analyzed microsatellites in segregating BC1 progeny.

Localization of a gene for autosomal dominant inherited hemolytic uremic syndrome to chromosome 1q. *P. Warwicker, J.A. Goodship, Y. Pirson, A. Nicholls, P. Turnpenny, and T.H.J. Goodship, Department of Medicine and Department of Human Genetics, Newcastle upon Tyne, England, United Kingdom; Renal Unit, Cliniques Universitaires St Luc, Brussels; Renal Unit and Clinical Genetics Service, Royal Devon and Exeter Hospital, Devon, England, United Kingdom.* Non infective hemolytic uremic syndrome (HUS) usually affects adults and often carries a poor prognosis. An important although rare subgroup comprises the familial form of the disease. Approximately 20 families have been reported in the literature, with both a dominant and recessive form of inheritance. We

have carried out a linkage study in 3 families with the inherited form of the disorder using microsatellite polymorphisms in the region of the potential candidate genes. A 26 cM cluster of markers located at 1q32, gave a significant lod score (Z max. = 3.95, Θ = 0) in the three families. Using 2 novel polymorphisms we have identified in the factor H gene, we have been able to confirm that it maps within our region of linkage. A role for complement in the pathogenesis of HUS has been suspected for several years, and hypocomplementemia, reflecting activation of the alternative pathway, is often noted in case reports. Factor H, a major plasma protein, occupies a central role in the regulation of this pathway. In summary, we describe genetic linkage between inherited HUS and an area of chromosome 1, which we have shown to contain the gene for factor H and related proteins.

LDL particle size—A vascular risk factor in proteinuria? *C.J. Deighan, M.J. Caslake, M. McConnell, J.M. Boulton-Jones, and C.J. Packard, Renal Unit and Department of Pathological Biochemistry, Glasgow Royal Infirmary University NHS Trust, Glasgow, Scotland, United Kingdom.* Heavy proteinuria is associated with marked abnormalities of lipoprotein metabolism and increased risk of atherogenesis. LDL exhibits heterogeneity with small, dense LDL III particles being more atherogenic. The aim of this study was to investigate LDL subfractions in patients with heavy proteinuria. LDL subfractions were measured by density gradient ultracentrifugation in 12 patients with 1° glomerular disease and 24 hour albuminuria >2.5 g, along with VLDL subfractions and post-heparin lipases. These were compared to 23 age- and sex-matched normolipemic controls randomly extracted from the database in the Department of Pathological Biochemistry. Total LDL concentrations were similar (patients 285 ± 28 mg/dl, control 334 ± 18 mg/dl, $P = 0.1$). Each subfraction, however, was significantly different with LDL I and LDL II being lower in the proteinuric group (32 ± 7 mg/dl vs. 62 ± 5 mg/dl, $P = 0.011$ and 121 ± 23 mg/dl vs. 193 ± 16 mg/dl, $P = 0.041$), whereas atherogenic LDL III (small dense) was higher in the proteinuric group (135 ± 18 mg/dl vs. 75 ± 15 mg/dl, $P = 0.0016$). Total VLDL concentrations and VLDL subfractions were all increased in the patients with proteinuria (VLDL total 339 ± 80 mg/dl vs. 103 ± 13 mg/dl, $P = 0.014$, VI 192 ± 51 mg/dl vs. 58 ± 9 mg/dl $P = 0.028$, V2 147 ± 34 mg/dl vs. 45 ± 5 mg/dl $P = 0.049$). Post-heparin hepatic and lipoprotein lipase levels were similar. These findings suggest that atherogenic LDL is significantly more common in patients with heavy proteinuria. Since small dense LDL has a lower affinity for the LDL receptor, the altered nature of the lipoprotein in proteinuria may decrease its clearance by the receptor mediated pathway and contribute to the reduced clearance of LDL observed in this population. This may contribute to the accelerated vascular disease found in patients with heavy proteinuria.

Targeted prescription dialysis delivery and quality of life—A prospective controlled intervention trial. *W.D. Plant, K.J. Craig, S.W. Walker, R.J. Prescott, and R.J. Winney, Department of Renal Medicine and Clinical Biochemistry, Royal Infirmary of Edinburgh NHS Trust; Medical Statistics Unit, Edinburgh University, Edinburgh, Scotland, United Kingdom.* **Study objective:** To evaluate if introduction of an operational strategy to deliver targeted prescription dialysis leads to improved patient quality of life (QoL) or impacts upon nutritional status, morbidity and survival. **Study design:** Prospective controlled intervention trial over 12 months. The Control (C) group was provided by minimization of the variance of imbalance for selected levels of 24 sociodemographic, co-morbid medical, treatment and QoL variables. Dialysis prescription was adjusted proactively in the intervention (I) group to attempt delivery of a target dialysis dose [Kt/V of 1.2 per treatment for hemodialysis and total weekly creatinine clearance (WCC) of 50 l/week/1.73 m^2 for peritoneal dialysis]. Changes from baseline in the McMaster Health Index Questionnaire score, the Hospital Anxiety and Depression Scale, the Karnofsky Scale, and serum albumin levels were assessed. Number of days of hospitalization was used as a surrogate measure of intercurrent morbidity. The study has 80% power at the 5% level to detect significant changes in most variables of interest. **Subjects:** One hundred fourteen clinically stable ESRD patients, treated by dialysis for more than 6 months, entered the study. Eighty-two (72%) remained on dialysis for 1 year. Fifty-seven (50%) completed all stages of the protocol. **Results:** Eighteen percent of patients died. Nine percent were transplanted. The proportion achieving target dialysis dose rose from 30% to 43% in group (I), and fell from 47% to 27% in group (C). Median (IQR) inpatient days were 6(28) versus 5(23), respectively. Mean (95% CI) changes in serum albumin were +0.80 (−0.2, +1.9) versus −1.0 (−2.5, +0.5) g/liter respectively. Changes from baseline in all QoL scores were modest and did not differ between groups. No change from baseline in any of these outcomes of interest achieved statistical significance. **Conclusion:** With the operational strategy employed in this study, an attempt to introduce target prescription dialysis delivery did not lead to any improvement in a number of important outcome variables after 1 year.

Lupus nephritis, renal dysfunction and associated anticardiolipin antibody and intraglomerular thrombi. *S. Bhandari, A.M. Brownjohn, J.H. Turney, P. Harnden, Renal Unit, Leeds General Infirmary, Leeds, England, United Kingdom.* The presence of intraglomerular capillary thrombi (ICT) has been described in association with anti-cardiolipin (ACA) positivity in patients with systemic lupus erythematosis (SLE). However, controversy remains as to its significance in progression of renal disease. We performed a retrospective study of 50 patients with SLE (10 male, 40 female), mean age 37 years, and clinically evident nephritis confirmed by renal biopsy. Analysis of biopsies revealed that the percentage of sclerosis, crescent formation and necrosed glomeruli were greater in both specimens positive for thrombi and from patients with positive serum ACA. An increase in serum anti-DNA and ANA antibodies and a reduction in C3 and C4 were significant in ACA+ patients, with a strong relationship to disease activity when compared with changes in ACA negative patients ($P < 0.05$ in all cases). No significant difference occurred when patients were separated according to the presence or absence of thrombi. The presence of ICT correlated with positivity for ACA ($P = 0.0015$), ACA positivity was associated with renal dysfunction at presentation ($P = 0.042$), while the presence of ICT was significant at conclusion ($P = 0.007$). In patients with both thrombi and positivity for ACA, renal progression was worse ($P = 0.0054$). Hypertension and nephrotic range proteinuria were more common in thrombi positive or ACA positive biopsies. Twenty-one thrombotic episodes occurred in 14 patients of which 13 were ACA+. In conclusion, ACA is a strong predictor of the presence of intraglomerular thrombi in SLE patients with renal involvement. Thrombi presence indicates a worse long-term renal outcome. ACA positivity is a strong predictor of vascular thrombotic complications. In patients with SLE, ACA status and biopsy screening for the intraglomerular thrombi is important.

An intact tissue RAS is required for the transition to malignant hypertension. *C. Whitworth, H. Montgomery, L. Kiernan, S. Fleming, T. Ungar, P. Gohlke, J. McEwan, J. Mullins, Leicester University, Leicester, and UCLMS, London, England, United Kingdom; Department of Pathology, University of Edinburgh and Centre for Genome Research, Edinburgh, Scotland, United Kingdom; and Universität zu Kiel.* An intrinsic role for the RAS in the genesis of MH in transgenic *Ren*-2 rats has been suggested by previous studies, but it is not known whether this is independent of direct pressure. The ACE inhibitor, Ramipril, was administered to male *Ren*-2 heterozygotes from 28 days of age at a dose of 5 μg/kg/day. SBP was measured by tail cuff plethysmography in treated ($N = 24$) and untreated controls ($N = 40$) from 30 to 120 days of age (×3/wk). SBP did not differ in the treatment period, reaching means of ~250 mm Hg by 50 days of age in both groups. Sixty-three (25/40) untreated rats died with MH (confirmed by light microscopy) compared with only 4% (1/24) treated rats. Histological examination showed medial hyperplasia, but no fibrinoid necrosis in the treatment group. Features of chronic hypertensive renal damage were common in untreated survivors, but less frequent or severe in the ACEI treated group. Tissue ACE activity was significantly reduced in resistance vessels, myocardium and kidney, in treated rats while *in vitro* plasma ACE activity rose by 52%. In conclusion, ACE inhibition at a non-hypotensive dose protected rats from developing MH and reduced the severity of chronic renal damage, suggesting that an intact tissue RAS may be involved in hypertension-induced renal injury.

Relationship between plasma Leptin, fat and diet in chronic renal failure. *G.A. Young, G. Woodrow, S. Kendall, B. Oldroyd, L. Tompkins, J.H. Turney, and A.M. Brownjohn, Renal Research Unit, General Infirmary, Leeds, England, United Kingdom.* Loss of appetite contributes to malnutrition in chronic renal failure. Recent studies suggest that Leptin, the ob gene protein, has an endocrine function for lowering food intake and adiposity. Increased plasma concentrations in obesity may be ineffective due to resistance or reduced brain uptake. We have compared plasma

leptin with body composition, measured by DEXA and other techniques, in subjects grouped as undialyzed CRF ($N = 23$), PD (24), HD (22) and controls (24). Plasma leptin was higher for PD patients (35 ng/ml vs. 13.8 ng/ml in controls), although most had a similar percentage of fat to controls. Leptin was not significantly increased for HD and CRF groups, but both had less fat than controls. However, leptin was more than fivefold greater for some individuals in all patient groups. Higher leptin in females was due to higher body fat. Leptin was highly correlated with total, arm, leg and all other fat measurements, for example, Rs for leptin vs. % total fat was: CRF 0.88, PD 0.81, HD 0.93 and controls 0.83 ($P < 0.0001$ for all). Lean tissue was lower in dialyzed patients than in controls. Leptin correlated inversely with total lean tissue/height^2, that is, Rs = -0.38 ($N = 69$). Dietary intake was assessed in dialysed patients and leptin correlated inversely with energy Rs = -0.47, and protein intake, Rs = -0.54 ($P < 0.001$ for both). Leptin also correlated with cholesterol, C3, triglycerides and insulin. In dialyzed and non-dialyzed patients, leptin is higher than for normals with comparable body fat and probably contributes to loss of appetite and malnutrition. The close association with fat indicates its value as a marker of body composition.

Effect of nitric oxide synthase (NOS) inhibition on renal function and hemodynamics in endotoxemia. *C.G.M. Millar, C. Thiemermann, and J.R. Vane, The William Harvey Research Institute, St Bartholomew's and the Royal London School of Medicine and Dentistry, London, England, United Kingdom.* An overproduction of nitric oxide (NO) contributes to the hemodynamic alterations and organ dysfunction in septic shock. NOS inhibition improves hypotension and restores vascular responsiveness to vasopressor agents, but regional perfusion may be impaired. We have investigated the effect of the non-selective NOS inhibitor N^G-methyl-L-arginine (L-NMMA) on systemic and renal hemodynamics and renal function in a rat model of endotoxemia induced by lipopolysaccharide (LPS, 1 mg/kg over 30 min). Hemodynamic parameters included mean arterial blood pressure (MAP), renal blood flow (RBF) and cortical (C flux) and medullary (M flux) laser Doppler flow. Clearance of ^{3}H-inulin (C_{in}) was used as an index of glomerular filtration rate. All animals received NACI 0.9% at 3 ml/hr. Following a control period, vehicle ($N = 6$) or L-NMMA (50 μg/kg/min, $N = 7$) was infused.

		Control	Post-LPS 1–2 hr
MAP *mm Hg*	LPS + Vehicle	112 ± 3	102 ± 6
	L-NMMA	110 ± 2	112 ± 2
C_{in} *% control*	Vehicle	100	44 ± 7
	L-NMMA	100	65 ± 4
C flux *% control*	Vehicle	100	81 ± 3
	L-NMMA	100	73 ± 3
M flux *% control*	Vehicle	100	78 ± 6
	L-NMMA	100	80 ± 8

	Post-LPS 3–4 hr	Post-LPS 5–6 hr
MAP *mm Hg*	97 ± 6[a]	91 ± 4[a]
	108 ± 5	103 ± 4
C_{in} *% control*	47 ± 3	44 ± 2
	69 ± 5[b]	75 ± 6[b]
C flux *% control*	79 ± 4	75 ± 6
	62 ± 4[b]	69 ± 4
M flux *% control*	77 ± 9	70 ± 13
	61 ± 7	54 ± 6

[a] $P < 0.05$ vs. control
[b] $P < 0.05$ vs. vehicle

Following LPS, MAP was maintained until it fell at 4 hr. L-NMMA prevented this late fall. C_{in} fell immediately after LPS and remained reduced for 6 hr. In rats receiving L-NMMA, C_{in} recovered to 75% baseline by 6 hr. RBF did not change significantly. The reduction in C flux was enhanced by L-NMMA. In this model, M flux fell in both groups. Thus, L-NMMA (at a dose that does not affect total RBF) improves renal function in endotoxemia.

Visceral glomerular epithelial cell proliferation in experimental and human membranous nephropathy. *S. Harper, E. Bailey, F. Baker, P.N. Furness, D.J. Salant, and J. Feehally, Departments of Nephrology and Pathology, Leicester General Hospital, Leicester, and Richard Bright Renal Unit, Southmead Hospital, Bristol, England, United Kingdom; and Renal Section, Boston University Medical Centre, Boston, Massachusetts, USA.* Human membranous nephropathy (MN) is not a proliferative pattern of glomerular disease. However, visceral glomerular epithelial cell (vGEC) proliferation has been claimed as a feature of passive Heymann nephritis (PHN, a model of MN); these studies used proliferating cell nuclear antigen, a non-specific marker of cellular proliferation. We have used the non-isotopic *in situ* hybridization demonstration of histone mRNA, an S-phase specific marker, to study glomerular cell proliferation in PHN and MN. PHN was induced in 24 Sprague-Dawley rats by tail vein 0.75 ml injection of sheep antibody to Fx1A on days 1 and 2. Eight controls received saline or sheep serum, and 12 animals (PHN = 8, Saline = 2, Serum = 2) were sacrificed on day 5, the same number on day 10. Twenty-four hour urinary protein loss was quantified on day 0 and prior to sacrifice. Renal biopsies from 10 cases with MN and proteinuria, and 10 age- and sex-matched controls were also studied. Controls had normal biopsies and no proteinuria. Twenty-four hour PHN animals had heavy proteinuria by day 10 and demonstrated significantly more histone mRNA positive cells per glomerulus by day 5 compared to controls (0.53 ± 0.09 vs. 0.195 ± 0.045, $P < 0.01$). There was a significant increase in histone mRNA positive glomerular cells in patients with MN compared to controls (0.24 ± 0.07 vs. 0.04 ± 0.018 positive cells per glomerular cross-sectional area respectively, $P < 0.03$). In both PHN and MN studies, cells at the periphery of the glomerular tuft with typical morphology of vGECs were histone mRNA positive. Therefore, vGEC proliferation is a feature of PHN soon after the onset of complement-mediated vGEC injury. In addition, a significant increase in S-phase cells was seen in MN in a similar distribution to that in PHN. These data call into question the notion that mature vGECs are terminally differentiated and incapable of cell division.

Effects of angiotensin-converting enzyme inhibition on glomerular volume in experimental diabetes. *P. Mackin, K.E. White, R.W. Bilous, and S.M. Marshall, Department of Medicine, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, England, United Kingdom.* Experimental diabetes is associated with renal enlargement and hyperfunction. Total glomerular volume is increased by 30% after only 4 days of streptozotocin diabetes. Altered renal hemodynamics, resulting in raised interglomerular pressure, may play a role in the initial renal enlargement observed in diabetes. We investigated this hypothesis by studying the effects of trandolapril (0.01 mg kg^{-1} day^{-1}) on renal morphology, in the first week of experimental diabetes. This dose is sufficient to reduce tissue ACE activity by 86%, with only a minimal effect on systemic blood pressure. Five groups of male Wistar rats were studied: C (control); CTr (control + trandolapril); D (diabetic); DTr (diabetic + trandolapril) and DI (diabetic + insulin). Diabetic animals had marked hyperglycaemia throughout the study period (day 7 blood glucose values (mean ± SD) D = 22.2 ± 3.4 mmol l^{-1}; DTr = 24.1 ± 3.7 mmol l^{-1}, P = NS), and blood glucose levels were similar in groups C, CTr and DI(C = 5.3 ± 1.0 mmol l^{-1}; CTr = 4.8 ± 0.4 mmol l^{-1}; DI = 5.6 ± 1.6 mmol l^{-1}, P = NS). Animals were sacrificed on day 7. Kidney volume (C = 0.868 ± 0.061 mm^3; D = 1.169 ± 0.059 g; DTR = 1.059 ± 0.114 g) and weight (C = 0.902 ± 0.48 g; D = 1.202 ± 0.097 g; DTr = 1.15 ± 0.119 g) was increased in groups D and DTr ($P < 0.01$ vs. other groups). However, total glomerular volume was increased in group D only (37.3 ± 4.2 mm^3) and was similar in all other groups (C = 28.0 ± 2.3 mm^3; DI = 27.0 ± 2.4 mm^3; DTr = 26.7 ± 7.6 mm^3, $P < 0.05$ vs. D). These data demonstrate that amelioration of intraglomerular pressure by ACE inhibition does not prevent the increase in whole kidney size, but has a salutary effect on glomerular enlargement, suggesting that differential growth is occurring within the kidney.

Progression of diabetic nephropathy (DN)—Is diurnal BP rhythm more important than BP level? *C.K.T. Farmer, J. Cox, P. Dallyn, P. Sharpstone, J.C. Kingswood, J. Quinn, and D.J.A. Goldsmith, Trafford Department of Renal Medicine, Royal Sussex County Hospital, Eastern Road, Brighton, England, United Kingdom.* Factors that are implicated in renal functional decline in DN include diabetic control (HbA1c) and blood pressure. In chronic renal failure and diabetes, abnormal diurnal BP is commonly seen when ambulatory BP monitoring (ABPM) is performed;

this is thought to be an additional risk for end-organ damage. We studied 26 patients with known DN and declining renal function, who underwent 38 ABPM recordings between 1990 and 1996, to assess the relative contributions to progression of DN from: (a) overall BP levels; (b) diurnal BP rhythm; and (c) diabetic control. Diurnal BP was defined as normal if >/=10% fall in SBP at night (that is, "Dipper"). Results are in the table (Mann-Whitney U-test; $P < 0.05$):

	Dippers	Non-dippers	*P*
Number	13	13	
Age *years*	55.2	51.7	NS
SBP *awake, mm Hg*	144	152	NS
SBP *sleep, mm Hg*	126	144	<0.05
Creatinine *μmol/liter*	116	192	<0.05
24 hr protein *g*	2.7	3.5	NS
ΔGFR *ml/min/yr*	−2.9	−7.9	<0.05
HbA1c %	7.4	8.5	NS
IDDM/NIDDM	3/10	4/9	NS

These results demonstrate a relationship between BP and rate of renal functional decline in DN. There is a profound effect of dipping/non-dipping status, which is at least as important as the BP level. Diabetic control (HBA1c) is irrelevant, at least in this study. It may be that non-dipping BP status is a marker for other adverse factors, possibly autonomic dysfunction.

An epidemiological study of patients with renovascular disease in a single center. *P. Macdowell, P.A. Kalra, S. Cain, D.J. O'Donaghue, S. Waldek, I. Laing, and H. Mamtora, Department of Renal Medicine, Hope Hospital, University of Manchester, Manchester, England, United Kingdom.* Three hundred seventy-two angiographic studies for suspected RVD were performed between 1986–1993 in this unit. One hundred thirteen patients were found to have RVD. A retrospective analysis of the epidemiological characteristics was performed in 95 patients where data were available. Eighty percent had significant lesions (RAS > 50% or occlusion = RAO). The mean age at time of investigation was 62.9 years (range 27–88 years), male: female ratio was 1:1.1; 61.1% were smokers, 28.4% had peripheral vascular disease, 45.3% had significant hypertension (systolic BP > 170 mm Hg, diastolic BP > 100), and 15.8% were diabetic. Nineteen patients had RAS < 50% (17 unilateral, 2 bilateral). The angiographic findings and renal function of patients with significant RAS are shown below.

	Unilateral stenosis	
	>50%	RAO
Renal function	21 (R12L9)	23 (R13L10)
Normal	7	2
Mild-Mod.	11	15
Severe	3	6

	Bilateral stenosis				
	1 < 50% 1 > 50%	1 < 50% 1 RAO	2 > 50%	1 > 50% 1 RAO	Bilateral RAO
Renal function	2	7	11	4	8
Normal	1	1	1	0	0
Mild-Mod.	1	5	8	3	0
Severe	0	1	2	1	All RRT

Of the patients with anatomically insignificant stenosis, 12 had normal renal function and 7 mild-moderate renal impairment (creatinine 120–450 μmol/liter). As can be seen, 8 patients had bilateral occlusion, and all of these patients presented with dialysis-requiring renal failure. Twelve others had severe renal failure (creatinine ≥ 450 μmol/liter), of whom 8 had unilateral occlusion. Mean follow-up was 50.5 months to 1/1/96 or to death. Mortality was 36.8% during this period. This study highlights the relationship of RVD with PVD, smoking and renal failure and indicates the high mortality of these patients due to excess cardiovascular co-morbidity. Of the 23 patients with significant bilateral RVD, 8 needed RRT at presentation and 40% of the remainder progressed to ESRF over a mean period of 46.5 months.

Down-regulation of the erythropoietin receptor in uremia. *D.A. Allen, I.C. Macdougall, and M.M. Yaqoob, Anthony Raine Research Laboratories, St. Bartholomew's Hospital, and Department of Nephrology, King's College Hospital, London, England, United Kingdom.* Hyporesponsiveness to erythropoietin (EPO) in uremic patients is well-documented, but the cellular mechanism(s) remains to be elucidated. Previous studies have shown that sera from uremic patients with or without inflammatory disease cause a blunted bone marrow response to EPO *in vitro*. The aim of this pilot study was to determine the level of EPO receptor (EPOR) expression in an erythroid cell line, TF1, under normal and uremic conditions. TF1 cells were maintained in RPMI-1640 medium containing 10% FCS. 10^6 cells/ml were seeded in medium containing 1 U/ml EPO and either: (a) 10% normal human serum; or (b) 10% uremic human serum for 48 hours at 37° in 5% CO_2 in air. Cells were then washed and lysed in RIPA buffer containing protease inhibitors. After centrifugation at 15000g supernatants were removed and 10 μg of protein was subjected to SDS-PAGE and electroblotted onto nitrocellulose membrane. Membranes were blocked overnight and immunoblotted with rabbit anti-human EPOR polyclonal antibody (1:2000) followed by HRP-conjugated anti-rabbit IgG (1:3000). Signals were detected using the Amersham-ECL system. Blots were subjected to densitometric analysis and band density expressed as peak area (pA) and % control pA. Control TF1 cells express a 46 kDa EPOR protein, consistent with published data (pA = 3937; 100%). Cells cultured in 10% uremic serum expressed fewer EPOR when compared with controls (pA = 1814; 46%). This preliminary data suggests that EPOR is down-regulated by a factor(s) present in uremic serum. The effect of sera from uremic patients who are resistant to EPO on EPOR expression remains to be determined.

Urinary tissue factor levels in glomerulonephritis: A potential marker of glomerular injury? *B.A. Lwaleed, P. Bass, M. Chisholm, and J.L. Francis, University Department of Haematology and Department of Pathology, Southampton University Hospitals, Southampton, England, United Kingdom; and Hemostasis and Thrombosis Research Unit, Walt Disney Memorial Cancer Institute at Florida Hospital, Altamonte Spring, Florida, USA.* Coagulation activation and fibrin deposition are common events in human and experimental glomerulonephritis (GN). These phenomena occur by multiple pathways, but local expression of tissue factor (TF) by infiltrating monocytes/macrophages (mTF) and resident glomerular cells may be involved. TF is found in the urine (uTF) where levels may mirror glomerular activation. We have recently developed a highly standardized assay for uTF. Using this assay, we measured uTF levels in controls (healthy volunteers and patients with uncomplicated renal stones) and in patients with immune complex (IC) GN and non-IC GN. The uTF level was significantly higher in patients with GN compared to normals ($P < 0.01$) or renal stones ($P < 0.05$). uTF activity correlated with the Protein Creatinine Index (PCI, $r = 0.41$, $P < 0.001$) and 7 patients with GN and a PCI ≤ 0.1 g/mmol had elevated uTF levels. We then subdivided the GN patients into two groups depending on the PCI: <0.2 g/mmol creatinine (mild to moderate proteinuria, group I) and ≥0.2 g/mmol creatinine (heavy proteinuria, group II). Analysis of group I showed there was a significant difference between normals and the IC ($P < 0.01$) and non-IC ($P < 0.05$) groups. In group II, the IC group showed significantly higher uTF level compared to normals ($P < 0.001$) and renal stones ($P < 0.01$). A significant difference was also observed between non-IC and normals ($P < 0.01$). When the GN groups were divided into broad WHO subtypes, the significance level varied with the type of the GN. We conclude that uTF levels are significantly raised in patients with GN, particularly in heavy proteinuria. In addition the level of uTF may reflect the etiopathogenesis of GN.

Increased vascular permeability factor (VPF) mRNA expression in minimal change disease (MCD). *M.J. Bottomley, P.E.C. Brenchley, N.J.A. Webb, I.S.D. Roberts, J. Feehally, and S. Harper, Renal Research Group, St Mary's Hospital, Manchester and Department of Nephrology, Leicester General Hospital, Leicester, England, United Kingdom.* VPF is a disulphide linked homodimer of 34 to 42 kDa with potent capillary enhancing properties and has previously been implicated in the pathogenesis of the heavy proteinuria associated with MCD. *In situ* hybridization (ISH) and

immunohistochemical studies have shown VPF to be constitutively expressed by glomerular epithelial cells (GECs) in the normal adult kidney. Renal biopsies from 10 proteinuric adults with MCD were investigated for VPF expression. All had normal renal function at the time of biopsy. Ten age/sex matched non-proteinuric adults with normal renal function biopsied for microscopic hematuria acted as controls. All had normal biopsies with no evidence of foot process effacement, electron dense deposits or thin basement membrane at EM level. VPF mRNA expression was investigated using non-isotopic ISH. mRNA for all four alternatively-spliced VPF transcripts was detected using twelve 3Omer hapten labelled oligonucleotide probes spanning the entire VPF sequence. Alkaline phosphatase labeled Fab fragments to hapten and nitro blue tetrazoleum substate were used for the final detection step. Biopsies, reviewed by a single investigator blinded to the clinical histories, were scored according to the number of cells expressing VPF mRNA per glomerulus. No significant difference was detected in glomerular size between MCD and control biopsies. VPF mRNA was only detected in GECs consistent with the findings of other groups. The number of GECs expressing VPF per glomerulus was significantly higher in MCD than control biopsies ($P <$ 0.3). mRNA positive GECs were uniformly distributed within the glomerulus in normal biopsies, though clustered at the peripheries in MCD biopsies. There was no difference in glomerular histone mRNA expression between the two groups ($P = 0.03$). Analysis of urinary protein data revealed a significant correlation between 24-hour protein excretion at the time of biopsy and the number of VPF mRNA positive cells per glomerulus ($r = 0.80, P = 0.01$). Up-regulation of intrarenal VPF mRNA may be central to the pathogenesis of proteinuria in MCD.

Altered O-glycosylation of IgA1 in Henoch Schönlein purpura is restricted to subjects with nephritis. *A.C. Allen, F. Willis, S.J. Harper, T.J. Beattie, and J. Feehally, Department of Nephrology, Leicester General Hospital, Leicester and Renal Unit, Royal Hospital for Sick Children, Glasgow, Scotland, United Kingdom.* We have previously described abnormal O-glycosylation of IgA1 in IgA nephropathy (IgAN), detected by increased binding of the lectins *Vicia Villosa* (VV) and *Helix* aspersa (HA). Henoch Schönlein purpura (HSP) is a systemic vasculitis with IgA deposition in various sites, often including the kidney. The aims of this study were to investigate whether the O-glycosylation defect of IgA1 seen in IgAN is also found in HSP, and whether HSP patients without nephritis display the same abnormality. Serum was obtained from children with HSP with ($N = 28$) and without ($N = 22$) nephritis, and from adults with HSP nephritis ($N = 33$), IgAN ($N = 20$), and membranous nephropathy (MN) ($N = 8$). Age- and sex-matched controls for each group were also recruited. Ammonium sulphate precipitates were prepared from the sera and applied to anti-IgA coated immunoplates. Biotinylated VV or HA lectins were applied and their binding to the O-glycans detected with peroxidase-avidin and OPD substrate. The results were expressed as OD at 492 nm. In both IgAN and HSP in adults, IgA showed raised lectin binding as compared to controls (IgAN 0.56 ± 0.04 vs. 0.39 ± 0.03, $P =$ 0.001; HSP 0.53 ± 0.02 vs. 0.48 ± 0.02), but in MN, lectin binding was normal (0.45 ± 0.06 vs. 0.42 ± 0.06, $P =$ NS). Serum IgA from children with HSP and nephritis also showed significantly higher lectin binding than matched controls (0.53 ± 0.04 vs. 0.41 ± 0.01, $P < 0.004$). However, IgA from children with HSP but without nephritis had significantly lower binding than those with nephritis (0.41 ± 0.02 vs. 0.53 ± 0.04, $P < 0.005$), and did not differ from controls. These data show abnormal O-glycosylation of IgA1 in IgAN and in HSP with nephritis, but this is not seen in HSP without nephritis, nor in MN. Altered IgA1 O-glycosylation contributes directly to renal IgA deposition and the development of glomerular damage in IgA-associated renal disease.

Diagnostic value of standardized assays for ANCA in systemic vasculitis. *D.R.W. Jayne, G. Gaskin, C.D. Pusey, C.M. Lockwood, for the EC/BCR ANCA Standardisation Study Group (Co-ordinator: FW van der Woude), Division of Renal Medicine, St George's Hospital Medical School, and Renal Unit, RPMS, London; and Department of Medicine, University of Cambridge, Cambridge, United Kingdom.* ANCA are widely used as diagnostic markers for the primary systemic vasculitides, Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and renal-limited vasculitis (RLV). The current, standard ANCA test is indirect immunofluorescence (IIF). The diagnostic value of ANCA measurement by IIF and by antigenic specific ELISAs for antibodies to proteinase 3 (anti-PR3) and to myeloperoxidase (anti-MPO), was evaluated in patients with primary systemic vasculitis. Fourteen centers entered 174 new patients with WG, MPA or RLV, classified by pre-defined criteria, and the results compared to 184 disease, and 740 healthy, controls. IIF used a standard method, while the ELISAs were previously standardized between participants. Results for the sensitivity of each assay and for the assay combinations, cANCA/anti-PR3 positive or pANCA/anti-MPO positive were calculated and the specificity of the assays or their combinations compared to results from the disease controls.

	Sensitivity %			
	WG	MPA	RLV	Specificity %
cANCA (IIF)	68	23	36	95
pANCA (IIF)	19	58	45	81
anti-PR3 (ELISA)	69	26	50	87
anti-MPO (ELISA)	25	58	64	91
cANCA + anti-PR3	58	10	33	99
pANCA + anti-MPO	17	55	41	99

The value of IIF for ANCA detection can be greatly increased by the addition of antigen specific ELISAs, when the specificity of a positive combination rises to 99% at the expense of a loss of around 10% in sensitivity. A number of patients with primary systemic vasculitis remain ANCA negative.

Detection of renovascular disease (RVD) in elderly patients with congestive cardiac failure (CCF). *P. Macdowell, P.A. Kalra, D.J. O'Donaghue, S. Waldek, K. Brown, and H. Mamtora, Department of Renal Medicine, Hope Hospital, University of Manchester, Manchester, England, United Kingdom.* Occult RVD frequently occurs in the elderly, is associated with generalized atheromatous disease, and is increasingly recognized as a cause of end-stage renal failure. ACEI are widely used in treatment of congestive cardiac failure (CCF) in an aging population who are likely to have a high prevalence of RVD. Captopril renography (CR) is a useful test for the detection of significant renal artery stenosis (RAS ≥ 50% or occlusion) in selected hypertensive patients. Magnetic resonance angiography (MRA) allows the non-invasive detection of RAS. Seventy-three patients, aged 70 to 94 years (mean 79.4 years), presenting with CCF to the Medical unit were recruited. Serum creatinine was 75–289 μmol/liter (mean 185 μmol/liter). All underwent CR with MAG-3 prior to the initiation of ACEI therapy. Captopril 25 mg (oral) was administered 1 hour before the second renogram. Renograms were analyzed semiquantitatively. CR was considered positive if: (1) time to peak was delayed > 5 min; (2) split function changed > 5%; (3) there was > 30% cortical retention at 20 min; (4) significant deterioration in shape of the curve occurred. MRA was performed with a Siemens Magnetron Expert 1.0 tesla using 3-dimensional phase contrast studies. All patients with positive or equivocal renography and 40% of the 47 patients with negative renograms underwent MRA; renal artery anatomy was normal in the latter 19 patients. CR was positive in 26 patients (35.6%). Five patients had a suspected unilateral occlusion at CR (no isotope uptake) that was confirmed at MRA, and were able to tolerate ACEI without any decline in renal function. Five other patients were found to have significant bilateral RVD (strongly positive CR and RAS ≥ 50% at MRA) and were advised to avoid ACEI. Fourteen patients had unilateral RAS at MRA as predicted by CR appearance. Two patients had abnormal baseline renograms but had no change or improved post-captopril; these patients had normal MRA. Further analysis of the epidemiological characteristics demonstrate a strong link with peripheral vascular disease, smoking and renal impairment. The prevalence of occult RVD was 32.9% in this elderly population with CCF. CR had a sensitivity of 100% and a specificity of 92% for detecting RVD in this group, and may predict outcome after initiation of ACEI.

***In vitro* and *in situ* production of IL-8 in ANCA-associated vasculitis.** *P. Cockwell, C.J. Brooks, D. Adu, and C.O.S. Savage, Renal Immunobiology, CCRIS, Medical School, University of Birmingham, Birmingham, England, United Kingdom.* Tissue production of the CXC chemokine IL-8 directs tissue infiltration and activation of neutrophils. In contrast, intravascular IL-8 production may disrupt this orderly sequence. We examined (i) the ability of ANCA to stimulate neutrophil IL-8 production using an ELISA and (ii) renal IL-8 mRNA expression using *in situ* hybridization with a ^{35}S

labeled IL-8 riboprobe in patients with ANCA positive vasculitis. Neutrophils were isolated from normal healthy donors, primed using cytochalasin B and TNFα and stimulated with ANCA rich IgG isolated from the serum of patients with vasculitis. ANCA stimulated time and dose dependent IL-8 production, maximal at 200 μg/ml IgG, with peak levels at 4 to 8 hours. Four out of five ANCA rich IgGs (3/3 pANCA, 1/2 cANCA) stimulated IL-8 production (max 647 ± 72 pg/ml/5 × 10^5 cells). Normal human IgG was non-stimulating. IL-8 mRNA was expressed by proximal tubular epithelium and interstitial and glomerular associated inflammatory cell infiltrates in renal biopsies from patients with ANCA positive small vessel vasculitis. Renal biopsies from non-inflammatory glomerulonephritis were negative for IL-8 mRNA, as were the sense probes. While tissue IL-8 may direct neutrophil infiltration, we hypothesize that intravascular production of high levels of IL-8 by ANCA-activated neutrophils may oppose successful migration to extravascular sites. In this situation, frustrated transmigration of activated neutrophils may contribute to endothelial injury.

Role of T cell activation in the initiation and progression of chronic renal allograft dysfunction. *A. Chandraker, H. Azuma, K. Nadeau, M. Schaub, W.W. Hancock, N.L. Tilney, C.B. Carpenter, and M.H. Sayegh, Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston Massachusetts, USA. (Introduced by Dr. M. Edmunds, Walsgrave NHS Trust, Coventry, England, United Kingdom.)* Chronic rejection remains the most important cause of long-term renal allograft loss. The pathogenetic mechanisms of chronic rejection are elusive. We investigated the role of T cell costimulation in the initiation and propagation of chronic rejection in an experimental rat transplantation model. We used CTLA41g, a recombinant fusion protein which blocks the CD28-B7 T cell costimulatory pathway to inhibit T cell activation. We studied the effects of CTLA41g when administered early and late post-transplantation. The following groups were set up (N = 7–30/group):

Group 1	Received CsA (0.5 mg/kg/day, day 0–10)
Group 2	CTLA41g (0.5 mg on day 2)
Group 3	CsA plus CTLA41g (as above)
Group 4	CsA (0.5 g/kg/day, day 0–10 plus CTLA41g (0.5 mg at 8 weeks)
Group 5	Isograft controls

Outcome was measured in terms of survival, proteinuria, morphology and cytokine expression by RT-PCR. The survival rate was 72% at >100 days in group 1 compared with 92–100% in the other groups. Animals treated with CsA alone developed progressive proteinuria and typical changes of chronic rejection, namely cellular infiltration, glomerulosclerosis and graft arteriosclerosis. Proteinuria as well as morphologic changes of chronic rejection were significantly reduced by CTLA41g therapy. Grafts from CTLA41g treated animals (day 2 and 8 weeks groups) had significantly decreased transcript levels for activation and inflammatory cytokines and TGF-β. These data indicate that T cell activation is important in both the initiation and progression phases of chronic rejection. These observations should have clinical relevance for organ transplantation.

An association between CMV infection and transplant renal artery stenosis. *O.I. State, W. Wong, and B.M. Hendry, Renal Medicine, King's College Hospital, King's College School of Medicine and Dentistry, London, England, United Kingdom.* The stenosis of coronary arteries both after angioplasty and following cardiac transplantation has been linked to CMV infection. Similar mechanisms could contribute to the incidence of transplant renal artery stenosis (TRAS) after renal transplantation. We report a retrospective study of the relationship between TRAS and postoperative CMV infection in patients transplanted at our center in the period of 1978 to 1992. Review of all 917 transplanted patients identified 75 patients with TRAS diagnosed on renal arteriography whose records also allowed the incidence of CMV infection (between transplantation and diagnosis of TRAS) to be assessed. A case-control group of 75 transplant patients was identified without TRAS, matched for year of transplant, age at transplant, sex and number of previous grafts. The incidence of CMV infection (in the same period as their matched TRAS case) was also assessed in these patients. Using a combination of clinical and serological criteria, the patients were divided into those in whom CMV infection was highly probable (clinical episode, serologically confirmed), possible and negative. The incidence of CMV infection in the TRAS and control patients is tabulated:

	TRAS (N = 75)	Controls (N = 75)	P value
CMV highly probable	36 (48%)	12 (16%)	< 0.05
CMV possible	24 (32%)	30 (40%)	> 0.05
CMV negative	15 (20%)	33 (44%)	< 0.05

We have previously reported an increased incidence of rejection in the TRAS patients. Analysis of CMV infection in the subgroup of patients without rejection episodes further increased the relative excess in TRAS versus controls. The causal basis of these associations remains ill-defined, but the data support the hypothesis that CMV infection can contribute to the development of TRAS.

Zinc protoporphyrin assay aids in the identification of functional iron deficiency in patients on dialysis. *B. Wilson and M.K. Almond, Departments of Haematology and Medicine, Southend Hospital, Prittlewell Chase, Westcliff-on-Sea, Essex, England, United Kingdom.* The use of recombinant human erythropoietin (EPO) in patients on chronic dialysis corrects the anemia of chronic renal failure, providing the patient is not iron deficient. No single assay adequately identifies patients who are functionally iron deficient. The most commonly used assay (ferritin) has limitations that may be resolved by combining other assays to improve specificity and sensitivity. Zinc protoporphyrin (ZPP) is formed when zinc is substituted for the ferrous ion during haem synthesis when iron is not available. We investigated the possibility of combining an assay of erythrocyte ZPP with ferritin to identify those patients responding poorly to EPO who may have functional iron deficiency. Fifty-one hemodialysis patients from a single unit who had been receiving EPO for a minimum of 3 months were studied. Having excluded accepted causes for EPO resistance, patients were classified as good or poor responders according to hemoglobin and EPO dose (U/kg/week). Ferritin and ZPP assays were performed on all patients. No correlation existed between the ferritin and ZPP levels in either good or poor responders nor in the pooled data. Of the 13 poor responders, 11 had a high ZPP (> 40 μM ZPP/mol hem) with a normal ferritin (> 20 ng/ml) while 2 had both a high ZPP and a low ferritin. There was no significant difference in ZPP levels between the responders and non-responders (P = 0.06). There was a significant difference in ferritin levels between the responders and non-responders (P < 0.01). Combining the two assays improved the identification of poor responders. The normal range for ZPP in hemodialysis patients is not established. By adjusting the normal range of ZPP, sensitivity can be improved and in combination with a ferritin assay the specificity is increased.

Reticulocyte markers in patients with end-stage renal disease on hemodialysis. *S. Bhandari, J.H. Turney, and A.M. Brownjohn, Renal Unit, Leeds General Infirmary, Leeds, England, United Kingdom.* Measurement of the hemoglobin distribution with the reticulocyte population enables one to identify poorly hemoglobinized reticulocytes in advance of any change in the red cell population as a whole. Mean reticulocyte hemoglobin content (CHr) and mean reticulocyte hemoglobin cell concentration (CHCMr) are early indices of any change in recent erythropoiesis and may therefore be of benefit in the early diagnosis of functional iron deficiency (FID). In a cross-sectional study of 67 hemodialysis patients, mean age 62 years (range 20–63), mean hemoglobin 9.5 ± 0.16 g/dl on s.c. erythropoietin (EPO) and oral iron supplements, reticulocyte markers were compared with standard hematological indices. CHr correlated with mean cell volume (MCV) (r = 0.527, P < 0.001), mean hemoglobin concentration (MCH) (r = 0.545, P < 0.001) and red cell ferritin (RCFer) (r = 0.344, P = 0.004), while CHCMr correlated with MCH (r = 0.315, P = 0.009). There was no significant correlation of CHCMr or CHr with either serum ferritin (SF) or transferrin saturation (TS). The sensitivity of a reduced CHr (normal range 25.8–30.6 pg) to diagnose FID (SF < 100 ng/liter, TS < 20%) was 0.77 in both cases, respectively, while to diagnose absolute iron deficiency (AID) (RCFer <7 ag/ferritin/red cell) was 0.46. The sensitivity of a reduced CHCMr (normal range 23.5–28.7) to diagnose FID was 0.62 and 0.47, respectively, while to diagnose AID was 0.25. The sensitivity of an increase in percentage hypochromic red cells to diagnose FID was 0.69 and 0.48, respectively. Five patients with a normal SF and 3

with a normal TS had reduced CHr levels, while 13 patients with a normal SF and 17 with a normal TS had reduced CHCMr levels, suggesting FID. **Conclusion:** Reticulocyte measures provide evidence of iron deficient erythropoiesis despite oral iron supplementation as a result of EPO. Prospective analysis may indicate the potential usefulness of these measures in real time erythropoiesis during EPO therapy and enable more effective use of this therapy.

Effective utilization of EPO with intravenous iron therapy. *S. Bhandari, R. Kendall, D. Norfolk, J.H. Turney, and A.M. Brownjohn, Renal Unit, Hematology Unit, Leeds General Infirmary, Leeds, England, United Kingdom.* Iron replacement therapy reduces the demand for erythropoietin in dialysis patients. It has been postulated that iron supply to the bone marrow is a rate-limiting step in the process of erythropoiesis under erythropoietin stimulation. We evaluated the use of intravenous iron (Imferon) therapy for this purpose in hemodialysis patients in a prospective, non-blinded study of 22 patients (16 male, 6 female, mean age 62.4 years, range 24–80 years). All patients had a serum ferritin (SF) of ≤60 mg/liter despite oral iron therapy. Patients with high aluminum and PTH levels, underlying bleeding/hematological disorders or active inflammatory diseases were excluded. Patients were established on subcutaneous erythropoietin (EPO) and given Imferon over 7 consecutive dialysis sessions (total dose 1150 mg) with fortnightly monitoring for 4 months. Median EPO dose was 4000 U/week (mean 6,050 U/week) pre-treatment and 2,000 U/week (mean 3,700 U) post-Imferon therapy ($P = 0.03$). No serious adverse events occurred in the 154 treatment sessions of Imferon. Two patients experienced nausea and malaise, and one complained of dizziness, but all completed the course. Mean Hb response remained constant at 6 and 12 weeks ($P = 0.098$). SF ($P < 0.0001$) and red cell ferritin RCFer ($P = 0.004$) rose significantly while transferrin saturation remained static ($P = 0.08$). This coincided with a sharp increase in reticulocytes in the first 14 days after commencement of Imferon and a fall in percentage hypochromic red cells ($P < 0.0001$). An early decline in RCFer was apparent. There was no correlation between RCFer and SF. Imferon therapy is a safe and cost effective method of maintaining/improving Hb levels in patients with low SF levels despite oral iron therapy. RCFer is an early marker of functional iron depletion.

Outcome following peritoneal dialysis-related staphyococcal peritonitis. *S. Peacock, P. Howe, D. Crook, A. Berendt, and C. Winearls, Nuffield Department of Medicine and Public Health Laboratory, The John Radcliffe; Oxford Renal Unit, The Churchill; The Oxford Radcliffe Hospitals NHS Trust, Oxford, England, United Kingdom.* **Objectives:** To compare the outcome following continuous ambulatory peritoneal dialysis (CAPD)-related peritonitis caused by *Staphylococcus aureus* and coagulase-negative staphylococci. **Methods:** Patients were prospectively identified between July 1990 and November 1995. Endpoints evaluated were: (1) abdominal complications including abscess formation, need for laparotomy during infection, and development of adhesions; (2) attributable mortality; (3) seeding of staphylococci from the peritoneum to distant sites; (4) removal of the peritoneal dialysis catheter within 1 month of the onset of infection; (5) cumulative survival of CAPD as the mode of dialysis. **Results:** Outcome is shown in the table below.

Outcome event	*Staphylococcous aureus* peritonitis (78 episodes)	Coagulase-negative staphylococcal peritonitis (123 episodes)	*P* value
Abdominal complications	5	0	0.008
Attributable mortality	3	0	0.057
Metastatic seeding	0	0	—
PD catheter removal	20	8	<0.0003

Four months after the first recorded episode, 93% of patients remained on peritoneal dialysis following coagulase-negative staphylococcal peritonitis, compared with 63% following *Staphylococcus aureus* peritonitis. **Conclusions:** This study has demonstrated a clear distinction between peritonitis caused by *Staphylococcus aureus* and coagulase-negative staphylococci. These two diseases should be differentiated both during clinical management, and in future studies of natural history and treatment.

West Midlands regional audit of dialysis using renal association quality standards. *S.S. Dhillon and S.A. Smith, Birmingham Heartlands Hospital, Birmingham, England, United Kingdom.* We have developed a system of automated computer controlled audit of dialysis for end-stage renal failure which is based on the Renal Association guidelines. Most of the data is collected automatically using a PROTON clinical database with direct laboratory links. Current data from this database are analyzed using software enquiries, and reports can be generated in a few minutes. The program has been installed in six of the eight renal units in the West Midlands. During 1995 all eight West Midlands renal units contributed data on all patients receiving dialysis treatment for end-stage renal failure (1197 patients, 587 hemodialysis, 610 CAPD). The data collected included demographic information such as age distributions and death rates, performance against clinical standards defined by the Renal Association and information about use of dialysis techniques. The data were analyzed for presentation using an SPSS statistical software program. Results were presented in a form which allowed individual units to compare their performance to each other and to the regional mean. The computerized system enables virtually instantaneous, local collection of accurate performance data which can be repeated at any time interval. There is potential for expanding the database to include units outside the West Midlands.

***In vitro* age formation is reduced in PD fluid heat sterilized in a two-compartment bag.** *A. Dawnay, A.P. Wieslander, and D.J. Millar, Department of Clinical Biochemistry, St. Bartholomew's Hospital, London, England, United Kingdom; Gambro Group, Lund, Sweden.* Peritoneal membrane function may be impaired by advanced glycation endproduct (AGE) formation which is promoted in PD fluid by unknown factors in addition to glucose. Since AGE formation is more rapid in PD fluid after heat sterilization, the factors may be highly reactive glucose degradation products. Their formation is substantially reduced if the glucose and electrolyte components are heat sterilized separately. The aim of this study was to assess whether AGE formation in such a fluid is reduced. PD fluid (1.5% glucose, Gambro Lundia AB) was heat sterilized in either a single bag (standard) or with the glucose and electrolytes separated in two compartments and then combined before use (PD-Bio) such that the final composition of each was identical. HSA (1 g/liter) was incubated in these fluids at 37°C, pH 7.4, for up to 30 days. HSA glycation was assessed by boronate affinity chromatography and RIA, and AGE formation by fluorescence (Ex_{350}, EM_{430}). The table shows fluorescence (F) in U/g HSA/l at each time point and fluorescence generation rate (FGR) per day for each interval.

	Days		
	0	1	2
Standard F	48	286	377
PD-Bio F	57	123	155
Standard FGR	238	91	
PD-BIO FGR	66	32	

	Days		
	5		10
Standard F	568		615
PD-Bio F	191		229
Standard FGR	64	9.4	
PD-BIO FGR	12	7.6	

	Days		
	20		30
Standard F	786		892
PD-Bio F	285		421
Standard FGR	17.1	10.6	
PD-BIO FGR	5.6	13.6	

AGE formation was increased ($P < 0.05$) in standard PD fluid compared with PD-Bio. This increase was due to an enhanced FGR in the first 5 days

of incubation, rates thereafter being similar between the two fluids. There was no difference ($P > 0.05$) in glycation rate. Our data are consistent with glucose degradation products being even more significant than glucose in promoting AGE formation in PD fluid.

Reactive oxygen species generation, membrane biocompatability and the role of vitamin E. *P. Carmichael, L. Gallivan, B. Walker, and A.M. Davison, Department of Renal Medicine, Clinical Oxidant Group, St. James's University Hospital, Beckett Street, Leeds, England, United Kingdom.* The process of contact to artificial surfaces during hemodialysis activates leukocytes leading to formation of reactive oxygen species (ROS) which oxidatively modify low-density lipoprotein (LDL) cholesterol; a fundamental process in the development of atherosclerosis. The aim of this study was to assess: (i) ROS production during hemodialysis using three filters of differing biocompatability; (ii) the effect of pre-dialysis treatment with vitamin E on ROS production during hemodialysis. A dose of 890 IU of vitamin E was administered 18 hours prior to each dialysis; the timing was chosen to coincide with maximal plasma levels. Blood sampling was performed at 0.5, 15, 30, 60 and 180 minutes into dialysis.

Patient	Cuprophane		Modified celluose	
Vitamin E	−	+	−	+
A	58.7%	51.7%	60.3%	55.9%
B	60.3%	45%	67.4%	58%

Patient	Polysulphone	
Vitamin E	−	+
A	63.9%	54.8%
B	59.8%	69%

The table shows percent reduction in plasma total anti-oxidant capacity (TAC) at 180 minutes compared to pre-dialysis value before (−) and after (+) vitamin E pre-treatment for two patients.

The results demonstrated a significant reduction in plasma TAC during dialysis and a transient increase in lipid peroxidation (LPO) values for all three filters. Pre-treatment with vitamin E resulted in a decrease in plasma TAC for all three membranes in one of the patients, and for two of the membranes in the other patient. Pre-treatment with vitamin E had no effect on LPO production. These studies are being extended to a further 4 patients. We conclude that vitamin E may have a protective role against long-term cardiovascular morbidity in the hemodialysis population.

Increased cell protein oxidation in hemodialysis. *J.G. Anderton, T.H. Thomas, and R. Wilkinson, Department of Medicine (Nephrology), University of Newcastle Upon Tyne, Newcastle Upon Tyne, England, United Kingdom.* Susceptibility to cell damage by reactive oxygen species (ROS) is increased in renal failure. Protein thiol (−SH) residues are of widespread functional importance: they are vulnerable to oxidation and may therefore be targets for damage by ROS. We have measured thiol complement in mononuclear leukocytes (MNL) from 14 normal controls (NC), 16 hemodialysis (HD), 13 peritoneal dialysis (CAPD), and 16 chronic renal failure (CRF) patients. Comparison was made with protein carbonyl levels as a corroborative marker of protein oxidation. At baseline, fast-reacting thiol was depleted in HD compared to NC (HD 2.46 ± 0.25 vs. NC 3.56 ± 0.24, $P = 0.003$. Values in nmol SH/10^6 cells ± SEM). After exposure to oxidant stress both total thiol and fast reacting thiol were lower in HD than NC (total thiol, fast-reacting thiol, rate of thiol recovery and protein carbonyl levels did not differ significantly between normal controls, CRF and CAPD patients. Cellular protein oxidation is increased in HD patients, accompanied by loss of thiol residues and a reduced capacity to recover thiols after oxidant stress. This may be a sequela of the oxidant stress imposed by hemodialysis, and may have important functional effects on thiol-dependent cellular proteins.

Increased antioxidant activity of uremic plasma. *J.G. Anderton, T.H. Thomas, and R. Wilkinson, Department of Medicine (Nephrology) University of Newcastle Upon Tyne, Newcastle Upon Tyne, England, United Kingdom.* Susceptibility to oxidant stress is increased in renal failure, and haemodialysis itself may exacerbate oxidant damage. Conventional antioxidants (eg vitamin C,E) are depleted, but other substances may contribute to the antioxidant capacity of plasma. We have used a chemiluminescent assay to measure the plasma antioxidant activity (AOA) of controls (NC), CRF (median creatinine 490 μmol/liter), CAPD and haemodialysis (HD) patients, before and after a dialysis session. Comparison was made with Trolox, a vitamin E analogue.

Subjects	Number	AOA (μM Trolox)
NC	16	418.8 ± 19.0
CRF	10	652.4 ± 56.0[a]
HD (pre)	15	556.3 ± 31.2[b]
HD (post)	15	384.8 ± 12.8[c]
CAPD	14	468.4 ± 27.2

Values are mean ± SEM; Student's t-test; [a] $P = 0.002$ vs. NC; [b] $P = 0.001$ vs. NC; [c] $P < 0.0001$ vs. HD (pre).

AOA was increased relative to NC in both CRF and HD patients, but was normal in CAPD. HD acutely reduced AOA to control levels [$P = 0.15$ HD (post) vs. NC]. Urate is a scavenging antioxidant which may be elevated in uremia and acutely reduced by HD. Although urate was an effective antioxidant in this assay (0.2 mM urate = 640 μmol Trolox/liter), the probability of a correlation between changes in AOA and urate ($r = 0.38$) was only $P = 0.1$. Plasma AOA is increased in uremia, and hyperuricemia is at most a minor factor in this increase. Acute hemodialysis adversely affects plasma antioxidant status.

Nutritional status and correction of acidosis by high bicarbonate dialysis. *A.J. Williams, I.D. Dittmer, J. Clarke, A. McArley, and R.A. Banks, Renal Unit, Gloucestershire Royal Hospital, Gloucestershire, England, United Kingdom.* Long-term metabolic acidosis is harmful to protein and bone metabolism in CRF. Standard bicarbonate dialysis cannot always correct acidosis adequately. This study was to investigate the longer-term efficacy of high bicarbonate dialysis on controlling acidosis, and its effect upon nutritional status. Forty-six stable hemodialysis patients, in two groups, were dialyzed in a single blind double crossover trial for two six month periods, using low (L.Bic, 30 mmol/liter) and high (H.Bic, 40 mmol/liter) dialysate bicarbonate concentrations. All patients had previously been dialyzed for at least 6 months using 35 mmol/liter bicarbonate dialysate. Predialysis arterial plasma pH (L.Bic, 7.38 ± 0.05, H.Bic, 7.43 ± 0.04) $P < 0.001$, bicarbonate (L.Bic, 22.4 ± 2.9, H.Bic, 27.4 ± 3.0 mmol/liter) $P < 0.001$, and pCO_2 (L.Bic, 5.14 ± 0.57, H.Bic, 5.57 ± 0.55, kPa) $P < 0.01$, all different significantly. Predialysis arterial pO_2 did not differ (L.Bic, 11.67 ± 1.69; H.Bic, 11.12 ± 1.39 kPa) neither did pO_2 values during 4 hours hemodialysis. No significant sustained differences in predialysis plasma calcium, phosphate or albumin were found. After 3 months of high bicarbonate dialysis, a predialysis venous bicarbonate of <20 mmol/liter was seen in one patient only. KT/V (L.Bic 1.27 ± 0.19, H.Bic 1.27 ± 0.25), UGR (L.Bic 1.99 ± 0.77, H.Bic 1.92 ± 0.77 mmol/min) and NPCR (L.Bic 1.04 ± 0.26, H.Bic 0.99 ± 0.28 g/kg/day) did not differ during treatment periods. Triceps skinfold thickness changed significantly (L.Bic 14.8 ± 6.9 to 11.8 ± 5.5, H.Bic 14.9 ± 6.3 to 15.8 ± 6.4 mm; $P = 0.018$) and the changes reversed following dialysate change (H.Bic 11.8 ± 5.5 to 13.3 ± 7.2; L.Bic 15.8 ± 6.4 to 13.8 ± 6.7 mm $P = 0.034$). Mid-arm circumference did not change. This study shows that alteration of acid base status can affect nutritional parameters in the longer-term, and that individual prescription of dialysate bicarbonate would be an effective way of controlling acidosis.

Renal transplant experience in Asian children. *N.E. Moghal, D.V. Milford, S.A. Hulton, and C.M. Taylor, Department of Nephrology, The Birmingham Children's Hospital, Ladywood, Birmingham, England, United Kingdom.* We undertook a retrospective study of renal transplantation between 1980 and 1995 in an Asian population (Indian, Pakistani, Bangladeshi). Asian children account for 7.4% of the total child population (0–15 years) in the West Midland Health Region served by the regional renal service at the Birmingham Children's Hospital. End-stage renal disease (ESRD) developed in 165 children (138 non-Asian, 27 Asian) during this time. The prevalence of ESRD for the non-Asian cohort was $15/10^5$ non-Asian child population and for Asians, $40/10^5$ Asian child population. Genetic diseases accounted for 26 (19%) in non-Asians and 12 (44%) in Asians progressing to ESRD ($P < 0.001$). Of the 147

grafts, 22 were Asian recipients (15%). The percentage grafted in the Asian cohort increased over the 15 year period: 1980–84, non-Asian 54% vs. Asian 0%; 1985–90, non-Asians 64% vs. Asians 50%; 1991–95, non-Asian 82% vs. Asian 86%. The distribution of blood groups in the two populations reflected the pattern in the respective general population as a whole: Asians O = 32%, A = 16%, B = 47%, AB = 5%; non-Asians O = 55%, A = 35%, B = 7%, AB = 3%. Time to transplantation was 1–44 months (mean 7 months). Despite the different distributions of blood groups between the two populations there was no significant difference in the time to transplantation (non-Asians mean 6 months, 95% CI 0.6–0.9; Asians mean 7 months, 95% CI 0.3–1.0). Asian patients had significantly ($P < 0.001$) more mismatches (≥ 3 or ≥ 4) compared to non-Asians. There was a higher prevalence of ESRD in the Asian child population, with genetic disease accounting for a significantly higher percentage compared to the non-Asian population. Differences in blood groups did not influence time to transplantation between the two populations, although Asians had significantly more mismatches compared to the non-Asians.

Indo-Asian dialysis and transplant rates. *R.M. Higgins, N. West, M.E. Edmunds, D.C. Dukes, H. Kashi, and F.T. Lam. Dialysis and Transplant Units, Walsgrave Hospital, Coventry, England, United Kingdom.* HLA matching between donor and recipient in cadaver renal transplantation has been increasingly utilized. Since 1988, 98.0% of the kidneys transplanted in our center have had only 0 or 1 DR mismatch between donor and recipient. We have analyzed our activity from 1988–1995 inclusive. We serve an adult population of 597,046 White Europeans and 26,869 Indo-Asians (1991 census). Dialysis and transplant rates have been calculated per million population per year (pmp/yr).

	Europeans *N, pmp/yr*	Indo-Asians *N, pmp/yr*	*P*
Started dialysis	395 (82.6)	71 (330.3)	<0.001
On transplant waiting list	254 (53.1)	51 (237.2)	<0.001
Transplanted	176 (36.8)	21 (97.6)	<0.001
Donor kidneys	192 (40.1)	2 (9.3)	<0.025

Indo-Asian patients registered for transplantation were less likely to receive a kidney than White European patients (31% vs. 58%, $P < 0.025$); those transplanted waited longer [mean times on list 403.4 (SEM 134.4) vs. 331.7 (SEM 33.3) days respectively, $P < 0.05$)] and were more likely to remain on the waiting list for over 750 days without being transplanted (33.3% vs. 19.4%, respectively, $P < 0.025$). In summary, allocation of kidneys by HLA matching and a kidney shortage both contributed to a relative disadvantage for individual Indo-Asian patients awaiting transplantation.

Early induction of TGF-β1 in transplantation. *M. Picton, S. Williams, I.S. Roberts, C.D. Short, R.W. Johnson, P.E. Brenchley, Renal Research Group, Manchester Royal Infirmary, Oxford Road, Manchester, England, United Kingdom.* The major cause of chronic vascular rejection (CVR) in renal transplantation is intimal thickening initiated by an accelerated inflammatory, fibro-proliferative response to injury and culminating in sclerosis of small arteries. Acute rejection, infection and hypertension may be implicated in this process in some patients and is probably modulated by raised circulating TGF-β1, secondary to deranged regulation of this pro-fibrotic cytokine. Therefore, we studied 82 sequential renal allograft recipients during the first 12 months following transplantation. Plasma samples were collected daily during hospitalization and at every subsequent out-patient clinic visit. Plasma TGF-β1 levels were determined by immunoassay. Biopsy material from 14 patients was processed for immunostaining. Thirty-one of the patients (37.8%) had detectable TGF-β1 (plasma >100 pg/ml) and were positive over a range of 1–134 days with a mean duration of 36 days. There was a higher incidence of CMV disease ($P > 0.05$) and other infections ($P > 0.01$) in the TGF-β1 positive patients. There was no significant difference in the number of acute rejection episodes or episodes of ATN between the TGF-β1 positive patients and the negative group. The rise in TGF-β1 was associated with acute rejection (1), chronic rejection (1), CMV infection (4), other infection (7), surgery (7), ATN (2), CSA toxicity (1) or unknown (8). Immunostaining for TGF-β1 and TGFβRII protein was no different from normal. Thus, raised levels of TGF-β1 have been documented in association with a number of events following renal allograft transplantation. Long-term sequential follow-up will be necessary to determine whether this has an adverse outcome with regards to allograft function.

Can cyclosporin failure be converted to tacrolimus success in renal transplantation? *S.S. Dhillon, M.E. Edmunds, D.C. Dukes, H. Kashi, F.T. Lam, and R.M. Higgins, Dialysis and Transplant Units, Walsgrave Hospital, Coventry, and Birmingham Heartlands Hospital, Birmingham, England, United Kingdom.* In the pre-tacrolimus era, failure of cyclosporine to prevent acute rejection required augmentation of immunosuppression with high dose steroids or protein immunosuppression, which was often associated with either graft failure or the adverse side-effects of treatment. We have reviewed our use of tacrolimus for biopsy proven rejection in 13 patients on primary cyclosporine immunosuppression. The mean time to conversion after transplantation was 461 days (range 46–1718), and the mean creatinine level at conversion was 367 μmol/liter (range 190–823). The mean cyclosporine trough level through which rejection occurred was 188 mmol/liter. Graft survival after conversion is currently 70% with a mean follow-up in functioning grafts of 211 days (range 38–370). The 3 failures were patients already on dialysis, due to graft rejection, at the time of conversion. Six cases had received courses of OKT3 before conversion. Conversion was associated with only a levelling in plasma creatinine levels, presumably due to nephrotoxicity. Twenty days after conversion the mean creatinine level in 11 functioning grafts had risen from 310 μmol/liter (range 190–554) to 320 μmol/liter (range 162–503), a mean change of +3.2%. The most recent creatinine levels were 298 μmol/liter (range 151–444) in 10 functioning grafts, a mean change of +4% compared to pre-conversion levels in these grafts. There was 1 case of intestinal lymphoma which has gone into spontaneous remission and 1 case of CMV pneumonitis associated with graft failure; both patients had received OKT3. In summary, we have identified renal transplant patients in whom cyclosporine has failed. Conversion to tacrolimus has been associated with stabilization of renal function in otherwise failing grafts, but also with possible nephrotoxicity.

T-cell adhesion processes in ANCA positive glomerulonephritis. *S.J. Chakravorty, A.J. Howie, P. Cockwell, D. Adu, and C.O.S. Savage, Renal Immunobiology, CCRIS and Department of Pathology, Medical School, University of Birmingham, Birmingham, England, United Kingdom.* The focal segmental necrotizing glomerulonephritis of ANCA positive small vessel vasculitis (SVV) is associated with glomerular and tubulointerstitial T-cell infiltrates. Interactions of the T-cell expressed α4β1 integrin (VLA-4) and its dual tissue ligands, CS-1 containing fibronectin and vascular cell adhesion molecule-1 (VCAM-1), are believed to play key roles in T-cell trafficking in immune and inflammatory disorders. We have developed a modified Stamper-Woodruff assay to produce a functional system to assess leukocyte adhesion requirements in inflammatory renal diseases. We studied SVV (6) and normal tissue (4). T lymphocytes (Jurkat cell line) in suspension were added to renal biopsy sections in the absence and presence of blocking antibodies to VLA-4 (Max 68) and CS-1 (90.45). Cells and tissue were co-incubated and adherent cells were visualized by an anti-CD3-HRP conjugate. There was minimal binding of unstimulated cells to normal and vasculitic tissue or of preactivated cells to normal tissue. Preactivated cells bound to vasculitic sections within glomeruli, to parietal epithelium, interstitium and to the luminal aspect of proximal tubular epithelium. Binding was inhibited by anti-VLA-4 (32.4 ± 8.2%) and anti-CS-1 (58.4 ± 6.3%), with anti-CS-1 preferentially inhibiting interstitial binding. The two antibodies in combination produced a dramatic reduction (75.5 ± 8.9%), but not complete inhibition of T-cell binding. These data suggest that in SVV: (i) VLA-4 mediated adhesion processes play a pivotal role in T-cell infiltration; (ii) T-cell activation is a requirement for adhesion in this assay system; and (iii) VLA-4 independent adhesion processes also have a role in T-cell trafficking.

ANCA stimulates IL-1β and IL-6 production from TNF-α primed neutrophils. *C.R. Myers, D.J. O'Donoghue, and P.E.C. Brenchley, Department of Renal Medicine, Hope Hospital, Salford; Renal Research Group, St. Mary's Hospital, Manchester, England, United Kingdom.* Anti-neutrophil cytoplasmic antibodies (ANCA) have the potential to interact with primed neutrophils (PMN) through Fab binding to antigen displayed on the plasma membrane and through Fc binding to the FcγRII. ANCA are known to induce production of reactive oxygen species and degranulation of primed PMN. ANCA may modulate other neutrophil functions. This

study investigated the potential modulation of neutrophil cytokine production in response to ANCA stimulation. PMN were isolated from six healthy volunteers, to a purity of >99% and cultured At 37°C and 5% CO_2 at 0.5×10^6/ml. Cells were primed with 20 ng/ml TNF-α for 20 minutes then stimulated with 100 μg/ml IgG isolated from serum from biopsy proven vasculitis patients (10 pANCA, 6 cANCA) and from normal individuals (10) for 6 hours. Supernatants were assayed by ELISA using chemiluminescent detection for IL-1β, IL-6 and IL-8 production. ANCA induced significantly higher production of IL-1β and IL-6 than did normal IgG but no significant difference in production of IL-8.

	IL-1β pg/ml	IL-6 pg/ml	IL-8 pg/ml
ANCA IgG (16)	147 ± 95	67 ± 23	2283 ± 375.5
Normal IgG (12)	87 ± 36.8	41.5 ± 14.8	2074 ± 426.8

2-tailed Student t-test: $P = < 0.05$, $P = < 0.005$, $P = < 0.191$.
Values = mean and SD.

pANCA IgGs stimulated higher levels of IL-1β and IL-6 than did cANCAs (230.7 pg/ml ± 115 vs. 125.5 pg/ml ± 105, $P < 0.001$ and 81.43 ± 30 pg/ml vs. 64.2 pg/ml, $P > 0.005$, respectively). Although ANCA activity was present in all IgG subclasses, IgG2 and IgG4 predominated and a significant relationship between the presence of IgG4 and IL-6 production ($P = 0.007$, 2 tailed Student t-test) was apparent. $F(ab)'_2$ ANCA preps failed to stimulate detectable IL-1β and IL-6 production, indicating dependence on FcR interaction. We propose that ANCA induced cytokine production may prolong neutrophil survival and enhance neutrophil mediated tissue damage, sustaining the bias toward a pro-inflammatory environment.

Expression of intracellular and secreted recombinant proteinase 3 recognized by ANCA. *M.E. Griffith, A.N. Turner, J. McVey, and C.D. Pusey, Renal Unit, Department of Medicine, and MRC CSC, Royal Postgraduate Medical School, London, England; Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, Scotland, United Kingdom.* PR3 is the major autoantigen of cANCA in systemic vasculitis. However, it is difficult to purify from human neutrophils, and we have therefore attempted to produce recombinant protein which retains its antigenicity. RNA was extracted from human bone marrow and reverse transcribed. PR3 specific cDNA was then amplified by PCR, cloned into pUC19, and sequenced. Different expression systems were then investigated. The baculovirus/insect system was initially used for expression. Full length cDNA for PR3, including that encoding the prepropeptide, was cloned into PVL 1392. This was contransfected with BACPAC 6 into SF9 insect cells, and recombinant viral clones identified by PCR with PR3 specific primers. Cytospins of cells infected with virus for 72 hours were made and incubated with test antibodies. There was positive immunofluorescence in the plasma membrane of cells infected with recombinant virus following incubation with 2/3 anti-PR3 monoclonal antibodies, and with 4/5 cANCA positive sera, but not with control sera. Cells infected with wild type baculovirus were negative on immunofluorescence with all test antibodies. Thus, PR3 was expressed in an antigenic form, but was not secreted. Secondly, using an *E. coli* expression system, cDNA for PR3 was cloned into a vector encoding the STII enterotoxin secretory leader. This results in expression of a secreted product which is cleaved from the secretory peptide as it is exported from the cell, and which tends to remain soluble. Following induction with IPTG, cells transformed with this recombinant vector produced a secreted protein of 30 kDa. This was identified on Western blotting by cANCA positive sera from 4/4 patients, but not by control sera. It was also recognized by 1/3 anti-PR3 monoclonal antibodies. This product contains the prepropeptide of PR3, which may have affected binding of the monoclonals. Nonetheless, PR3 was expressed and secreted in an antigenic form. In summary, we have produced recombinant PR3 recognized by ANCA in both insect cells and *E. coli*. It should be possible to isolate the secreted form in large quantities. This will facilitate basic research in vasculitis as well as being potentially useful for clinical assays.

Peripheral mononuclear cells express VEGF and the VEGF receptor flt-1. *N.J.A. Webb, C.J. Watson, M.J. Bottomley, and P.E.C. Brenchley, Renal Research Group, St. Mary's Hospital, Manchester, England, United Kingdom.* Vascular endothelial growth factor (VEGF) is a potent modulator of capillary permeability, an endothelial cell mitogen, and is a chemoattractant for monocytes. Peripheral mononuclear cells (PMCs), central to the pathogenesis of many glomerulopathies, have been shown to express VEGF and flt-1 mRNA, though protein production has not previously been reported. PMCs were isolated from healthy adult volunteers (N = 11) by Ficoll centrifugation, resuspended in Iscoves/10% FCS at 2×10^6/ml and stimulated with PHA (0–25 μg/ml) and LPS (0–10 ng/ml) at 37°C for varying time intervals between 0 and 96 hours. Free VEGF levels in the culture supernatants were measured using a novel sensitive chemiluminescent assay which utilizes soluble r-VEGF receptor flt-1 for capture and a rabbit polyclonal anti-VEGF antibody for detection, and thus measures free VPF but not VEGF-flt-1 complexes. VEGF-flt-1 complexes were measured by a second assay utilizing antibodies to flt-1 and VEGF. Total RNA isolated from cell pellets was submitted for semiquantitative RT-PCR studies for all three common VEGF splice variants ($VEGF_{121}$, $_{165}$ and $_{189}$) and also both membrane bound and soluble variants of the VEGF receptor flt-1. PMCs stimulated with LPS showed significantly increased VEGF production compared with unstimulated controls (∅) at all doses though maximal at 1 ng/ml (561 pg/ml vs. 213 pg/ml at 24 hours, $P = 0.018$), with peak production at 72 hours [965 pg/ml (1 ng/ml) vs. 415 pg/ml (∅), $P = 0.0002$]. PHA stimulated cells also showed increased protein production compared with ∅, with the maximum response occurring with 0.1 μg/ml at 72 hours (633 pg/ml vs. 113 pg/ml, $P = 0.01$). LPS stimulated shedding or secretion of flt-1 with peak VPF-flt-1 complex levels at 72 hours. Semiquantitative RT-PCR studies identified up-regulation of mRNA in LPS stimulated cells for all three VEGF splice variants and also for both membrane bound and soluble flt-1 variants at 8 hours compared with control samples. Thus, PMCs may play a pivotal role in the production of VEGF and the control of its bioactivity via flt-1 secretion.

Heterogeneity of endothelin receptors and endothelin production in a renal cell line. *A.F. James, N.A. Parkinson, and B.M. Hendry, Renal Research Group, Department of Medicine, King's College School of Medicine and Dentistry, London, England, United Kingdom.* Endothelins (ETs) have been implicated in the progression of renal failure and may be involved in tubulo-interstitial fibrosis. However, the role of direct actions of ETs on tubule cells remains unclear. We have recently defined the actions of ETs on Ca homeostasis in MDCK cells, a cell line from canine renal tubule. This report concerns ET production and ET receptor expression in MDCK cells. Cells were grown to confluence in 24-well culture plates. Immunoreactive (i.r.) ET released into the culture medium was assayed by ELISA. Specific binding to cultured cells was measured after incubation with [^{125}I]ET-1 (74 TBq/mol) at 37°C for 2 hours. Two subtypes of MDCK cells were identified according to their production of ETs and receptor-binding properties. MDCK-a cells produced low, but significant, levels of i.r. ET; 12.2 ± 5.4 fmol/well/24 hours. Binding of 30 pM [^{125}I]ET-1 to these cells was concentration-dependently displaced by ET-1 > BQ-123 > ET-3, suggesting binding to ET_A type receptors. In contrast, MDCK-b cells produced 13-fold more i.r. E.T (161.9 ± 16.7 fmol/well/24 hours) than MDCK-a cells. Binding of 30 pM [^{125}I]ET-1 could not be detected in untreated MDCK-b cells. However, treatment of MDCK-b cells with the non-selective endothelin-converting enzyme inhibitor, phosphoramidon (4 mM, 48 hours), increased the binding of 500 pM [^{125}I]ET-1 to these cells from 354 ± 8 cpm/well to 4480 ± 459 cpm/well, suggesting that ET produced by these cells may bind to and mask the ET receptors present. ELISA confirmed that phosphoramidon treatment had reduced the i.r. ET of the culture medium from these cells by a factor of 5. Binding of 500 pM [^{125}I]ET-1 to these MDCK-b cells was inhibited by both ET_A-selective BQ-123 (1 μM; 36.5%) and ET_B-selective BQ-788 (10 nM; 74%), suggesting the existence of both ET_A and ET_B receptors on MDCK-b cells. Production of i.r. ET by MDCK-b cells was not affected by 24-hour incubation in the presence of either BQ-123 (1 μM) or BQ-788 (10 nM). These results show that phenotypic variation in this cell line includes the expression of both ET_A and ET_B receptors and suggests that responses to exogenous ETs may be inhibited by receptor-bound endogenous ET.

Inhibition of diabetic hyperfiltration via peptide antagonism of the type 1 IGF receptor. *H.M. Hickling, J.L. Haylor, C.A. Hardisty, and A.M. El Nahas, Sheffield Kidney Institute and Diabetic Centre, Northern General Hospital Trust, Sheffield, England, United Kingdom.* Insulin-like growth factor I (IGF-I), IGF-I binding proteins and IGF-I receptor proteins are all modified early on in the diabetic kidney, and hence IGF-I has been

proposed as an important mediator of diabetic kidney growth. IGF-I infusion results in an elevated GFR, which would also suggest a role for this growth factor in diabetic hyperfiltration. Seven days after the induction of diabetes by i.v. streptozotocin (45 mg/kg), diabetic hyperfiltration was measured using an isolated perfused rat kidney (IPRK) preparation. GFR was assessed as the clearance of [^{14}C] inulin. The role of IGF-I was established using JB1 (1 μg/ml), a 12 amino acid peptide antagonist of the type 1 IGF receptor. The use of the IPRK model avoided the systemic metabolism of JB1 and also distinguished between the kidney and the systemic circulation as a source of IGF-I. Diabetic rats had a two-fold higher GFR throughout the perfusion period ($P < 0.01$, $N = 6$) when compared to non-diabetic controls. In kidneys perfused from diabetic rats, the addition of JB1 (1 μg/ml) to the perfusate produced a significant fall in GFR of 44% (1.08 $\pm$ 0.16 to 0.64 $\pm$ 0.17 ml/min, $P < 0.01$, $N = 6$) similar to the level detected in kidneys obtained from non-diabetic rats following JB1 administration. In the absence of JB1, kidneys perfused from diabetic rats showed only a small decrease in GFR (12%) throughout perfusion. The effectiveness of JB1 as an antagonist for the type 1 IGF receptor was verified by inhibition of the renal vasodilator response to exogenous recombinant human IGF-I. In conclusion, the results suggest a role for renal IGF-I in mediating diabetic hyperfiltration, independent of changes in kidney growth.

Low density lipoprotein enhances monocyte bonding to human mesangial cells. *R.S. Channa and D.C. Wheeler, Department of Nephrology, University Hospital NHS Trust, Queen Elizabeth Hospital, Birmingham, England, United Kingdom.* Glomerular monocyte infiltration is a recognized early histopathological feature of lipid-mediated renal injury in cholesterol-fed rats and other animal models of glomerulosclerosis. Interactions between infiltrating leukocytes and mesangial cells might contribute to glomerular injury. To determine whether lipoproteins might enhance this process, binding of U937 monocytes to low density lipoprotein (LDL)-prestimulated human mesangial cells was studied *in vitro* by colorimetry of nuclear staining using crystal violet. LDL enhanced adhesion in a time- and concentration-dependent manner. Maximum binding was observed after 24 hours prestimulation with 100 μg/ml lipoprotein when mean $\pm$ SD monocyte adhesion was increased by 268 $\pm$ 5.5% compared to unprimed cells (100%) ($P < 0.005$). Similar results were obtained using minimally modified LDL (MM-LDL) that had been mildly oxidized by prolonged storage. Under the same experimental conditions, adhesion to cells pre-stimulated with 100 U/ml tumor necrosis factor α (TNFα) was 387 $\pm$ 4.2% of control. In an attempt to elucidate the mechanisms of enhanced monocyte binding, mesangial cell expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) was studied using an ELISA. While LDL had no effect, a small increase in ICAM-1 but not VCAM-1 was detected after pre-stimulation with MM-LDL. At a concentration of 100 μg/ml MM-LDL and after 8 hours pre-stimulation, ICAM-1 was increased to 130.0 $\pm$ 8.8% of control ($P < 0.005$), a modest effect when compared to that of 100 U/ml TNFα (349 $\pm$ 9.9%). When cells were primed with TNFα prior to lipoprotein stimulation, both LDL and MM-LDL led to an increase in ICAM-1 but not VCAM-1 expression. These results suggest that lipoproteins might promote mesangial cell-leukocyte interactions and that enhancing expression of cell surface adhesion molecules may at least in part facilitate this process.

Analysis of urinary proteins by 2D-gel electrophoresis. *P.S. Williams, and M. Longlands, Department of Medicine, North Manchester General Hospital, Crumpsall, Manchester, England, United Kingdom.* The magnitude of proteinuria is the strongest independent risk factor for progression of renal failure. However, little is known of the type of proteins excreted. Urine was obtained from 45 (28M, 17F) adult patients for flat bed 2D electrophoresis, desalted using Millipore Ultrafree filter units and redissolved in urea solution. The first dimension was run between pH 3 and 10.5 using Pharmacia Immobiline Dry strips, the second on Pharmacia Excel Gel SDS gradient 8–18. Visualisation was by silver stain. Sixty-three gels were run. Renal pathology was glomerulonephritis (19), diabetic nephropathy (6), vasculitis (3), chronic pyelonephritis (3), polycystic kidneys (3), primary tubular disorders (7), others (4). Proteinuria was 1.8 (0.2–6.4) g/liter and plasma creatinine 184 (57–570) μmol/liter (mean range). Twenty-eight (5–65) individual proteins were identified, with MW 10–200 kDa. Higher MW (>50 kDa) proteins exhibited a reproducible, consistent pattern. Little or no immunoglobulin was seen in 4 patients with minor glomerular lesions but also in membranous GN (1), IgA GN (2), hypertensive nephrosclerosis (2), and diabetic nephropathy (1). Neither the level of proteinuria nor plasma creatinine influenced the pattern. Proteins >170 kDa were seen in vasculitis (3) but also in idiopathic GN (3). Urine from patients with primary tubular disorders contained low MW proteins and albumin only, but patients with chronic pyelonephritis and renal impairment had patterns similar to those seen in idiopathic glomerulonephritis. The pattern of proteinuria therefore was independent of the underlying renal pathology and was unrelated to renal function or the magnitude of proteinuria. If proteinuria is pathogenic it appears independent of the type of protein excreted.

ACE gene polymorphisms and disease severity in HSP nephritis. *J.A. Dudley, A. Gardiner, E.J. Tizard, and M.E. McGraw, Department of Paediatric Nephrology, Southmead Hospital, Bristol, England, United Kingdom.* It has recently been reported that the rate of progression in renal disease in patients with IgA nephropathy is significantly worse in patients homozygous for the deletion (D) polymorphism in intron 16 of the gene for angiotensin-converting enzyme. We are currently investigating the influence of insertion (I) and deletion (D) polymorphisms in patients with Henoch-Schönlein nephritis seen in our unit over the last 13 years. Twenty-two patients have been investigated to date. DNA was obtained from buccal scrapings using a dry sterile cytotak brush. ACE genotype was established by PCR reaction. The distribution of genotype was as follows: II:4, ID:12 and DD:6 patients. Mean age at presentation in these groups was 3 years 4 months, 7 years 1 month and 7 years 5 months, respectively. Length of follow-up is 6 months–13 years. Severe onset with nephrotic edema, hypertension and crescent formation on renal biopsy was seen in 7/12 patients with ID genotype, 1/6 patients with DD genotype and 0/6 patients with II genotype. In the ID group, 2 patients have received a renal transplant; 3 have persistent proteinuria 3, 5 and 9 years after presentation; one of whom is hypertensive. The remaining patients have made a complete recovery or have microscopic hematuria alone. All the patients in the II and DD group have either no urinary abnormalities or have microscopic hematuria at follow-up of 6 months to 13 years. These preliminary results suggest that the ID genotype is more likely to be associated with more severe renal disease than the II and DD genotype.

Interleukin-12 modifies mercuric chloride induced T-helper lymphocyte type 2 autoimmunity. *M.J. Gorrie, C.J. Whittle, K.M. Gillespie, E.M. Bolton, and P.W. Mathieson, Western Infirmary Renal Unit, Glasgow, and Academic Renal Unit, Southmead Hospital, Bristol, England, United Kingdom.* Interleukin-12 (IL-12) promotes Th1-type T helper cell responses (cell-mediated immunity) and has therapeutic potential in diseases featuring excessive activity of the reciprocal Th2 subset. Mercuric chloride ($HgCl_2$) induced autoimmunity in Brown Norway (BN) rats is characterized by selective Th2 activation, and we tested the effects of IL-12 in this model. Recombinant murine IL-12 (1 μg intraperitoneally for 5 days) was given to $HgCl_2$ treated BN rats. Controls received IL-12 alone, $HgCl_2$ alone or no treatment. Serial measurements of serum IgE, IgG1 antilaminin antibodies and proteinuria were made and animals were killed at various time points for preparation of splenic and renal RNA and analysis of cytokine gene expression by semiquantitative polymerase chain reaction. $HgCl_2$ alone induced IL-4 mRNA by day 8 and peak serum IgE was on day 14. Co-administration of IL-12 delayed IL-4 up-regulation to day 14 and peak serum IgE to day 21. Interferon-γ (IFNγ) mRNA expression was increased by day 3 in animals receiving IL-12 or $HgCL_2$ and this was markedly enhanced in animals receiving both agents. There were no differences between the groups in IL-12 gene expression, IgG1 antilaminin antibodies or proteinuria. The pattern of cytokine gene expression was similar in both spleen and kidney. Thus, IL-12 enhanced IFNγ gene expression and markedly delayed the Th2 response. Further experiments with more prolonged administration of IL-12 are required to test whether the Th2 response can be abolished altogether. This may have implications for the treatment of human autoimmune disease including types of glomerulonephritis.

Renal myofibroblasts predict response to treatment in membranous nephropathy. *N.A. Tamimi, P.J. O'Donnell, E.C. Muchaneta-Kubara, P.G. Strange, and A.M. El Nahas, Kent and Canterbury Hospital, Department of Biosciences, University of Kent, Kent, King's College Hospital and Sheffield Kidney Institute, Sheffield, England, United Kingdom.* We have analyzed by immunohistochemistry (avidin-biotin-peroxidase) the renal biopsies of 21

patients with membranous nephropathy (MN) in order to determine whether the presence of myofibroblasts (identified by their cytoplasmic expression of α-smooth muscle actin/α-SMA or Vimentin/V) predicts the response to treatment. Patients treated by immunosuppression (steroids) were divided into progressors (P, $N = 6$) and non-progressors (NP, $N = 15$) depending on a rise of serum creatinine >20% over an observation period ranging from 2 to 15 years. Clinical parameters such as hypertension or proteinuria and conventional histological ones such as the severity of interstitial infiltrate or fibrosis did not differentiate P from NP. Vascular sclerosis was statistically more severe in P ($P = 0.0053$). Glomerular α-SMA was significantly higher in P (0.36 ± 0.22) than in NP (0.17 ± 0.07), $P = 0.033$. Interstitial α-SMA was also higher in P but did not reach significance. By contrast, interstitial V was higher in P (0.208 ± 0.07); NP = 0.087 ± 0.05, $P = 0.0019$). These data suggest that the expression of glomerular α-SMA and interstitial vimentin predicts the response to treatment in MN.

Abnormal membrane lipid-cytoskeleton interactions in adult polycystic kidney disease. *K. Vareesangthip, T.H. Thomas, R. Wilkinson, Department of Medicine (Nephrology), University of Newcastle upon Tyne, Newcastle upon Tyne.* We have previously shown that the red blood cell (rbc) membrane fluidity in adult polycystic kidney disease (APCKD) patients is higher than that in normal controls. This difference was found only in intact rbcs but not in ghost membranes indicating that the cytoskeleton is abnormal in APCKD. The dynamics of the membrane lipid bilayer are mainly controlled by the lipid-cytoskeleton interactions. Five APCKD patients and 5 normal controls (NC) were studied by measuring fluorescence anisotropy of the rbc membrane after incubating with liposomes (Lp) containing antibodies to actin (anti-actin) and ankyrin (anti-ankyrin). Superficial and core region anisotropies were phobed by trimethyl ammonium-diphenylhexatriene (TMA-DPH) and 1,6-diphenyl-1,3,5-hexatriene (DPH) respectively. Results are shown in the Table.

	NC ($N = 5$)	
	TMA-DPH	DPH
Untreated rbc	0.277 ± 0.002	0.222 ± 0.001
Lp	0.277 ± 0.002	0.222 ± 0.001
Anti-actin/Lp	0.262 ± 0.002[b]	0.194 ± 0.001[b]
Anti-ankyrin/Lp	0.262 ± 0.002[b]	0.196 ± 0.002[b]

	APCKD ($N = 5$)	
	TMA-DPH	DPH
Untreated rbc	0.264 ± 0.001[a]	0.198 ± 0.001[a]
Lp	0.265 ± 0.001[a]	0.198 ± 0.001[a]
Anti-actin/Lp	0.264 ± 0.001	0.197 ± 0.001
Anti-ankyrin/Lp	0.263 ± 0.001	0.197 ± 0.001

Values are mean ± SEM. [a] $P < 0.001$ for NC and APCKD patients; [b] $P < 0.001$ for untreated rbc and after incubation with anti-actin/Lp or anti-ankyrin/Lp.

Disturbance of cytoskeletal actin and ankyrin clearly caused alterations of membrane fluidity in NC but not in APCKD patients. This indicates that the interactions between the lipid bilayer and the cytoskeleton of erythrocyte membranes are abnormal in APCKD.

Transglutaminase intracellularly crosslinks tubule cells and stabilizes the ECM in kidney fibrosis. *T.S. Johnson, M. Griffin, G.L. Thomas, B. Yang, J. Skill, A. Cox, B. Nicholas, C. Kubara, and A.M. El Nahas, Sheffield Kidney Institute, Northern General Hospital Trust, Sheffield; Department of Life Science, Nottingham Trent University, Nottingham, England, United Kingdom.* Transglutaminases (Tg) are a family of calcium-dependent enzymes that catalyse the cross-linking of polypeptide chains, including those of extracellular matrix (ECM) proteins, through the formation of ε (γ-glutamyl) lysine bonds. This leads to the formation of protein polymers that are highly resistant to degradation. Consequently, tissue Tg have been associated with ECM protein deposition in fibrotic diseases such as pulmonary fibrosis and atherosclerosis. We investigated the involvement of Tg in the development of kidney fibrosis in adult male Wistar rats submitted to subtotal nephrectomy (SNx). Groups of 4 to 6 rats were sacrificed on days 7, 30, 90 and 120 after SNx. These rats developed progressive glomerulosclerosis and tubulointerstitial fibrosis. The tissue level of ε (γ-glutamyl) lysine crosslink (as determined by exhaustive proteolytic digestion followed by cation exchange HPLC) increased from 347 ± 94 pm/mg protein (mean ± SEM) in controls to 1324 ± 143 pm/mg 90 days after SNx, $P < 0.01$. Levels of crosslink correlated well with the renal fibrosis score throughout the 120 observation days ($r = 0.84$, $P < 0.01$). Tissue homogenates, when corrected for surviving tubule mass showed significant changes in overall Tg levels as determined by activity (^{14}C putrescine incorporation assay) increasing from 5.77 ± 0.35 to 13.93 ± 4.21 U/μg DNA ($P < 0.01$), while Western blot analysis showed increases in Tg antigen from 829 ± 199 to 5256 ± 2163 U ($P < 0.01$). Immunohistochemistry (avidin-biotin-peroxidase) demonstrated that this large increase in crosslink and Tg was predominantly in the cytoplasm of tubular cells. Immunofluorescence showed low levels of ε (γ-glutamyl) lysine bonds in the extracellular environment in scarred tissue. Some of the cells high in crosslink and Tg displayed morphology typical of a cell undergoing apoptosis, although the majority of these did not show DNA cleavage (*in situ* end labelling) normally associated with apoptosis. We postulate a dual role for tissue transglutaminases in the development of experimental renal fibrosis by stabilizing extracellular matrix and the intracellular crosslinking of proteins leading to cell death.

Effects of verocytotoxin-1 on human renal epithelial cells. *J.M. Williams, D.V. Milford, and C.M. Taylor, Department of Nephrology, The Children's Hospital, and Renal Research Laboratories, University of Birmingham, Birmingham, England, United Kingdom.* Infection with verocytotoxin-producing *E. coli* causes hemolytic uremic syndrome in which endothelial damage is central to the pathogenesis of the disease. Verocytotoxin-1 (VT1) is cytopathic to certain immortalized cell lines and renal microvascular endothelial cells in culture. Uncertainty surrounds the localization of VT1 receptor (Gb3) in the kidney. Lingwood (1995) found Gb3 was localized to the glomerulus and distal convoluted tubule in pediatric renal sections, whereas both Siegler (1994) and Oosterwijk (1991) found staining was confined to renal tubular epithelial cells. We provide evidence that VT1 inhibits protein synthesis in primary cultures of glomerular and proximal tubular epithelial cells. These cells are more sensitive to the protein synthesis inhibitory effects of VT1 than Vero cells (for example, 10 pg/ml reduced protein synthesis to 21.75% over 24 hours). However, unlike Vero cells apoptosis is not apparent at 24 hours.

Percentage of basal protein synthesis in the presence of VT1

Time (h)	4	10
Glomerular epithelial cells		
1 pg/ml	87.0 ± 4.7	54.8 ± 4.2
10 pg/ml	29.1 ± 2.8	14.9 ± 2.0
100 pg/ml	5.7 ± 0.6	2.2 ± 0.5
Proximal tubular epithelial cells		
1 pg/ml	92 ± 2.7	81.6 ± 2.0
10 pg/ml	50.6 ± 1.0	16.1 ± 0.6
100 pg/ml	3.3 ± 0.2	0.7 ± 0.01

Time (h)	16	24
Glomerular epithelial cells		
1 pg/ml	45.5 ± 6.7	47.6 ± 4.1
10 pg/ml	7.4 ± 0.9	4.9 ± 0.5
100 pg/ml	1.4 ± 0.3	1.5 ± 0.1
Proximal tubular epithelial cells		
1 pg/ml	74.1 ± 1.8	76.0 ± 10
10 pg/ml	9.4 ± 0.4	7.1 ± 1.1
100 pg/ml	0 ± 0	0.7 ± 0.2

Mean ± SD; $N = 3$

We conclude that VT1 induces profound protein synthesis inhibition in glomerular and proximal tubular epithelial cells without early cytocidal effects.

Multilevel modelling of the determinants of acceptance on to renal replacement therapy in England. *P. Roderick, S. Clements, N. Stone, D. Martin, and I. Diamond, Departments of Public Health, Social Statistics, Geography, Southampton University, Southampton, England, United Kingdom.* **Background.** The National Renal Review in England found an overall unmet need in renal replacement therapy provision and substantial variation in age, sex, ethnic and district health authority rates. The higher ethnic rates found may be partly confounded with the proximity of ethnic minority populations to renal services and DHA rates partly explained by "need" based factors such as age, sex, and ethnicity. However, little is known of the influence of socio-economic factors or the effect of access to or supply of renal services. Multilevel modelling techniques allow analysis of individual and real characteristics at the same time. This has been applied to the Review dataset and this paper will present data on the independent effects on acceptance rates of need, access and supply factors. **Methods.** The Review collected information on each adult patient accepted onto RRT in England in 1991–1993: 6046 patients were accepted in 53 renal units, 5715 (94.5%) had valid postcodes and were assigned to their Census electoral ward. Multilevel modelling with Poisson regression were used with ward acceptances as the dependent variable. Level 1 dependent variables were age and sex. Level 2 ward factors included percent Asian or Black, deprivation indices, and accessibility (crow fly distance or private transport time from ward centroid to renal units) and supply (nephrology beds or hemodialysis stations/catchment population). **Results and conclusion.** The modelling results presented will show the independent effects of age, sex, ethnicity, social deprivation, travel time and distance and supply on the RRT acceptance rate for England and their effects in explaining DHA variations. The implications for understanding the epidemiology of ESRF and for the planning of renal services will be discussed.