GBM heparan sulfate alterations in experimental diabetic nephropathy. J. van den Born, A.A. van Kruis, M.A.H. Bakker, K.J.M. Asmann, H.B.P.M. Dijkman, I.W.M. van der Laak and J.H.M. Berden, Departments of Nephrology and Pathology, University of Nijmegen, Nijmegen, The Netherlands. Heparan sulfate (HS) is the strongly anionic side chain of heparan sulfate proteoglycan (HSPG), the major proteoglycan within the GBM, responsible for the charge selective permeability of the GBM. To investigate the possible role of HS in diabetic nephropathy (DNP), male Wistar-Münch rats (N = 15) were made diabetic by an i.v. injection of 55 mg streptozotocin/kg body wt. Rats were treated by a low dose of insulin to maintain blood glucose levels between 20-25 mmol/l. Age matched rats (N = 5) were used as controls. Albuminuria was measured by rocket immunoelectrophoresis, the selectivity index was defined as the clearance of IgG clearance of albumin. After eight months kidneys were removed for histology and isolation of the glomeruli. 4-hydroxyproline (4-HP, collagen content) was measured colorimetrically after hydrolysis of the glomeruli. OHM US was analyzed using two different methods: (a) quantitation of the HS content in glomerular extracts by inhibition-ELISA using anti-GBM HS mAb JM-403; (b) quantitation of the HS-associated anionic sites in the GBM by cuproin blue staining and quantitation by a computerized image analyzer. The HS specificity of the staining was investigated by preadsorption with the HS mAb. OHM US was decreased after eight months in diabetic rats compared to control rats (P < 0.05). Recently, we found in biopsies of human lupus nephritis an associated anionic sites has been implicated in the development of albuminuria. The expression of USPO-core protein was inhibited by the AT1 receptor antagonist DuP-753 (10^-6 M). These data suggest that Ang II induces an inhibition of HS production by AMC in vitro and that AT1 receptors may play a role in this process.

Decrease of heparan sulfate staining in the glomerular basement membrane in murine lupus nephritis. M.C.J. van Bruggen, C. Kramers, M.N. Hylkema, J. van den Born, M.A.H. Bakker, K.J.M. Asmann, R.J.T. Smeenk, and J.H.M. Berden, Departments of Nephrology and Pathology, University Hospital Nijmegen, Nijmegen, and Department of Autoimmune Diseases, CBL, Amsterdam, The Netherlands. Loss of heparan sulfate (HS) associated anionic sites has been implicated in the development of albuminuria. Recently, we found in biopsies of human lupus nephritis a nearly complete loss of HS staining in the GBM. To clarify the relationship between HS staining and albuminuria in lupus nephritis, we studied MRL/lpr mice with short (<7 days) or prolonged duration of albuminuria (14-21 days) and compared them with age matched controls without albuminuria (N = 8 per group). Kidney sections were stained for mouse Ig, HS and heparan sulfate proteoglycan (HSPG)-core protein by immunofluorescence (IF). In mice with prolonged albuminuria HS staining in the glomerular capillary loops had almost completely disappeared, whereas staining was unaltered in non-albuminuric mice. In mice with short duration of albuminuria, there was a tendency towards a decrease of HS staining (P = 0.06). The expression of HSPG-core protein was unaltered in all groups. HS staining correlated inversely with albuminuria (r = -0.55; P < 0.001) and with staining of Ig deposits in the capillary loops (r = -0.74; P < 0.001). Despite the nearly complete loss of HS staining in the GBM in mice with prolonged albuminuria, there was no...
change in glomerular HS content as assessed with an inhibition ELISA in extracts of pooled isolated glomeruli in the different groups. Results are given in the Table [mean ± SD or median (range)].

<table>
<thead>
<tr>
<th></th>
<th>No albuminuria</th>
<th>Recent albuminuria</th>
<th>Prolonged albuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IF staining GBM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse Ig</td>
<td>1.3 ± 0.7*</td>
<td>1.8 ± 0.8</td>
<td>2.4 ± 0.7*</td>
</tr>
<tr>
<td>HS</td>
<td>2.7 ± 0.6</td>
<td>1.9 ± 0.8</td>
<td>0.5 ± 0.2*</td>
</tr>
<tr>
<td>HSPG-corc</td>
<td>3.3 ± 0.3</td>
<td>3.0 ± 0.3</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>HS content pg/glomerulus</td>
<td>81</td>
<td>105</td>
<td>87</td>
</tr>
<tr>
<td>albuminuria</td>
<td>52</td>
<td>1700</td>
<td>4800</td>
</tr>
<tr>
<td>μg/18 hours</td>
<td>(20–104)</td>
<td>(1200–21400)</td>
<td>(2100–23800)*</td>
</tr>
</tbody>
</table>

* P < 0.05 compared with non-albuminuric age matched controls

We conclude that the development of albuminuria in MRL/lpr mice is paralleled by a loss of HS staining in the GBM which is probably due to masking of HS by deposits of Ig.

Glucomerular pressure (P_{GC}) and tubuloglomerular feedback (TGF) in Nagase anabuminic rats (NAR). B. Braam, J.A. Joles, J. Grond, and H.A. Koomans, Department of Nephrology, University Hospital Utrecht, Utrecht, Department of Pathology, University Hospital Groningen, Groningen, The Netherlands. The ∆ NAR as compared to ∆ NAR displays severe hyperlipidemia. The ∆ uninephrectomized (UnX) NAR develops proteinuria and glomerulosclerosis (GS), in contrast to the ∆ UnX NAR. As increased P_{GC} is generally considered to be a major risk factor in the development of GS, we evaluated glomerular hemodynamics and TGF responsiveness in two-kidney (2K) and UnX, ∆ and ∆ NAR at a moment shortly before proteinuria develops; 13–15 weeks following UnX or sham-operation, rats were anesthetized (pentobarbital, 50 mg/kg body wt) and prepared for micropuncture. Late proximal tubular fluid samples were collected and stop-flow (SFP), star vessel and free flow pressure were assessed. Furthermore, maximum TGF-mediated decreases in SFP (ASF-P_{max}) were evaluated by late proximal perfusion with artificial tubular fluid (at 40 in 2K and 80 nI/mm in UnX rats).

Plasma cholesterol was 7.1 ± 0.6* and 7.1 ± 0.3* in UnX and 2K and 5.5 ± 0.3* and 4.2 ± 0.3 mm in UnX and 2K ∆ rats. Plasma triglycerides were 5.5 ± 0.7* and 6.5 ± 1.4* in UnX and 2K ∆ and 1.8 ± 0.2 and 1.5 ± 0.3 mm in UnX and 2K ∆ rats. Hyperfiltration, glomerular hypertrophy, and arterial pressure were comparable in ∆ and ∆ NARs. In the ∆ UnX rats, no increase in P_{GC} could be demonstrated. TGF behavior in response to UnX differs between ∆ and ∆ UnX rats. The present study, together with previous data, suggests that in ∆ UnX NAR, plasma lipid composition forms a major risk factor for the development of GS.

Identification of Rf/1, a gene responsible for renal failure in the fawn-haired (FHH) rat. A.P. Provoost, D.M. Brown, M.J. Daly, E.S. Lander, and H.J. Jacobs, Department of Pediatric Surgery, Erasmus University, Rotterdam, the Netherlands, Cardiovascular Research Center, Massachusetts General Hospital/Harvard Medical School, Charlestown, Massachusetts, and Whitehead Institute for Biomedical Research/Massachusetts Institute of Technology Genome Center, Cambridge, Massachusetts, USA. End-stage renal disease (ESRD) is a serious potential complication of hypertension, although only a minority of hypertensive patients develop ESRD. We have investigated the relationship between hypertension and ESRD using the genetically hypertensive fawn-haired (FHH) rat. Previous studies in the FHH have suggested that systemic and/or glomerular hypertension was the primary cause of renal failure as defined by proteinuria and focal glomerular sclerosis. We approached the interaction between hypertension and renal failure using molecular genetics. Linkage analysis was performed in a genetic cosegregation study using the FHH and the normotensive non-proteinuric ACI rat. A total of 54 genetic markers, covering at least 99% of the rat genome, were evaluated in the male backcross F1 (FHH × ACI) × FHH progeny. These animals were analyzed for linkage with a macroscopic renal sclerosis score (RSS), proteinuria, and systolic blood pressure (SBP). Strong evidence was found for a locus, named renal failure 1 (Rf/1) on chromosome 1, that is responsible for 55% of the total variance in RSS, and 36% of the variance in proteinuria. Interestingly, the region was found to have no significant effect on SBP. We did, however, locate another locus accounting for 15% of the variance in SBP, which also mapped to chromosome 1. This locus appeared to contain the S_{α} gene, but it failed to account for any variance in the renal failure phenotypes. These results seem to imply that development of hypertension-associated renal damage does not result simply from the effects of systemic hypertension on the kidney, but also involves the presence of other genetic susceptibility factors. In this respect, our study is the first to demonstrate that the genetic predisposition to renal failure appears to be a disease within a disease.

Effects of Ca^{2+} channel blockers, low Ca^{2+} medium and glycine on cell Ca^{2+} and cell injury in anoxic rabbit proximal tubule cells. U.M. Rose, R.J.M. Bindels, and C.H. van Os, Department of Physiology, University of Nijmegen, The Netherlands. L-type Ca channel blockers (CCBs) have been shown to be protective against ischemia-induced injury of the kidney, suggesting that increased intracellular Ca^{2+} levels ([(Ca^{2+})]) play an important role in the pathogenesis of ischemic cell injury. To assess the role of [(Ca^{2+})], in anoxic injury of the proximal tubule (PT) and the protective effect of CCBs, [(Ca^{2+})], was monitored in individual PT cells during 60 minutes of anoxia. PT cells were attached to Cell-Tak® coated coverslips and subsequently loaded with fura-2 AM and mounted in an anoxic chamber at 37°C. [(Ca^{2+})], was measured using digital imaging fluorescence microscopy. During anoxia, [(Ca^{2+})], started to rise within 10 minutes, reaching a maximal level between 30–45 minutes of anoxia. The onset of this increase and the maximal levels reached varied markedly among individual cells. The mean values for initial and maximal anoxic [(Ca^{2+})], were 109 ± 2 and 422 ± 14 nm, respectively. Methoxyverapamil (D600; 1 μM) significantly reduced anoxic [(Ca^{2+})] to 122 ± 5 nm (P < 0.05). Removal of extracellular Ca^{2+} completely abolished anoxia-induced increases in [(Ca^{2+})], confirming that these increases in [(Ca^{2+})], result from Ca^{2+} influx. During 60 minutes of anoxia, PT cells showed a gradual decrease in cell viability to 54 ± 2%. D600 (1 μM) significantly increased cell viability to 64 ± 3% (P < 0.05). Low Ca^{2+} medium only protected when 0.1 La^{3+} was included, which condition increased cell viability to 82 ± 5%. La^{3+} did not enter PT cells and probably protects via a membrane-stabilizing effect. Glycine (5 mM), however, increased cell viability to 77 ± 4% without a significant reduction in anoxic [(Ca^{2+})]. The combination of glycine and La^{3+} did not further increase the protection. In conclusion, D600 almost completely prevented anoxia-induced increases in [(Ca^{2+})], by blocking Ca^{2+} influx via L-type Ca^{2+} channels. Since D600 only partly protected PT cells against anoxic injury, [(Ca^{2+})], unrelated cell injury, which is attenuated by glycine, is a more prominent factor in anoxia-induced cell injury in rabbit PT cells.

The dihydropyridine calcium entry blocker felodipine and its non-calcium channel blockers, dihydropyridine derivative H186/86 attenuate hypoxia-induced cell injury in isolated rat proximal tubules. S.M.A. Peters, M.J.A. Tijen, R.J.M. Bindels, R.A.P. Koene, C.H. van Os, and J.F.M. Wetzel, Department of Physiology, University of Nijmegen, and Department of Nephrology, University Hospital Nijmegen, The Netherlands. The hypothesis that calcium influx into hypoxic renal proximal tubules participates in the development of cell injury is mainly based on the observations of the protective effects of the phenylephrine-converting enzyme inhibitor captopril. We have studied the effects of the dihydropyridine calcium entry blocker felodipine. To dissociate the protective effects from the calcium blocking properties, we also studied the effects of the felodipine derivative H186/86, which has no calcium-antagonistic properties, on hypoxia-induced injury. Freshly isolated rat proximal tubules were incubated in a modified Krebs-Henseleit buffer (calcium 1.0 mM) under normoxic (95% O_{2}, 5% CO_{2}) or hypoxic
conditions (95% N₂, 5% CO₂) for 30 minutes. Hypoxia caused significant cell injury as reflected by the release of LDH (control 26.6 ± 2.2%, hypoxia 67.6 ± 2.0%; means ± SEM, P < 0.01). Both felodipine (100 μM) and H1186/86 (100 μM) attenuated cell injury (LDH release 38.2 ± 3.0% and 50.3 ± 2.5% respectively, P < 0.01 vs. hypoxia). No protection was observed with 10 μM felodipine (LDH release 72.0 ± 3.0%). The protective effect of felodipine was accompanied by a better preservation of cell potassium under hypoxic conditions. Lowering of extracellular calcium (10 μM) did not prevent but rather enhanced hypoxic cell injury (LDH release 78.2 ± 4.0% vs. 68.2 ± 2.0%, P < 0.05). Both felodipine and H1186/86, in protective concentrations, increased intracellular potassium levels in control normoxic tubules (control 200 ± 9, felodipine 250 ± 17, H1186/86 260 ± 9 mmol/mg protein, both P < 0.01). During normoxia, felodipine, but not H1186/86, caused cell injury (LDH release: control 26.2 ± 2.2%, felodipine 43.8 ± 3.7%, H1186/86 28 ± 2.9%). In conclusion, the dihydropyridine calcium entry blocker felodipine protects isolated rat renal tubules against hypoxic cell injury only at very high concentrations.

Together with our observations that the derivative H1186/86 also affords protection, and that low medium calcium is not protective at all, this strongly suggests that the protective effect of felodipine is not mediated by blockade of calcium entry. Preservation of cell potassium might contribute to the effects observed.

Renin-angiotensin system components in the cardiac interstitial fluid. Uptake from plasma and local production. L.M. De Lannoy, A.H.J. Danser, R.G. Schoemaker, P.R. Saxena, and M.A.D.H. Schalekamp, Departments of Internal Medicine I and Pharmacology, Erasmus University, Rotterdam, The Netherlands. To develop a Langendorff model. This model allowed us to collect separately the coronary effluent (CE) and interstitial fluid (1SF), which drips from the coronary microcirculation. Angiotensinogen (Ao), Ang I (Ang I) and Ang II (Ang II), using a modified Langendorff method. This model allowed us to collect separately the coronary effluent (CE) and interstitial fluid (ISF), which drips from the heart muscle. Each sample was collected in separate 1SF fractions and (ISF and 1SF) were continuously collected. Prior to their infusion, Ang I and Ang II were below the detection limit in CE and ISF. Ang II entered the ISF slowly during its infusion, like other serum proteins (steady-state levels in >25 minutes). In contrast, Ang I reached steady-state levels within 10 minutes and could still be detected in ISF, but not CE, 10 minutes after Ang I infusion had been stopped. In addition, the Ang II levels in 1SF were 2–3 times those in CE, whereas Ang I in ISF rose to 75% of the level in CE. Infused Ang I and Ang II reached steady-state levels in ISF within 2–3 minutes and disappeared within 2–3 minutes after the infusion had been stopped. During Ang I infusion, the steady-state level of Ang II in ISF was 75% of that in CE, and during Ang II infusion the steady-state level of Ang I in ISF was 55% of that in CE. Ang I infusion resulted in the appearance of Ang II in ISF and CE, and during Ang I infusion the steady-state Ang II levels in ISF were slightly higher than those in CE. During R infusion Ang I and Ang II appeared in ISF in concentrations 5–10 times higher than in CE. The results indicate that: (1) in vivo part of Ang I and Ang II in the cardiac interstitium is produced in the heart; (2) renin is rapidly taken up by the heart from the circulation, possibly by an active process; and (3) intracardiac Ang I production depends on renin from the kidney.

Does the renin-angiotensin system determine the renal and systemic response to sodium in essential hypertension? P. van Pausen, D. de Zeeuw, and P.E. de Jong, University Hospital Groningen, Groningen, The Netherlands. Many patients with essential hypertension (EH) respond to a high dietary sodium intake with a rise in blood pressure (BP). Experimental evidence suggests that the renal hemodynamic response to HS determines, at least partially, this rise in BP. To clarify the role of the renin-angiotensin system (RAS) in the renal and systemic hemodynamic adaptation to a change in dietary sodium, we studied mean arterial pressure (MAP), mean arterial pressure (MAP), effective renal plasma flow (ERPF, ml/min/1.73 m²), body weight (body wt, kg), and immunoreactive renin (IR, pg/ml) in 17 EH and 15 normotensive controls (NT), on a sodium restricted (50 mmol, LS) and a sodium replete diet (200 mmol, HS), both for three weeks (random crossover, placebo controlled), as well as after renin-inhibition by Remikiren (REM, 600 mg, single oral dose) during the high sodium diet. In NT, HS had no effect on MAP (90 ± 1 to 88 ± 2) or body wt (72 ± 2 to 72.6 ± 2), whereas ERPF increased (490 ± 19 to 535 ± 21, P < 0.05) and IR decreased (31.8 ± 5.6 to 14.1 ± 1.4, P < 0.05). In EH, HS induced a heterogenous response of MAP (110 ± 2 to 114 ± 2, NS), ERPF (424 ± 21 to 451 ± 25, NS) and irR (18.1 ± 3.1 to 10.1 ± 1, P < 0.05), with an increase in body wt (81.3 ± 1.9 to 82.5 ± 2, P < 0.05). Interestingly, the patients with a distinct rise in MAP showed a blunted ERPF response and irR response to HS (r = 0.72, P < 0.01 and r = 0.70, P < 0.01, respectively). Moreover, the change in ERPF correlated negatively with the change in irR (r = 0.71, P < 0.01). After REM a varying rise in ERPF (451 ± 25 to 464 ± 23, NS) was observed: the patients with the blunted ERPF response to HS showed the largest ERPF rise (r = 0.69, P < 0.01). The REM-induced rise in ERPF correlated (r = 0.68, P < 0.01) with the fall in MAP (114 ± 2 to 110 ± 2). In conclusion, in EH a rise in blood pressure in response to a high sodium intake appears to partially be the result of an insufficient renin vasodilatation. This seems to be the result of an inadequate (intrarenal?) RAS response.

Prognostic significance of severe interstitial edema with minimal infiltrate in renal allograft biopsies. M.I.J.J. Bogman, Ph. M.M. Dooper, A.J. Hoitsma, K.J.M. Assmann, and R.A.P. Koene, Departments of Medicine and Pathology, University Hospital Nijmegen, Nijmegen, The Netherlands. Percutaneous renal allograft biopsies show in 5–10% of the cases severe localized or diffuse interstitial edema with minimal T-cell infiltrate. Based on diagnostic features such as intimal arteritis, tubulitis, and/or infiltrate in other areas of the biopsy in most cases a histological diagnosis of acute rejection (AR) can be made. In cases with diffuse edema in which no arteries are found, the diagnosis often remains uncertain. We tested whether severe edema with little or no infiltrate in one or multiple cortical areas was associated with an unfavorable response to treatment and a higher incidence of graft failure. For this we screened consecutive allograft biopsies, taken over a 3-year period, for the presence of severe interstitial edema, defined as a cortical area in which at least 10 adjacent tubuli are separated by intertubular spaces of more than 1 tubular diameter in width, and filled with scurvy fluid in which no or very few mononuclear cells are seen. In a series of 292 adequate renal cortical graft biopsies, 26 cases (8.9%) showed severe edema that was localized in 20 cases, and diffuse (that is, over more than 80% of the biopsy area) in 6 cases. In 24/26 cases the histological diagnosis was AR grade I–III (Banff Classification). The 2 remaining cases showed borderline changes with minimal tubulitis, in 1 case accompanied with membranous nephropathy and transplant glomerulopathy, and in both cases with interstitial fibrosis suggestive of chronic rejection. In 13/26 biopsies with edema medium-sized or larger arteries were seen, and in 12 cases (92%) these showed intimal fibrosis, that in 9/12 was accompanied with acute intimal arteritis. One biopsy contained arteries without pathological changes. Apart from the case with membranous nephropathy all patients received anti-rejection therapy. In the edema group the percentage of graft failure to date (39%) was significantly higher than in the AR group (21), P < 0.01. Most importantly, the patients with severe edema and little or no infiltrate had a 71.4% increased risk of graft failure compared to patients with diffuse edema and T-cell infiltrate (21, 7, P < 0.05). The difference in graft survival rates between the two groups was statistically significant (log rank test, P = 0.02). These findings underscore the importance of recognizing early diffuse edema in allograft biopsies and initiating an aggressive treatment to prevent graft failure.
Abstracts

Renal hemodynamics and the renin-angiotensin system in psoriasis patients during cyclosporine treatment. M.A. van den Dorpel, R.J.A. de Bruin, G.J. Wenting, F. Boomsma, F.H.M. Derlenc, and M.A.D.H. Schalekamp, Department of Internal Medicine I, Erasmus University, Rotterdam, The Netherlands. Cyclosporine (CsA) treatment in transplant patients often leads to hypertension and impaired renal function. Studies of the underlying mechanism in these patients may be confounded by preexisting renal failure and cardiovascular disease, graft denervation, or the use of concomitant medication. We therefore studied the effects of CsA in psoriasis patients (N = 12) who were otherwise healthy. During the first 3 months CsA dose was 5 mg/kg/day (high dose), followed by 6–18 months of 2 mg/kg/day (low dose). Mean arterial pressure (MAP), glomerular filtration rate (GFR), effective renal plasma flow (ERPF), renal vascular resistance (RVR) and hormones were measured at baseline, at the end of both CsA dosage regimens and 3 months after cessation of CsA treatment. Results were as follows (mean ± SD):

<table>
<thead>
<tr>
<th>Study/Period</th>
<th>MAP (mm Hg)</th>
<th>GFR (ml/min/m²)</th>
<th>ERPF (ml/min/m²)</th>
<th>RVR (dyn-s·cm⁻⁵)</th>
<th>Active renin (µIU/ml)</th>
<th>Prorenin (µIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>88 ± 8</td>
<td>135 ± 36</td>
<td>684 ± 243</td>
<td>11,675 ± 3,929</td>
<td>22.2 ± 8.3</td>
<td>231 ± 131</td>
</tr>
<tr>
<td>High</td>
<td>97 ± 10</td>
<td>106 ± 15</td>
<td>516 ± 120</td>
<td>15,958 ± 4,258</td>
<td>15.3 ± 5.4</td>
<td>357 ± 132</td>
</tr>
<tr>
<td>Low</td>
<td>93 ± 8</td>
<td>109 ± 20</td>
<td>494 ± 132</td>
<td>15,867 ± 3,935</td>
<td>17.4 ± 8.5</td>
<td>235 ± 61</td>
</tr>
<tr>
<td>After</td>
<td>84 ± 18</td>
<td>126 ± 23</td>
<td>550 ± 149</td>
<td>13,523 ± 3,678</td>
<td>28.2 ± 13.6</td>
<td>207 ± 75</td>
</tr>
</tbody>
</table>

* P < 0.05, Kruskal-Wallis one-way ANOVA

CsA treatment increased arterial pressure and reduced GFR and ERPF. Effects were still present 3 months after CsA had been stopped. CsA significantly reduced renin but increased prorenin. Our results demonstrate marked blood pressure elevation and intrarenal vasoconstriction by CsA in subjects with normal cardiovascular and renal function. This effect is not caused by renin stimulation. CsA treatment may interfere with prorenin to renin conversion.

Renal function after lung transplantation. J.G. Navis, H. Pathoog, W. van de Bij, G.P.M. Manners, W.J. de Boer, and P.E. de Jong, Division of Nephrology and Pulmonology, Department of Internal Medicine and Department of Thoracic Surgery, University Hospital, Groningen, Groningen, The Netherlands. In patients with terminal respiratory failure, lung transplantation (LTX) improves life expectancy. The nephrotoxicity of the therapeutic regimen (including cyclosporine A, ATG and co-trimoxazole) has not been well-defined for these patients. We therefore included renal function measurements (clearances of ³¹¹-Hippuran and ¹²⁵-Iothalamate, for ERPF and GFR, respectively) in the pre- and post-LTX evaluation of LTX patients. Of 32 patients transplanted between April 1991 and December 1993, 2 patients died from primary graft failure, and 3 patients died from pulmonary causes after 3, 6, and 7 months, respectively. The results of the patients available for renal follow-up are listed below:

<table>
<thead>
<tr>
<th>Study</th>
<th>GFR (ml/min/1.73 m²)</th>
<th>ERPF (ml/min/1.73 m²)</th>
<th>FF</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-LTX</td>
<td>30</td>
<td>103</td>
<td>391</td>
<td>0.27</td>
</tr>
<tr>
<td>Post-transplantation % change from pre-LTX value</td>
<td>1 month</td>
<td>30</td>
<td>-10b</td>
<td>0</td>
</tr>
<tr>
<td>6 months</td>
<td>20</td>
<td>-21b</td>
<td>-12</td>
<td>-70b</td>
</tr>
<tr>
<td>12 months</td>
<td>16</td>
<td>-31b</td>
<td>-13</td>
<td>-57b</td>
</tr>
</tbody>
</table>

* P < 0.05; b P < 0.01, paired Wilcoxon test vs. pre-transplant value

Thus, a decline in GFR was apparent 1 month after LTX, with a further fall after 6 and 12 months. Urinalysis remained normal in all patients. Antihypertensive treatment was needed in 8 patients after 12 months. Pre-LTX GFR was mildly impaired (60–90 ml/min), without other signs of renal disease in 8 patients. Pre-LTX GFR and ERPF were negatively correlated with the post-LTX changes in GFR (r = −0.70; P < 0.01) and ERPF (r = −0.87; P < 0.001), respectively. Thus, the renal function decline after LTX was less pronounced in patients with pre-LTX renal function impairment. This contrasts with the common finding of greater renal vulnerability in the presence of impaired renal function in other populations. Post hoc analysis of our data suggests that this is due to a particular feature of the renal functional impairment in pre-LTX patients: in all patients with impaired pre-LTX GFR intense renal vasoconstriction was present, that was partially reversible after LTX. In conclusion, a significant decline in renal function and a rise in blood pressure occur after LTX, presumably due to nephrotoxicity of the therapeutic regimen. Nevertheless, pre-LTX renal function impairment, characterized by intense renal vasoconstriction and the absence of urinary abnormalities, should not be considered a contra-indication for LTX.
Role of TNF receptors and protein kinase C (PKC) in the induction of the verocytotoxin receptor, Glb3, in human endothelial cells. N.C.A. van de Kar, T. Kooistra, L.A.H. Munnens, and V.W.M. van Hinsbergh, Gauthius Laboratorium TNO-PG, Leiden, and Department of Pediatrics, Sint Romboud Hospital, Nijmegen, The Netherlands. Infections with verocytotoxin (VT) producing E. coli infections have been strongly implicated in the epidemic form of hemolytic uraemic syndrome (HUS). Endothelial damage plays a central role in the pathogenesis of HUS. In vitro studies have shown that VT can damage endothelial cells after interaction with a cellular receptor, which has been identified as globotriaosylceramide (Gb3). TNFa and IL-1 potentiate the toxic effect of VT by inducing an increase in VT receptors (Gb3) on endothelial cells. The TNFa-induced increase in VT-receptors was prevented by the protein synthesis inhibitor cycloheximide. This study further investigates the mechanisms underlying the increase in endothelial VT receptors induced by TNFa. To investigate which proteins were involved in this induction, endothelial cells were incubated with and without TNFa in the presence of 3H-Galactose or 3H-glucose. TLC analysis of the glycolipid extracts of these cells demonstrated a markedly enhanced incorporation of 3H-Galactose and in GB3 and Gb3 and other galactose-containing glycolipids, suggesting that TNFa enhanced the expression of 2 galactosyltransferase activities (i.e., Gb3 and Gb3). To examine the role of the 2 recently cloned TNF receptors (TNFR75 and TNFR55) in the TNFa-induced increase in Gb3 in human endothelial cells, cells were incubated with TNFa or a TNFa mutant that only recognizes and stimulates TNFR55. The effect of TNFa, determined by binding experiments with 125I-VT-1, could be largely, but not completely, mimicked by the TNFa mutant, indicating that the receptor-mediated increase in VT receptors is not completely present during the first 4 hours of incubation. A role for PKC activity in the signaling pathway for TNFa was investigated. Activation of PKC by phorbol ester could partly mimic the effect of TNFa. The involvement of PKC in the TNFa-mediated increase in VT receptors was further demonstrated by the specific PKC inhibitor Ro31-8220. Our results indicate that the increase in VT receptors on endothelial cells by TNFa mainly occurs via the TNFR55 and depends on PKC activity.

The production of monocyte chemoattractant protein-1 by human proximal tubular epithelial cells is up-regulated by interleukin-1z and tumor necrosis factor a. W. Prodjusudjadi, J.S.J. Gerritsma, N. Klar-Mohamad, A.F. Gerritsen, J.A. Brujin, M.R. Daha, and L.A. van Es, Departments of Nephrology and Pathology, University Hospital Leiden, Leiden, The Netherlands. Impairment of renal function in various types of glomerular disease is associated with infiltration of mononuclear cells in the interstitium. The role of mesangial and tubulointerstitial cells in these processes is unknown. Mononuclear cells may play an important role in the damage of renal tissue through the production of various cytokines and growth factors. The mechanism of mononuclear cell infiltration in the interstitium is not fully understood, but it is likely that chemotactic factors and adhesion molecules are involved. Monocyte chemoattractant protein-1 (MCP-1) has recently been reported to have a high degree of specificity as a chemotactic factor for monocytes. We analyzed the presence of MCP-1 in renal biopsies of patients with various forms of glomerulonephritis and demonstrated that MCP-1 expression is increased in renal tubular epithelial cells during disease. To further analyze whether renal tubular epithelial cells are able to produce MCP-1, proximal tubular epithelial cells (PTEC) were obtained from 6 different kidneys and were cultured in vitro under serum-free conditions. To examine the role of macrophages, we studied the effect of TNFa and INF-gamma on MCP-1 production. PTEC in culture produced 2.23 ± 0.87 ng/24 hours/10^6 cells of MCP-1 and the presence of IL-1a and TNFa enhanced the production by each cell line in a dose- and time-dependent manner. Optimal levels of MCP-1 production were found with 250 pg/ml and 250 U/ml of IL-1z and TNFa, respectively. PTEC cultured in medium containing 250 pg IL-1z/ml or 250 U TNFa/ml enhanced the production of MCP-1 up to 6.42 ± 0.05 ng/24 hours/10^6 cells and 5.19 ± 1.03 ng/24 hours/10^6 cells as measured by radioimmunoassay. De novo synthesis of MCP-1 was demonstrated by blocking MCP-1 synthesis with cycloheximide. The molecular weight of MCP-1 produced by PTEC was determined by HPLC gel filtration and found to be 13 kD. Taken together, these findings indicate that PTEC are a source of MCP-1 and the increased expression of MCP-1 in renal tubular epithelial cells in various types of glomerulonephritis may directly influence the degree of inflammation in the kidney.

Complement in serum and dialysate in children on CAPD. R.E. Reddingius, C.H. Schröder, M.R. Daha, A.M. Koster, and L.A.H. Munnens, Department of Pediatrics, University Hospital Nijmegen, Nijmegen, and Department of Nephrology University Hospital Leiden, Leiden, The Netherlands. During chronic ambulatory peritoneal dialysis (CAPD) activation and consumption of complement in the peritoneal cavity could theoretically occur, with inappropriately high or low levels of complement components in dialysate as a consequence. Low levels of complement in the peritoneal cavity could impair host defense. Fifteen children treated with CAPD were studied. Median age of these children was 8.1 years (range 2.1—13.2). They had been on CAPD for a median period of 39.7 months (range 0.4—89.1). The children did not suffer from peritonitis or other significant infections. Seven complement factors (C1q, C3, C4, C5d, B, D, P) were simultaneously measured in dialysate and serum. Four non-complement proteins (β2-microglobulin, albumin, IgG, α2-macroglobulin) were also measured. Assuming a linear relationship between the log base 10 of molecular weight and the log base 10 of the dialysate/serum ratio for these non-complement proteins, the expected levels of the complement factors were determined. Possible effects of molecular structure or charge were neglected. The actual and expected dialysate/serum ratios were compared with a modified t-test, taking into account the inaccuracy of the estimate. The ratios of factor D (P < 0.001) and C3d (P = 0.02) were elevated, whereas those of C3 (P < 0.001), C4 (P < 0.001), and factor P (P < 0.01) were decreased. Relatively low dialysate levels of C4, C3 and factor P could be caused by intraperitoneal consumption of complement. High dialysate/serum ratios of C3d are a logical consequence of consumption of C3. High ratios of factor D indicate intraperitoneal production of factor D. These results provide evidence for activation of complement in the peritoneal cavity in children on CAPD.

In vivo characterization of muscarinic receptor subtypes in the forearm vascular bed of patients with primary hypertension. T.A. Bruning, P.C. Chang, M.G.C. Hendriks, E.A.P. Kuypers, and P.A. van Zwieten, Department of Pharmacotherapy, Academic Medical Centre, Amsterdam, and Departments of Nephrology and Hospital Pharmacy, University Hospital Leiden, Leiden, The Netherlands. Cholinergic vasodilation has been reported to be attenuated in patients with primary and secondary hypertension. We have recently presented evidence for the muscarinic type-3 (M3) receptor to predominate in the cholinergic vasodilator response in the forearm of healthy volunteers. To investigate the role of muscarinic receptor subtypes in the forearm resistance vasculature of 6 male patients with primary hypertension and 6 matched normotensive controls (both groups age 47 ± 4 years; mean ± SD) we infused methacholine (MCh) in the presence of saline, and the antagonists atropine (non-selective), pirenzepine (M1-selective), and AF-DX 116 (M2-selective), using venous occlusion plethysmography. Affinity constants (Kp values) were determined from calculated plasma concentrations of the infused compounds and resulting EC50 values. Sodium nitroprusside (SNP) was given as an endothelium-independent control. We found no differences between the groups for SNP- and MCh-induced vasodilation, with apparent EC50 values (~log molar; mean ± SD) of 7.32 ± 0.13 and 7.51 ± 0.21 (hypertensives), and 7.37 ± 0.13 and 7.45 ± 0.02 (controls), respectively. The concentration-response curve of MCh was shifted to the right by atropine, pirenzepine, and AF-DX 116, with apparent Kp values of 8.63 ± 0.29, 6.81 ± 0.13 and 5.51 ± 0.08 (hypertensives), and 8.62 ± 0.10, 6.98 ± 0.08 and 5.49 ± 0.09 (controls), respectively. Again, there were no statistical differences between the groups. The affinity constants and rank order of potency—atroipine > pirenzepine > AF-DX 116—confirm that cholinergic vasodilatation in this vascular bed is predominantly mediated by the M3-receptor subtype. The vasodilator responses to both SNP and MCh, as well as the functional characteristics (pKp values) proved unchanged by the hypertensive state.

Inhibition of sodium transport in rabbit cortical collecting system by extracellular ATP depends on protein kinase C activation. H.P.G. Koster, C.H. van Os, and R.J.M. Bindels, Department of Cell Physiology, University of Nijmegen, Nijmegen, The Netherlands. Cells from the rabbit connecting tubule and cortical collecting duct were isolated by immunodissociation, subsequently cultured on permeable filters and placed in Ussing chambers in which short-circuit current (Isc) and transepithelial conductance (G)
were measured. In all experiments, cells were pretreated for 15 hours with 10^{-5} M aldosterone, which doubled I_{EC} and G when compared to controls (I_{EC} 12.3 ± 3 vs. 27 ± 3 μA cm^{-2} cm^{-2}, G 1.8 ± 0.3 vs. 3.1 ± 0.4 mmol·cm^{-2}·s^{-1}). The Na^+ channel blocker benzamil dose-dependently inhibited I_{EC} (IC_{50} = 5 × 10^{-8} M). The benzamil-sensitive component of I_{EC} correlates with active trans epithelial Na^+ transport. Extracellular ATP (10^{-7}−10^{-3} M, apical side) reduced active transcellular Na^+ transport dose-dependently, with a maximal inhibition of 60 ± 5%. The rank order of potency of nucleotides analogues for inhibition of Na^+ transport was UTP > ADP > AMP > adenosine, which suggests that the ATP effect is mediated by P_{2y}-purinoceptors. At the cellular level, ATP (10^{-7}−10^{-3} M) induced a dose-dependent transient increase in intracellular [Ca^{2+}]_{cyt} ([Ca^{2+}]_{cyt}), followed by a sustained elevated level. Preloading the cells with the Ca^{2+} chelator BAPTA completely prevented the ATP-induced Ca^{2+} transients, but ATP was still able to inhibit Na^+ transport by 60%. This dissociation between Ca^{2+} transient and Na^+ transport inhibition is explained by ATP mediated activation of protein kinase C (PKC). Activation of PKC by the cell permeable 1,2-dioctanoyl-sn-glycerol mimicked ATP-induced inhibition of transcellular Na^+ transport. The inhibitory effect of ATP was completely abolished in PKC down-regulated cells. In conclusion, extracellular ATP binds to an apically located P_{2y}-purinoceptor, which activates phospholipase C and generates inositol1,4,5-triphosphate (IP_{3}) and diacylglycerol. For inhibition of Na^+ transport only activation of PKC by diacylglycerol is needed, since the IP_{3}-induced Ca^{2+} release and activation of a Ca^{2+} entry mechanism were not required.

Proteinuria and glomerular apolipoprotein (apo) deposition in uninephrectomized female analbuminemic rats. J.A. Joles, H. van Goor, H.A. Koomans, and A. van Tol, Department of Nephrology, University Hospital Rotterdam, The Netherlands. Female analbuminemic rats (NAR), as compared to male NAR, are known for their hyperlipidemia. It is still unclear whether hyperlipidemia per se contributes to the development of proteinuria in hyperfiltrating kidneys. To answer this question, NAR and e NAR were uninephrectomized (UnX) or sham-operated (2K). The NAR developed comparable degrees of hyperfiltration: 1 hour EDTA and 24 hours creatinine clearances increased to values between 140 and 175% vs. 2K: ap < 0.05; UnX: 2K 9 NAR. Gd) were longest in UnX e NAR. Thus, in the absence of excessive hyperfiltration and glomerular hypertrophy, pre-existent hyperlipidemia appears to contribute to proteinuria and GS after UnX in female NAR.

Cryopreserved bone marrow cells after allogeneic kidney transplantation and European anti-thymocyte globulin do not induce tolerance in non-human primates. A.A.M.J. Hollander, L. de Waal, H. van Bockel, M. Jonker, F.H.J. Claas, M. van der Voort Maarschalk, J.A. Bruin, and F.J. van der Houwe, University Hospital Leiden, Leiden, and CLB, Amsterdam and TNO-ITRI, Rijswijk, The Netherlands. According to Thomas et al, long-term tolerance after allogeneic kidney transplantation can be achieved in Rhesus monkeys with a 5 day course of anti-thymocyte globulin (ATG) followed by the infusion of fresh donor bone marrow (BM) cells. The best graft survival was found when MHC class II negative BM cells were infused. If this same procedure were used in the postmortal donor setting, donor BM cells would have to be stored for several days after the death of the donor. To test whether tolerance induction is also possible with stored (cryopreserved) donor BM cells in Rhesus monkeys after allogeneic kidney transplantation, 9 female monkeys, which shared 1 DR antigen with the male donor, received a kidney transplant. From days 0 to 4, ATG (Fresenius) was given (50 mg/kg body wt). BM cells were MHC-DR-matched and magnetic beads labeled with the monoclonal anti-thymocyte globulin AT234. The BM cells were frozen and stored in liquid nitrogen. On day 5 the thawed donor-specific BM cells were infused in 5 kidney recipients (1.3 to 9.8 10^12 viable cells/kg body wt). The control group (4 monkeys) did not receive BM cells. No difference in graft survival was found between the two groups. Mean graft survival in the BM group was 23.4 days versus 19.8 days in the control group. All grafts were lost due to rejection: interstitial rejection in 3 control and 2 BM monkeys, vascular rejection in 2 BM monkeys and interstitial and vascular rejection in 1 control and 1 BM monkey. In both groups cytotoxic T lymphocyte precursor frequency (CTLp) against donor cells was low after transplantation and thus did not predict rejection. In conclusion, cryopreserved BM cells did not induce tolerance in the Rhesus monkey-ATG-BM model. Before applying this model in postmortal kidney transplantation in humans the effect of other ATG preparations and methods of storage of donor BM cells need to be further investigated.
vasodilation occurs at plasma DDAVP levels similar to plasma AVP levels under pathological conditions with stimulated AVP release.

Hantavirus nephropathy (HVN): Not just an arcane zoosnoisis. J. Clement, P. Colson, Ph. Darnoiseaux, P. McKenna, J. Coeck, J. Neyts, H. Leirs, and R. Verhaegen, Queen Astrid Military Hospital, Brussels, C.S. des Fagnes, Chimay, C.H. de Dinant, and RUCA, Antwerp, Belgium. During vasodilation occurs at plasma DDAVP levels similar to plasma AVP levels peritonitis and in some patients on long-term CAPD. This is likely to be conclusion, these findings indicate that mesothelial cell mass is negatively related to duration of CAPD treatment, but that the mesothelial cells probably do not play an important role in transport kinetics.

Effects of endothelin-1 (ET-1) and nitric oxide (NO) stimulation on renal function in humans. J.A. Bijlsma, A.J. Rabelink, H.A.H. Kaasjager, and H.A. Koornmans, University Hospital Utrecht, Utrecht, The Netherlands. Introduction: We recently demonstrated profound renal vasoconstrictive and antiinflammatory effects of pathophysiological dosages of ET-1 in humans. In isolated resistance arteries and in coronary arteries, NO is a potent inhibitor of the vasoconstrictive effects of ET-1. We therefore investigated whether stimulation of endogenous NO by L-Arginine (L-Arg) could also inhibit the renal effects of ET-1. Methods: Studies were performed in 6 healthy subjects. Each subject underwent 3 clearance studies, during which 90 minute infusions of ET-1 (2.5 mg/kg/min), L-Arg (5 mg/kg/min) and a combination of these were administered. This dosage of L-Arg is an established NO stimulation test. Clearances were calculated every 30 minutes. Parameters were mean arterial blood pressure (MAP), renal vascular resistance (RVR), electrolyte and acid excretion, and urinary NO2 excretion as an index of renal NO stimulation. Results: MAP mm Hg |

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\begin{array}{llll}
\text{ET-1} & 95.4 \pm 2.0 & 102.4 \pm 2.3 & 78 \pm 3 & 124 \pm 7 \\
\text{L-Arg} & 96.8 \pm 1.4 & 93.0 \pm 1.4 & 80 \pm 3 & 71 \pm 4 \\
\text{ET + L-Arg} & 97.8 \pm 1.5 & 94.0 \pm 2.1 & 82 \pm 3 & 114 \pm 5 \\
\end{array}
\]

Sodium excretion [\mu mol/min] |

\[
\begin{array}{llll}
\text{ET-1} & 178 \pm 30 & 83 \pm 11 & 123 \pm 28 & 58 \pm 11 \\
\text{L-Arg} & 180 \pm 28 & 309 \pm 69 & 149 \pm 24 & 553 \pm 84 \\
\text{ET + L-Arg} & 170 \pm 43 & 130 \pm 38 & 126 \pm 27 & 370 \pm 47 \\
\end{array}
\]

All observations during infusion were significantly different from baseline, \(P < 0.05\).

L-Arg prevented the increase in MAP during ET-1 infusion. However, while L-Arg alone caused renal vasodilation and increased urinary NO2 excretion (from 62.21 to 405 to 169 pmol/min), L-Arg could not prevent the increase in RVR during ET-1, despite a comparable increase in renal NO2 excretion (from 112.30 to 465 to 190 pmol/min). In addition, ET-1 still caused sodium retention in the presence of L-Arg, while L-Arg alone was natriuretic. In contrast, chloride excretion increased markedly during ET-1, both with and without L-Arg. This is explained by excretion of acid, secondary to metabolism of Arg-HCl. Conclusion: In humans, endogenous NO stimulation by L-Arg prevents the increase in blood pressure, but cannot prevent the renal vasoconstriction caused by pathophysiological amounts of ET-1.

Effect of sex and age on kidney graft survival. Essential role of the number of nephrons at the start of transplantation. E.P.M. van Steenberge, P.G. Mulder, J.N. IJzermans, and W. Weimar, Department of Internal Medicine I, Department of Epidemiology and Biostatistics and Department of General Surgery, University Hospital Rotterdam, Rotterdam, The Netherlands. To assess pretransplant prognostic factors associated with long-term kidney transplant outcome, we analyzed all 591 consecutive first cadaveric kidney transplantations performed between July 1972 and July 1992 at our center, with follow-up completed to December 31, 1993. Proportional hazards analysis was used to estimate the relative risks of the covariates associated with functional graft, patient and overall graft survival. Factors having no influence on any outcome included: cause of death of the donor, warm and cold ischemia times, the method of kidney preservation, recipient sex and recipient blood group. The factors associated with functional graft failure were: donor-recipient age difference > 0 (relative risk 1.63, \(P = 0.07\)), and the female sex of the donor (relative risk 1.48, \(P = 0.013\)). The female sex of the donor (relative risk 1.78, \(P = 0.004\)) and each additional year in recipient age (relative risk 1.07, \(P < 0.001\)) provided the highest risk of patient death. The recipients of relatively older donor kidneys had the highest risk of overall graft failure (relative
The sex of the donor (relative risk 1.56, P < 0.001), each additional mismatch on the HLA ABDR loci (relative risk 1.12, \( P = 0.045 \)), and recipient age (relative risk 1.02, \( P = 0.01 \)) also had significant, HLA independent, effects on the overall graft survival. It is likely that both vulnerability to ischemia and aging factors of the female donor kidneys reduce the number of functioning nephrons and are responsible for the donor sex effect. Matching for age could improve the graft outcome of the high risk female donors.

**Sodium homeostasis and dopamine excretion in HLA-identical kidney donors and recipients.** J.N.M. Barendregt, L. van Nispen, and P.C. Chang, Department of Nephrology, University Hospital Leiden, Leiden, The Netherlands.

Intrarenal dopamine (DA) synthesis may be involved in sodium homeostasis. In 7 HLA-identical kidney recipient-donor couples and 1 unrelated pair, we investigated hemodynamic and hormonal parameters and excretion of catechols and sodium at 50 (LoSo), 150 (NoSo), and 300 (HiSo) mmol·day⁻¹ of dietary sodium intake. We infused trimethaphan to mimic acute denervation, DOPA (0.15 µg·kg⁻¹·min⁻¹) to study DOPA to DA conversion and tyramine in suppresor and pressor doses (3.3 and 10 µg·kg⁻¹·min⁻¹) to induce norepinephrine release. Blood pressure was higher in the recipients (\( P < 0.05 \)) and was not influenced by sodium intake. UNaV did not differ between recipients and donors at LoSo, NoSo, and HiSo (37 ± 5, 132 ± 7, and 312 ± 19 mmol·day⁻¹). UDAV did not increase on HiSo in both groups. The UDA/UDOPA ratio was higher in donors than in recipients (\( P < 0.01 \)) and suppressed in both groups on HiSo (\( P < 0.05 \)). Trimethaphan decreased RVR and increased UNaV only in the donors (\( P < 0.05 \)), while GFR increased in both groups. DOPA infusion increased UDAV 4—5 fold but did not change UNaV in either group. Tyramine increased the UDA/UDOPA in donors (\( P < 0.05 \)). The absence of a natriuretic response to DOPA and the absence of an increase in UDA on HiSo indicate that intrarenal DA generation plays only a small role in sodium homeostasis. Evidence regarding the presence of functional renal reinnervation in grafted kidneys remains inconclusive.


Sixty-one kidney allograft recipients were randomized in a prospective, double-blind, placebo-controlled trial of BT563 (a murine IgG, monoclonal antibody directed against the 55 kDa chain of the interleukin-2 receptor) in the prophylaxis of rejection. The study medication was given in a dosage of 10 mg i.v. during the first 10 days after transplantation. Maintenance immunosuppression consisted of cyclosporine and prednisone. Clinical tolerance was excellent: no patient experienced significant side effects. In the first 4 weeks after transplantation an acute rejection was diagnosed in 7/30 (23%) of placebo-treated patients compared to 1/27 (4%) of BT563-treated patients (\( P < 0.05 \)) and no rejections were found. Another rejection was found in the placebo-group between week 5 and week 12 and 2 rejections in the BT563-group were found in this period. All rejections in the BT563 group were reversible with treatment (steroids and/or r-ATG), whereas in the placebo group rejection led to graft loss in 2 cases. Infectious complications were comparable in both groups and consisted mainly of urinary tract infections (12 in placebo vs. 16 in BT563 group in first 12 weeks) and CMV disease (3 in placebo vs. 0 in BT563 group in first 12 weeks). In the BT563 group, 3 grafts were lost due to renal artery thrombosis. One placebo-treated patient died at day 32 post-Tx of acute necrotizing pancreatitis, and another placebo-treated patient had a non-fatal myocardial infarction at the second post-operative day. We conclude that the use of the anti-IL-2R monoclonal BT563 leads to a significant reduction in the incidence of acute rejections after kidney transplantation, without side effects or an increased incidence of infectious complications. The complete absence of acute rejections after BT563 administration may be the result of combining cyclosporine and anti-IL-2R MoAb, a combination that was shown to have a synergistic immunosuppressive effect in animal models.

**Compassionate treatment of Wegener's granulomatosis with rabbit anti-thymocyte globulin (ATG).** E.C. Hagen, R.J.W. de Keizer, W.P.L. van Boven, K. Andrassy, J.A. Bruijn, L.A. van Es, and F.J. van der Woude, Department of Nephrology, Ophthalmology and Pathology, University Hospital Leiden, Leiden, St. Elisabeth Ziekenhuis, Tilburg, The Netherlands; and Department of Nephrology, University of Heidelberg, Heidelberg, Germany.

This study aimed to evaluate the effect of treatment with ATG in patients with Wegener's granulomatosis, untreatable with cyclophosphamide and steroids. Five patients with active Wegener's granulomatosis who were either not responsive to standard therapy, or who could not tolerate alkylating agents were treated with a single course of rabbit ATG. Four out of 5 patients showed a favorable response to treatment, with partial or complete remission of disease activity, with a follow-up period of 6 to 13 months. One patient had progressive retroorbital granuloma, which resulted in enucleation of the eye. Side effects were mild, with chills and fever during the first infusion of ATG, and development of serum sickness in 2 patients. Two patients had labial herpes simplex shortly after the start of treatment. No other infectious complications were seen. We conclude that ATG treatment seems to be an effective treatment for patients with severe Wegener's granulomatosis.