infection of the recipients and a multidisciplinary laboratory approach to establish an etiological diagnosis. The pathology and IHC staining of transplant-associated LCMV infection is most reminiscent of Lassa fever. The contributory role of immunosuppression in the pathology and pathogenesis of LCM in these patients deserves further study.

1220 Howell-Jolly Body-Like Inclusions in the Neutrophils of HIV Positive Patients Are Associated with Low CD4 Counts

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Background: Howell-Jolly body-like inclusions are discrete intracytoplasmic inclusions which have been previously observed in the neutrophils of HIV positive individuals. The inclusions are morphologically and structurally similar to red blood cell Howell-Jolly bodies; consisting of remnant nuclear material and appearing as small, densely basophilic inclusions. Little is known of their clinical significance, although it has been suggested that the appearance of Howell-Jolly body-like inclusions is related to antiviral medications. We evaluated the incidence of the inclusions in HIV positive individuals and correlated these results with clinical parameters including CD4 count, which has not previously been reported.

Design: Peripheral blood smears from 18 consecutive HIV positive patients and 20 consecutive HIV negative patients who underwent bone marrow biopsy were reviewed. The entire thin portion of the peripheral blood smear was reviewed for the presence of neutrophil cytoplasmic inclusions. A Feulgen reaction was performed to confirm that the inclusions consisted of nuclear material. Concurrent clinical information was collected including: HIV status, therapeutic regimen, viral load, and CD4 count.

Results: Of the 18 HIV positive patients, neutrophil inclusions were identified in 10 individuals (55%). CD4 counts were significantly lower ($P<0.05$) in inclusion-positive cases (mean 12.86 cells/mm3) versus the inclusion-negative cases (mean 226.7 cells/mm3). HIV RNA viral loads were not significantly different between the inclusion-positive (mean 178,200 copies/ml) and inclusion-negative groups (mean 119,400 copies/ml). Antiretroviral therapy did not show a statistical relationship with the presence of inclusions (30% of inclusion-positive vs. 63% of inclusion-negative receiving antiretroviral therapy). No inclusions were identified in the 20 HIV negative patients.

Conclusions: The findings suggest that Howell-Jolly body-like inclusions are not uncommon in HIV positive individuals. It appears that the inclusions are not related to antiviral medication or viral load. A relationship does appear to exist between lower CD4 counts and the appearance of inclusions. This shows that in HIV positive individuals, Howell-Jolly body-like inclusions correlate with the degree of immune suppression.

1221 Correlation of Histology, Human Papillomavirus Detection, and Viral Load in Laryngeal Papillomas in Childhood

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Background: Laryngeal papillomas are lesions in children associated with human papillomavirus (HPV) infection that have a high recurrence rate. The histologic correlates of these lesions as well as viral load is not well understood.

Design: The purpose of this study was to analyze laryngeal papillomas in children for HPV by in situ hybridization (for productive infection) and PCR in situ and correlate this with the histologic findings.

Results: HPV DNA was detected by in situ hybridization (Ventana Medical Systems) in 29/47 cases (62%); all cases were HPV 6 or 11 positive. We compared the presence of keratohyaline granules, non-uniform perinuclear halos, marked papillomatosis, and marked acanthosis in the HPV positive and negative cases. There was a statistically significant increase in the presence of keratohyaline granules (22/29 -76% vs 9/18 - 50%), non-uniform perinuclear halos (20/29 – 69% vs 3/18 – 17%), and marked papillomatosis (22/29- 76% vs 6/18 – 33%) in the viral positive cases. The viral load was low (defined as less than 10 positive cells per tissue with a corresponding weak signal) in 18/29 (62%) of the viral positive cases; in comparison a high viral load was evident in 17/20 (85%) vulvar condylomas. The viral negative cases were tested for HPV by PCR in situ hybridization using primers for HPV 6 and 11. The detection of HPV increased to 38/47 (81%) after PCR amplification.

Conclusions: It is concluded that larygeal papillomas in childhood are characterized, in general, by a relatively low HPV viral load and that the viral positive cases are associated with keratohyaline granules, non-uniform perinuclear halos, and marked papillomatosis.

1222 Histopathology of the Liver in Untreated Human Immunodeficiency Virus and Hepatitis C (HIV/HCV) Coinfected Patients in a County Hospital

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Background: Previous studies show that patients with HIV and HCV coinfection are at increased risk for progression to cirrhosis compared with patients with HCV infection alone, but the causes are unclear. CD4 cell depletion, cholestatic hepatitis and certain antiretroviral therapies in HIV/HCV patients, and interleukin producing plasma cells in chronic viral hepatitis are suggested as possible causes for fibrosis progression. We studied histopathologic and relevant clinical features of untreated HIV/HCV coinfected patients seen in a county hospital.

Design: Liver biopsy material from 2001 to 2004 was available from 29 HIV/HCV patients. Data obtained from those biopsies were compared to the results of biopsies obtained from 67 HCV patients. Clinical data included CD4 count and HIV RNA load. Sections were stained with H&E, trichrome and methyl-green-pyronin (for easier visualization of plasma cells). All biopsies were assessed for grade, stage (Ludwig and Batts), and other significant pathologic findings. Type and distribution of inflammatory infiltrate with plasma cell count was performed in 26 HIV/HCV patients and 17 HCV patients.

Results: Comparison of HIV/HCV group vs HCV group: 22/29 vs 36/67 males (75.9% vs 53.7%); mean age of 45.9 vs 50.7; 51.7% vs 61.2% African Americans, 34.5% vs 19.4% Hispanics and 13.8% vs 19.4% White. Mean HIVRNA load was 17556.2 copies/ml; mean CD4 count was 372.2 cells/μl (ranged from 50-945). HIV/HCV group had significantly less inflammatory activity (lower grade than HCV group alone, $p=0.023$), while there was no difference in degree of fibrosis. Due to depletion of lymphocytic infiltrate in the coinfected group, plasma cells were more apparent in HIV/HCV group. However, number of plasma cells per portal area was significantly lower in HIV/HCV group than in HCV alone (14.13 ± 10.02 vs 23.7 ± 10.4, $p=0.04$). There was no correlation between CD4 count or HIVRNA load and grade, plasma cell number and degree of fibrosis. None of the HIV/HCV patients had an opportunistic infection. No cholestatic hepatitis is seen in either group.

Conclusions: HIV coinfection with HCV is associated with less inflammatory activity (lower grade) due to depletion of lymphocytic and plasma cell infiltrate. There is no correlation between CD4 count and degree of inflammation or fibrosis. This suggests that the mechanism of hepatic fibrosis in HIV/HCV coinfection is independent of HIV disease status, inflammatory activity / grade, or plasma cell number.

1223 Pathologic Studies of a Variant Creutzfeldt-Jakob Disease Case in the United States

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Background: A 25-year-old Florida woman with variant Creutzfeldt-Jakob disease (vCJD) died in June 2004. The patient had lived in the United Kingdom during the period of highest risk for human exposure to the agent causing bovine spongiform encephalopathy and was believed to have been infected between 1980 and 1992 before moving to the United States. The patient was the first known person with vCJD to reside in the United States.

Design: A full autopsy was performed by a team from Centers for Disease Control and Prevention (CDC). Precaution and decontamination procedures were followed and tissue samples were collected according to guidelines established by the World Health Organization and CDC. Tissue samples from the central nervous system, all major organs, and other sites, including lymph nodes, tonsil, and muscle, were collected for histopathologic evaluation, immunohistochemical assays (IHC), and other studies.

Results: The neuropathologic changes were distinctive and differed markedly from those caused by non-variant CJD endemic in the United States. Gross examination revealed that the brain was markedly atrophic, with a softened cortical ribbon that sloughed off easily; the white matter was considerably firmer. No significant gross finding was noticed in other major organs. Microscopic examination of brain tissue revealed that the loss of neurons was near complete throughout the cerebral and cerebellar cortices, with numerous "florid" plaques, which are typical of vCJD. These plaques consist of a central amyloid core surrounded by a peripheral rim of spongiform change. IHC using two different antibodies demonstrated diffuse confluent sheets of proteinase-resistant prion protein throughout the cerebral and cerebellar cortices. Focal immunostaining was also observed in lymphoid organs, such as tonsil and spleen. Pathologic studies on other tissue samples from this case-patient are ongoing.

Conclusions: The patient described in this report represents the first vCJD case in a U.S. resident. Although vCJD is a rare disease, pathologists should be familiar with its histopathologic features and diagnostic modalities because of their critical role in diagnosis of and surveillance for this disease. Further pathologic studies should provide valuable insight in understanding the pathogenesis of vCJD.

Kidney

1224 Detection of BK Virus in Laser Capture Microdissected Kidney Biopsies Using Real Time PCR

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Background: The BK virus (BKV) is a major source of infection among renal allograft recipients, and may play an adverse role in long term graft survival. Previous reports of BKV detection using molecular techniques have suffered from poor sensitivity and specificity. There is therefore a need to explore new technologies that could be utilized to improve sensitivity and specificity of BKV detection in tissues. We applied real-time PCR technology to the detection of BKV in H&E stained kidney biopsies, utilizing laser capture microdissection. We believe this represents the first description of the use of real-time PCR for this purpose.

Design: Renal allograft biopsy specimens from a patient with the histologic diagnosis of BKV infection were retrieved. Diagnostic inclusion-bearing cells were microdissected by laser capture microscopy as were normal-appearing glomerular cells. DNA was extracted and real time amplification performed using primers that targeted the large "T" and small "t" regions of the BKV genome. Tubular epithelial and glomerular cells from a control case with no evidence of BKV infection were used as negative controls in a similar reaction.

Results: The presence of BKV was demonstrated in the epithelial cells containing typical viral inclusions, as well as from glomerular cells that appeared normal by histology. The materials from the negative control were completely negative.

Conclusions: Real-time PCR technology can be used to detect BKV in H&E stained, paraffin-embedded tissue sections. In this case, this technique was able to detect the BKV in tubular epithelial cells in renal allograft biopsy specimens and in the cells that lack diagnostic viral inclusions by routine examination. To the best of our knowledge, this is the first report of detecting BKV in laser capture microdissected renal biopsies.

1225 Global Proteomics in Dysplastic and Normal Fetal Kidneys

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Background: Proteomics approaches utilizing two-dimensional difference in-gel electrophoresis (2D DIGE) and tandem mass spectrometry (MS/MS) may be used to identify and quantitate proteins in a wide range of tissues, but the proteome of the developing and dysplastic kidney is unknown.

Design: Kidneys from 3 normal mid gestation autopsy fetal kidneys and 3 dysplastic kidneys were frozen in OCT and histologically assessed. Fifty micron thick sections were used from each block for total protein extraction. A high throughput proteomics approach was used to profile protein changes. Proteins were labeled with Cy dyes, co-mingled, then separated by 2-D DIGE. Images from individual fluorescent dyes were analyzed (using DeCyder image analysis software) to determine fold differences between normal and dysplastic tissues. Selected spots were excised from the gels, and trypsinized in situ before analysis by matrix-assisted laser desorption ionization-time-of flight (MALDI-TOF/TOF) or by LC MS/MS mass spectrometry.

Results: Histologically, two dysplastic kidneys had hydronephrosis with segmental fibrosis and focal dysplastic collecting ducts; the 3rd dysplastic kidney had global scarring, islands of cartilage and extensive loss of nephrons. A total of 48 differentially expressed proteins spots were identified; 35 were selected for identification. By comparison to normal fetal kidney, dysplastic kidneys with segmental scarring showed a 1.25-4.5 fold increase for heat shock proteins (HSPA1L, HSPA2) that regulate cellular homeostasis and apoptosis, albumin and NADH and aldolase reductase enzymes. Several structural proteins were decreased including α and β tubulin and topomyosin. In the globally scarred kidney a unique set of proteins was increased >10fold, including lumican, vimentin, osteoglycin, extracellular matrix proteins such as collagen type I and IVα1, and α and β tubulin which were increased by >30fold. A different set of enzymes composed of M2-pyruvate kinase and Glyceraldehyde-phosphate dehydrogenase were decreased.

Conclusions: Our results show that increased HSP, albumin and NADH and decreased structural proteins (α, β tubulin) predominate in segmentally scarred hydronephrotic kidneys in contrast to increased structural proteins and decrease in a different set of enzymes that appear in the proteome of globally scarred kidneys. These findings may lead to identification of biomarkers that will shed more light in the natural history of this disease and may be useful in identifying molecular endpoints for therapeutic intervention.

1226 C4d and the Renal Allograft Biopsy: A Different Perspective

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Background: C4d has become recognized as a marker for detecting humoral rejection in renal allograft biopsies. Staining of peritubular capillaries for C4d is reported to be associated with the presence of anti-donor antibodies, and may be seen as an independent finding or may be associated with histologic findings of vascular or tubulointerstitial transplant rejection. Published data indicate rates of C4d positivity averaging 25-30%. The rate of C4d positivity in our laboratory has been significantly lower. We report our experience with C4d staining of renal allograft biopsies.

Design: A review of our case files was performed in search of renal allograft biopsies on which C4d staining had been performed. 184 such cases were identified from 3-7-03 htrough 8-31-04. C4d staining on all cases was performed by immunofluorescence according to standard protocol.

Results: Three of the 184 cases (1.6%) showed diffuse peritubular capillary staining for C4d with histology showing acute vascular transplant rejection (2 cases) and transplant glomerulitis (1 case). Two cases showed focal peritubular capillary C4d positivity of 5% or less associated with chronic allograft nephropathy, and interstitial acute inflammation. Glomerular capillary loop C4d staining was present in 2 cases of membranous glomerulopathy and 2 cases of glomerular thrombotic microangiopathy. Tubular basement membranes stained for C4d in a case of recurrent lambda light chain deposition disease. Mild C4d positivity of glomerular mesangial regions was normally present, providing an internal positive control. Biopsy diagnoses in C4d negative cases included: chronic allograft nephropathy (41 cases), acute tubulointerstitial transplant rejection (39 cases), mild non-specific changes (22 cases), acute vascular transplant rejection (19 cases), acute tubular injury (12 cases), focal segmental glomerulosclerosis (9 cases), glomerular thrombotic microangiopathy (4 cases), borderline tubulointerstitial transplant rejection (5 cases), medullary tissue only (5 cases), polyoma virus nephritis (4 cases), acute transplant glomerulitis (3 cases), renal infarct (3 cases), diabetic nephropathy (2 cases), cholesterol emboli (2 cases), IgA nephropathy (2 cases), recurrent crescentic glomerulonephritis (1 case), and changes suggestive of calcineurin inhibitor toxicity (1 case).

Conclusions: The rate of C4d positivity in renal allograft biosies in our laboratory is drastically lower than that reported in the literature. We propose that this finding may be a reflection of organ procurement and transplantation practices among our submitting physicians.

1227 Quantitative Assessment of Glomerular Infiltrates in Chronic Allograft Glomerulopathy

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Background: Chronic allograft glomerulopathy (CAG) is a distinctive pathologic manifestation of chronic renal allograft rejection seen in about 15% of patients with chronic allograft nephropathy. It may be associated with proteinuria and reduced graft survival. The pathophysiology is poorly understood. We sought to define the immune cell populations in glomeruli of patients who developed CAG.

Design: A database search of our renal transplant recipients identified all patients diagnosed in the last 5 years with CAG. All biopsies from such patients were stained with antibodies to CD3, CD4, CD8, CD20, CD45 and CD68. Positively stained cells in each glomerulus were counted and an average count for each biopsy obtained. Pre- and post-reperfusion biopsies served as controls.

Results: A total of 94 baseline biopsies were reviewed. The glomerular cell counts were (mean +/- SD): CD3: 0.12 +/- 0.42; CD4: 0.08 +/- 0.35; CD8: 0.06 +/- 0.33; CD20: 0.01 +/- 0.09; CD45: 1.13 +/- 1.7; CD68: 0.71 +/- 1.7 cells/glomerulus. Sixteen patients with a total of 75 biopsies were identified with at least 1 biopsy with a Banff score of cg2 (14 biopsies) or cg3 (12 biopsies). The mean time to diagnosis of CAG was 541 days post-transplant. CAG was preceded by acute rejection in 5, BK infection in 1, and acute glomerulitis in 3 patients, 2 of whom had humoral rejection. Glomerular immune cell counts in biopsies with cg2 or cg3 were (mean +/- SD): CD3: 1.38 +/- 1.87; CD4: 2.95 +/- 3.36; CD8: 1.44 +/- 1.44; CD20: 0.02 +/- 0.06; CD45: 7.43 +/- 5.93; CD68: 8.23 +/- 6.45 cells/glomerulus. These were all significantly elevated above baseline, except for CD20 ($p < 0.0001$, unpaired t-test). The mean ratio of CD68-(+) macrophages to CD3-(+) T-cells was 6.74 in CAG biopsies and was greater than 2 in 63%. In 13 patients, there were biopsies preceding the diagnosis of CAG that showed mean CD45 or CD68 cell counts more than 2 standard deviations above baseline.

Conclusions: Glomeruli in cases of CAG show increased immune cell infiltrates of both T-cells and macrophages. Macrophages outnumbered T-cells in both baseline and CAG biopsies. Histologic CAG is often preceded by biopsies showing increased numbers of immune cells. These data support the idea that CAG is immune-related and may be mediated by long-term colonization of glomeruli by T-cells and macrophages.

1228 Granulomatous Interstitial Nephritis: Analysis of 46 Cases in Single Institution

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Background: While interstitial nephritis is commonly seen in kidney biopsies of patients with acute renal failure, granulomatous form is rarely encountered. Reports and reviews of granulomatous inflammation in the kidney point to a profile of causes that differ from causes of granulomatous inflammation in the lungs, upper respiratory tract or skin. We review all cases of granulomatous interstitial nephritis (GIN) in our institution in an eighteen-year period to determine a profile of our patient population and to subcategorize them according to different causes; this is the largest review of single institution experience with this diagnosis in the literature so far.

Design: We identified cases of GIN through a computer search of 7227 kidney biopsies collected between 1986 and 2004. Using clinical information system, we investigated most likely causatives of granulomatous inflammation in identified cases and divided them into different causative categories.

Results: Granulomatous interstitial nephritis was identified in 46 patients. Insufficient clinical data were found in eight patients. Seventeen of 38 patients (45%) were classified as drug-induced. Renal sarcoidosis was responsible for 30% of cases. There were two cases of Wegener's granulomatosis and two cases of foreign body giant cell reaction to a polarizable foreign material. In one case, the patient had received intravesical bacillus Calmette-Geurin (BCG) therapy for bladder cancer and necrotizing granulomatous nephritis was found on the nephrectomy specimen for renal transitional cell carcinoma that this patient consequently developed. One patient had a diagnosis of milliary tuberculosis and was placed on multi-drug treatment including rifampicin and ciprofloxacine; the patient developed GIN which we subcategorized as drug induced GIN, in the absence of caseating necrosis and with negative acid fast bacilli stain. Xanthogranulomatous nephritis was diagnosed in one patient. Detailed clinical investigation failed to reveal any known cause of GIN in four patients (11%), classified as idiopathic. Available results of special stains and microbiological data were reviewed in all cases and no fungal or bacterial causatives were found in any of these cases.

Conclusions: Drug-induced interstitial nephritis and sarcoidosis are responsible for three quarters of GIN in our series. In significant proportion (11%) of cases, the cause remain unknown. Remaining 14% of cases include Wegener's granulomatosis, xanthogranulomatous nephritis, foreign body giant cell reaction and BCG-induced GIN.

1229 CD20+ Lymphocytic Aggregates in Biopsies for Acute Rejection Are Not Associated with Increased Renal Allograft Loss in Adults

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Background: Current histopathologic methods are notoriously poor at stratifying episodes of acute renal allograft rejection to reliably predict allograft loss. A recent study utilizing DNA microarray profiling of pediatric biopsies suggested a strong correlation between CD20+ lymphocytic aggregates (LA) and graft loss in episodes of untreated acute rejection (AR). The current study examines this correlation in adult biopsies.

Design: A total of 114 adult renal allograft biopsies including 44 cases of untreated AR obtained between 2002-2004 were examined. The mean allograft age of the AR cases was 33 months (range 1-228). CD20 immunohistochemistry was performed on biopsies with LA by light microscopy, defined as a lymphocytic cluster filling at least 1/2 a 40X field. In-situ hybridization for Epstein-Barr virus was performed on all CD20+ cases. C4d immunofluorescence was performed in 19 of the AR cases. Poor outcome was defined as failure of serum creatinine to return to baseline within a month and graft loss as reversion to dialysis or retransplantation.

Results: LA were identified in 22 of 44 cases of AR, and 33 of 65 other biopsies with follow-up. LA were often multiple and had a variable plasmacytic component. The lymphocytes forming the aggregate(s) were predominantly B-cells in 9 of the 22 cases and the largest clusters consisted of approximately 150-500 B-cells each. 4 of 21 allografts one year or less post-transplantation and 5 of 22 allografts greater than one year post-transplantation had B-cell aggregates. None of the cases with B-cell aggregates was EBV positive and viral inclusions were absent from all cases of AR. Acute humoral rejection was present in 3 cases based on clinical and light microscopic findings, together with C4d positivity. See Table 1 for outcome and Table 2 for graft survival in AR cases.

Outcomes in Acute Rejection Cases

Acute Rejection Cases (n=44)	CD20+ Clusters Present	CD20+ Clusters Absent
Good Outcome*	2	22
Poor Outcome	7	13

*Return of serum creatinine to baseline within one month

Graft Survival in Acute Rejection Cases

Acute Rejection Cases (n=44)	CD20+ Clusters Present	CD20+ Clusters Absent
Graft Survival	3	22
Graft Loss*	6	13

*Reversion to dialysis or retransplantation

Conclusions: Outcomes of AR episodes were marginally worse in the presence of CD20+ B-cell aggregates (P=0.57). Allograft survival was not significantly worse in the presence of B-cell aggregates (P=0.14).

1230 A Clinicopathologic Study of Thrombotic Microangiopathy in the Setting of IgA Nephropathy

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Background: IgA nephropathy (IgAN) is the most common glomerulonephritis in the world. Thrombotic microangiopathy (TMA) occurs in a number of clinical settings, including but not limited to thrombotic thrombocytopenic purpura/hemolytic uremic syndrome, malignant hypertension (HTN), anti-phospholipid antibody syndrome, and radiation nephropathy. Renovascular complications, such as TMA, in the setting of IgAN may be overlooked and their significance as a concomitant histologic finding is unclear. We conducted a clinicopathologic study to understand the possible relationship between IgAN and a concurrent TMA injury process.

Design: We reviewed the renal pathology files at the University of Washington Medical Center (Seattle, WA) from 1998-2004 and identified 10 patients with an established diagnosis of IgAN by renal biopsy and concurrent findings of TMA based on light microscopy (LM) and/or electron microscopy (EM). We correlated the histologic findings with relevant clinical information.

Results: All 10 patients in the series had HTN, five presented with clinical signs of malignant HTN, and 3 others had severe HTN (>100 mm Hg, diastolic). None had signs or symptoms of Henoch-Schonlein purpura. 9 patients had occasional arteriolar thrombi identified by LM and prominent glomerular subendothelial space widening by EM, while one patient demonstrated only ultrastructural features of TMA. Other possible etiologic causes of TMA were not identified with the available clinical information. 2 of 10 biopsies demonstrated glomerular crescent formation and/or segmental necrosis, but vasculitis involving arterioles or small arteries was not identified in any biopsy. 2 cases revealed focal features of collapsing glomerulopathy. 9 cases showed advanced and extensive global glomerulosclerosis with diffuse and marked interstitial fibrosis and tubular atrophy. One IgAN case revealed mild increase in mesangial matrix and only subendothelial space widening by EM, correlating with a milder degree of HTN.

Conclusions: Our study suggests that a TMA injury, when present, is usually found in advanced stages of IgAN. The TMA injury process may be related to the clinical presence of severe HTN, perhaps a consequence of the disease progression of IgAN. The findings also suggest that such hypertensive renal injury contributes to disease progression in IgA nephropathy, and that anti-hypertensive therapy may impede disease progression. The identification of renovascular complications, such as TMA, in the setting of IgAN is likely underdiagnosed.

1231 Development of Polycystic Kidney Disease in a Gene Targeting Animal Model Indicates Multiple Genetic Interactions Involved in the Pathogenesis

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Background: Polycystic kidney disease (PKD) is the most common heritable cystic renal disease. The autosomal recessive form (ARPKD) has variable clinical presentations ranging from perinatal death to milder progressive disease. While the mutations of PKHD1 gene are responsible for ARPKD, other genetic defects might account for the less well-defined PKD in human. Mice that are homozygously deficient for Bcl-2 develop PKD with variable onset and severity, resembling the human disease.

Design: The main function of Bcl-2 is to regulate apoptosis. If apoptosis contributes to the development of PKD in the Bcl-2 knockout mice, concurrent knockout of Bid, a Bcl-2 interacting pro-death molecule, should correct the phenotype. We thus created Bcl-2/Bid double knockout mice. We also bred the mice into two different genetic backgrounds, the C57BL/6 and 129/SvJ, to test whether the variations in disease presentation are related to the genetic background. We then collected kidneys from these mice at different ages for histologic examination. We developed a scoring system with maximum score of 7.0 representing the most severe cystic kidney changes. Serum BUN was quantified and compared to the histological results.

Results: Genetic background is found to influence the development of PKD in the Bcl-2-deficient mice. Severe PKD are found at birth in C57BL/6 background (average score of 5.8). While in 129/SvJ background, half of the mice showed nearly normal kidney morphology (average score of 2.5). Serum BUN levels were consistent with the above findings, with an average of 88.3 mg% in the C57BL background and 45 mg% in the 129/SvJ background. Interestingly, crossing the Bcl-2-deficient mice to Bid-deficient mice did not seem to rescue the phenotype.

Conclusions: The autosomal recessive presentation of the PKD in Bcl-2-deficient mice seems to be most significantly affected by the genetic background, suggesting that there are other genetic factors modifying the disease process. However, our results from the Bid/Bcl-2 doubly deficient mice indicate that the pro-death Bid molecule does not seem to be such a factor. Since the Bcl-2-deficient mice also display smaller auricles and gray hair, it suggests that bcl-2 may be involved during the morphogenesis where inductive interactions between epithelium and mesenchyme are important. Our findings thus suggest that human ARPKD may also involve the dysfunction of these processes, which can be modified by multiple genetic elements.

1232 Evaluation of "Penetrating" Electron-Dense Deposits To Distinguish Lupus and Idiopathic Membranous Glomerulonephritis

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Background: Membranous glomerulonephritis may occur as part of systemic lupus erythematosis (SLE) or it may be idiopathic. Both diseases show diffuse subepithelial electron-dense deposits by electron microscopy. Some deposits have been observed in membranous lupus nephritis (MLN) that appear to extend through the lamina densa, "penetrating" the glomerular basement membrane (GBM). These penetrating deposits have been put forth as characteristic of MLN and not present in idiopathic membranous glomerulonephritis (MGN). Because renal disease may precede a clinical diagnosis of SLE, recognition of a feature characteristic of SLE on renal biopsy would be helpful. The presence of penetrating deposits as characteristic of MLN, versus idiopathic MGN, has not been systematically studied.

Design: Electron micrographs from 32 renal biopsies with a diagnosis of MLN (13 cases) or idiopathic MGN (19 cases) were examined, without knowledge of the diagnosis, for the presence of penetrating deposits. Penetrating deposits were defined as subepithelial deposits that penetrate the full thickness of the capillary loop GBM. All available electron micrographs for each case were examined, with an average of 9.7 (range, 4-20) micrographs per case. Clinical follow-up data on idiopathic MGN patients were obtained by contacting clinicians and reviewing available chart and laboratory data.

Results: All cases showed subepithelial deposits within the GBM. Of 13 MLN cases, 10 showed penetrating deposits (77%). Among 19 cases with a diagnosis of idiopathic MGN, 8 showed penetrating deposits (42%). Clinical follow-up data were available in 4 of these 8 idiopathic MGN cases with penetrating deposits: two developed clinical SLE, one developed a positive ANA in three years, and the other showed no evidence of SLE (follow-up period 13-18 years, average 15 years). Among idiopathic MGN patients without penetrating deposits, clinical follow-up data were available in 4 of the 11 patients, none of whom developed laboratory or clinical evidence of SLE (follow-up period 3-27 years, average 12 years).

Conclusions: Penetrating deposits are typically present in MLN and if found in idiopathic MGN are suggestive but not diagnostic of MLN. In this small series, the majority of patients with idiopathic MGN whose renal biopsies showed penetrating deposits later developed clinical or laboratory features of SLE.

1233 Screening and Diagnosis of Polyomavirus Allograft Nephropathy. Comparative Value of Urine Cytology (UC), Urine Pcr (UPCR) and Viremia (VLB)

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Background: Polyomavirus allograft nephropathy (PVAN) significantly shortens renal allograft survival. The specific factors determining ultimate graft outcome are not known, but patients with histologically advanced PVAN in the initial biopsy have higher rates of graft loss and more difficulty overcoming the infection after immunosuppression is decreased. PVAN is as a rule preceded by a period of clinically silent viruria. Evaluation of urine cytology for decoy cells (UC), quantitative determinations of viruria (UPCR) and quantitative viremia (VLB) have been proposed as surrogate markers of PVAN.

Design: In this study we present the experience with the concurrent evaluation of UC, UPCR and VLB in 349 patients (940 sets of samples). Results were correlated with each other and with a previous, concurrent or subsequent biopsy diagnosis of PVAN. All patients were followed for at least 12 months post-transplantation and had two or more concurrent UPCR, VLB and UC. A positive UC was defined by the presence of any amount of well preserved decoy cells. UPCR and VLB were considered negative if <1000 BK viral copies/ml were identified. Patients with exclusive JC viremia were excluded from the study. The test results were specifically correlated with a previous, concurrent or subsequent biopsy diagnosis of PVAN after a mean follow up of 27 months post- transplantation (range 12 months to 147 months, median 18 months).

Results:

DIAGNOSTIC VALUE OF LABORATORY TESTS FOR DIAGNOSIS OF PVAN

TEST	UC	UPCR	VLB
Sensitivity	100%	100%	70%
Specificity	65%	52%	98%
Neg Predictive Value	95%	100%	85%
Pos Predictive Value	52%	47%	93%

Conclusions: Urine cytology and quantitative viruria are excellent screening methods due to their high sensitivity. The absence of decoy cells or polyomavirus in UPCR rules out polyomavirus nephropathy in 95-100% of cases.The simultaneous performance of both UC and UPCR does not add useful clinical information. In patients with positive UC performance of UPCR is necessary to allow for the distinction between BK and JC viruses. Quantitative measurement of viremia is not indicated in patients lacking viruria since no patients with PVAN present with this combination of findings. In patients with viruria, positive viremia strongly correlates with PVAN. Quantitative viremia is nearly 100% specific for polyomavirus nephropathy, however, this test cannot be used as a screening tool due to its low sensitivity.

1234 Morphological Spectrum of Light Chain Deposition Disease of the Kidney in 45 Patients. Experience in a Multiple Myeloma Center

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Background: Light Chain Deposition Disease (LCDD) is a systemic disease and usually occurs in patients with plasma cell dyscrasias or other lymphoproliferative diseases. It is characterized by deposition of monotypic immunoglobulin light chain (LC) in several organs. The morphologic features of LCDD have been previously presented in only two series of biopsies and autopsy specimens with 23 and biopsy series of 34 cases, respectively. This study examines morphologic features of renal biopsies in 45 LCDD cases and expands the available literature on this disease entity.

Design: The files at University of Arkansas for Medical Sciences, Little Rock, from 1997 to September 2004 were searched retrospectively. Renal biopsies of 45 consecutive cases with LCDD were found and re-evaluated by light (LM), immunofluorescence (IF) and electron microscopy (EM). Fourty-one patients had multiple myeloma; 1 had monoclonal gammopathy of unknown significance (MGUS) and in 3 patients, no lymphoproliferative disease was identified. Among 45 patients, only 2 patients with light and heavy chain deposition (LHCDD) were identified. Ten patients had LCDD with coexistent myeloma cast nephropathy (MCN) and one patient had LCDD with amyloidosis.

Results: The LM lesions most suggestive of LCDD, nodular glomerulosclerosis (NS), and thickening and wrinkling of tubular basement membranes (TBM) were present in 11 cases, only GBM and TBM thickening in 9 cases , mild to moderate mesangial matrix increase in 7 cases and unremarkable glomeruli and tubules in 18 cases. Forty patients showed LC immunoreactivity (25 kappa, 15 lambda). Among these, 2 cases had both LC and HC. Twenty-four cases were positive by IF and EM. Two cases were negative by IF, but had characteristic mesangial deposits by EM. In 11 cases with immunoreactivity to LC (10 Kappa, 1 lambda), no EM deposits were identified. Three cases did not have kidney tissue for IF studies, but had deposits by EM. In 5 cases with positive IF, no EM was performed due to lack of kidney tissue.

Conclusions: This study shows the spectrum of changes by LM, IF and EM in LCDD of the kidney. There is a lack of consistency between deposits detected by IF and EM, creating a difficulty for definitive diagnosis without LM, IF and EM.

1235 Lupus-Like Glomerulonephritis in HIV-Infected Patients: Pathologic and Clinical Features of 14 Cases

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Background: While the most common glomerular lesion associated with HIV infection is collapsing focal-segmental glomerulosclerosis (HIV-associated nephropathy, or HIVAN), immune complex-mediated forms of glomerulonephritis (GN) have been increasingly reported in HIV-positive patients. One form of GN that appears to be relatively unique to the HIV-infected population is lupus-like GN, characterized by histologic, immunohistologic, and ultrastructural features resembling lupus nephritis, but occurring in patients without serologic and clinical evidence of systemic lupus erythematosus (SLE). Data regarding clinical outcomes in patients with this form of GN are very limited.

Design: We reviewed pathology reports for all native renal biopsies from HIV-positive patients processed at our center from 1/99 - 12/03. Of 77 total specimens, 14 met the following criteria for lupus-like GN: 1) Immunofluorescence microscopy showed granular glomerular staining for IgG, IgA, IgM, C3 and C1q, with at least 1+ (0 to 4+ scale) staining for C1q, and 2) The serum was negative for anti-nuclear antibodies (ANA) [11 cases], or weakly positive (titer not greater than 1:80) for ANA and negative for anti-double-stranded DNA [3 cases].

Results: Clinically, 10 of the 14 patients with lupus-like GN presented with nephrotic syndrome, all had microscopic hematuria, 11 had hypertension, and 9 had serum creatinine >3.0 mg/dl. All but 1 were African-American. Histologically, 7 biopsies showed diffuse proliferative GN, 6 focal proliferative GN, and 1 membranous nephropathy. All but 2 biopsies showed moderate or severe chronic change, and 3 showed concurrent HIVAN. EM was performed on 12 biopsies, with all showing mesangial and subendothelial immune complex deposits and tubulo-reticular inclusions; 9 showed subepithelial deposits. Of 12 patients for whom clinical follow-up was available, 10 developed end-stage renal disease (ESRD) within one year of the biopsy. Nine of these 10 patients presented with proteinuria of >5 g/24 h and nephrotic syndrome, while the 2 patients who did not develop ESRD had proteinuria <1.5 g/24 h.

Conclusions: Lupus-like GN, as defined by immunohistologic features and absence of serologic evidence of SLE, is not an uncommon form of glomerular disease in HIV-infected patients. The renal outcome in these patients was found to be generally poor, although this may be due in large part to most patients presenting with advanced disease and severe proteinuria.

1236 Renal Injury in the Subtotal Nephrectomy Model (5/6 Nx) in the Mouse Is Ameliorated by Angiotensin Receptor Blockade

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Background: The rat 5/6 Nx model has been extensively used to study mechanisms of progression. However, application of this model to mice has been challenging. We have previously demonstrated that glomerulosclerosis can be induced by a modified 5/6 Nx approach in the susceptible 129 mouse strain. We now investigated whether this mouse model is sensitive to angiotensin inhibition (ARB), as has been shown in the rat.

Design: 129 strain male mice (10 wk old, n=33) underwent 5/6 Nx by removal of the right kidney and ligation of lower branch of left renal artery and cauterization of upper part of left kidney. After 8 wks, 10 mice were sacrificed and the 23 remaining mice were divided into three groups: CONT (n=9), no further treatment; LOS-80, the ARB losartan 80 mg/L drinking water (DW)(n=9) or LOS-500, losartan 500 mg/L DW (n=5). Treatment was continued for 4 wks, and mice were sacrificed at wk 12. Systolic blood pressure (SBP) by tail cuff method, 24 hour urine albumin (Ualb), and body weight were measured at baseline and every 4 wks. GFR was measured by inulin clearance at baseline and the time of sacrifice. Sclerosis was assessed in each glomerulus on a 0-4+ scale, and average sclerosis index (SI) was calculated. In separate mice, combined ARB antagonizing AT1 and AT2 receptors was given, starting at 8 wks as above (n=22).

Results: SBP and Ualb increased after Nx (SBP at 0 wk 116.6±2.1, at 8 wk 149±1.7, at 12 wk 153.8±6.6 mmHg, Ualb at 0 wk 25.6±6.7, at 8 wk 495.7±120.0, at 12 wk 587.8±127.6 mg/24h, $p<0.05$ vs baseline). After 4 wks of Los-80 or Los-500 treatment, functional parameters were almost normalized. SBP at 12 wks in these mice was markedly decreased (101.4±2.9 in LOS-80 and 87±4.6 mmHg in LOS-500, $p<0.05$ vs CONT 12 wk). Ualb was 40.3±7.6 in LOS-80, 24.3±9.6 mg/24h in LOS-500 ($p<0.05$). GFR was decreased after Nx and significantly increased after LOS-80 (0 wk 371.9±30.7, 8 wk CONT 114.3±18.1, 12 wk CONT 110.3±6.7, 12 wk LOS-80 143.8±5.7 ml/min, $p<0.05$ vs CONT). Sclerosis data available from AT1+AT2 ARB mice showed SI 1.54±.21 at 8 wks, vs 2.68±.26 at 12 wks in CONT, vs 1.26±.29 in AT1+AT2 blockers mice, and 1.65±.11 in AT2 blocker alone mice ($p<0.05$, ARB vs CONT 12 wks).

Conclusions: These data suggest that ARB is protective in the mouse renal injury induced by 5/6 Nx, with reversal of hypertension, albuminuria, decreased sclerosis and increased GFR. These data indicate that the mouse model shares key characteristics with the rat model, and thus will be useful to evaluate novel treatments.

1237 The Alternative Complement Pathway Plays a Role in Mediating Glomerulonephritis Induced by Anti-MPO IgG

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Background: Intravenous injection of mouse anti-MPO IgG into wild type B6 mice results in the development of a pauci-immune focal necrotizing glomerulonephritis with crescents that closely mimics human ANCA glomerulonephritis. Although appear to be the primary effector cells, the details of the cellular and molecular events that cause the necrosis are not fully elucidated. In the process of evaluating the importance of various molecular pathways in the mediation of the injury, we investigated the role of the complement system in this model.

Design: Mouse anti-MPO IgG (derived from MPO-/- mice that had been immunized with murine MPO) was injected into the following groups of mice: wild type B6 (n=5), C4-/- mice (n=6), Factor B-/- mice (n=8), and C5-/- mice (n=3). Serum anti-MPO and urine protein and blood were monitored. After 6 days, mice were euthanized and kidneys examined by light and immunofluorescence microscopy.

Results: All wild type B6 mice developed glomerular necrosis (8.7% of glomeruli) and crescents (20.1%), and all C4-/- mice developed glomerular necrosis (8.7%) and crescents (17.6%). Whereas none of the Factor B-/- mice and none of C5-/- mice developed glomerular necrosis or crescents in spite of comparable levels of circulating anti-MPO.

Conclusions: These studies demonstrate the unexpected finding that defects in the alternative complement activation pathway (but not the classical pathway) abrogate disease induction by anti-MPO antibodies. Thus, these data suggest a role for the alternative complement pathway in the induction of ANCA disease, which is a novel concept. Given the paucity staining for immunoglobulin and complement at the sites of injury, complement activation probably is through an innate immune inflammatory pathway that does not involve initiation by substantial immune complex accumulation at the site of injury.

1238 Matrix Composition Alters Matrix Metalloproteinase Expression in Mesangial Cells Incubated with Glomerulopathic Light Chains

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Background: Mesangial cell behavior is partly modulated by their immediate extracellular matrix (ECM) components. Therefore, shortly after initial ECM alterations, mesangial cell function is altered, eventually leading to changes; sometimes irreversible, in the mesangium. It is known that during the initial phases of light chain-mediated glomerulopathies collagen I and tenascin accumulate in mesangium. The effects of these glomerulopathic light chains on an altered mesangium however,

have not been critically examined. The purpose of the present study is to evaluate the influences of various matrices in altering mesangial cell behavior when incubated with glomerulopathic light chains; leading to a better understanding of the progression of light chain deposition disease (LCDD) and AL-amyloidosis (AL-Am).
Design: Human mesangial cells (HMCs) were grown on collagen IV, collagen I and tenascin matrices and then incubated with glomerulopathic light chains purified from the urine of patients with LCDD and AL-Am for up to 96 hours. The supernatants were then analyzed for MMP-pro 1, 2, 3, 7 and 9 protein expressions by Western blots and ELISA. Mesangial cells growing on these matrices without light chains were used as controls.
Results: MMP-2, and 7 expressions were decreased in HMCs incubated with LCDD-LCs on tenascin and collagen I matrices at 72 vs. 96 hours compared with control. MMP-7 inhibition in HMCs grown on tenascin and treated with LCDD-LCs was the most striking. In contrast, HMCs incubated with AL-Am-LCs showed significantly enhanced expressions of MMPs 3, 7 and 9 on collagen I, while MMP-2 showed no significant alterations, except for increased stimulation from tenascin which enhanced all MMP expressions. Only collagen IV matrix failed to significantly stimulate MMP-3 and 7 in AL-Am-LC treated HMCs.
Conclusions: The initial alterations in the mesangial matrix in light-chain mediated glomerulopathies further enhance the deleterious effect of these light chains resulting in incremental damage. In LCDD the reduction in expression of MMPs, especially MMP-7, and the concomitant accumulation of tenascin, potentiates matrix increase enhancing nodular glomerulosclerosis. In contrast, over expression of virtually all MMPs when HMCs were cultured in the various matrices and incubated with AL-Am-LC highlights how the initially altered mesangium can contribute to potentiate matrix destruction leading to eventual replacement by amyloid.

1239 Heterozygous Mice for TGF-βIIR Gene Are Resistant to the Progression of Streptozotocin-Induced Diabetic Nephropathy

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Background: Transforming growth factor-β (TGF-β) receptor complex and its downstream Smad signaling intermediates constitute an extracellular matrix (ECM) accumulation pathway.
Design: In the present study, we examined whether decreased expression of TGF-β type II receptor (TGF-β RII) in TGF-βRII gene heterozygous (TGF-βRII$^{+/-}$; HT) mice could inhibit the Smad signaling pathway and subsequent progression of renal lesions when streptozotocin (STZ)-diabetes is induced.
Results: At the end of the 28th week after STZ injection, wild type (WT) diabetic mice showed severe glomerular hypertrophy and mesangial matrix accumulation occasionally featuring nodular glomerulosclerosis. In contrast, mean glomerular area and mesangial volume density were significantly decreased in the HT diabetic mice as compared with the WT diabetic mice. Immunostaining for phosphorylated Smad2/Smad3 and TGF-βRII in the glomerular cells was also significantly reduced in the HT diabetic mice. Southwestern histochemistry using Digoxigenin-labeled CAGA sequence probe showed that localization of labeled probe to the nuclei of glomerular cells in the HT diabetic mice was significantly less frequent than that in the WT diabetic animals. Northern blot analysis showed that α1(IV) collagen mRNA levels were significantly reduced in the kidney tissue of HT diabetic mice as compared with that of WT diabetic mice.
Conclusions: These results suggest that decreased expression of TGF-β RII in the HT diabetic mice can inhibit the progression of diabetic renal injury by inhibiting the downstream Smad signaling pathway and subsequent ECM gene expression. Thus, TGF-β RII appears to play an important role in the progression of diabetic nephropathy by mediating the intracellular Smad signaling.

1240 Interstitial Nephritis in HIV Patients Is Associated with an Increased Density of Lymphatic Vessels

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Background: Interstitial nephritis (IN) is a frequent cause of renal dysfunction common to many disease conditions. IN is one of a constellation of findings in both transplant rejection and HIV associated nephropathy (HIVAN). The physiologic and molecular mechanisms that govern the influx and egress of these inflammatory infiltrates are incompletely understood. Studies in renal transplant rejection have linked the presence of nodular infiltrates to an increased density of lymphatic vessels and suggest a special role for both macrophages and podoplanin (PODO), a specific marker for lymphatics, in the evolution of IN. To our knowledge, lymphatic neoangiogenesis in the setting of HIVAN associated IN has not been described.
Design: 43 renal H&E biopsies from HIV positive pateints were reviewed and the degree of IN was scored from 1(minimal) to 4(dense). Immunohistochemical staining of paraffin embedded 2 micron tissue sections with antibodies to PODO, D2-40 (both specific lymphatic endothelial markers), and KP-1 (macrophage marker) was performed on the 14 HIVAN biopsies that scored 4 for IN and on normal renal tissue (control) from 3 tumor nephrectomies. A mean lymphatic vascular density (MLVD), expressed as the number of PODO or D2-40 positive vascular profiles per high power field (40x), was calculated from 5 to 10 consecutive cortical fields per specimen. The percentage of KP-1 positive inflammatory cells was scored as 1+(<5%), 2+(5-20%), 3+(20-30%), 4+(>30%).
Results: The MLVD by PODO and D2-40 on all controls was 0.4 (range 0.1 to 0.7) and 0.9 (range 0.8 to 1.0) respectively. The MLVD by PODO and D2-40 for all HIV cases was 4.3(range 2.0 to 7.2) and 4.5(range 2.7 to 7.6) respectively. KP-1 scores for HIV cases were as follows: two 1+, and four cases each of 2+,3+, and 4+. The MLVD for KP-1(1+) HIVAN cases was 3.5 and 3.9 for PODO and D2-40 respectively. The MLVD for KP-1(4+) HIVAN cases was 5.9 and 5.4 for PODO and D2-40 respectively.
Conclusions: All HIVAN biopsies with severe (4+) IN examined had a higher MLVD by both PODO and D2-40 than controls. Generally, HIVAN cases with higher MLVD were associated with higher KP-1 scores. These results indicate that, as in nodular infiltrates found in renal graft rejection, these cases of HIVAN are associated with a substantial increase in the density of lymphatic vessels. This study also demonstrates a quantatative relationship between macrophage infiltrate and lymphangiogenesis; further study is needed to determine any causal relationship between these features.

1241 Cholesterol Embolization in Renal Allografts: A Clinicopathologic Study of 12 Cases

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Background: Cholesterol embolization (CE) in native kidneys is uncommon and is a well-established cause of renal failure requiring dialysis. In kidney allografts, CE is a rare occurrence, the natural history and prognostic significance of which is poorly characterized in the literature. We studied the clinicopathologic features and outcome of the largest known series of CE in renal allografts.
Design: We reviewed the surgical pathology files of the University of Pittsburgh Medical Center (UPMC) from 1997 to September 2004 to identify renal allograft biopsies (RAB) with CE. All pathology material related to such biopsies including recipient non-CE and donor kidney biopsies, if available, were examined and correlated with clinical and follow-up information. The most probable source of CE was determined based on review of the available pathology as well as the recipient and donor's age, sex, and coexisting medical conditions that predispose to atherosclerosis.
Results: Among 5435 RAB, a total of 21 biopsies from 14 patients had CE (incidence = 0.0039), of which 19 biopsies belonging to 12 patients originated from UPMC. Of the 12 cadaveric transplant recipients, there were 7 males and 5 females with a median age of 63 years (IQR = 17.8 years). Of the donors, there were 9 males and 2 females with a median age of 47 years (IQR = 40.5 years). One donor's age and sex was unknown. The two most common native renal diseases were hypertension and diabetes mellitus (75% of cases). The most probable source of CE was recipient in 7 cases, donor in 3 cases, and uncertain in 2 cases. Out of 12 cases, 5 had a rapid rise in creatinine without associated acute cellular rejection (ACR); 5 had 2 or more biopsies with CE; and 4 had failed renal allografts, 2 of which were considered primary non-functioning grafts complicated by CE and moderate-to-severe ACR. The other 2 allografts failed due to BKV nephropathy and chronic rejection. All the remaining functioning grafts showed variable chronic allograft nephropathy (CAN). Of the 19 RAB, the most frequent coexisting diagnoses were CAN (63%), none or borderline changes suspicious of ACR (63%), ACR (32%), and preservation/ischemic injury (21%). The median graft survival (from initial RAB showing CE) was 661 days (IQR = 1269 days).
Conclusions: CE in renal allografts: (a) is most often correlated with recipient cardiovascular disease; (b) may present with a rapid rise in creatinine, (c) usually does not result in acute graft loss, (d) is not incompatible with prolonged graft survival, and (e) may contribute to the development of CAN.

1242 Thymosin β4 Is a Marker or Possibly Even a Contributor to Glomerulosclerosis

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Background: Progression of focal segmental glomerulosclerosis (FSGS) is postulated to develop from early podocyte injury preceding overt sclerosis. In our previous study, we found that proteomic patterns can accurately distinguish normal, non-sclerotic and sclerotic glomeruli in a rat FSGS model. Thymosin β4 was one of the key differentially expressed proteins identified by tandem mass spectrometry increased in sclerotic glomeruli vs. non-sclerotic glomeruli in FSGS or normal glomeruli. We therefore aimed to investigate the expression and role of thymosin β4 in sclerosis.
Design: We assessed 5/6 nephrectomy rat tissues and cultured cells. Four small/short interfering RNAs (siRNAs) specific for thymosin β4 and control siRNAs were designed. Glomerular endothelial cells (GEN), derived from SV40 mice, were cultured and transfected with these siRNAs (Si) or control siRNAs (ContSi) for 48 hours. GEN were also stimulated with angiotensin II (Ang II, 10-6 M) for 24 hours, with or without concomitant transfection with siRNA or control siRNA. Normal GEN were used as baseline control (Baseline). The expression of thymosin β4 and plasminogen activator inhibitor 1 (PAI-1), a promoter of fibrosis, were detected by Western blots and normalized to β-actin.
Results: Immunohistochemistry of rat 5/6 nephrectomy model kidney showed no thymosin β4 in normal glomeruli and increased expression in sclerotic glomeruli in endothelial cells. Western of cultured podocytes and GEN confirmed thymosin β4 expression only in the latter. Thymosin β4 was successfully knocked down about 90% using siRNA. Neither siRNA or control siRNA affected baseline PAI-1 expression (Si 0.53 ±0.05, ContSi 0.54±0.01, Baseline 0.57±0.05, pNS). Ang II dramatically upregulated PAI-1 in normal GEN (1.54±0.03, $p<0.001$ vs Baseline). Treasfection with siRNA markedly dampened this Ang II induction of PAI-1 (Ang + siRNA 0.57±0.05, $p<0.001$ vs Ang II alone). Control siRNA had no effect (Ang + ContSi 1.48±0.12, pNS vs Ang II alone).
Conclusions: Our results demonstrate that over-expression of PAI-1 induced by Ang II is modulated by thymosin β4. These findings imply that thymosin β4 is not only a marker but potentially a contributor to glomerulosclerosis. Further, inhibition of thymosin β4 might provide a novel target for treatment of glomerulosclerosis.

1243 Overexpression of Plasminogen Activator Inhibitor –1 (PAI-1) Potentiates the Response of Glomerular Endothelial Cells (GEN) to High Glucose

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Background: Increased PAI-1 has been linked to fibrosis and diabetes. However, the functional roles of PAI-1 in collagen expression in GEN, and especially during hyperglycemia are unknown. We therefore investigated the direct effect of PAI-1 overexpression by adenovirus mediated gene transfer on collagen expression in GEN.

Design: GEN were infected with adenovirus expressing human PAI-1 (Ad-PAI-1) for 3 hours, then incubated in medium containing normal glucose (NG, 5.5 mmol/L), high glucose (HG, 25 mmol/L), or 5.5 mM glucose+19.5 mM mannitol (M) for 24 hours. Collagen I mRNA expression was assessed by semiquantitative RT-PCR.

Results: PAI-1 mRNA was overexpressed about 3.5-fold in GEN after Ad-PAI-1 infection vs baseline normal GEN. Collagen I mRNA expression in response to HG was significantly increased vs NG (collagen I mRNA/β-actin mRNA: GEN+HG 1.80±0.03 vs GEN+NG 0.73±0.01, $p<0.01$). PAI-1 overexpression in GEN increased collagen I mRNA expression about 2-fold under normal glucose conditions (vs GEN+NG, $p<0.01$). Collagen I mRNA expression in GEN under HG was further significantly upregulated following PAI-1 overexpression (3.13±0.21 vs GEN+NG or GEN+HG, $p<0.01$, separately). Control adenovirus encoding β-gal (Ad-Lacz) had no effects on collagen I mRNA expression under normal glucose conditions.

Conclusions: We conclude that PAI-1 overexpression contributes to extracellular matrx accumulation by directly promoting collagen expression in glomerular endothelial cells. Increased PAI-1 may predispose vascular endothelial cells to accumulation of extracellular matrix under hyperglycemic conditions. We postulate that modulation of existing PAI-1 levels in vascular endothelial cells may play an important role in protection against extracellular matrix accumulation in diabetic nephropathy.

1244 Glomerular Number and Size in Rats of the Milan Hypertensive and Normotensive Strains

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Background: Abnormalities in glomerular number and size, and hence of glomerular mass, have been implicated in the development of hypertension and progressive renal disease in several animal models. In rats of the Milan hypertensive strain (MHS), hypertension has been ascribed to a congenital defect of sodium tubular reabsorption and occurs without significant renal disease. In contrast, rats of the Milan normotensive strain (MNS) develop spontaneous glomerulosclerosis. Structural analysis, including morphometric computation of glomerular size and number, was applied to dissect the divergence between propensity to hypertension and renal damage in MHS and MNS rats.

Design: MHS, MNS rats and progenitor Wistar rats were investigated at age 9 weeks and 9 months. Classical morphometric methods were complemented by the dissector/ fractionator technique to count glomeruli.

Results: At 9 weeks, when nephrogenesis was completed and hypertension established, MHS rats exhibited significantly lower kidney weight, cortical volume, glomerular number and volume than coeval MNS rats. In Wistar rats, these parameters were similar to those of MNS rats, except for lower glomerular volume. At 9 months, MHS rats showed significantly lower expansion of glomerular volume than MNS or Wistar rats. MNS rats had 10% sclerotic glomeruli, reduced renal function, and heavy proteinuria; conversely, sclerosis was rare in coeval MHS and Wistar rats.

Conclusions: These data indicate that structural changes other than a tubular defect could play a role in the development of hypertension in MHS rats. The lack of significant glomerular hypertrophy and damage in this strain, despite reduced glomerular number, could be related to their (hemodynamic) protection from hypertensive renal disease. The larger size of glomeruli of MNS rats may be linked to their susceptibility to glomerulosclerosis.

1245 Hyaline Sclerosis of the Renal Vasa Recta in Chronic Calcineurin-Inhibitor Toxicity

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Background: Nodular hyaline sclerosis of the afferent arteriole is commonly observed in calcineurin-inhibitor toxicity (CIT), but morphologic changes in the post-glomerular microcirculation have not been described in this context. Pathological abnormalities in the vasa recta (VR), which arise from the efferent arterioles of the juxtamedullary glomeruli are rarely described, mostly related to systemic vasculitis. VR perfuse the loop of Henle and collecting ducts, and are believed to be the primary site of differential regulation of the medullary circulation. Our sporadic observations of hyaline sclerosis in the VR in renal transplant biopsies and biopsies from patients with focal glomerulosclerosis (FSGS) prompted this study.

Design: We examined 373 renal biopsies for VR hyaline sclerosis (VRHS). 143 cases were excluded due to inadequate medulla sampling. The biopsies were scored semi-quantitatively for VRHS, as well as glomerulosclerosis, arterial fibrointimal thickening, arteriolar hyalinosis, tubular atrophy, cortical and medullary fibrosis. The sample included baseline transplant biopsies and other early post-transplant biopsies, chronic allograft nephropathy (CAN), calcineurin-inhibitor toxicity (CIT), FSGS, IgA nephropathy (IgAN), diabetic nephropathy (DM), hypertensive nephrosclerosis, and various types of glomerulonephritis.

Results: Significant VRHS was frequently found in biopsies from patients with chronic CIT (24/29), and was often associated with nodular arteriolar hyalinosis. It was found less often in CAN (11/35), early post-transplant biopsies (6/34), FSGS (8/22), IgA nephropathy (8/33), and diabetic nephropathy (6/24). Mild changes were rarely seen in baseline transplant biopsies (2/23) and in biopsies from various types of glomerulonephritis (3/20). None of 10 biopsies with nephrosclerosis showed VRHS. Marked VRHS was most often seen in association with CIT.

Conclusions: VRHS occurs at significant frequencies in late transplant biopsies, particularly in association with evidence of CIT. It is seen less frequently in native kidney FSGS, IgA nephropathy, and diabetic nephropathy. It can occur before other vascular or parenchymal lesions. Increased transmitted blood pressure is a likely mechanism, however toxic drug effects and post-inflammatory scarring may also play a role. VRHS is an under-recognized lesion and may have significant functional correlates.

1246 Differences in Colocalization of IgA and Complement C3 in the Immune Complexes (IC) of Renal Biopsies from IgA Nephropathy (IgAN) Patients with Mild and Severe Renal Tissue Injury

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Background: IgA and complement C3 are major components of IC present in the IgAN patients. The properties of these IC have been studied using image analysis.

Design: Frozen remnant renal biopsy tissues from 13 random IgAN patients containing IgA and C3 were double-stained with Cy3-conjugated anti-IgA and Cy5-conjugated anti-complement C3 fluorescent antibodies. The staining protocol and antibody dilutions were identical for all biopsies. Distribution, size and shape of IC were examined with a confocal laser scanning microscope. Using image analysis software IP Lab 3.6, colocalization of IgA and C3 in glomeruli was expressed as Pearson's coefficient (Pc=1 in maximal colocalization). Further, lines were drawn through diameters of 10 IC of each biopsy to obtain line-intensity profiles. Results were compared with electron microscopy and histologic findings.

Results: Different types of IC were found in mesangium. Diffusely distributed mesangial IC had irregular surface and variable size up to 25 μm. Intensity profiles showed predominant IgA in 7 biopsies and predominant/codominant C3 in 6 biopsies. Interestingly, mesangial IC adjacent to capillary wall were linear with smooth surface, variable in length, however with uniform average width of 6 μm. In these linear IC, IgA predominant profile was found in 11 biopsies, one biopsy was C3 predominant, and one biopsy did not show linear IC. Electron microscopic examination confirmed the distribution of IC in glomeruli. Assessment of histology was performed prior to this study and was blinded to IC measurement. Eight of the 13 renal biopsies showed progressive tissue injury characterized by segmental glomerular sclerosis, moderate to severe tubular atrophy, interstitial fibrosis or atherosclerosis. Seven of these 8 biopsies had mesangial IC with predominant IgA intensity profile (Pc=0.50). In contrast, all 5 biopsies with mild renal tissue injury had predominant/codominant C3 (Pc=0.67).

Conclusions: Mesangial IC showed different IgA and C3 intensity profiles. IgA predominant profile and lower colocalization (Pc) value in mesangial IC were associated with more severe renal tissue injury compared to C3 predominant/ codominant profile and higher Pc in mild injury. Mesangial IC adjacent to capillary wall had mostly IgA predominant intensity profile independent of histologic findings.

1247 Renal Tubulo-Interstitial Changes Following Internal Irradiation with Alpha Particle Emitting Actinium-225 Daughters

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Background: Radiation nephropathy as a consequence of radionuclide therapies is an emerging problem. Although external beam radiation-induced renal pathological changes are well documented, few cases of radionuclide therapy associated nephropathy are reported. A systematic study of time-dependant renal tubulo-interstitial changes following administration of radiolabeled antibodies is not known.

Design: A chronological (10-40 weeks) study of the functional and morphological changes in mice kidneys (light and electron microscopy) after injection with Actinium-225 nanogenerator, a molecular-sized, antibody-targeted, in vivo generator of alpha particle emitting elements, was conducted.

Results: Renal irradiation from free, radioactive daughters of Actinium-225 (primarily Bismuth-213 and Francium-221), led to time-dependant changes in renal function manifesting as increased blood urea nitrogen. The histopathological changes corresponded with the decline in renal function. Glomerular, tubular and vascular endothelial cell nuclear hyperchromatism/pleomorphism, focal tubular cell injury/ lysis and karyorrhexis were observed as early as 10 weeks. Progressive thinning of the cortex due to widespread, varying degrees of tubulolysis and collapse, degenerative and reactive tubular lining cells, glomerular crowding, decrease in glomerular cellularity, mild, active interstitial inflammation and an elevated juxtaglomerular cell index were noted at 20 - 30 weeks post treatment. By 35 - 40 weeks, considerable regeneration of simplified tubules, atrophy and loss with focal mild interstitial fibrosis had occurred. A lower juxtaglomerular cell index with focal cytoplasmic vacuolization, suggesting increased degranulation, was also observed in this period. There is a focal increase in tubular and interstitial cell TGF-ß1 expression starting at 20 weeks, peaking at 25 weeks and later progressively declining in intensity, with mild increase in the extracellular matrix deposition.

Conclusions: These findings suggest that internally-delivered alpha particle irradiation-induced damage to tubular epithelial cells triggers a chain of adaptive changes, leading to progressive morphological damage accompanied by a loss of renal function.

1248 Clinical Correlation of BKV Viral Load with BKV Nephropathy in Renal Transplant Patients

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Background: BKV associated nephropathy (BKVAN) is an increasingly recognized complication following kidney transplantation. BKV has been associated with diseases varying from BKVAN, ureteric stenosis and tubulointerstitial nephritis, to asymptomatic hematuria. BKVAN occurs at approximately 44 weeks after transplantation. Quantitative real time PCR testing has been advocated for diagnosis and monitoring of patients, but data demonstrating its efficacy is limited. The purpose of this study was to evaluate the efficacy of BKV viral load to identify kidney transplant patients who will develop BKVAN

Design: A total of 160 kidney transplant patients were evaluated as part of this study from Nov '02 to Jun '04. We analyzed 476 plasma and 374 urine samples that were collected during routine clinic visits and hospitals admissions. Renal failure and diagnosis of BKVAN was confirmed by laboratory data, pathology reports and chart reviews. Patients were of various ethnic backgrounds including 99 African Americans, 57 Caucasians, 1 Hispanic and 1 Asian. Male to female ratio was 1.5:1. Graft rejection was experienced by 30/160 patients of whom 24 developed acute, 6 chronic rejection and 1 both. BKVAN occurred in 7/160 patients of whom 3 developed graft rejection. A rapid real time PCR assay with primers against the Large T antigen was developed for plasma and urine specimens.

Results: A total of 100 transplanted patients had all samples undetectable for BKV. None of the patients developed BKVAN during the study period. Sixty patients had at least one sample detectable for BKV with a broad range of BKV viral load across samples. Of these 60 patients, 7 had documented BKVAN. Three of 7 patients with BKVAN developed graft rejection, 2 had acute and 1 had both acute and chronic rejection. Viral load for patients with documented BKVAN typically had higher viral loads than patients without BKVAN (mean values: $5.0x10^4$ vs $1.03x10^3$ copies/plasma). BKV viremia and viruria in asymptomatic patients was generally lower than values for patients with BKVAN, but there was some overlap more typical of BKVAN. Resolution of nephropathy led to a decrease in viremia and viruria.

Conclusions: BKV viral load measurement provides a useful tool for monitoring the course of BKV infection.

1249 Spironolactone Ameliorates Extracellular Matrix (ECM) Accumulation in Unilateral Ureteral Obstruction (UUO) by Downregulation of Plasminogen Activator Inhibitor-1(PAI-1)

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Background: Glomerular and tubulointerstitial fibrosis underlie progressive kidney failure. Aldosterone has previously been linked to glomerulosclerosis through interactions of the renin-angiotensin-aldosterone system (RAAS) with PAI-1, which inhibits ECM degradation. The aim of this study was to determine whether aldosterone also plays a role in interstitial fibrosis. Therefore, we studied the effects of aldosterone and angiotensin inhibition on UUO-induced fibrosis.

Design: Adult male wild type (WT 129) mice underwent UUO and were sacrificed at day 5 or 14 after no treatment (Cont) or aldosterone antagonist spironolactone (0.15mg/hr) or losartan, an angiotensin type 1 receptor antagonist (80mg/L DW), or combined inhibition (n=5 each group). Kidneys were processed for morphological and molecular analyses. Fibrosis was assessed by point-counting. Overall injury, including interstitial expansion and tubular dilatation, was scored on a 0-4+ scale. Tissue collagen levels were assessed by HPLC, and PAI-1 mRNA expression was determined by Northern blot.

Results: Point-counting of obstructed kidneys at 5 days showed decreased ECM accumulation in losartan (2.4±0.5%) and combined group (2.6±1.1%) relative to Cont (10.2±7.3%). Total injury scores at day 5 were higher in spironolactone (1.7±0.2) and combined group (1.9±0.2) vs. cont (1.4±0.1) or losartan (1.3±0.1), largely reflecting increased tubular dilatation in the former. At 14d, all treatments had less injury score than control, at this time largely reflecting less interstitial fibrosis. HPLC collagen content was significantly lower with combined inhibition (3.8±1.1 %collagen/total protein) than in control (7.3±2.3) and trended lower with spironolactone (5.0±1.2) ($p<0.05$, $p=0.077$ respectively vs. control) at 5 days. PAI-1 levels, expressed as a densitometric ratio relative to GAPDH were decreased from control (mean=3.92) in losartan (2.29±0.24), with further decreases in spironolactone (1.64±0.52) and combined inhibition (1.18±0.26).

Conclusions: We conclude that early tubular dilatation/injury was not improved, and even increased by spironolactone. However, ECM and PAI-1 were decreased by spironolactone, culminating in decreased chronic injury scores, predominantly reflecting decreased ECM by day 14. We speculate that the divergent effects of spironolactone on tubular dilatation and ECM arise from its diuretic properties increasing tubular dilatation while its antifibrotic actions are linked to PAI-1.

1250 Comparison of Immunofluorescence and Immunohistochemical Methods for C4d Staining in Renal Allograft Biopsies

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Background: Immunostaining of human renal allograft biopsies with antisera directed against C4d has become an important diagnostic tool in the recognition of humoral mediated graft rejection. The majority of studies have been performed on frozen tissue sections, using one of several commercially available antibody reagents. Studies evaluating these reagents, or comparing the results of immunofluorescence with immunohistochemical staining of formalin fixed, paraffin embedded tissue have not been published.

Design: We directly compared two different staining methods in 107 renal allograft biopsies obtained between February 2003 and September 2003 at Stanford University Medical Center; biopsies included protocol and clinically indicated biopsies from adult and pediatric patients. We tested a monoclonal antibody (Quidel, San Diego, CA) on frozen tissue sections with indirect immunofluorescence (IF), and a polyclonal antibody (Biomedica Gruppe, distributed by ALPCO, Windham NH) applied to formalin fixed, paraffin embedded tissue with immunohistochemical detection (IHC).

Results: The study group included 58 biopsies performed for clinical indication and 49 protocol biopsies. Of these, 33 biopsies had evidence of acute rejection (ranging from Borderline to IIB per Banff classification), and 74 biopsies had no evidence of acute rejection. There was complete agreement between staining methods in 104/107 cases (97%); two cases were positive by IF but negative by IHC, while one case was identified by IHC but was negative by the IF method. Nine of 107 cases were positive with either method, representing 8.4% of all allograft biopsies tested, 15% of clinically indicated biopsies, and 26% of biopsies with a histologic diagnosis of acute rejection.

Conclusions: Given comparable staining results, we recommend the IHC method on formalin fixed tissue. The advantages include preservation of tissue morphology, automation of staining procedure, conservation of tissue specimen, and availability of a permanent slide record.

1251 BK Polyoma Virus in Renal Transplants. Role of Electron Microscopy and Immunostains in Detecting Early Infection

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Background: Reactivation of BK polyoma virus (BKV) is increasingly recognized as a cause of failure of renal allografts. Currently no specific treatment is available for this viral infection and early diagnosis is the key to prolong graft survival. In the initial phase of viral infection in renal transplants, minimal or no histologic viral changes may be seen in renal biopsies. Electron microscopy (EM) and immunostains can be utilized effectively to identify the disease process at an early phase.

Design: 44 consecutive renal transplant biopsies performed over a two year period were selected. CD3, CD20, BK immunostain and EM studies were performed. BK polyoma virus immunostain was interpreted as follows: negative-no staining, positive-tubular cell nuclei staining, equivocal- only cytoplasmic/background nuclear staining. Using EM, the nuclei of tubular cells (range: 67-254 nuclei) were examined for viral particles. CD3/CD20 % was scored in cases with inflammatory infiltrate.

Results: Histologic diagnosis of 42 biopsies were as follows: 3-ATN, 6-Banff borderline, 26-IA, 4-IB and 3-IIA. Nine cases (20.5%) showed polyoma virus particles on EM. 3 of 5 cases (60%) with identifiable viral particles on EM showed positive immunostain while only one out of 15 cases (6.7%) with negative immunostain showed viral particles on EM.

Comparing BK virus immunostain and electron microscopy results

BK virus immunostain (n=36)	EM positive	EM negative
Positive (n=5)	3	2
Equivocal (n=16)	3	13
Negative (n=15)	1	14

Immunostain could not be performed in 8 cases and two of these 8 cases had viral particles on EM.

All cases with viral particles identified by EM showed an intense CD20+ inflammatory infiltrate (60-80% of all inflammarory cells). Of the two cases that were EM negative but had positive BK staining, one showed 80% and the other 20% CD20 staining. Of cases that had both negative BK virus immunostain and EM, all but two cases showed predominant CD3 positivity.

Conclusions: None of the transplant cases exhibited classical histologic viral changes. Viral particles were seen by EM in 20.5%, and was confirmed by immunostain in 33.3% of these cases. CD20 rich inflammatory infiltrates predominated in cases in which either positive BK stain and/or viral particles were identified by EM. EM has a definitive role in detecting early viral infections in renal biopsies. Early diagnosis in order to attempt viral clearance and thereby limit graft damage should be the primary treatment goal.

1252 Correlation of Cylex™ Immune Cell Function Assay with Findings on Renal Transplant Biopsy

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Background: The Cylex ™ Immune Cell Function Assay (ICA) utilizes quantitation of ATP generation in stimulated CD4 lymphocytes as a measure of cell mediated immunity. ATP is measured with a chemiluminescence assay and reported as ng/ml. The ICA has been proposed as a measure of effectiveness of immunosuppression in the transplant setting. The current study was designed to assess the correlation of ICA with concomitant findings on renal transplant biopsies. We anticipated a direct correlation between ICA and severity of histologic rejection.

Design: Cylex™ ICA was performed on stimulated peripheral CD4 lymphocytes from whole blood taken at the time of protocol or clinically indicated renal transplant biopsy. ATP levels were classified as previously defined: low responder: <225 ng/ml, moderate: 225-524 ng/ml; high: >525 ng/ml. One hundred twenty-seven patients underwent renal biopsy for evaluation with concomitant ICA determination. The population consisted of 93 men and 34 women. One hundred sixty-nine biopsies were evaluated according to the Banff '97 criteria. Patients with serial ICA/biopsy data were assessed for significance of pattern of change of ICA level and biopsy findings.

Results: There was no direct correlation of the ICA level and histologic rejection. Average ICA (ng/ml) was: No rejection (n=84) - 258.34; Borderline (n = 38) - 221.03; Ia (n = 18) ICA = 228.82; Ib rejection (n = 8)189.59. The ICA in Polyoma virus infection (n = 5) was 228.51 ng/ml. The distribution of the rejection grades among the

ICA response classes was similar, with no trend toward increased rejection in high responders, as follows: High responders (no rejection 86%, 1a 14%); Medium responders (no rejection 49%, borderline change 25%, 1a 12%, 1b 3%); Low responders (no rejection 51%, borderline 24%, 1A 15%, 1B 7%). Serial ICA was noted to be variable in stable non-rejecting patients.
Conclusions: The Cylex ™ ICA does not directly correlate with allograft biopsy findings. ICA levels show variability in stable patients without histologic rejection. Our study indicates that individual and serial ICA measurements are not a sensitive indicator of histologic rejection in renal allografts and should not replace the serial histologic monitoring.

1253 Absence of Integrin β_6 Worsens Glomerulosclerosis in Remnant Kidney by Modulating Tubuloglomerular Feedback

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Background: The heterodimeric integrin αvβ6 activates transforming growth factor-β (TGFβ) locally. Integrin αvβ6 is expressed in the tubular epithelium and the juxtaglomerular apparatus (JGA) in the kidney. We have previously observed that absence of integrin β6 decreases interstitial fibrosis but worsens glomerulosclerosis in remnant kidney mice. The aim of the present study was to investigate the mechanism of these changes.
Design: Adult male wild type (WT 129) and β_6-/- mice on 129 background underwent 5/6 nephrectomy (Nx). Kidneys were harvested for morphologic, P-Smad2, renin, neuronal nitric oxide synthase (nNOS) and cyclooxygenase-2 (COX-2) analyses by western and immunohistochemistry at week 0 and 12.
Results: At baseline, β_6 -/- did not show altered expression by western blot of P-Smad2, nNOS, renin or COX-2 (WT vs β_6 -/-, P-Smad2 0.055±0.005 vs 0.065±0.002; nNOS 0.139±0.016 vs 0.172±0.015; renin 4.020±0.854 vs 4.253±1.484; COX-2 0.909±0.160 vs 1.087±0.341; pNS). At week 12 after Nx, β_6 -/- mice had decreased P-Smad2 by 5.7-fold and nNOS by 2.4-fold, while renin expression increased 1.8 fold vs WT Nx (WT vs β_6 -/-, P-Smad2 0.205±0.053 vs 0.036±0.007; nNOS 0.194±0.035 vs 0.082±0.023; renin 0.363±0.058 vs 0.653±0.089; $p<0.05$). There was no difference in COX-2 expression (WT 0.544±0.030 vs β_6-/- 0.585±0.046, pNS). By immunohistochemistry, nNOS and renin were observed mainly in JGA at week 0 with increased renin extent and intensity and decreased nNOS intensity in β_6 -/- vs WT at week 12. Increased COX-2 was detected in macula densa/cTALH and medullary interstitial cells after 5/6 nephrectomy in both WT and β_6 -/-.
Conclusions: We conclude that absence of integrin β_6 worsens glomerulosclerosis after 5/6 nephrectomy, with disproportionally milder tubulointerstitial fibrosis at least in part by increasing renin and decreasing nNOS expression in the JGA. We speculate that dysregulated tubuloglomerular feedback due to absent JGA β_6 expression in the remnant kidney might be involved in this augmented sclerosis.

1254 AAV-Mediated Gene Therapy for Systemic Lupus Erythematosus (SLE)

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Background: SLE is a systemic autoimmune disease characterized by autoantibody production and immune complex-mediated glomerulonephritis(GN). By blocking the interaction between autoreactive B cells and T cells, costimulatory blockade using CTLA4Ig and anti-CD40L mAb was able to suppress lupus in NZB/W F1 mice, a SLE murine model. However, for such a chronic disease long-term intervention is necessary and gene therapy may be advantageous. In this study, we tested whether recombinant adeno-associated viral vector serotype 8 (rAAV8)-mediated gene delivery can prevent and suppress lupus in NZB/W F1 mice.
Design: To prevent lupus, rAAV8-CTLA4Ig or rAAV8-CD40Ig was delivered separately or co-injected into the peritoneum of newborn mice. In the therapy group, rAAV8-CTLA4Ig was injected into the spleen of lupus mice that had already developed autoantibody. Serum anti-dsDNA IgG titer and CTLA4Ig level were measured by ELISA. Proteinuria was monitored with Albustix. Kidney cryosections were stained with FITC-anti-IgG and anti-C3; paraffin sections were examined histologically.
Results: In rAAV8-CTLA4Ig-treated group, serum CTLA4Ig level reached 522.72±93.79ug/ml in 1 month and remained at 317.23±96.24ug/ml at 8 months after injection. Serum anti-dsDNA IgG titers were significantly lower in rAAV8-CTLA4Ig-treated and co-injection groups than control group. The elevated anti-dsDNA IgG titer in therapy group decreased after gene delivery. While all untreated control mice developed severe proteinuria and died by the age of 10 months, only 14.29% mice in rAAV8-CTLA4Ig-treated, 30% mice in therapy group, 80% mice in rAAV8-CD40Ig-treated group had proteinuria, surprisingly consistent with the mortality. Moreover, all mice in the co-injection group were symptom free and alive for at least 10 months. Kidney pathology showed no immune deposits and only minor glomerular changes in co-injection group.
Conclusions: rAAV8-mediated gene delivery was able to achieve long-term, stable transgene expression. Both rAAV8-CTLA4Ig and rAAV8-CD40Ig suppressed autoantibody production, delayed proteinuria onset and extended the life span in NZB/W F1 mice. Moreover, a strong synergistic effect on lupus prevention was detected in two vector combination regimen. Importantly, rAAV8-CTLA4Ig reversed disease progression in lupus mice. Our results suggest that rAAV-mediated gene therapy can not only efficiently prevent disease onset and but also inhibit its progression in the murine model of SLE.

1255 Subclassification of Focal and Segmental Glomerulosclerosis (FSGS)

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Background: A new subclassification scheme has been proposed for focal and segmental glomerulosclerosis (FSGS) in an effort to better define prognosis and treatment for this heterogeneous and poorly understood nephrotic disease (D'Agati et al, Am J. Kid. Dis. 43: 368-382, 2004). We have applied this classification to renal biopsies from a population in Bronx, NY, in which FSGS is the most frequent biopsy diagnosis.
Design: Seventy two cases of FSGS biopsied between 2001 to 2004 were retrieved from the Montefiore Medical Center slide files. All cases were evaluated with H&E, PAS, Masson-trichrome, and PASM slides, immunofluorescence and electron microscopy. All diagnostic glomeruli were used for classification into the five subtypes of D'Agati et al: collapsing, cellular, tip, perihilar, and not otherwise specified (NOS). Subtypes were then correlated with age and clinical presentation as measured by serum creatinine (sCr), proteinuria (UP), hypertension (HT) and hematuria (HM).
Results: 30/72 cases (42%) were classifiable as a specific subtype, while 42/72 (58%) remained as FSGS NOS. 12 cases (17%) were classified as collapsing, 13 as cellular (18%), 4 as perihilar (6%), and only 1 as tip (1%). 46/72 (64%) were from children and 26/72 (36%) from adults, with virtually identical percentages of pediatric and adult cases (41% and 42% respectively) classifiable as specific subtypes. Each subtype was also represented in similar percentages in children and adults. Initial sCr and UP were lower in cellular in comparison to either collapsing or NOS subtypes (sCr: 0.9 vs 2.4 and 3.2; UP: 221 vs 338 and 333). Numbers of patients with HT and HM were also lower in cellular vs collapsing or NOS subtypes (HT: 0 vs 1 and 6; HM: 2 vs 5 and 8). Again, there were no significant differences between adults and children. Perihilar and tip subtypes were represented by too few cases for analysis.
Conclusions: For the patient population in the Bronx, a significant number (but not a majority) of FSGS biopsies can be classified into one of the specific subtypes. Of these, the cellular subtype presents initially with less severe clinical findings than either collapsing or NOS subtypes. Pediatric and adult cases have the same relative percentages represented by each subtype, and there appears to be no significant difference between pediatric and adult clinical presentation as a function of subtype. The NOS subtype may still be heterogeneous and in need of another criterion for subclassification. It may also be that some currently undefined large number of glomeruli is necessary for accurate subclassification.

1256 COX2-Dependent Osmolyte Accumulation and Gene Expression in Medullary Interstitial Cells

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Background: A recent study has shown that hypertonicity-induced osmolyte accumulation in mouse medullary interstitial cells (MMICs) was dramatically reduced by the COX2-specific inhibitor SC58236 *in vivo* and *in vitro* (JBC 2003, 19352). The purpose of this study was to investigate the effect of COX2 products PGE_2 and PGI_2 on osmolyte accumulation, the osmolyte regulating transcription factor TonEBP, osmolyte transporters and osmolyte enzyme gene expression in cultured MMICs. A further aim of our study was to determine ERK1/2-dependent COX2 expression in MMICs following hyperosmotic conditions.
Design: Primary culture MMICs were slowly adapted to 400 mosmol/L medium tonicity with and without PGE_2 (1 μM), PGI_2 (20 μM) or ERK-inhibitor PD98059 (20 μM). Organic osmolyte accumulation in MMICs was quantitated using isocratic HPLC. Betaine transporter (BGT1) and sodium *myo*-inositol transporter (SMIT) mRNA levels and aldose reductase (AR) mRNA levels were determined using Northern blot analysis. COX2 protein levels and transcription factor TonEBP levels were analyzed using Western blot.
Results: As expected, MMICs accumulated the organic osmolytes inositol, betaine and sorbitol following exposure to 400 mosmol/L. COX2, TonEBP, SMIT, BGT1 and AR increased two-fold at 400 mosmol/L compared to isotonic controls. PGI_2 further augmented the increase of TonEBP, SMIT, BGT1 and AR (5fold, 4 fold, 3 fold and 3fold respectively). Surprisingly, both PGE_2 and PGI_2 stimulated SMIT, BGT1 and AR mRNA expression under isotonic conditions. ERK inhibitor PD98059 dramatically reduced hypertonicity-induced TonEBP and COX2 expression.
Conclusions: Our study shows that hyperosmotic culture conditions not only induce COX2 and the osmolyte transcription factor TonEBP in MMICs, but also that the COX2 products PGE_2 and PGI_2 stimulate the expression of Ton EBP as well as the major osmolyte transporter SMIT, BGT1 and the sorbitol-forming enzyme aldose reductase. Furthermore, the observation that PGE_2 and PGI_2 stimulate TonEBP, BGT1 and SMIT independantly from hypertonic stress indicates that COX2 products are important mediators in the compatible osmolyte response. Finally, the dramatic reduction of hypertonicity-induced TonEBP and COX2 stimulation by PD98059 indicates that ERK1/2 is the predominant signaling pathway that stimulates COX2 and TonEBP upregulation following exposure to hyertonic stress. In conclusion we speculate that the protective osmolyte response following hyperosmotic stress in MMICs is induced by ERK1/2 and mediated by the COX2 products PGE_2 and PGI_2.

1257 Acute Renal Rejection Predominated by Monocytes Is a Severe Form of Rejection in Human Recipients with or without Campath-1H (Alemtuzumab) Induction Therapy

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Background: Campath-1H has been used successfully for induction of immunosuppression and has resulted, in combination with various postoperative immunosuppressive regimens, in a low rate of acute rejection in renal transplantation. This study was undertaken to investigate the extent of monocyte involvement in acute renal transplant rejection, with or without Campath-1H induction. Design: Renal specimens with or without Campath-1H treatment were stained routinely

(including HE, PAS and Trichrome stains). Using Dako Autostainer, representative renal sections were immunohistochemically stained for CD3 and CD68 to identify T lymphocytes and monocytes, respectively. Results: Statistically, cases with grade Ib or higher rejection (n = 11) had a significantly higher percentage of CD68 positive monocytes (66.9 ± 6.4 %*) than the cases with Ia rejection (n = 11, 40.0 ± 5.2 %, *p<0.05 vs Ia rejection, using student t test), regardless of the status of Campath-1H induction. Conversely, the percent of CD3 positive T lymphocytes from the cases with grade Ib or higher rejection was significantly lower (27.2 ± 7.1 %*), when compared to the cases with Ia rejection (56.4 ± 5.8 %). Furthermore cases of acute rejection, following Campath-1H induction, appear to demonstrate a "pure form" of monocytic acute rejection, whereas monocytes were mixed with many other types of inflammatory cells in the cases of acute rejection in the absence of Campath-1H induction. In addition with Campath-1H induction, the cases of monocyte-predominant acute rejection were found to uniformly exhibit a good response to corticosteroid treatment. Conclusion: Our findings suggest that monocyte-predominate acute rejection is often a severe form of acute rejection either with or without Campath-1H induction, although monocytic acute rejection, following Campath-1H induction, appears to be more obvious on the CD68 stained section.

Liver & Pancreas

1258 Histologic Abnormalities Are Common in Protocol Liver Allograft Biopsies from Patients with Normal Liver Function Tests

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Background: The utility of protocol liver allograft biopsies remains controversial, particularly in patients with normal liver function tests (LFTs). However, evaluation of these biopsies provides an opportunity to examine the types and severity of liver disease that occur in livers with normal clinical and laboratory function.

Design: We studied 165 protocol allograft biopsies taken from 100 liver transplant patients at the time of normal LFTs at 3-8 mos (n=36), 1 yr (n=52), 2-3 yrs (n=54), and 4-5 yrs (n=23). Biopsies were classified as normal, minimal/nonspecific changes (e.g., nonaggressive portal or lobular inflammation, steatosis <10%), mild steatosis (10-33%), fatty liver disease (steatosis >33% or active steatohepatitis), recurrent primary liver disease, and transplant-related disease (portal-based rejection or central venulitis, an inflammatory pattern encompassing perivenular hepatocyte dropout, mononuclear inflammation, and pigment-laden macrophages).

Results: In these 100 patients, 165 (42%) of a total of 394 protocol biopsies were performed at the time of normal LFTs. 121 (73%) biopsies were normal or showed minimal changes. 44 (27%) showed histologic abnormalities that included mild steatosis (n=9), fatty liver disease (n=10; steatosis range 25-50%, grade 1/3 steatohepatitis in 7, stage 1/4 fibrosis in 1), recurrent primary biliary cirrhosis (n=8; all stage 1/4), recurrent hepatitis C (n=6; grade 0/4 in 1, grade 1/4 in 5, stage 0/4 in 4, stage 1/4 in 1, and stage 2/4 in 1), recurrent sarcoidosis (n=1), Ito cell hyperplasia (n=3), central venulitis (n=10; with mild zone 3 fibrosis or central vein obliteration in 5 and central-portal bridging fibrosis in 1), and mild acute portal rejection (n=2). We judged the histologic changes to be of clinical significance in 19 (11.5%) cases.

Conclusions: Despite normal clinical and laboratory liver function, a significant fraction of protocol allograft biopsies show histologic (27%) and clinically significant (11.5%) abnormalities. Common liver diseases that occur in the absence of clinical or laboratory dysfunction include steatohepatitic liver disease, low-grade/low-stage hepatitis C and primary biliary cirrhosis, and central venulitis (including cases with early or advanced fibrosis). These results 1) support the performance of protocol biopsies to assess the status of the allograft, and 2) provide insight into the types and severity of liver disease that can smolder in transplant (and probably also native) livers with apparent normal function.

1259 Will the Real Mucinous Carcinoma of the Pancreas Please Stand Up?: A Reappraisal of the Terminology, Classification and Differential Diagnosis of "Mucinous" Carcinomas in the Ampullo-Pancreatobiliary Region

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Background: The definition of "mucinous carcinoma" varies from organ to organ. In the pancreas, most tumor types are of ductal nature, and thereby form mucin. This study investigates the types of tumors of the ampullo-pancreatobiliary region that are referred to as mucinous by pathologists, and attempts to formulate an approach for a more accurate conceptualization of these neoplasms.

Design: Pathology material from 65 pancreatic specimens with 1° or 2° carcinomas, which had been designated as "mucinous" in a database of 908 cases, was reviewed. The cases were classified by an algorithmic approach based on the distribution of mucin and presence or absence of invasion (inv). Each case was designated a specific clinicopathologic entity according to current concepts.

Results: I. Inv ca with intracellular and/or intraluminal mucin (n=33). A. Without a pre-inv mass. 1. Inv ductal. (n=11). 2. Inv ductal: morphologic variants (8 foamy gland, and 2 signet ring). 3. CBD ca (n=2). 4. Secondary from GI-tract (n=3), and ovary (n=1). *These were all highly aggressive neoplasia*. **B. With a pre-invasive mass.** 5. Inv ductal associated with IPMN (n=3), or MCN (=2). 6. Intestinal type of ampulla/duodenum (n=1) with adenoma. *These were relatively less aggressive.* **II. Inv ca with stromal mucin deposition (n=25). A. Cells confined to (floating within) mucin** 7. Colloid ca (n=8), 4 with IPMNs. *These had indolent behavior.* **B. Non-mucinous type inv present.** 8. Mixed mucinous (n=7): Colloid admixed with intestinal type. *This group had moderately aggressive behavior.* 9. Mucinous ductal (n=10):Usual ductal ca with abundant stromal mucin deposition. *These were highly aggressive.* **III. Intraductal and cystic (non-invasive) ca with intraluminal (and cytoplasmic) mucin (n=7). *A. Without ovarian stroma.*** 10. IPM ca (in-situ), n=5: *4 intestinal and 1 pancreatobiliary type papillae*. **B. With ovarian stroma.** 11. Mucinous non-invasive cystadenoca (in-situ), n=2. *These were indolent, although 1 from each behaved aggressively.*

Conclusions: In the pancreas, the term "mucinous carcinoma" has been applied to a plethora of neoplastic entities, ranging from in-situ to inv to secondary tumors. Pancreatic carcinomas with mucin formation should be classified as one of the specific entities described above, as each has different clinicopathologic characteristics and outcomes. An algorithmic approach based on the distribution of mucin and invasiveness of the tumor is useful in the differential diagnosis.

1260 Ampullary Carcinoma: Role of DNA Mismatch Repair Gene Defects in Pathogenesis

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Background: While microsatellite instability (MSI) secondary to DNA mismatch repair (MMR) gene abnormality is known to be the underlying molecular defect in about 15% colorectal carcinomas, the frequency and significance of MSI in ampullary carcinomas remain to be defined. We and others have previously shown that immunohistochemistry (IHC) using antibodies against the MLH1 and MSH2 proteins is a simple and specific method for detecting MMR defects, and in the case of colorectal carcinoma, certain morphological features, such as tumor infiltrating lymphocytes (TIL), poor differentiation, and medullary type and mucinous histology, also carry a significant predictive value in identifying MMR abnormalities. The purpose of this study was therefore to evaluate the frequency of MMR abnormality in ampullary carcinomas using IHC, and to investigate the presence or absence of any association of morphological features with MMR defects in these tumors.

Design: Pathologic review and IHC with anti-MLH1 (clone G168-728, PharMingen) and anti-MSH2(clone FE11, Oncogene Research Products) antibodies were performed on a series of 60 ampullary carcinomas treated at Memorial Sloan-Kettering Cancer Center from 1986 to 1994. Morphological features analyzed included tumor differentiation, histological subtypes (medullary, mucinous and signet ring cell), and number of TILs per 10 high power field.

Results: All 60 tumors were adenocarcinomas, 45 moderately differentiated, 8 poorly differentiated and 7 mucinous. No tumors showed typical medullary carcinoma morphology. Focal presence of signet ring cells was noted in only 1 tumor. TILs were noted in 39 tumors, ranging from 1/10 hpf to 62/10 hpf. All 54 cases that were tested by IHC showed positive staining for MSH2, and 53 of 54 showed positive staining for MLH1. Of the positive cases, the staining intensity was scored as weak for MSH2 in 5 tumors and for MLH1 in 18 tumors, whereas the remaining positive cases showed moderate to strong intensity. No significant correlation was detected between any of the morphologic features and the staining intensity. The one MLH1-negative tumor was a moderately differentiated adenocarcinoma with no apparent TILs.

Conclusions: DNA mismatch repair gene defect does not appear to play a significant role in the pathogenesis of ampullary carcinoma, and morphological features that are shown to be associated with microsatellite instability in colorectal tumors do not carry the same implication in ampullary carcinomas.

1261 p53, Ki-67, VEGF and EGFR Expression in Liver Transplant Patients with Hepatocellular Carcinoma (HCC) with and without Subsequent HCC Recurrence

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Background: Recurrence is the main factor influencing the prognosis of HCC. The aim of this study is to determine the expression of p53, Ki-67, VEGF, and EGFR in explanted livers with HCC and correlate the expression pattern with tumor recurrence.

Design: The study population consisted of 23 liver transplant recipients at UICMC who had HCC of the liver explant. The subjects were divided into two groups: 1. Non-recurrent: HCC patients with at least 1 year recurrence-free survival (n=14), and 2. Recurrent: HCC patients with known recurrence within the first year post-transplant (n=9). We performed standard immunohistochemical staining for p53, Ki-67, VEGF and EGFR on the cirrhotic liver and the tumor. Results were independently analyzed by two observers. Nuclear staining for p53 was interpreted as positive. Ki-67 was interpreted as percent positive nuclear stain per 100 cells. A diffuse cytoplasmic staining for VEGF was interpreted as positive. A membranous staining for EGFR was interpreted as positive. For both VEGF and EGFR, the percentage of positive cells were multiplied by the intensity of staining (grade 0=no staining to 3+=strongest intensity of staining) to provide an index.

Results: While p53 expression in the tumor of the non-recurrent vs. recurrent group did not show any significant difference (χ^2 test), a negative p53 in the cirrhotic liver correlated significantly with absence of HCC recurrence ($p \leq 0.05$ χ^2 test). High percentage of Ki-67 expression (>10%) correlated significantly with HCC recurrence when observed both in the tumor ($p \leq 0.05$ χ^2 test) or the cirrhotic liver ($p \leq 0.025$ χ^2 test). A high VEGF index in the cirrhotic liver was not seen in any of the cases that recurred. While VEGF index was stronger in the tumor compared to the cirrhotic liver in the recurrent group ($p \leq 0.008$ Wilcoxon paired test), there was no difference in the VEGF index of the cirrhotic liver compared to tumor in the non-recurrent group ($p \leq 0.25$ Wilcoxon paired test). EGFR indices were higher in the tumor compared to the cirrhotic liver in both the recurrent ($p \leq 0.008$ Wilcoxon matched-pairs signed-ranks test) and non-recurrent group ($p \leq 0.014$ Wilcoxon matched-pairs signed-ranks test).