Transplantation

D1 Basic, experimental

M695 DONOR BONEMARROW DERIVED STEM CELL INFUSION IN THYMUS AND PERIPHERY: AN INTEGRATED APPROACH TO ACHIEVE TOLERANCE IN CADAVER RENAL ALLOGRAFT RECIPIENT

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We designed a prospective, randomized clinical trial to evaluate immune response of donor bone marrow derived stem cell (DBMC) infusion in thymus and periphery to create immune tolerance in cadaver renal allograft recipients.
We studied 24 patients divided into two equal groups. In treated group (A), 350 mL of un-fractionated bone marrow (BM) was aspirated from anterior iliac crest of cadaver donors. Two ml of concentrated marrow was infused into thymus, 100 ml into BM before surgery; remaining 250 ml was infused peripherally post-transplantation. Recipients were lymphocytotoxicity cross-match negative in both groups. Group B (controls) received kidney and no donor bone marrow cells. Group A received low dose Prednisolone and Cyclosporine; Azathioprine was added in controls. Average mean cell count of thymic inoculum was 3.3 x 10^4 cells/cmm and in periphery, 8.6 x 10^7 cells/kg body wt.
Over a mean follow-up of 369 days of both groups, group A had significantly better graft function with no acute rejection (AR) episode/CMV infection, mean serum creatinine (S.Cr) of 1.18 mg.% ($p<0.005$) and no graft/patient loss. Group B with mean S.Cr of 2.03 mg % had 3 patients with single AR episode each, 2 patients had CMV infection and 1 patient was lost. Graft survival was 91.7% in controls.
This novel integrated un-fractionated donor bonemarrow derived stem cell infusion approach into thymus and periphery to create tolerance is safe, efficacious, gives significantly better graft function without any AR/CMV disease with minimum immunosuppression as compared to controls on standard triple drug therapy.

M696 INTRACELLULAR CYTOKINE RESPONSE TO IMMUNOSUPPRESSIVE AGENTS IN PATIENTS WITH END STAGE RENAL FAILURE AWAITING TRANSPLANTATION

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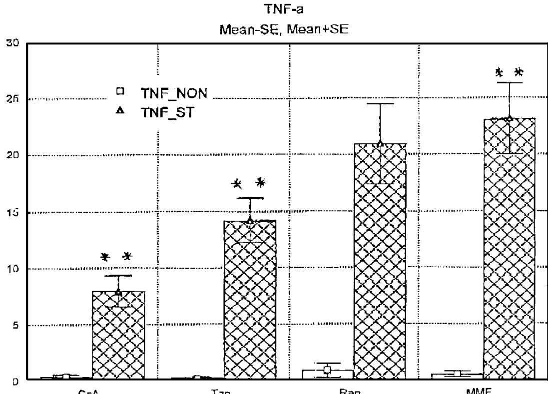
Immunosuppressive drugs used in transplantation may polarize the T-cell response by altering the balance of cytokine expression.
The aim of this study was to investigate the intracellular cytokine response to cyclosporine A (CsA), sirolimus (Sir), tacrolimus (Tac) and mycophenolate mofetil (MMF) in 15 haemodialysis patients awaiting transplantation. Each patient was given a single immunosuppressive drug sequentially for two weeks at a standard dose used for renal transplantation, followed by a two-week washout period before the introduction of the next immunosuppressive drug.
T-cells in peripheral blood were phenotyped and analysed for the ability to produce the Th1 cytokines IL-2, IFN-gama, TNF-alpha, the Th2 cytokines IL-4, IL-10 and the Th3 cytokine TGF-beta before, during and after the administration of each drug. The percentage of CD3+ cells producing intracytoplasmic cytokines were determined by flow cytometry analysis before and after 6 hours mitogen stimulation (PMA + ionomycin).
After performing analysis of variance we observed that Tac and Rap differ in their effect on mitogen stimulated TGFb (2.59±0.59; vs. 15.51±11.13; p=0.04) and spontaneous TNFa (0.57±0.07; 1.49±0.60; p=0.02) production. Spontaneous IL-10 production was significantly higher in patients treated with CsA (2.17±0.40) when compared to Tac (1.19±0.21; p= 0.02), Rap (1.29±0.33; p=0.47) and MMF (0.79±0.34; p=0.003). TNFa production on MMF (23.12± 3.13; p=0.0002) was higher compared to CsA (7.95±1.39; p=0.0002) and on Tac (14.18±1.94; p=0.021).



These results suggest that immunosuppressive drugs differ in their effect on T-cell cytokine production and may differentially polarise the immune response. This might contribute to understanding of side effects such as nephrotoxicity, which in the long term is partly related to the up-regulation of IL-10. Of interest, although there is evidence that sirolimus reduces fibrosis, it was associated with increased production of TGF-beta – this observation requires further investigation.

M697 EXPRESSION OF THE GRANZYME B INHIBITOR PI-9 IN HUMAN RENAL ALLOGRAFTS EXPLAINS STABLE RENAL FUNCTION IN SPITE OF CYTOTOXIC INFILTRATES

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Granzyme B-positive T-lymphocytes infiltrate renal allografts during acute cellular rejection and cause graft injury by inducing apoptosis of tubular cells. We hypothesized that expression of Protease Inhibitor 9 (PI-9), an intracellular serpin that inhibits granzyme B, protects renal tubular cells from cytotoxicity by granzyme B-positive T-lymphocytes. Hence, we compared the expression of granzyme B and PI-9 in renal transplant biopsy specimens from patients with acute cellular rejection or with subclinical rejection.
Renal allograft biopsy specimens were obtained from patients with a subclinical rejection, i.e., with a mononuclear cell infiltrate in the graft without deterioration of renal function (n=9), acute rejection type I (n=6), acute rejection type II (n=3), acute rejection type III (n=6), or no rejection (n=9). Granzyme B and PI-9 expression was assessed with monoclonal antibodies (Ab), and scored as 0-4.
Granzyme B was expressed by infiltrating mononuclear cells in all biopsies with cellular infiltrates. PI-9 was expressed by renal tubular cells in all patients with subclinical rejection; in most patients with acute rejection, although more focally and at low level; and in some patients without rejection. To estimate the balance between granzyme B and PI-9 expression, for each biopsy PI-9 score for tubuli was subtracted from that for granzyme B in inflammatory cells. This resulted in a median calculated score of 0.0 (range: -1.0-0.0) for the subclinical rejection group; 1.0 (range: 0.0-4.0) for the acute rejection group; and -0.5 (range: -1.0-1.0) for the group with stable function ($p< 0.01$).

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These data support the idea that PI-9 expression in renal tubular epithelial cells protects human renal allografts from rejection in spite of inflammatory cell infiltrates.

M698 SELECTIVE INHIBITION OF JUN N-TERMINAL KINASE (JNK) IN ALLOREACTIVE HUMAN T CELLS IS PRIMARILY IMMUNOSUPPRESSIVE, BUT ENHANCES ACTIVATION-INDUCED CELL DEATH IN CONJUNCTION WITH mTOR TARGETING

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Jun N-terminal kinase (JNK), a member of the mitogen-activated protein kinase (MAPK), is a serine threonine kinase that phosphorylates c-Jun, a component of the transcription factor AP-1, which regulates the transcription of numerous genes including IFN-g, IL-2, TNF-a, immunoglobulins, etc. In this study we evaluated the consequences of selective JNK-targeting by means of the recently developed JNK inhibitor SP600125. We show that JNK inhibition results in downregulation of CD3/28 as well as alloantigen-driven T cell proliferation (MLC). Likewise, surface marker expression (CD69, CD25, CD71) and cytokine production (IL-2, IFN-g) were profoundly blunted. Interestingly, no suppressive effect was observed in T cells treated with phorbol esters and calcium ionophors indicating inhibition of T cell activation by JNK targeting only at low-intermediate T cell activation strength. To elucidate the functional consequences of JNK ablation MLC analysis was done with/without calcineurin and mTOR inhibitors. While Cyclosporine led to a synergistic downregulation of the proliferative response in allogeneic T cells, secondary responses where only affected when T cell activation was performed in conjunction with rapamycin. The underlying basis of this phenomenon was a profound induction of activation-induced cell death by JNK plus mTOR inhibition. Collectively, these data indicate a new and promising strategy to eradicate alloreactive T cells by selective MAPK-targeting together with mTOR inhibition, which could be of particular benefit in clinical transplantation.

M699 ❋ DELIVERY OF NFκB DECOY OLIGONUCLEOTIDES USING ULTRASOUND WITH MICROBUBBLES INTO TRANSPLANTED RAT KIDNEY

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Nuclear factor-κB (NFκB) plays a pivotal role in the coordinated transactivation of series of cytokine and adhesion molecule genes that are highly involved in the onset of acute rejection of transplantation. We hypothesized that synthetic double-stranded oligonucleotides (ODN) with high affinity for NFκB introduced in vivo as decoy cis elements bind to the transcription factor and block the activation of genes mediating acute allogenic response, thus providing the effective therapy for renal acute rejection. To transfect ODN into the kidney, we employed a novel non-viral vector system using ultrasound irradiation with microbubbles, as safety issue using viral vector systems remains in relation to human gene therapy despite of high transfection efficinency. FITC-labeled decoy ODN could be successfully transfected into rat kidney using ultrasound with microbubbles (an contrast agent (Optison)) in the tubular cells and glomeruli, while ultrasound alone or decoy ODN alone demonstrated little fluorescence. Donar kidney transfected with NFκB decoy ODN orthotopically transplanted into bilaterally nephrectomized Lewis recipients. In the control group, the marked destruction of renal tissue with the mononuclear cell infiltration occurred at day 4, accompanied by the increased cytokine production and adhesion molecule expression in the grafted kidneys. All animals died of renal failure by 10 days after operation. In contrast, the recipients of donar kidneys transfected with NFκB decoy ODN exhibited a significant prolongation in the death rate as compared to other groups ($p<0.01$). Especially, graft function as well as histological appearance was well reserved in grafted kidney transfected with NFκB decoy ODN exhibited a significant prolongation in the death rate as compared to other groups. Especially, graft function as well as histological appearance was well preserved in grafted kidney transfected with NFκB decoy ODN, accompanied by the decrease in the expression of inflammatory cytokines such as IL-1, MCP-1 and TNF-α, and an adhesion molecule such as ICAM-1. Overall, a novel gene trasfer method using ultrasound with microbubbles remarkably enhanced thetransfection efficiency of NFκB decoy ODN into the rat kidney grafts. Using this new gene transfer method, transfection of NFκB decoy ODN significantly improved the survival rate of transplanted kidney associated with the inhibition of cytokine and adhesion molecule expressions in the kidney. This new approach may provide a novel therapeutic strategy for renal allograft.

M700 DEVELOPMENT OF A NOVEL GENE TRANSFER METHOD ITNO RAT KIDNEY MEDIATED BY ULTRASOUND WITH MICROBUBBLES

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For the clinical gene therapy, the gene transfer method is often one of the limiting steps for sucessful trials. Successful gene transfer techniques that target the kidney can provide a powerful and attractive tool for revealing the mechanism of renal disease and may be applicable to a therapeutic intervention to renal diseases. Recently, it has been reported that ultrasound irradiation can enhance gene expression in vitro vascular cells. In this study we tried to establish a novel gene transfer method into rat kidney by ultrasound with microbubbles. A catheter was inserted into the aorta from the left femoral artery. Then, mixture of luciferase or GFP plasmid, and microbubbles was injected into the left kidney. First of all, we examined the effect of the duration of ultrasound irradiation on luciferase gene expression in the transfected kidney. Then, the dose-dependent effect of microbubbles at different concentrations (0, 5, 10, 25, 50 and 100% of microbubbles) was examined. The localization of luciferase and GFP gene expression was also examined. Finaly, side effects of luciferase gene transfer were examined by measuring blood parameters and histology in HE-stained sections. Ultrasound with microbubbles significantly enhanced luciferase gene expression in the kidney on days 1, 3, 7, 14 and 21 after gene transfer ($p<0.01$). A significant dose-dependent effect of microbubbles on gene expression was also observed on day 4 ($p<0.01$). Moreover, we detected luciferase gene expression in glomerui, tubules and interstitial tissues of the transfected kidney on day 7 after gene transfer. This result was also supported by GFP plasmid transfer. Similarly, GFP gene expression was detected in glomeruli, tubules and interstitial tissues. On the other hand, there was no significant changes in blood tests and no damage was observed in HE-stained kidney sections due to gene transfer mediated by ultrasound with microbubbles. Of course there was no significant damages in other organs and no expression was observed in other organs such as the lung, the heart, the liver and the spleen. Taking together, this novel gene transfer method into the kidney mediated by ultrasound with microbubbles enhanced transfection efficiency into the kidney with no apparent side effects. This novel gene transfer method may be useful in clinical gene therapy for renal diseases.

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M701 MONITORING OF NFAT REGULATED GENE EXPRESSION THE PERIPHERAL BLOOD OF KIDNEY- AND LIVER ALLOGRAFT RECIPIENTS – A NEW PERSPECTIVE OF INDIVIDUALIZED IMMUNOSUPPRESSION

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With the introduction of Cyclosporin A (CsA) longterm allograft function has significantly improved. The problems of limited therapeutic margins and the toxicity of CsA remain unsolved. Up to now there is no reliable marker to measure the biological activity (intensity of immunosuppression) of CsA in vivo.
Employing quantitative real-time RT-PCR we studied the CsA induced inhibition of NFAT-regulated genes (IL-2, IFN-gamma, GM-CSF) in PMA/Ionomycin stimulated circulating lymphocytes in healthy controls and renal-or liver transplanted patients pre-dose and 2 hours after oral uptake of CsA. In 7 patients a complete pharmacokinetic profile (12h) was assessed. Renal- (n=29) and liver allograft recipients (n=14) were studied, baseline data are summarized in Table 1.

Table1

	Renal allograft recipients	Liver allograft recipients
Median time of follow-up (mths)	79; 65-198	108; 24-180
Predose CsA (mg/l)	108; 65-198	93; 34-169
2h CsA (mg/l)	667; 242-1098	542; 168-1044
CsA dosage (mg/kg bw)	1.85; 0.9-3.8	2; 1.2-2.8
Methylprednisolon (mg)	2; 1-4	prior steroid withdrawal
C-reactive protein (mg/dl)	<3.4	<3.4

In renal transplant patients stimulation of lymphocytes from CsA treated patients showed a 70% reduction of IL-2 gene expression at pre-dose as compared to lymphocytes of healthy controls. IL-2 gene expression decreased by a factor of 4 to 280, 2 hours after oral CsA uptake. Individual IL-2 gene expression and the 2 hour CsA levels were in a close relation (r^2=0.42). Six hours after oral CsA uptake IL-2 gene expression reached the same values as pre-dose IL-2 expression values and progressed to an even further increase prior to the next oral CsA dose (rebound). In all patients intra-individual measurements (at different days) showed similar results. In CsA alone (steroid withdrawal) treated liver allograft recipients pre-dose IL-2 gene expression was comparable to healthy controls. The correlation of 2h CsA level and the inhibition of IL-2 gene expression was even stronger (r^2= 0.76) compared to renal allograft recipients. Similar results were found for interferon-gamma and GMCSF.
The quantitative assessment of IL-2, IFN-gamma, GM-CSF gene expression in CsA treated patients is a new method to determine precisely the biological activity of CsA based immunosuppression. The present method allows an adaptation to individual immunosuppressive intensity, which will guide to an optimal immunosuppression with reduced toxicity.

M702 ♦ EPITHELIAL CHIMERISM IS AN EARLY AND PERSISTENT EVENT AFTER RENAL TRANSPLANTATION

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Recent studies demonstrated a migration of donor derived stem cells into solid organ allografts with differentiation into organ specific and locally functional cells, resulting in an intragraft microchimerism. Whether or to which extent this intriguing finding is influencing function and survival of the allograft has still to be elucidated.
To detect epithelial chimerism in renal transplants from 36 patients epithelial tubular cells were harvested from formalin-fixed and paraffin embedded sequential allograft biopsies (n=72) by Laser-Microdissection. To prevent contamination by donor lymphocytes a preceding immunohistochemical stain for CD45 was done. DNA was isolated following standard protocols and PCR assay was performed for analysis of highly polymorphic short tandem repeat marker at the human β-actin-related pseudogen (SE33, heterozygosity 93%). In cases with sex-mismatched (donor female to male recipient) transplantation chimerism was detected by in situ hybridization for the Y-chromosome, too. Findings in epithelial chimerism were correlated to morphological diagnosis in allograft biopsies and to clinical course of the patients.
Epithelial chimerism was detectable as early as 8 days after transplantation and lasted for nearly 8 years in our collective. 91,6% (33/36) of our patients showed an epithelial chimerism after renal transplantation. 25 from 36 (69%) patients had a stable chimerism in sequential biopsies (time between biopsies: 3-96 months; mean 16,6 months). 4 patients had no epithelial chimerism in two sequential biopsies. 3 patients showed a late onset of chimerism. In one patient a loss of chimerical epithelial cells was found. 3 pre-implant biopsies served as controls and showed no chimerism. In 3 patients due to technical reasons only one biopsies could be evaluated. Semiquantitative evaluation of Y-chromosome in situ-hybridization (8 patients) revealed low percentages of chimerical tubular epithelial cells (<5%). No correlation to morphological changes was found. Chimerism was detectable in inconspicuous protocol biopsies, cases with drug toxicity, as well as in rejecting allografts with and without chronic changes in their course. No correlation was found to graft function.
In summery, in our patients epithelial microchimerism is an early and persistent event after renal transplantation. Low percentages of donor derived epithelial cells were detectable showing no correlation to morphology or function of the allograft.

M703 ANTI- HLA ANTIBODIES PRODUCTION AND NITRIC OXIDE LEVEL IN THE SERUM AND URINE OF POST TRANSPLANT PATIENTS IN RELATION TO GRAFT FUNCTION AND SURVIVAL

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The purpose of this work was to study the production of HLA-Abs and nitric oxide level in serum and urine of post transplant patients, and their correlation to incidence of rejection, renal allograft function and survival. Twenty patients with renal transplantation for the first time (group I) and twenty healthy control subjects (groupII) were included in the study. Anti HLA –Abs class I and class II were detected by enzyme-linked immunosobant assay using Tarasaki trays (ELIZA), serum and urinary nitrate and nitrite and urinary N acetyl-B-D-glucosaminidase (NAG) were measured. Using duplex Doppler ultrasonography, renal hemodynamics and the renal vascular resistance were studied by calculating the resistive and pulsatility indices (RI and PI). All patients before transplantation had a negative cross match and were compatible. After transplantation anti HLA-Abs class I were detected in 7 patients (35%) and class II in 3 patients (15%), all the control subjects were negative for both anti HLA-Abs Class I and class II. Three patients (43%) with positive anti HLA-Abs class I and all the three patients (100%) with positive anti HLA-Abs class II had acute rejection episodes, but none of the negative anti HLA-ABs classI and classII. In renal allograft recipients there was a significant increase of serum and urinary nitrite than the controls and an insignificant difference as regards serum and urinary nitrate, urinary NAG, RI and PI. Patients with rejection episodes had a significant increase in serum and urinary nitrate, serum nitrite, RI and PI and a significant decrease in urinary NAG than transplant patients without rejection. The RI and PI returns to normal after treatment with anti-rejection therapy. In renal transplant patients the urinary nitrate was positively correlated with serum creatinine, serum nitrate, serum nitrite, RI and PI ($p<0.05$). Urinary NAG was negatively correlated with serum creatinine, serum and urinary nitrate, RI and PI ($p<0.05$). The RI and PI were positively correlated with serum creatinine and serum nitrite ($p<0.05$). The presence of anti HLA-Abs of either class I or class II were correlated with the occurrence of graft rejection (Fischer's exact test 0.031, <0.001 respectively). From this study it can be concluded that anti HLA-Abs can be de novo appear in the post transplant period and can

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lead to acute or chronic rejection. The detection of these antibodies in the serum of post transplant patients, allows identification of high risk patients with poor outcome, which could provide guidance for immunosuppressive therapy. The increase of urinary nitrate and the decrease in urinary NAG can be a marker for detection of graft rejection episodes and differentiate it from other causes of graft dysfunction. Also, the increase in serum nitrate and nitrite during rejection episodes could be a compensatory mechanism for the renal hemodynamic changes with the increase of intrarenal vascular resistance and renal vasoconstriction.

M704 INTERFERON-ALPHA IS A POWERFUL PREDICTOR OF ACUTE REJECTION IN KIDNEY TRANSPLANTS

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Previously we described a significant association between acute rejection of kidney transplants (KTX) and IL-18 synthesis by cultures of fine-needle aspiration biopsy (FNAB) samples. While IL-18 on its own does not seem to be able to initiate an alloimmune response, IL-18 in conjunction with IFN-a may play an important role in T helper 1 deviation, substituting for IL-12. We analysed IFN-a synthesis by FNAB sample cultures in KTX.
Fifty KTX, were treated CsA-AZA/MMF-Pred and divided into I (acute rejection, n=16) and II (stable KTX, no rejection during the first year, at least, n=34). FNAB were done on the first day of acute rejection in I and on day 7 post-KTX in II. Every episode of rejection was confirmed by a classical biopsy and they occurred during the first two weeks post-surgery. FNAB samples were diluted into a $5'10^4$cells/200ml concentration and incubated for 48h with 10U/ml of rIL-2 (Boheringer) and 10% of autologous serum. At the end, culture supernatants were collected and kept at -70°C. IFN-a was measured by ELISA from R&D. Statistics by Kruskal-Wallis ANOVA. Every patient gave his informed consent.
We did not find any significant difference on comparing the demographic data of donor-recipient pairs from I versus II, neither blood CsA levels were different. Among I, we measured 212.3±216.6 pg/ml (lower quartile 105 and upper 178); among II, we measured 75.1±14.4 pg/ml (lower quartile 62.4 and quartile 87); I versus II, $P<0.0001$. Empirically we set as cut-off a value of IFN-a = 100 pg/ml, resulting in a sensitivity 76.9%, specificity 100%, positive predictive value for rejection 100% and negative predictive value 94.1%.
Our study shows that IFN-a synthesis by cultures of FNAB samples rises significantly with acute rejection in KTX. These results, together with those we found for IL-18 production by FNAB cultures (Nephron 92:622-628, 2002), surmise that the combined effects of IFN-a with IL-18 may constitute one of the main determinants for T helper 1 deviation substituting for IL-12, which we did not find to be different when comparing stable versus acute rejection (Nephron, 91:637-645, 2002).

M705 NATURAL KILLER CELL ACTIVITY AFTER HUMAN RENAL TRANSPLANTATION IN RELATION TO KIR AND HLA MISMATCH

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Natural Killer cells are emerging as an important component in the rejection process. They use their inhibitory receptors such as Killer Ig-like receptors (KIR) which bind to self class I MHC molecules to prevent killing of autologous cells. Mismatched allografts not expressing self MHC can therefore be potential targets for NK cell killing. The genes for these KIRs are on chromosome 19 and therefore inherited independently of HLA (chromosome 6). In our living related/unrelated renal transplantation programme, donor recipient pairs can share some of the HLA as well as KIR genes. We hypothesise that NK cells are activated following renal transplantation and that recipients expressing the appropriate KIR genes to inhibit killing of donor cells will show lower anti-donor NK cytotoxicity. NK cells were purified from recipient peripheral blood mononuclear cells (PBMC) by negative magnetic beads sort and used in a 4hr cytotoxicity assay against donor PBMC at different effector:target (E:T) cell ratios 2 days before, on the day of and 3 days after transplantation. HLA and KIR typing were performed by PCR. Mean NK cell cytotoxicity against donor cells was 13.7% (E:T ratio 25:1) before transplant and was unaltered on the day of transplant after the recipient had been loaded with cyclosporin for 2 days (13% n=21). However, it rose significantly in all but 6 recipents 3 days after transplantation (mean cytotoxicity for all 21 patients=21%, $p<0.04$). Recipient KIR genotyping was performed and correlated to donor HLA based on our current knowledge of HLA binding to specific KIRs. The 15/21 recipients exhibiting increased NK cytotoxicity against donor after transplantation were found to express fewer number of inhibitory NK receptors but more stimulatory receptors that recognise donor class I MHC molecules when compared with those who did not demonstrate an increase. Amongst the 15 recipients who showed a rise in anti-donor cytotoxicity, 10 of them expressed at least one activating KIR that binds to donor HLA (Fisher's exact test $p<0.04$). NK cells are activated after transplantation despite quadruple immunosuppression, suggesting that recipient NK cell cytotoxicity against donor may be a prevously unnoticed area of the rejection process:-the unrecognised enemy, especially in poorly matched donor/recipient pairs where the recipient may not express the correct repertoire of inhibitory receptors to prevent killing of donor cells.

M706 EFFECTS OF EXOGENOUS OR INTRINSIC HEME OXYGENASE-1 (HO-1) ON APOPTOSIS, CASPASE-3 EXPRESSION AND DIFFERENTIATION OF MONOCYTES: IMPLICATIONS FOR ACUTE REJECTION OUTCOME

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Heme oxygenase-1 (HO-1) is involved in the oxidative stress response catalyzing the initial and rate-limiting step in the oxidative degradation of heme to biliverdin, iron and carbon monoxide. HO-1 has protective effects e.g. in ischemic or inflammatory diseases and acts as an antagonist of apoptosis. Therefore we investigated mechanisms of signaling used by HO-1 in regulation of monocyte apoptosis in-vitro and its implications for acute rejection in an animal renal transplantation model in-vivo.
Freshly isolated human monocytes were stimulated by LPS or hemin, an inducer of HO-1. HO-1 was measured at mRNA level by semiquantitative RT-PCR and at protein level by flow-cytometry. Apoptosis was determined by flow cytometry, DNA-electrophoresis and TEM.
LPS and exogenous hemin induced HO-1 mRNA in human monocytes in a dose- and time-dependent manner. Monocytes undergoing apoptosis induced by serum starvation were protected from apoptosis after induction of HO-1 by LPS or hemin. Interestingly, also hypoxia as a pathophysiological condition acting during transplantation up-regulated monocyte HO-1. Although apoptosis was prevented by HO-1 this effect was paralleled by the induction of caspase-3, a main effector of apoptosis. Proving this observation protection from apoptosis was increased by additionally using an inhibitor of caspase-3. After longer culture periods HO-1 led to differentiation of monocytes into macrophages and up-regulation of the costimulatory molecule CD86 (B7-2).
For studying the clinical significance we transplanted LBN allografts into Lewis rats which developed severe acute rejection within four days. Induction of HO-1 within renal grafts was achieved by injecting hemin into LBN donor rats i.p. without improving the grade of rejection. This could be explained by the increased influx and survival of invading leukocytes, further monocytic differentiation and CD86-dependent allogenic immune activation.
This study shows that HO-1 inducable by hypoxia during graft harvesting or transplantation is an important effector of survival, differentiation and costimulation of monocytes.

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M707 CHANGES OF THE URETER MICROCIRCULATION PATTERNS DURING NEOIMPLANTATION TECHNIQUES IN ANIMAL MODEL

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Various types of ureter neoimplantation methods can be used in diseases that affect the distal part of the ureter. The most common techniques are the Cohen and the Politano. When the ureter is dilatated tapering of the enlarged ureter is necessary during neoimplantation using excisional or plication techniques. The aim of the study was to compare the effect of these different methods on the ureter tissue microcirculation in animal model and to answer the question which technique has better outcome.
The authors investigated tissue microcirculation patterns of ureter segments under surgical conditions employing Laser Doppler flowmetry in dogs. Different surgical procedures were compared in the function of tissue microcirculation as measured in the affected ureter segments. Measurements both in the intra-and postoperative periods were performed to compare different methods of ureter neoimplantation (Cochen vs. Politano-Leadbetter) and tapering (excisional technique, i.e. tailoring vs. plication of the lower ureter). The status of the ureter microcirculation was compared before and after catheterisation. 9 cases of Politano-Leadbetter and 9 cases of Cohen procedure as well as ureter tailoring in 7 and plication in 6 cases were performed in mongrel dogs. The microcirculation patterns of the ureter were detected intraoperatively and postoperatively using Laser Doppler Flowmetry device. The influence of catheter insertion on ureter microcirculation was examined in each case.
Among the neoimplantation methods the Cohen procedure whereas among the tapering procedures the plication of the ureter were found to affect the least the microcirculation of the ureter tissue. The catheter insertion had significantly worsened the blood supply.
Based on these results better outcome can be expected with the Cohen neoimplantation method and plication of ureter as compared with others.

M708 MAP KINASE SIGNALLING AFTER RENAL TRANSPLANTATION AND REJECTION IN RATS

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After renal transplantation, the expression of multiple extracellular signals which stimulate MAP kinase cascades in vitro is increased acutely and with chronic rejection. Whether or not the different MAP kinases are differentially regulated in vivo after renal transplantation has not been studied yet. We set out to elucidate whether or not interval since transplantation and type of rejection are relevant for expression of MAP kinases.
Two rat rejection models were used to investigate the regulation of MAP kinases after renal transplantation and rejection. Acute rejection was provoked by transplanting LBN kidneys into Lewis rats while chronic rejection was induced by grafting Lewis rats with a kidney from Fischer rats. Former were removed 1, 2, 4, and 5 days (acute), latter 10 weeks after transplantation (chronic). Semiquantitative PCR and western blotting were used to investigate expression of the ERK. GAPDH served as reference signal.
With acute rejection, mRNA expression was drastically enhanced for ERK1 and MEK1on day 1 and for SAPK on day 5. Regarding protein expression, MEK1 and SAPK, were increasingly upregulated with acute rejection. The phosphorylated state of MEK1 protein was enhanced from day 1 to day 5 after transplantation in comparison to the non-transplanted controls and that of p38 was increased from day 2 to day 5. The phosphorylated states of ERK2 and SAPK were not altered. In the chronic rejection model, expression and phosphorylation of the MAP kinases studied were not significantly altered.
After renal transplantation, mRNA and protein expression as well as phosphorylation of mitogen-activated protein kinases are differentially affected. The respective changes are dependend on type of rejection (acute or chronic) and interval since transplantation.

M709 COMPARISON OF GENE EXPRESSION PROFILES BETWEEN SYNGENEIC AND ALLOGENEIC TRANSPLANTS

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This study compares profiles and kinetics in the expression of genes in two models of murine, heterotopic, cardiac transplantation.
cDNA transcripts of 80 different genes were measured using real-time PCR technology in syngeneic (B6 donor, B6 recipient) and allogeneic (BALB/c donor, B6 recipient) heart grafts at 15 time-points during the first 7 post-transplant days (first day intervals of 3 hrs, then of 24 hrs). At each time-point 3 BALB/c and 3 B6 donor hearts were analyzed, 6 untransplanted hearts served as controls. GAPDH was used as an endogeneous reference. The target genes were selected from results of microarray studies and covered the following classes: complement components (n=13), acute phase proteins (n=10), cytokines (n=11), effector molecules (n=5), Toll-like receptors (n=9), metabolic markers (n=13), cytoskeletal genes (n=3), stress markers (n=3), pteridines (n=8), and MHC-antigens (n=4). Hierarchical clustering methods were used for the data analysis.
1. The baseline gene expression in the control hearts differed significantly between the functional classes. High mRNA levels were measured for stress, cytoskeletal, and metabolic genes, low levels for cytokine, effector, acute phase, and pteridine genes (mean expr. of 40% vs. 0.1% rel. to GAPDH, resp.). 2. Genes with low control levels showed characteristically a high up-regulation during the post-transplant course (mean fold-change of 8.6), whereas genes with high expression changed in the average only 1.6-fold and frequently displayed a down-modulation. 3. Syngeneic and allogeneic heart tissues showed a strong innate immune response during the first three post-operative days. 4. From day 4 onwards inflammatory responses were seen almost exclusively in the allogeneic model, comprising genes of innate and adaptive immunity. 5. Genes with highest up-regulation (> 50-fold) were the acute phase reactant SAA-3, the cytokine IL-6, the effector molecule granzyme B, and the MHC-antigen I-A-b. 6. Cytokine and stress genes showed the earliest up-regulation (already after 3 hrs) followed by acute phase and complement genes. 7. Pro-inflammatory cytokine genes showed a biphasic response, peaking at 3 to 6 hrs and at d 6 and 7 in the allogeneic transplant model. 8. Metabolic genes were those most significantly down-regulated. 46% of these genes showed a more than 5-fold down-modulation, starting within the first 24 hrs and being most pronounced during the late phase of the alloimmune response.
Genes with a high baseline expression show smaller changes in up-regulation than those with low expression in the control tissue. Daily measurements would miss the early dynamics of expression in cytokine and stress genes. The overall response shows similar gene profiles in syngeneic and allogeneic transplants during the early post-transplant course. A distinct alloimmune response is detectable from day 4 onwards, characterized by a shift from genes associated with metabolism, cell structure, and stress towards genes of the innate and adaptive immune response.

M710 ADOPTIVE TRANSFER OF ALLOSPECIFIC Th2 CELLS RESULTS IN TOLERANCE AFTER FULLY ALLOGENEIC KIDNEY TRANSPLANTATION AND INVOLVES SPECIFIC EXPRESSION OF T CELL RELATED ANTIGENS

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We recently reported the in vivo functional/regulatory capacities of Th1/Th2 clones generated from allografts of rejecting (Th1 type) and tolerant (Th2 type) animals using donor-derived immunodominant class II MHC allopeptide (RT1.D$^{u\beta}$ 20-44). Injection of Th1 cell clones accelerated rejection while Th2 clones induced specific tolerance (WF→LEW recipients). In this study we analyzed the expression of T cell related antigens in Th1/Th2 cell clones treated animals. The animals were divided into: Gp

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1, controls with short-term cyclosporine (CsA, 1mg/kg day 0-3, 5mg/kg day 4-7) therapy resulting in prolongation of graft survival (121.7±34.6 days). Gp 2, animals with adoptive transfer of Th2 cell clones ($30x10^6$ i.p., day 0) showing prevention of allograft rejection (>250 days, tolerance proven by acceptance of donor specific skin). Gp 3, animals injected with Th1 clones chronically rejecting their allografts in an accelerated manner (65.0±20.4 days). Allografts were harvested at 250 days or endstage graft failure. Antigen expression was demonstrated by FACS or immunohistology. Enrichment of regulatory T cells (FACS) producing Th2 type cytokines (ELISPOT) was observed in tolerant animals (Gp 2). Immunohistological analysis showed intragraft staining for the transcription factor GATA-3, important in Th2 cytokine gene expression and/or Th2 differentiation, and the Th2 marker, T1/ST2 in Gp 2 allografts (not seen in Gp 1 and 3). Double immunostaining of molecules important in T cell activation was as follows:

Combinations	WF→LEW+CsA (Gp 1)	WF→LEW+CsA+Th2 cell clones (Gp 2)	WF→LEW+CsA+Th1 cell clones (Gp 3)
α/βTCR/class II MHC	++ *	+	++ *
ICOS/CD4	++ *	+	++ *
ICOS/CD8	+	(+)	+
CD28/B7-1	+ *	(+)	+ *
CD28/B7-2	+ *	(+)	+ *
CTLA4/B7-1	+	(+)	++
CTLA4/B7-2	+	+	++
CD4/CD25	0	+	(+)
CD4/CD45RC	+ *	(+)	++ *
CD8/CD45RC	(+) *	0 - (+)	+ *
CD40/CD40L	+	0 - (+)	+

(+) sporadic; + mild; ++ moderate; * ubiquitous

Our data demonstrates the pattern of expression of a variety of molecules important in T cell activation and regulation in transplanted animals injected with allospecific Th1 or Th2 cell clones, and indicates that most of these molecules are downregulated in the Th2 clones treated animals with the exception of GATA-3, T1/ST1 and CD25+CD4+ T cells.

M711 DELETION OF PROTEIN KINASE C EPSILON IN KIDNEY TRANSPLANTS IMPROVES ALLOGENIC TRANSPLANT SURVIVAL

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Protein Kinase C (PKC) contributes to vascular reactivity in response to hypoxia and is involved in different signaling pathways which are relevant for ischemia reperfusion injury and transplant rejection. The isoform PKC epsilon regulates various physiological functions including the activation of inflammatory and immunological reactions. The role of PKC isoenzymes in acute kidney transplant rejection has not been studied so far.

Allogenic kidney transplantation was performed after removal of the right native kidney. Donor kidneys from 12-16 weeks old PKC epsilon knock out (-/-) donor mice (mixed background: C57Bl6/SV129) were transplanted into BalbC recipients. The contralateral kidney was removed at day 4. Donor kidneys from littermates (C57Bl6/SV129) served as wild type (WT) controls. Animals were sacrificed 24 hours and 6, 14, 21 and 28 days after transplantation. Plasma creatinine was measured spectrophotometrically. Kidneys were examined by western blot analysis and by immunohistology for infiltrating cells, adhesion molecules and MHC expression.

PKC epsilon -/- mice displayed normal renal function and histology. Renal function and pathology deteriorated rapidly in rejecting kidneys from WT but surprisingly not from PKC epsilon -/- donors. Recipients from WT donors died within 7-10 days after transplantation, whereas recipients from PKC epsilon -/- mice survived 4 weeks until the end of the planned observation period. Recipients from PKC epsilon -/- mice had only 2 fold creatinine elevation 6 days after transplantation and kidney function remained almost stable within the next 4 weeks. WT recipients suffered from severe loss of kidney function with 5 fold creatinine elevation 6 days after transplantation. The number of infiltrating monocytes and macrophages in recipients from PKC epsilon -/- donors was reduced compared to WT donors. The number of CD8 T-cells in recipients from PKC epsilon -/ donors was higher than in the control group, whereas CD4 infiltration was slightly reduced. ERK 1/2 activation was comparable in both groups.

Our results indicate that PKC epsilon deletion in donor kidneys protects renal allografts from severe rejection and loss of renal function.

M712 CLASS II MHC SPECIFIC ALLOPEPTIDES MODULATE THE IMMUNE RESPONSE AFTER EXPERIMENTAL KIDNEY AND SMALL BOWEL TRANSPLANTATION VIA THE INDIRECT PATHWAY OF ALLORECOGNITION

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T cells recognize intact allo-MHC molecules on the surface of donor cells via the direct pathway or as processed allopeptides on self-APCs via the indirect pathway of allorecognition. There is evidence that indirect allorecognition plays an important role in allograft rejection. We studied the functional role of class II MHC-specific allorecognition after orthotopic small bowel (SBTx) or kidney transplantation (KTx) in the WF→LEW ($RT1^u$,$RT1^l$) rat combination. LEW recipients were primed on day -7 by subcutaneous footpad immunization with a synthetic class II MHC peptide derived from the $RT1.D^u$(WF)β chain (residues 20-44), (group 1) or with control peptide $RT1.B^{u\beta}$(20-44), (gp 2). The animals were transplanted with either SB- or kidney allografts and injected intraperitoneally with $RT1.D^{u\beta}$(20-44) (gp 1) or $RT1.B^{u\beta}$(20-44) (gp 2) allopeptide (200mg/kg day 0) (n=8/gp). Gp 3 and 4 recipients with SB- or kidney grafts were treated with cyclosporine A (CsA, SBTx: 20mg/kg from day 0-13, KTx: 5mg/day 0-4) and challenged with $RT1.D^{u\beta}$(20-44), (gp 3) or $RT1.B^{u\beta}$(20-44), (gp 4) peptide on days 20 and 27 after Tx or left without immunization (gp 5) or without CsA and immunization (gp 6). Animals were sacrificed during endstage graft failure. Proliferation assays were performed with splenic and lymph node (LN) lymphocytes and representative cytokines were analyzed from supernatants by ELISA. Cytokine expression in SB- and kidney allografts was determined by RNase protection assay. The graft survival is shown below:

groups	peptide application	CsA	KTx (days)	SBTx (days)
1	RT1.Du (20-44), d -7, 0	no	4	3.4
2	RT1Bu (20-44), d -7, 0	no	5.6	4
3	RT1.Du (20-44), d 20, 27	yes	37±3.6	33.7±4.1
4	RT1.Bu (20-44), d 20, 27	yes	49.5±11.2	44±14.2, one >100
5	no	yes	51.2±9.2	>250
6	no	no	7.5	5.3

Gp 5 SBTx controls showed a mean survival of >250 days (no signs of chronic rejection, secondary transplanted WF heart and skin were specifically accepted) whereas gp 3 and 4 animals rejected their grafts acutely. Splenic/LN lymphocytes (gp 1 and 3) demonstrated significant reactivity to the specific allopeptide. Rejected allografts showed upregulation of IL-2, -4, -6, IFN-γ, TNF-α/β (gp 1 and 3), whereas gp 4 and 5 animals expressed significantly higher levels of IL-4. This data demonstrates that T cells primed in vivo via the indirect pathway can mediate a specific immune response independent of the type of transplanted organ and point out the importance on the early ongoing of this process. This may therefore have implications in terms of novel therapeutic strategies especially after SBTx.

M713 EVIDENCE FOR THE EARLY INVOLVEMENT OF INTERLEUKIN 17 IN HUMAN AND EXPERIMENTAL RENAL ALLOGRAFT REJECTION

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Inflammatory processes can stimulate renal cells to release, chemoattractants and matrix proteins into the interstitium, thus contributing to intestitial injury during acute allograft rejection. To test the role of interleukin 17 (IL-17) in this process, cultured human renal epithelial cells (hRECs)

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Abstract M715 – Table

	p16INK4a mRNA [units]	p16INK4a in tubules [% of nuclei]	p16INK4a in glomeruli [% of nuclei]	p16INK4a in interstitium [% of nuclei]
normal young (3 mo)	6.5 ± 3.0	8.4 ± 6.6	2.8 ± 1.6	2.1 ± 0.5
tx young (3 mo)	18.1 ± 10.8†	23.9 ± 4.3†	24.2 ± 9.6†	17.3 ± 5.0†
normal old (18 mo)	82.0 ± 35.9*	44.3 ± 7.1*	9.1 ± 7.0	7.3 ± 2.7*
tx old (18 mo)	148.2 ± 96.9**	56.5 ± 8.4	36.3 ± 5.5‡	18.8 ± 6.0

* <0.05 vs. normal young; **<0.05 vs. all groups; †<0.05 vs. normal young; ‡<0.05 vs. normal old

were first established and treated with or without human IL-17 (hIL-17) for 2,4,8 and 10h in vitro. Significant elevations of IL-6 and IL-8 levels were noted in the supernatants in a dose-dependent and time-dependent manner, as also IL-6 mRNA expression. Secondly, using a rat acute allograft rejection model, the correlation between IL-17 expression and histopathological changes was serially studied. The results demonstrated that increased expression of IL-17 protein on infiltrating mononuclear cells (MNCs) was detectable on day 2. This corresponds to the borderline change of acute rejection according to the Banff classification, and it increased progressively to day 5. Serial study of IL-6, IL-8 and IL-17 mRNA expression of the renal allograft confirmed IL-17 mRNA expression in the allograft early on post-transplant day 2, whereas IL-6 and IL-8 expression started on day 3. Thirdly, IL-17 expression was observed in human renal allograft and urinary sediment. IL-17 protein expression was found in human subclinical (borderline) rejection renal allograft biopsy tissue and none in biopsy tissue not showing any evidence of rejection. There was also a 100% detectable rat of IL-17 mRNA expression in the MNCs of urinary sediment of patients with subclinical borderline rejection. These results demonstrate that hRECs exposed to IL-17 can produce inflammatory mediators with the potential to stimulate early alloimmune responses, which may also serve to give warning of acute renal allograft rejection.

M714 EXPRESSION OF SILENCER OF DEATH DOMAIN AND DEATH-RECEPTOR-3 IN NORMAL HUMAN KIDNEY AND IN REJECTING RENAL TRANSPLANTS

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We have previously reported the pattern of cellular expression of tumor necrosis factor receptors (TNFR) in human kidney and their altered expression in transplant rejection. We have extended our studies to examine the expression of Silencer of Death Domain (SODD), a protein that binds to the cytoplasmic portion of TNFR1.to inhibit signalling in the absence of ligand. In normal human kidney SODD is expressed in glomerular endothelial cells where it colocalizes with TNFR1. During acute rejection both SODD and TNFR1 are lost from glomeruli, but we found strong expression of SODD on the luminal surface of tubular epithelial cells and microvascular cells. This occurs in the absence of detectable TNFR1 expression, suggesting that SODD could interact with other proteins at this site. Several other members of the TNF superfamily, including, Fas and death receptors (DR)-3, -4, and –5, also contain intracellular death domains, but SODD only interacts with the death domain of DR3. We therefore studied the expression of DR3 in human kidney, and report that this death receptor is upregulated in renal tubular epithelial cells and microvascular cells, in parallel with SODD, during acute transplant rejection. These data confirm that TNF receptor family members are expressed in a regulated manner during renal transplant rejection, and identify DR3 as a potential inducible mediator of tubular inflammation and injury

M715 EVIDENCE FOR INDUCTION OF EPITHELIAL CELL SENESCENCE IN ACUTE REJECTION

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Donor age is the major predictor of function and survival of kidney transplants. This could represent the effect of cell senescence due to age, coupled with accelerated senescence due to transplant stresses such as rejection. In the present experiments we sought robust markers for somatic cell senescence *in vivo*, and examined the effect of rejection on those markers.

We surveyed the expression of genes previously associated with cell senescence *in vitro* in mice of different ages (n=37). The genes were $p16^{INK4a}$, $p19^{ARF}$, regucalcin, metallothioneins 1 and 3, HIC-5, TGF-β and Hsp70 with expression measured by real-time RT-PCR. Only $p16^{INK4a}$ was very low in development and strongly induced in aging, making it a unique candidate marker for somatic cell senescence. $P16^{INK4a}$ expression increased significantly with age in nuclei of tubules, with less interstitial and glomerular staining.

We then studied how acute rejection affected $p16^{INK4a}$ expression in young (3 months, n=4) vs. old (18 months, n=3) donor kidneys. In rejecting kidneys at day 7 (CBA into B6), $p16^{INK4a}$ mRNA and protein increased in young and old donors. Increased $p16^{INK4a}$ protein were found in tubular, interstitial and glomerular cells. The increases in glomerular staining were striking since this was rare in normal aging. Interstitial staining probably reflects infiltrating lymphocytes that show $p16^{INK4a}$ expression, suggesting that these lymphocytes underwent senescence in the graft with no difference between young and old donor kidneys.

Conclusion: Acute rejection induces the senescence marker in cells of young and old kidneys, adding to senescence due to age. Overall $p16^{INK4a}$ expression was highest in rejecting kidneys from old donors. Thus, older kidneys not only show more age-related senescence, but the number of senescent cells increases with acute rejection. This may limit their capability to repair peritransplant injuries and to maintain organ function and mass.

Free Communication June 9

M716 INTERFERON-INDUCIBLE PROTEIN-10 (CXCL10/IP-10) AND ITS RECEPTOR CXCR3 ARE CANDIDATE TARGETS FOR BLOCKING ALLOGRAFT REJECTION IN THE CLINICAL SETTING

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Despite the hypothesized redundancy of chemokines and chemokine receptors, solitary blockade or enforced deletion of IP-10 or deletion of its receptor CXCR3 results in a lack of recruitment of pathogenetic effector T cells, prevention of target organ damage and in the prolongation of survival time of experimental allografts in mice (Hancock et al. J Exp Med 2000; Hancock et al. J Exp Med 2001; Frigerio et al. Nature Medicine 2002). Inspired by these elegant pre-clinical studies, we, in our bench-to-bedside investigation, explored the role of IP-10 and CXCR3 in human recipients of renal allografts. We investigated whether IP-10 and CXCR3 are hyperexpressed during an episode of acute rejection (AR). With the aid of real-time quantitative PCR assay, we measured mRNA for IP-10 and CXCR3 (and

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constitutively expressed 18S gene) in 66 urine samples collected from 59 renal allograft recipients. Our data demonstrate heightened expression of IP-10 and CXCR3 in urinary cells obtained at the time of AR. Log transformed mRNA copy numbers (mean ±SE) per one μg of total RNA are shown.

Type of mRNA	AR (N=19)	Other Findings (N=15)	Chronic Allograft Nephropathy (N=9)	Stable (N=23)	P (ANOVA)
IP-10 mRNA *	12.2±0.3	9.4±0.8	8.3±0.3	9.0±0.6	<0.0001
CXCR3 mRNA **	11.4±0.4	8.8±1.1	10.5±0.7	7.6±0.6	0.0006
18S rRNA	24.7±0.3	23.8±0.5	24.0±0.5	23.6±0.3	0.13

* $P<0.01$ by Dunnetts multiple comparison test between AR and each of the other 3 groups; ** $P<0.05$ by Dunnetts test between AR and Other group and between AR and Stable group; $P>0.05$ between AR and chronic allograft nephropathy group.

Analysis involving the receiver operating characteristic curve (ROC) showed that IP-10, at a cut-off value of 9.11, predicted AR at a sensitivity of 100% and a specificity of 75%. ROC analysis also showed that CXCR3, at a cut-off value of 9.69, predicted AR at a sensitivity of 95% and a specificity of 50%. Further analysis showed that CXCR3s low specificity of 50% was due to heightened expression of CXCR3 in patients with chronic allograft nephropathy. Our observations, in addition to emphasizing the diagnostic value of measuring mRNA for IP-10 and CXCR3 in urinary cells, advance the idea that IP-10 and CXCR3 are candidate targets for preventing AR in the clinical setting.

M717 UP-REGULATION OF CA2+ ACTIVATED K+ CHANNELS IN T-LYMPHOCYTES DURING ACUTE RENAL ALLOGRAFT REJECTION

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Acute rejection is a major prognostic factor for long term renal transplant survival. A reliable, sensitive, and specific early marker for acute rejection is still missing. In the cellular mechanism of the immune response, up-regulation of K^+-channel functions is an early and crucial step in the activation of T-lymphocytes by promoting membrane potential-driven Ca^{2+}-influx and cell proliferation. In the present study we tested whether K^+ channel function is altered after renal transplantation and could serve as prognostic marker for acute renal allograft rejection.
77 patients were studied longitudinally after renal transplantation for a follow-up period of 21 days. 12 of these patients developed acute renal allograft rejection as determined by histologic analysis of renal biopsies. Functional expression of the K^+ channels in T-lymphocytes from peripheral blood was analysed before transplantation, and daily up to 21 days after transplantation, and during acute rejection by use of the patch-clamp technique.
T-lymphocytes expressed two types of K^+-channels with the characteristics of the voltage-gated K^+-channel (Kv1.3) and the intermediate-conductance Ca^{2+}-activated K^+-channel (hIK1). The Kv1.3 current was found to be the predominat K^+-current (54± 2 pA/pF) whereas the hIK1 current component was low (6 ± 1 pA/pF). After renal transplantation Kv1.3 and hIK1 functions remained constant in T-lymphocytes from patients without acute renal allograft rejection. In contrast, in T-lymphocytes from patients with acute renal allograft rejection, we observed a significant threefold increase in hIK1-currents (13 ± 2 pA/pF; $p < 0.01$). Moreover, this up-regulation of hIK1 functions was already present 3-5 days before conventional histological diagnosis. Kv1.3-currents were not changed in these patients. HIK1 function returned to normal levels after immunsuppressive therapy.
In conclusion, the steep up-regulation of hIK-currents in T-lymphocytes from patients developing acute renal allograft rejection could serve as a new early diagnostic marker. Moreover, blockade of the hIK1 by highly selective inhibitors might represent a new pharmacotherpeutical strategy in the prevention of acute renal allograft rejection.

M718 ESTIMATION OF SERUM AND URINARY TRANSFORMING GROWTH FACTOR-BETA$_1$(TGF-β_1) AND PLATELET DERIVED GROWTH FACTOR (PDGF) IN RENAL ALLOGRAFT RECIPIENTS

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Chronic allograft nephropathy (CAN) is an important cause for graft failure after the first year of renal transplantation. Recent data confirmed the involvement of the key fibrogenetic cytokines such as transforming growth factor-beta 1 (TGF-β_1) and platelet derived growth factor (PDGF) in the pathogenesis of CAN. In the present study, we evaluated the potential contribution of TGF-β_1 and PDGF in the development of renal allograft dysfunction as well as the impact of cyclosporine A (CsA) therapy on serum and urinary levels of these cytokines.
Serum and urinary TGF-β_1 and PDGF were determined by enzyme-linked immunosorbent assay (ELISA) in 10 renal allograft recipients for more than one year with normal renal function (group I),10 renal allograft recipients for more than one year with impaired renal function (group II),10 patients with chronic renal failure (CRF) under conservative therapy (group III) and the results were compared with the levels of 10 healthy controls (group IV).
Serum and urinary TGF-β_1 and PDGF levels in the transplanted groups with normal or impaired renal function (group I,II) as well as in CRF patients (group III) were significantly increased compared to healthy controls ($P<0.01$). The impact of cyclosporine A and immune stimulation in the transplanted cases was manifested by higher levels of serum and urinary TGF-β_1 and PDGF in the transplanted group with normal kidney function (group I) when compared to healthy controls ($P<0.01$). Interestingly, serum and urinary TGF-β_1 and PDGF levels were significantly elevated among transplanted cases with impaired renal function (group II) compared to transplanted cases with normal renal function (group I) ($P<0.01$) confirming the role of these profibrotic cytokines in the developement of chronic allograft nephropathy. Serum and urinary TGF-β_1 and PDGF levels showed significant positive correlations with serum creatinine levels in the study groups ($P<0.001$).
Our data confirm the crucial contribution of the profibrotic cytokines TGF-β_1 and PDGF in the development of chronic graft dysfunction that could be further augmented by cyclosporine A therapy. Future studies are needed to examine the effect of manipulation of immunosuppressive regimen on the extent of profibrotic gene expression as well as the long term renal allograft survival.

M719 DIFFERENTIAL EFFECTS OF GLYCOSAMINOGLYCANS ON RENAL INFLAMMATION DURING ACUTE AND CHRONIC RENAL ALLOGRAFT REJECTION AND ISOLATED ISCHEMIA-REPERFUSION INJURY IN RATS

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Long-term treatment with a low-molecular weight heparin (LMWH) improves the course of chronic renal allograft rejection in rats. The aim of the present study was to investigate the influence of the LMWH reviparin (RE) and the hypersulfated heparin-fragment LU51198 (LU) on renal inflammation during acute and chronic renal allograft rejection and during isolated ischemia-reperfusion injury. Allogeneic transplantation was performed in the Fisher-Lewis rat model, whereas ischemia-reperfusion injury was studied in Fisher rats. In rats with chronic rejection, treatment with RE for 32 weeks markedly reduced overall renal allograft infiltration with monocytes and MHC II expression ($p<0.001$), whereas this could not be observed in rats treated with LU. However both glycosaminoglycans reduced glomerular inflammation down to the level of isografted rats. In opposition, RE and LU, administered for five days after transplantation, did not improve vascular and tubulointerstitial damage caused by acute allograft rejection, whereas these changes were completely prevented by a short course of cyclosporine A (5 mg/kg/d s.c. for five days). Finally, administration of

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RE and LU for five days after ischemia-reperfusion injury reduced renal infiltration with monocytes (RE: $p<0.05$), T-cells (RE: $p<0.0001$; LU: $p<0.05$), and renal MHC II expression (RE: $p<0.001$; LU: $p<0.05$). Our data show that glycosaminoglycans are able to prevent renal inflammation in chronic allograft rejection. Nephroprotection is most prominent in the glomerular compartment. The effects of glycosaminoglycans are independent from any influence on acute allograft rejection and may be related, at least in part, to an improvement of initial ischemia-reperfusion damage.

M720 DIFFERENTIATION OF REJECTION AND CYCLOSPORINE TOXICITY IN PATIENTS WITH KIDNEY TRANSPLANTATION: AN IMMUNOFLUORESCENCE MICROSCOPIC STUDY

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Renal allograft loss from chronic rejection or cyclosporine toxicity (CsAT) is characterized by progressive interstitial fibrosis. These two conditions sometimes aren't distinguished by kidney biopsy and light microscopy. Identification of extracellular matrix (ECM) protein by immunofluorescence (IF) microscopy may be useful in differentiating of rejection from CsAT. 24 patients with kidney transplantation and 16 patients with glomerular disease were performed for kidney biopsy and definite diagnoses were established by clinicopathology. All of the above specimens were stained with monoclonal antibodies (anti laminin-1, antifibronectin, anticollagen-1). At the end, the clinicopathological diagnoses were compared to IF microscopic findings.

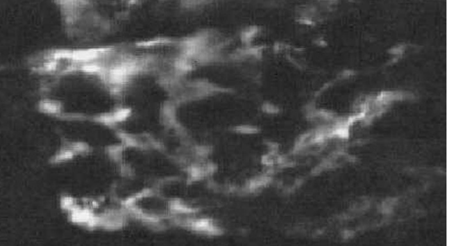

Figure 1.

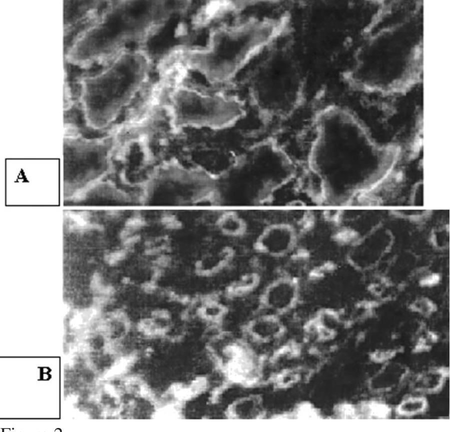

Figure 2.

4 patients had definite diagnoses of CsAT. 16 patients had definite diagnoses of rejection. 16 patients had glomerular diseases who didn't have CsAT or rejection. Renal biopsy specimens showed three differences of immunofluorescence patterns. Pattern 1 showed no change in the composition of ECM and contained linear laminin-1 in the tubular basement membrane and scanty fibronectin in the interstitial space (figure 2A). Pattern 2 showed generalized accumulation of collagen I in the interstitium that was the sign of CsAT (figure 1). Pattern 3 showed faint and discontinuous staining of the tubular basement membrane for laminin-1 and new intracellular collection of laminin-1 that was the sign of rejection (figure 2B). Extended fisher exact test was used for interpretation of results and a p-value =0.624 was found for relation between pattern of IF and clinincopathological diagnosis.

Table 1. Association between clinicopathologic and immunofluorescence pattern

	Immunofluorescence pattern		
Diagnosis	1	2	3
CsAT	3	1	0
Rejection	13	2	1
Glomerular diseases	12	4	0

p-value=0.624

These data suggest that there is no relation between specific changes in the ECM with a specific diagnosis. However more studies in this field for definite interpretation is necessary.

M721 ANALYSIS OF THE CELLULAR COMPONENT AND EXPRESSION OF GRANZIME B IN RENAL AND PANCREAS ALLOGRAFT BIOPSIES

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Kidney-pancreas transplantation is an established procedure for the treatment of diabetic patients with chronic renal insufficiency. Allograft rejection represents an important complication in this modality of transplantation. In pancreas transplantation, acute rejections are more frequent and severe. Therefore, pancreas has been described as an immunogenic organ. However, the cellular and the immune mechanisms involved in pancreas allograft rejection have not been entirely investigated. The aim of the present study was to evaluate the cellular component and the expression of granzyme B in pancreas allograft biopsies compared to renal allograft biopsies.

Patients were classified into two groups, according to clinical and histological criteria: acute rejection (AR) and chronic rejection (CR). Renal allograft biopsies (n=59) and pancreas allograft biopsies (n=26) were included in this study. Immunohistochemical techniques using paraffin sections were employed to identify macrophages (MØ), lymphocytes, myofibroblasts, mast cells, and the expression of granzyme B.

Results (mean±SEM; *=$p<0.05$ vs kidney AR; #= $p<0.05$ vs kidney CR)

	Kidney AR	Pancreas AR	Kidney CR	Pancreas CR
MØ (cells/mm^2)	235±52	147±55	31±4*	76±28
Lymphocytes (cells/mm^2)	303±25	79±28*	107±15*	20±10#
Myofibroblasts (%)	13.0±1.4	1.5±0.4*	21.5±3.4	4.0±1.7#
Mast Cells (cells/mm^2)	13±3	17±3	27±5	22±5
Granzyme B (cells/mm^2)	35±11	12±4	12±4	6±2

In kidney and pancreas biopsies, the number of MØ, lymphocytes and the expression of granzyme B was higher in acute rejection, reflecting an active process of the immune response. While lymphocytes constitute the most predominant cellular component in kidney rejection, in pancreas specimens macrophages predominate. This predominance of macrophages may play a role in the severity of the pancreas allograft rejection. On the other hands, the increased number of myofibroblasts and mast cells in Chronic Rejection suggest that these cells are involved in the chronic inflammatory process and development of interstitial fibrosis. Although the incidence of rejection is higher in pancreas transplantation compared to kidney transplantation the inflammatory reaction and the production of granzyme B are not higher in pancreas compared to renal allograft. These results suggest that the severity of pancreas rejection maybe related to other mechanisms than citotoxicity.

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M722 THE IMPORTANCE OF GLOMERULAR DEPOSITS OF VON WILLEBRAND FACTOR IN HUMAN RENAL ALLOGRAFTS

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The aim of this study was to evaluate the role and prognostic importance of glomerular endothelial cell alteration in human renal allografts with deteriorated function following transplantation.
We examined 72 patients with renal transplantation among whom 40 were in acute rejection (AR) and 32 in chronic rejection (CR). All biopsies were immunostained with monoclonal antibodies specific for Von Willebrand factor (vWF), fibronectin (FN) and CD68. Glomerular staining for vWF and FN was graded in a semiquantitative manner using the 1-3+ scale. All follow-up biopsies of patients with AR were re-evaluated for the presence of interstitial fibrosis and transplant glomerulopathy.
In the CR group there was markedly increased expression of vWF and FN in the glomeruli, whereas only 8 of 40 AR cases showed intense (grade 3) glomerular vWF. All of the 8 patients with intense glomerular vWF were showed type II vascular rejection. A significant difference was found between patients with AR and CR in regards of glomerular vWF deposition ($p<0.01$). A positive correlation between the degree of glomerular vWF deposition and early interstitial fibrosis was found in cases with AR in follow-up biopsies ($p<0.05$). The presence of transplant glomerulopathy significantly found earlier in cases with intense glomerular vWF deposition in AR group ($p<0.01$). We found strong correlation between both intraglomerular FN expression and macrophage infiltration with the degree of glomerular vWF deposition both in AR and CR group ($p<0.05$). The outcome for grafts that showed intense (grade 3) glomerular vWF was significantly worse than the outcome noted for other grafts with grade 1 and 2 glomerular vWF during the follow-up ($p<0.001$).
Increased glomerular vWF deposition in cases with AR is in risk of early transplant glomerulopathy, early interstitial fibrosis and early graft loss. In addition intense glomerular vWF deposition was observed in almost all biopsies of cases with CR. In conclusion it may be beneficial to use new therapeutic approaches such as anticoagulant medicine for the treatment of AR and CR in future.

M723 MORPHOMETRIC AND IMMUNOPATHOLOGIC STUDY OF RENAL ALLOGRAFT BIOPSIES AND CORRELATIVE IMPLICATIONS OF CLASSIFICATION

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Purpose of the study: The purpose of this study is to quantitatively analyse the histopathologic criteria of cellular rejection by morphometry and correlate with the immunohistochemical expression of T cell markers CD2,CD4,CD8 and IL_2 receptor (CD25).A subgroup of these patients were treated with humanised Interleukin-2 receptor antibody and subsequent renal biopsies were studied for IL_2 expression.The immunohistochemical data were then correlated with the histologic grade as per the Banff-97 classification and compared with the NIH- CCTT classification.
Methods: Two subgroups of patients were studied. (a) In a pilot study, renal allograft biopsies from 35 patients were quantitatively assessed by manual morphometry with particular reference to the features of cellular rejection and specifically the area of infiltrate.Immunohistochemical expression of CD2, CD4, CD8 and CD25 (IL_2 receptor antibody) were studied using the Alkaline phophatase anti alkaline phosphatase (APAAP) technique. (b) Subsequently 50 renal allograft biopsies were analysed by a computerised Olympus morphometric system using Image Analysis Software Version 4.0.The relative merits of the Banff 97 and the NIH-CCTT classification were comparatively analysed. Immunohistochemical expression of IL_2 receptor antibody by APAAP technique was studied in the cellular infiltrates and correlated with followup biopsies in the subgroup of patients who underwent immunosuppressive therapy with humanised IL_2 receptor antibody.
Summary: Histomorphometric evaluation helped in studying the relative merits of the Banff97 and the NIH-CCTT classification:
The ratio of the area of the infiltrate to the total area of the biopsy was lowest in the patients with no rejection. The mean ratio in patients diagnosed as Borderline by Banff and as type I by CCTT was lower than the mean ratio of Banff 1A and CCTT type 1.
Correlation with IL_2 receptor antibody expression shgowed that the CCTT is a simpler, robust and clinically useful classification
Moderate expression of CD2 and CD 4 were noted in the presence of Type 1 rejection. CD8 expression inversely correlated with severity of the disease. IL_2 receptor antibody expession was seen in 37 out of 39 allograft biopsies diagnosed to have evidence of clinical rejection.
Conclusions: A) Morphometry helps quantify the grade of cellular rejection and significantly correlated with expression of IL2 receptor antibody expression. B) NIH-CCTT classification was found to be a simple and clinically sound classification. C)Expression of Tcell markers and IL_2 receptor antibody correlated with degree of rejection.

M724 THREE-MONTH RENAL ALLOGRAFT PROTOCOL BIOPSIES: LOW RATE OF SUB-CLINICAL ACUTE REJECTION USING CYCLOSPORINE, MMF AND PREDNISONE

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Background: Chronic Allograft Nephropathy (CAN) is the commonest cause of late allograft loss. Its pathogenesis is multi-factorial, with both allo-dependent and independent factors being implicated in its development. One potential mechanism for the development of CAN is sub-clinical acute rejection (SC-AR), the incidence of which has previously been reported at 30% in patients on cyclosporine-based immunosuppression.
Methods: Forty-five patients underwent a renal allograft biopsy at 3 months after transplantation. Of these, 3 were diagnostic while the remaining 42 were protocol biopsies. All patients except for one were on cyclosporine, mycophenolate mofetil and prednisone. The incidence of biopsy-proven clinical Acute Rejection was 14%. Biopsies were analysed using Banff '97 criteria.
Results: Most biopsies were normal. Only one biopsy showed SC-AR (2.3%), while the incidence of borderline AR was 7.1%. Eight biopsies (19%) however showed evidence of CAN and 4 (9.5%) biopsies had lobular hyalinosis consistent with calcineurin phosphatase inhibitor nephrotoxicity. There was no difference in measures of renal function between the groups (Table 1). Similarly there was no difference in the incidence of clinical AR, antilymphocyte antibody use, delayed graft function, age, gender, or donor source between those with CAN and those with normal biopsies at three months.

Renal Function at 3 month protocol biopsy according to histological diagnosis

	subclinical AR	borderline AR	CAN	Hyalinosis	Normal
creatinine mmol/L	0.18	0.11	0.17	0.17	0.13
GFRml/min	51.9	64.4	53.2	55.4	60.1

Conclusion: This is the first study to show a very low rate of both subclinical and clinical AR in patients on cyclosporine based immunosuppression. Despite this, 19% of patients had histological evidence of CAN in protocol biopsies at 3 months after transplantation.

M725 HISTOLOGICAL FINDINGS IN PROTOCOL BIOPSIES PERFORMED IN STABLE RENAL ALLOGRAFTS ARE INFLUENCED BY IMMUNOSUPPRESSIVE SCHEDULE

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Background: Protocol biopsies performed in stable renal allografts show different degrees of acute and chronic lesions. Histological findings in protocol biopsies have been related with graft outcome. We evaluated histological lesions observed in protocol biopsies performed in patients under different immunosuppressive schedules.
Patients and methods: From June 1988 a protocol biopsy at approxi-

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matelly 4 months has been performed in cadaveric kidney transplant recipients who fullfilled the following criteria: serum creatinine at the time of biopsy < 300 μmol/L, stable renal function defined as a variability of serum creatinine during the month before and after biopsy of less than 10%, and proteinuria < 1g/day. Histological lesions were evaluated according to 1997 Banff criteria. We have considered for the present study the following immunosuppressive regimens: I) concomittant induction therapy with antilymphocytic antibodies (ALG or OKT3) associated with cyclosporine (CsA) and prednisone (P), II) CsA, micophenolate mofetil (MMF) and P, III) tacrolimus, MMF and P.Chi-square for categorical variables and ANOVA for continuous variables were employed for statistical analysis.

Results: During the study period we have performed 495 protocol biopsies obtaining sufficient sample for evaluation in 454. For the present study we have considered 346 biopsies performed in 342 patients. Clinical characteristics of patients and renal function at the time of biopsy are shown in the following table

Clinical characteristics

Variable	Group I	Group II	Group III	p
N	186	111	49	
Age	43±13	46±14	45±13	ns
Sex (male/female)	114/72	71/40	33/16	ns
Donor age	33±16	41±17	38±14	0.001
DGF (%)	21	18	20	ns
Acute rejection (%)	20	16	12	ns
Creatinine (μmol/L)	167±109	139±47	128±37	0.001
Proteinuria (g/day)	0.39±0.37	0.24±0.36	0.20±0.29	0.001

Banff diagnosis show that patients receiving tacrolimus have a higher proportion of normal biospies (p<0.001).

Histologic diagnosis

Banff category	Group I	Group II	Group III
Normal	40,8	44	55,1
Borderline	12,9	12,6	6,1
Acute rejection	0	7,2	2,0
CAN	25,2	21,6	30,6
CAN + borderline	16,2	9,9	4,2
CAN + acute rejection	4,8	4,5	2,0

Conclusions: Protocol biopsies in stable renal allografts show an association between histological findings and immnuosuppressive treatment.

M726 ROLE OF POST-TRANSPLANT ANTI-HLA ANTIBODY IN RENAL TRANSPLANTATION

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It has been accepted that pre-transplant anti-HLA antibody has impact on the outcome of renal transplantation (RT). However, the role of post-transplant anti-HLA antibody on the renal allograft outcome remains unclear. We have conducted this study with the aim to know whether post-transplant anti-HLA plays an important role in the outcome of renal allograft. This study was performed in cross sectional design. Anti-HLA antibodies of both class I and class II were performed in 41 renal transplanted patients. Fifteen patients were living-related RT and 26 were cadaveric RT. All patients have been transplanted for more than 6 months. The correlation of post-transplant anti-HLA antibodies of both classes I and class II, and renal allograft function, in term of GFR, were analyzed. In renal transplant patients who had GFR of more than 60 ml/min, anti-HLA antibody showed positive results in 2 patients for class I and 9 for class II. Contrastly, in renal transplants with GFR of less than 60 ml/min, anti-HLA antibody was positive in 3 patients for class I and 19 for class II. Post-transplant anti-HLA antibody of class II had a tendency to be positively correlated with GFR (r = –0.31, p = 0.05). This preliminary result of this study indicated that post anti-HLA antibody of class II HLA might be one mechanism of chronic allgraft rejection and confirmed the important role of HLA matching in the outcome of renal transplantation.

M727 THE FACTOR VIII OF COACULATION AS VASCULAR REJECTION MARKER

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The level of Willebrand's factor as vascular rejection marker after kidney transplantation in two groups have been analyzed. The 1st group included 20 patients (M:F 11:9), in which the 3-component immunosuppressive scheme was used (CsA,Aza,GK), the 2 [nd] group included 20 patients (M:F 14:6) where four-component immunosuppressive scheme was used. The fourth component induced was "Bromergon", the prolactin inhibitor. In the group 2 the dosage of CsA was 4 mg/kg/d instead 12 mg/kg/d in 1 group. Also have been used biopsy and other lab parameters. Samples of blood plasma, which had been taken 10 days after the transplantation (to avoid any influence on Willebrand's factor of post operational stress factors) were examined.

Lab parameters of cells and vascular rejection in two groups

Parameters	Acute cell rejection		Vascular rejection	
	group 1	group 2	group 1	group 2
CD4/CD8	3,0±0,09	2,78±0,1	2,0±0,3*	2,1±0,4
β-lyzins, %	24,0±4,3	41,0±2,4	25,3±2,3	33,9±3,6
TNF–α, pg/ml	27,6±7,5	25,0±3,7	12,3±2,3	13,6±0,8*
Willebrand's factor, %	98,5±2,4	87,8±4,5	189,8±4,3**	193,5±3,5**

*p<0,01, **p<0,001

The determination of Willerbrand's factor activity level was implemented by means of immunoenzyme analysis method using test-system of "Stago" company. The activity of Willebrand factor in plasma is 80-150%, providing normal condition of haemostasis.

The comparison has shown that the main authentic marker of vascular rejection is the level of Willebrand's factor.

M728 QUANTIFICATION OF DONOR-DERIVED DNA IN SERUM OF RENAL TRANSPLANTED RATS BY REAL-TIME PCR: A NEW APPROACH FOR ACUTE REJECTION DIAGNOSIS

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Introduction: The diagnosis of acute rejection in renal transplantation is usually performed by the invasive procedure of allograft needle biopsy. This raises the need for alternative non-invasive screening approaches to avoid potential biopsy complications. In a fully MHC-mismatched, rat orthotopic DA to Lewis kidney transplant model acute rejection occurs as a result of cell-mediated immunity which results in microchimerism present in body tissues, peripheral blood and plasma after organ transplantation. We used this model to quantitate released donor-specific DNA in sera or urine to establish an alternative screening procedure for an early diagnosis of acute rejection.

Methods: In an allogeneic rat model without immunosuppression, kidneys from male DA rats were transplanted to female LEWIS rats (n=4). Syngeneic kidneys from male DA rats transplanted to female DA rats (n=4) served as controls. Urine and sera samples were collected daily from the recipients and the genomic DNA was prepared. Rat male-specific Y-chromosomal primers (SRY) were designed for quantification purposes. Both SRY and the constitutively expressed housekeeping gene beta-actin for normalization were measured by real-time PCR. On day 7 the animals were sacrificed and kidneys were harvested for histological examination. Creatinine and protein data were obtained from urine.

Results: SRY-specific DNA expression in the sera of all female Lewis rats with kidneys from male DA donors was not detectable in the first 4 days but then significantly increased prior to acute rejection (day 6±1) on day 5. In contrast, none of the female DA rats from the control group displayed measurable levels of SRY-specific DNA from their male DA kindney donors during the whole screening period (d=7). In the urine no SRY-specific DNA expression was verifiable. The histological analysis revealed in the rejected allogeneic kidneys high infiltration of mononuclear cells, damaged tubuli

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and necrosis in contrast to the minor structural changes in the isografts. The creatinine and protein levels in the urine showed no significant differences between both groups.

Conclusion: These results suggest, that donor-derived DNA is present in the serum of renal transplanted recipients prior to rejection. The quantification by real-time PCR of this specific DNA may lead to the establishment of an alternative fast and reliable monitoring strategy for the diagnosis of acute rejection.

M729 NONINVASIVE DIAGNOSIS OF ACUTE ISLET ALLOGRAFT REJECTION BY PERIPHERAL BLOOD CELL mRNA PROFILING

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Diabetes mellitus is a major cause of end stage renal disease, and renal -pancreas/islet transplantation is an ideal treatment for the type 1 diabetic patient with irreversible renal failure. Pancreas or islet transplantaion may also prevent the development of renal disease and other complications of type 1 diabetes. Currently, pancreas or islet transplantaion is severly limited by our inability to diagnose allograft rejection prior to the development of hyperglycemia and increased insulin requirements. However, these endpoints for assessing rejection are inadequate since a significant loss of islet cell mass has to occur prior to the development of hyperglycemia. Thus, there is a critical need for reliable biomarkers that would predict rejection prior to the onset of significant graft dysfunction. In view of the role of cytotoxic attack molecules and inflammatory cytokine/chemokines in islet graft rejection and destruction, we explored the hypothesis that there is an increase in the levels of peripheral blood cell cytotoxic, cytokine and chemokine gene expression prior to clinical islet graft rejection as reflected by hyperglycemia. To test our hypothesis, we used an islet cell transplant model wherein diabetic C57BL/6 mice are transplanted with either C57BL/6 islets (syngenic islet grafts) or DBA/2 islets (allogeneic islet grafts). In this model the mean±SE survival time of allogeneic DBA/2 islets is 10±1 days. We collected blood samples at day 4 post-transplantation when all of the recipients of islet grafts are normoglycemic. We measured mRNA levels of cytotoxic gene perforin, inflammatory cytokine TNF-alpha and chemokine IP-10 with the use of real time quantitative PCR assay.

Type of Islet Graft	Mean mRNA copy number per one microgram of RNA		
	Perforin	TNF-alpha	IP-10
Syngeneic	4.23E+05	4.07E+05	7.31E+03
Allogeneic	1.29E+06	1.69E+06	1.62E+05
P	0.02	0.03	0.01

Our studies demonstrated up-regulation of mRNAs for perforin, TNF-alpha and IP-10 prior to clinical rejection. Our findings support the idea that the status of clinical islet/pancreas grafts can be accurately predicted noninvasively by measurement of transcripts for cytotoxic attack molecules and mRNAs encoding inflammatory cytokines and chemokines in peripheral blood cells. Moreover, candidate target molecules (e.g., IP-10) for blocking graft rejection prior to full graft damage are also advanced by our investigation.

M730 THE PREDICTIVE VALUE OF THE INTERFERON-GAMMA DEPENDENT CHEMOKINE MIG TO DIFFERENTIATE RENAL ALLOGRAFT REJECTION FROM OTHER CAUSES OF GRAFT DYSFUNCTION

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Monitoring patients following renal transplantation is based on clinical evaluation and classical parameters of systemic and renal function and inflammatory markers, e.g. leukocyte counts, CRP, creatinine. Together with their clinical experience physicians have to rely on these parameters to safely recognize and separate from each other acute or subacute rejection processes, interstitial or vascular organ failure, immunosuppressant adverse reactions, and intercurrent viral or bacterial infections. Given that recent investigations showed a prominent role of interferon-gamma dependent chemokines in allograft rejection in animal models, we examined the ligands of CXCR3, which in itself is a marker of activated T cells, monocytes and NK-cells. Urinary MIG (CXCL9) and IP-10 (CXCL10) were determined in comparison to clinical parameters and urinary MCP-1.

Urinary specimen were taken consecutively from patients within the first 3 months after renal transplantation or during rejection episodes and measured by a modified ELISA. Clinical data and laboratory parameters of patients with respect to infection, delayed graft function, and rejection (e.g. kidney biopsies) were monitored. So far, more than 500 samples from 22 patients have been measured. Four groups of patients can be characterized: Six (6) patients with an uneventful posttransplant course with no significant elevation of chemokines in the urine, three of those having received a living donor kidney with very short ischemia; eight (8) patients with delayed graft function and/or complications during surgery showed no significant elevation of MIG, however IP 10 and especially MCP1 were elevated in the early phase. Bacterial infections did not affect urinary MIG levels. Most importantly in five (5) patients with a confirmed diagnosis of acute allograft rejection MIG was elevated significantly ($p < 0.01$; 3-30x above the cut-off level of 400ng/ml) during rejection. MIG values preceded elevation of serum creatinine by 1-3 days and returned earlier to normal levels after successful rejection therapy. In addition urinary MIG was elevated in two (2) patients with high panel reactive antibodies (PRA) without the diagnosis of rejection and in one (1) patient with insufficient immunosuppression due to lack of absorption. MIG normalised under antibody treatment applied according to the routine immuosuppressive prophylaxis protocol for highly immunised patients or under adequate immunosuppression. From these preliminary results we conclude that urinary MIG might become a useful early and very sensitive marker for acute allograft rejection and successful rejection therapy, being unaffected by bacterial infections. Moreover MIG helps to differentiate between delayed graft function (DGF) due to reperfusion injury only and DGF combined with allograft rejection.

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M731 RESISTANCE INDEX PREDICTS LONG-TERM RENAL ALLOGRAFT SURVIVAL

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Background: Most renal transplants fail because of chronic allograft nephropathy or death of the recipient. Various factors influence long-term allograft survival; however, no reliable parameter predicting long-term outcome is available. We tested whether or not increased renal arterial resistance index (≥80) predicts long-term allograft survival.

Methods: We measured the renal segmental arterial resistance index (percent reduction of end diastolic flow compared to systolic flow) in 601 patients at least 3 months after transplant between August 1997 and November 1998. All were followed for >3 years. The main combined endpoint was decreased glomerular filtration rate ≥50 percent, allograft failure, or death.

Results: 122 (20 percent) patients had a resistance index >80. In this group, 69 percent decreased their renal function by >50 percent (n=84), 47 percent (n=57) required dialysis, and 30 percent (n=36) died. In contrast, only 12 percent (n=56) of patients with resistance index <80 decreased their renal function, only 9 percent (n=43) required dialysis (all $P<0.001$), and only 7 percent (n=33) died ($P<0.001$). 88 percent (n=107) of the resistance index ≥80 patients reached the combined endpoint, compared to 17 percent (n=83) of the resistance index <80 patients ($P<0.001$). The multivariate odds ratio for graft loss with resistance index ≥80 was 37 (95

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percent confidence interval 18 to 73). In contrast, the next best parameters cold ischemic time >12 h, proteinuria >1 g/day, and glomerular filtration rate <30 ml/min/1.73 m^2 after transplantation, provided odds ratios of 19 (2 to 154), 8 (4 to 18), and 4 (2 to 7), respectively.

Conclusion: A resistance index ≥80 measured at least 3 months after transplantation may predict poor allograft performance and death.

M732 DOES URINARY IL-8 mRNA-EXPRESSION REFLECT THE ISCHEMIA/REPERFUSION INJURY (IRI)? EVALUATION OF LIVING DONOR DERIVED AND CADAVERIC KIDNEY TRANSPLANTS

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IRI has been identified as a pivotal risk factor for poor renal allograft survival. Cytokine release and infiltration of polymorphs are initial steps promoting IRI. Elevated levels of the potent neutrophil chemoattractant IL-8 have been detected in ischemia induced acute renal failure. A strong correlate with both the degree of IRI and deteriorating graft function has been seen in lung and liver grafts. The aim of this study was to investigate the relationship between urinary IL-8 mRNA expression and cold ischemia time (CIT) as a predictor for IRI and graft function in renal transplantation. Urine samples (12-14 per patient) were obtained from the first day post surgery onwards for 4 weeks in patients with transplants from living (n=13, group 1) or cadaver (n=12, group 2) donors. Simultaneously, serum creatinine was monitored. CIT was 2.4 ± 0,24 h in the living-donor group compared to 22 ± 11,5 h in patients receiving a cadaveric graft. Patients had no delayed graft function or rejection episodes during the study period. Transcripts were quantitated in duplicates by real-time PCR using an external standard and GAPDH as a housekeeping gene for normalization. The results are expressed as the mean of IL-8 expression levels. Unexpectedly IL-8 mRNA was higher in the living donor group than in the cadaver transplants when looking at the first 48 h (group 1: 134,99 ± 64,5 vs. group 2: 12,2 ± 15,8; p < 0,005) as well as in the periods of day 3 to 10 (group 1: 194,5 ± 186,9 vs. group 2: 20,5 ± 28,7; n.s.) and day 11 to 20 (group 1: 144,9 ± 129,5 vs. group 2: 15,6 ± 15,4; n.s.). In both groups IL-8 levels declined gradually over the first three weeks. No correlation was found between IL-8 levels and serum creatinine or between IL-8 levels in the first 48 h and serum creatinine at three or six months. In conclusion this study shows that urinary IL-8 transcript levels were higher in living donors. Thus, in contrast to other solid organ transplants IL-8 expression does not seem to mirror the extent of IRI in kidney allografts. Since IL-8 is an instantly upregulated gene, it might be possible that with long CIT (cadaveric donors) IL-8 peaks within only a few hours after the injury and is already on a decline when the first sample was obtained. Short CIT (living donors), though, might lead to a gradual rise of IL-8 expression reflecting a delayed response to IRI. Pronounced fluctuation of IL-8 levels within each patient in the time course could be caused by subclinical otherwise undetected inflammatory processes.

M733 CD25 mRNA LEVELS IN URINARY CELLS PREDICT ACUTE REJECTION

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Humanized monoclonal antibodies (mAbs) directed at the interleukin-2 receptor-alpha (CD25) are currently used as induction biologicals and for the prevention of the development of acute rejection (AR). Indeed, US FDA approved humanized anti-CD25 mAbs for anti-rejection prophylaxis and the first dose is administered prior to transplantation. Because resting T cells express very few copies of CD25 on their cell surface and because CD25 is a physiologically relevant marker of activated T cells, we wondered whether a strong and mechanistic rationale for considering anti-CD25 mAbs for the treatment of an ongoing AR could be developed. Toward this objective, we determined if CD25 is expressed at a higher level during AR compared to no AR status. We were particularly interested in detecting the trafficking of CD25 expressing cells through the allograft. We measured with the use of real-time quantitative PCR assay, the level of mRNA for CD25 (and 18S rRNA-a constitutively expressed gene) in urinary cells obtained from renal allograft recipients and investigated whether CD25 mRNA expression is higher during an episode of AR compared to levels during other renal allograft diagnoses. We collected 102 urine specimens from 90 renal allograft recipients. 36 urine samples were from 32 patients at the time of biopsy proven AR, 21 were from 20 patients with biopsy confirmed chronic allograft nephropathy (CAN), and 20 from 19 patients whose biopsies were classified as Other (Banff 97). The remaining 25 urine specimens were from 19 patients deemed clinically stable (Stable). Our data demonstrated heightened expression of CD25 mRNA but not that of constitutively expressed 18S rRNA in urinary cells obtained at the time of AR.

Transcript Levels (median)

	AR (N=36)	Other (N=20)	CAN (N=21)	Stable (N=25)	P (K-W One Way ANOVA)
CD25 mRNA	61050	7885	8620	6800	0.0005
18S rRNA	5.80E+10	2.40E+10	4.30E+10	5.30E+10	0.3

CD25 mRNA levels were significantly (P<0.05) higher in AR group compared with each of the other group by Dunns multiple comparison test.

Our finding, in addition to documenting allograft trafficking of CD25+ cells during AR, support the use of anti-CD25 mAbs for the treatment of AR. In this regard, we suggest strategies that kill CD25+ cells rather approaches that block CD25 signaling since T cell apoptotic signals conducive to allograft protection might be interfered with.

M734 MOLECULAR SIGNATURES OF URINARY CELLS DISTINGUISH ALLOGRAFT INFILTRATES ASSOCIATED WITH ACUTE REJECTION FROM INFILTRATES THAT DO NOT CAUSE PROXIMATE ACUTE REJECTION

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Acute cellular rejection is defined by the presence of inflammatory infiltrates occupying greater than 25% of allograft parenchyma and associated moderate tubulitis. It is unclear if interstial infiltrates with mild tubulitis (borderline changes) represents a benign process or an immunologically active process. Herein, we explored the molecular profiles of urinary cells obtained from patients without infiltrates in their allograft biopsies, patients with infiltrates but without tubulitis, and patients with borderline changes and compared these mRNA profiles with the molecular signature of acute rejection. The chemokine IP10 is important for recruitment of TH1 type T cells and these cells express the chemokine receptor CXCR3. The integrin CD103 is critical for intraepithelial localization of CD8+CTL while granzyme B is an important cytotoxic T cell effector molecule. Thus, we measured mRNA levels of IP10,CXCR3, CD103, and granzyme B in urine specimens obtained from patients without infiltrates, patients with infiltrates but without tubulitis, and patients with borderline changes and compared the mRNA profiles with those of acute rejection. Total RNA was isolated from urinary cells and reverse transcribed to cDNA. mRNA copy number was measured using gene specific primers and probes in the real-time quantitative PCR assay and expressed as mean copy number per one microgram of total RNA. Our data demonstrate that molecular signatures of urinary cells distinguish allograft infiltrates associated with acute rejection from infiltrates that do not result in acute rejection.

Analysis involving ANOVA and Bonferroni multiple comparison testing demonstrated: (1) no significant differences among the urine mRNA profiles of patients without infiltrates, patients with infiltrates but without

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mRNA Profiles of Urinary Cells

Type of mRNA	No Infiltrate	Infiltrate	Borderline	Acute Rejection
	(N=9)	(N=10)	(N=6)	(N=31)
GB	14527	4367	22152	395599
CXCR3	3643	3424	28667	277276
IP-10	2862	6317	11610	420146
CD103	111	306	967	8968

tubulitis, and patients with borderline changes (P>0.05), and (2) significant difference in the urinary mRNA profile of patients with acute rejection compared to the profiles of patients without infiltrates, patients with infiltrates but without tubulitis, and patients with borderline changes (P<0.001). Our studies provide a molecular basis for the differential clinical outcome of cellular traffic into the allograft, resolve the controversy regarding the significance of borderline changes and support the idea that borderline changes do not merit anti-rejection treatment.

M735 DETECTION OF SUBCLINICAL REJECTION AFTER RENAL TRANSPLANTATION

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Protocol biopsies during the first 6 months after kidney transplantation have shown a 30% incidence of Acute Rejections without any alterations in graft function; such rejections were called sub-clinic rejections (SCR), and could be related to the lack of long term improvement in graft. Furthermore, detection and treatment of SCR has been associated to a better graft function two years after transplantation. The high incidence of SCR episodes, and the importance of their treatment in kidney physiology, have led some authors to suggest that protocol biopsies should be carried out in the first six months in order to detect them.

To prospectively analyse the incidence of SCR in kidney transplant patients and evaluate their influence on the post-treatment survival of both graft and patient.

This prospective cohort included fifty-seven transplant recipients who agreed to participate and signed an informed consent term. Patients were submitted to a percutaneous kidney biopsy on the 1st, 2nd, 3rd, and 6th months after renal transplantation. The definition of SCR was based on the absence of creatinine elevation during the last two weeks before the biopsy, and Banff's classification were used for the quantification of inflammation. Fifty-seven patients were submitted to protocol biopsy, in a total of 174 biopsies. Our HLA distribution was: HLA I/22 – 38.59%, HLA II/12 – 21.05%, HLA III/4 – 7.01% and cadaver donor/19 – 33.33%. In our sample, we found a 2.08% incidence rate of SCR – biopsy in the first month after transplantation, HLA II, Banff Ia. The incidence of Acute Rejection was 14.03%.

We did not observe any benefit of sequential protocol biopsy for detection of SCR. Our results differ from other groups results, regarding the incidence of SCR and a study with a larger number of patients will be necessary to determine the its incidence.

D4 Outcomes, cardiovascular morbidity and mortality

M736 EFFECTS OF FLUVASTATIN ON RENAL TRANSPLANT FUNCTION AND GRAFT LOSS IN RENAL TRANSPLANT PATIENTS IN ALERT (ASSESSMENT OF LESCOL IN RENAL TRANSPLANTATION)

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Background: Hyperlipidemia is a risk factor for cardiovascular morbidity and mortality and long-term renal transplant dysfunction; however, no studies have demonstrated that lipid-lowering strategies significantly reduce CV or renal events in this population. We therefore conducted the first large-scale, randomized, double-blind controlled trial comparing the effects of a statin vs placebo on CV and renal outcomes.

Methods: ALERT was an investigator-initiated, multicenter, randomized, double-blind, placebo-controlled trial conducted to assess the effect of fluvastatin on cardiac and renal outcomes in renal transplant recipients. Eligible patients were male and female renal transplant recipients aged 30-75 years with total cholesterol 4.0-9.0 mmol/L (155-348 mg/dL) and GFR > 20ml/min who had received a renal transplant more than 6 months before enrolment and currently were receiving immunosuppressive therapy containing cyclosporine. The primary endpoint of the study was time to first major adverse cardiac event (MACE). Renal endpoint included graft loss or doubling of serum creatinine and GFR was measured 3 times over the course of the study in a subset of patients. Renal biopsies were optional for all subjects. Baseline risk factors analyzed for renal endpoints included donor age, hypertension, elevated serum creatinine, proteinuria, previous acute rejection episodes, HLA-DR mismatch, elevated Apo B, low HDL cholesterol, smoking, diabetes and cold ischemia time.

Results: Between June 1996 and October 1997, a total of 2102 patients were randomly assigned to receive either fluvastatin or placebo. At baseline, subjects' average time post transplantation was 6 years and their average serum creatinine was 142 mmol/L. Over the course of the study, there was a linear increase in renal endpoints and deaths. Approximately 2 months prior to closing the database, a total of 435 combined renal events, including deaths, had occurred, and 215 graft losses had occurred. Data base lock will occur on February 6, 2003. Treatment results, including risk factor analyses, will be available for presentation at the Congress.

Free Communication June 11

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