ARE THERE ANY RELIABLE SERUM MARKERS FOR CARDIAC ALLOGRAFT REJECTION?

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The detection of cardiac allograft rejection still depends on serial routine myocardial biopsies with a certain procedural risk. Despite several biopsies per procedure false negative results due to sampling errors have to be considered. Therefore, an early sensitive and specific non-invasive marker for the detection of cardiac allograft rejection is highly warranted. A huge number of different laboratory markers have been tested for this purpose, including immunological parameters, markers of necrosis (troponin) and lately markers of ventricular dysfunction (B-type natriuretic peptide, BNP). Nonetheless, so far the results were disappointing. Immunological parameters cannot reliably discriminate between infection and rejection, troponins remain elevated for several weeks after transplantation even in the absence of histological signs of rejection. In addition troponins only increase with severe rejection, when myocardial necrosis occurs. The promising early report on BNP could only be partly confirmed in subsequent larger studies. Recently we tested the combination of BNP, cardiac troponin I, and neopterin for the detection of cardiac allograft rejection after the first month post transplantation.. However, at least based on a single testing on the day of myocardial biopsy also this pannel could not provide the desired solution, but serial sampling based on a preset regimen remains to be evaluated. In conclusion, according to preliminary results also the newer cardiac markers troponin and BNP do not appear to allow a reliable prediction of cardiac allograft rejection.

REJECTION IS INFLAMMATION - GENE EXPRESSION PROFILES IN MURINE HEART TRANSPLANTATION

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Background: Graft rejection is caused by the specific response against allo-antigens. However this adaptive immune response is accompanied by a multitude of innate immune responses. Little is known about the interplay and kinetics of innate and adaptive immunity.
Objective: Comparison of adaptive and innate immune responses by measuring profiles and kinetics in the expression of genes in a murine heart transplant model.
Materials and Methods: 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, b-actin, and 18S rRNA served as endogeneous references. 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). Descriptive statistics and clustering methods were used for the data analysis.
Results: The overall intra- and interspecies variability of the individual genes was low. The gene expression demonstrated a high correlation between the BALB/c and B6 strains. However expression of genes related to inflammation were higher in the B6 mice. 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 (mean expression of 40 % rel. to GAPDH). In contrast cytokine, effector, acute phase, and pteridine genes showed low baseline mRNA levels (mean expression of 0.1 % rel. to GAPDH). Genes with low control levels were characteristically highly up-regulated during the post-transplant course. In the mean they showed a mean fold-change of 8.6 in relation to their pre-transplant levels. Whereas genes with a high baseline expression changed in the average only 1.6-fold and frequently displayed a down-modulation.
Overall the individual genes showed a similar pattern of expression during the first 24 post-transplant hours in the syngeneic and allogeneic hearts. Syngeneic and allogeneic heart tissues showed a strong innate immune response during the first three post-operative days. From day 4 onwards inflammatory responses were seen almost exclusively in the allogeneic model, comprising genes of innate and adaptive immunity. 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. Cytokine and stress genes showed the earliest up-regulation (already after 3 hrs) followed by acute phase and complement genes. 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. 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.
Conclusions: 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 rejection response is detectable from day 4 onwards characterized by a strong up-regulation of genes associated with inflammatory responses.

PLASMA CYTOKINE LEVELS IN PATIENTS AFTER HEART TRANSPLANTATION

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Background: Heart transplantation induces systemic inflammatory response which is accompanied by a significant production and release of various kinds of cytokines during and shortly after weaning the cardiopulmonary bypass. It was demonstrated that some proinflammatory cytokines have adversary effects on endothelial and myocardial cells: they significantly influence myocardial contractility, and a direct relationship between the plasma levels of interleukins IL-6 and IL-8 and the development of left ventricular wall dyskinesia has been demonstrated, too. Therefore, the aim of this study was to investigate the release of some pro- and antiinflammatory acting cytokines in course of one month in patients after successful heart transplatation.
Patients and methods: In venous blood samples from 16 heart transplanted patients the plasma levels of proinflammatory (tumor necrosis factor - TNF and interleukins IL-6 and 8) as well as antiinflammatory (interleukin-10) cytokines were analyzed before and during the next four weeks after transplantation. The levels of cytokines were measured by enzyme-linked immunosorbent assays using commercially available diagnostic kits (R&D Systems Inc., Minneapolis, U.S.A.) and the measured values calculated with respect to the actual hematocrit values of individual patients (evaluated standard hematocrit values = 0.40).
Results: The most prominent finding was the simultaneous marked increase of plasma levels of two cytokines - of proinflammatory cytokine IL-6 and antiinflammatory acting cytokine IL-10 at the first post-transplant day (the level of IL-10 rose more than ten times, that of IL-6 even up to 18 times - both with statical significance p 0.001) followed with a strong level decrease on the third day after surgery. During the whole follow-up any of both cytokines did not reach the initial levels. The increase of plasma levels of other two analyzed cytokines (TNF and IL-8) was not so expressive as in the case of both formerly mentioned cytokines..
Conclusion: The results have shown that heart transplantation evokes systemic inflammatory reaction demonstrated by very strong increase in the production and release of proinflammatory cytokine IL-6 which was accompanied with simultaneous rise of release of an antiinflammatory cytokine IL-10. We suggest that this situation demonstrates a compensatory mechanism that may reflect the effort to maintain the balance between pro- and antiinflammatory acting cell mediators.

LIVER FUNCTION AFTER TRANSPLANTATION: ROLE OF THE DONOR HISTOLOGICAL FEATURES

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Background: Donor liver steatosis has been shown to influence early graft function but it is not clear if it can interfere with the long term histological features after liver transplantation (LT).
Aim: To evaluate the influence of donor graft histology on histological alterations after LT according to different etiology of primary liver disease.
Materials and methods: 151 consecutive liver transplants performed in 151 patients (98 M, 41 F; medium age 46.8 years, range 19-66) were studied. Donor liver biopsies were obtained after hepatectomy and before graft implantation; hepatocyte fatty infiltration, hydropic degeneration, glycogen content and lipofuscin/haemosiderin deposition were scored (0-3). All patients underwent protocol liver biopsy at 6, 12, 24, 36 months after LT. The recurrence of primary liver disease was evaluated by Ishak's score for HCV, Scheuer's score for HBV, International Group-Lancet 1981 score for alcohol and Scheuer's score for cholestatic liver disease. Chronic hepatitis was classified as mild, moderate or severe. Correlations between histological features of the liver before and after LT were obtained by Rho Spearman test. Patient survival up to 10 years was assessed by using the Kaplan Meyer method.
Results: A positive correlation was found between donor graft steatosis and histological alterations in HBV patients at 12 and 24 months (p=0.032) and in HCV-alcohol patients at 36 months (p=0.034) after LT. Donor graft steatosis did not correlate with 10 years cumulative patient survival.
Conclusion: steatosis in the donor graft may influence the liver histology in patients transplanted for viral and/or alcoholic liver disease without interfering however with the long term survival.

IS THERE STILL A PLACE FOR NEOPTERIN AS REJECTION MARKER?

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Macrophages play key regulatory functions in organ transplantation. Neopterin is secreted by interferon-activated human macrophages and therefore, a marker for the activation of cell-mediated immune response [1]. In 1983, Margreiter *et al* [2] described neopterin as a valuable immune activation marker in kidney graft recipients. Since then, numerous studies by various groups reported the same finding in many different transplantation settings.

Most previous reports studied the ability of neopterin in organ transplantation in a population-based statistical manner; i.e., the ability of neopterin to contribute to diagnoses such as rejection or infection episodes was judged for groups of patients by conventional statistical techniques. In slight contrast, we have investigated neopterin and other possible candidate markers with the aim to establish a basis for a non-invasive postoperative monitoring of graft recipients for individual patients, resting on a solid statistical foundation.

Laboratory measurements are recorded daily postoperatively. On every day, an experienced clinician classifies the patient according to on of several clinical states: stable function, acute rejection, viral infection, viral disease, bacterial infection. These data constitute the basis for a generalized likelihood ratio model [3] which allows to compute post-test probabilities for all chosen disease states, including pre-test probabilities for each of these states. Thus, for an individual patient, based on his or her individual constellation of laboratory markers on a certain day, we are able to estimate his or her probability to exhibit one of the given clinical states.

Application of this approach to a data basis stemming from 64 recipients of kidney allografts revealed that among the tested laboratory analytes, urinary neopterin is particularly well-suited to detect phases of viral infection / viral disease. In contrast, the day-to-day percent change of serum amyloid A (SAA) is better suited to diagnose acute rejection periods; finally, high absolute concentration of SAA indicates bacterial infection. A combination of these laboratory variables offers a useful model for differentiating these various disease states.

The model we propose, though computationally involved (which in these days of cheap high performance computers is not really a problem) offers wide flexibility because in principle it allows inclusion of multiple laboratory and other clinical information; it can handle multiple diagnostic categories and it allows the inclusion of pre-existing knowledge about the individual patient's probability of being in either of the diagnostic categories.

An extensive discussion of the model as well as data about the actual application in renal transplantation are available [4].

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CHARACTERISATION OF STEM CELLS

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In recent times much attention has been paid to the topic of stem cells including description of their characteristics, possible clinical applications and ethical questions arising from manipulation with them. Stem cells are precursor cells that have two important characteristics that distinguish them from other types of cells: They are unspecialized cells able of renewing themselves for long periods and they can differentiate into specialized cells and multiple tissue types.
There are two classes of stem cells: adult and embryonic stem cells. Adult stem cell is undifferentiated cells found in various tissues. Examples are hematopoietic stem cells from bone marrow, stem cells from umbilical cord blood and neural stem cells. Embryonic stem cells are primitive undifferentiated cells derived from 4 days old blastocysts, which have potential to become all kind of specialized cells.
Two methods of characterization for stem cells are used:
1. Detection of genes as markers for stem cells: Polymerase chain reaction is used to detect the presence of genes and transcription factors that are unique in stem cells. Replicates (mRNA) of stem cell samples are isolated, amplified probes are prepared by in vitro transcription and then hybridized to DNA microarrays. Recently a group of Harvard scientist established transcriptional profiles in embryonic, neural and hematopoietic stem cells: each stem cell type can clearly be identified by highly enriched genes that are not present in other stem cells. About 216 genes are active in each of the three types of stem cells. In hematopoietic stem cells 1977 genes have been identified, more than 600 of them show overlap with either embryonic or neural stem cells exhibiting 1787 or 2458 genes respectively.
2. Detection of surface markers by flow cytometry. There are several surface antigens presented by stem cells that give possibility to characterize and separate these cells. Hematopoietic stem cells express CD34, the adhesion structure which binds 2L-selectine, CD117, a receptor for stem cell factor (c- kit ligand), CD133 belonging to the family of mucoproteins and the panleukocyte marker CD45. However, several subpopulations, depending on presence or absence of mentioned CDs molecules can be identified. It is considered that CD133+/CD34- cells are the ones with the highest transdifferentiating capacity, which is dropping by decrease of CD133 expression and increase of CD34 expression. Isolation of hematopoietic stem cells from umbilical cord blood and bone marrow using a magnetic separation system against CD 133 antibodies results in 92,88 ± 4,75% purity. Flow cytometry analysis of purified samples showed that a majority of cells was CD34+, (90,02 ± 6,38%) and 1,34±1,27% of them were CD34-. The presence of CD117 on isolated cells surface was variable. Lineage markers were negative. Cells cultivated for four weeks in liquid medium containing serum, IL-3 and stem cell factor (SCF) showed a remarkable increase in cell number (2-20 fold) and a differentiation towards granulocytes and monocytes. From these preliminary in vitro data as well as from in vivo results it might be concluded that various cultivation conditions will enable engineering of stem cells towards major differentiated lineages to be used therapeutically for replacement of damaged cells.

EFFECT OF LIVER TRANSPLANTATION IN CEREBRAL BLOOD ALTERATIONS IN LIVER CIRRHOSIS OF DIFFERENT ETIOLOGY

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Background: brain imaging tecniques show impairment of cerebral blood flow in patients with liver cirrhosis. Few data on the role of etiology of liver disease on cerebral function impairment are reported. Liver transplantation (LT) should ameliorate brain function impairment.
Aims: 1. To evaluate whether the etiology of liver disease may produce different patterns of regional cerebral blood flow (rCBF) abnormalities. 2. To evaluate whether these alterations are reversed by successful liver transplantation.
Materials and Methods: 50 patients (28 males, 22 females, mean age±SD=44.6±8.8 years) with end stage liver disease were studied; 10 age matched subjects admitted to Neurology Department for headache were used as controls. Including criteria were absence of acute encephalopathy, focal brain lesions, severe brain atrophy or any abnormalities on CT scan suggesting other CNS diseases. rCBF assessed by using Single Positron Emission Tomography (SPECT) with 99m-Tc-hexamethylpropyl-eneamineoxime (99mTc-HM-PAO) as a tracer was performed in all patients and controls. The Mann Whitney U test was used for statistical analysis.
Results: The etiology of liver disease was as follows: alcoholic in 19 (A), viral (HBV, HDV, HCV) in 14 (V), mixed (A+V) in 5, cholestatic (PBC, PSC) in 12 (C) patients. SPECT showed significant reductions of rCBF in most cortical and subcortical regions in cirrhotic patients compared to controls. No statistically significant differences were seen among patients grouped according to the etiology of liver disease. When patients were divided according to previous alcohol intake (alcoholic group=A and A+V vs non alcoholic group=V and C), rCBF was significantly reduced in frontal superior ($p=0.05$), medial ($p=0.04$) and temporal superior ($p=0.02$) in the alcoholic compared to the non-alcoholic group.
Six patients died before LT, 5 are still in the waiting list. Thirty-nine patients underwent LT but 13/39 (33%) died within one year after surgery. Fourteen out of the remaining 26 long-term surviving patients repeated SPECT examination 12 months after LT. The etiology of previous liver disease in transplanted patients was alcoholic in 7 and non-alcoholic in 7. After LT there was a significant ($p<0.05$) increase in relative mean cortical tracer activity in most of the assessed areas, compared to pre-transplantation. In patients with previous alcohol intake rCBF was still impaired in the frontal inferior ($p<0.05$), frontal medial ($p<0.05$) and occipital superior ($p<0.05$) regions compared to non-alcoholic patients.
Conclusions: 1. Cerebral blood flow is impaired in patients with liver cirrhosis. 2. The viral or cholestatic etiology of liver disease does not produce different patterns of brain function abnormalities. 3. Alcohol intake is mainly associated with impairment of frontal areas which does not seem to be comletely reversed by liver transplantation.

THE ROLE OF CD25 REGULATORY CELLS IN AUTO- AND ALLO-IMMUNITY AND THEIR INTERACTION WITH THE INNATE IMMUNE SYSTEM

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The precise mechanisms that maintain immune tolerance towards autoantigens in healthy individuals (thus preventing autoimmune disease) or foreign alloantigens in transplant recipients (preventing graft rejection) remain incompletely understood. Experimental model systems have demonstrated that there are numerous non-mutually exclusive mechanisms that act on T lymphocytes and mediate such a state of unresponsiveness. These include deletion of the pathogenic T cells, T cell anergy, and immune regulation of effector T cells by soluble factors, or by professional regulatory cells. Understanding the contribution that each of these makes in human disease states is of fundamental importance for the development of strategies to achieve clinical tolerance, following its breakdown in autoimmune diseases or after transplantation.

Recently, a number of investigators have discovered a unique population of regulatory T cells, expressing both CD4 and CD25 (the IL-2 receptor α chain) which are hyporesponsive and have potent immune regulatory effects both *in vitro* and *in vivo*.

In animal models these cells can efficiently abrogate autoimmune diseases and prevent transplant rejection. Moreover, their counterparts have been described in healthy human subjects. Further characterisation of these cells has been attempted to identify a unique marker, since only a proportion of $CD4^+CD25^+$ cells mediate immune regulation. Recent data suggests that the foxp3 gene product may prove to be a specific marker of the regulatory cell subtype.

We have studied the role of these cells in human autoimmune renal disease and following renal transplantation. We have found that a proportion of patients have such a population, which can regulate the responses of T cells to auto- and allo-antigens, with some degree of specificity. The exact mechanism of action of these cells remains incompletely understood, and we have investigated the interaction of these cells with the innate immune system. Furthermore we have investigated whether these cells could be used therapeutically by expanding them in vitro, with a view to re-introducing them into patients.

OUTCOME OF LUNG TRANSPLANTATION AND LABORATORY ASSESSMENTS

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Lung transplantation (LTx) represents a "therapeutic" process which is necessary only when the lung is in an end-stage lung disease.
Bronchiolitis obliterative syndrome (BOS) is the major complication limiting the survival of lung transplant recipients. This chronic rejection is characterized by a progressive decline of the pulmonary function due to a high flow limitation caused by inflammation principally in the small air-ways usually referred to BOS.
The transplant outcome, giving early laboratory markers of rejection and therapy signals suitable for the correct treatment, are limited and mainly related to bronchoalveolar lavage (BAL) and lung biopsy (LB). Furthermore these investigations are not early predictive indicators.
Aim of present work is to review the potentials of clinical biochemistry markers suitable for testing lung function and the health of the transplanted patient. Up to now, in literature, the biochemical markers are restricted to very few and tentative researches. They are the measurement of exhaled nitric oxide (NO) and the metalloproteinase (MMPs), the tumor necrosis factor α (TNFα) and interterleukin-10 (IL-10) in sputum. Molecular biology has also been introduced into this field, but the gene polymorfisms in the development of BOS demonstrate a significant increase of this BOS risk.
Among the various potential biochemical signals the neopterin, oxidative stress markers, growth factors, circulating interleukin levels can be a suitable laboratory approach.
The traditional laboratory markers are not habitually in use and a suitable outline of the various potential biochemical investigations, also in the circulation, could be further improved by considering the potentiality that this study approach represents a new frontier of the LTx outcome. The potential role of these markers should also help in giving relatively low invasive intervention.

BONE MARROW STEM CELL TRANSPLANTATION FOR MYOCARDIAL REGENERATION IN POST-INFARCTION CABG PATIENTS

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Objective: The myocardium consists of terminally differentiated cells without clinically relevant potential for regeneration. However, experimental evidence suggests that adult stem cells may be able to regenerate infarcted myocardium, but feasibilty and safety of stem cell transplantation in human heart is unknown. We initiated a phase-I study of human bone marrow-derived stem cell transplantation in conjunction with coronary artery bypass grafting (CABG).
Methods: Fifteen patients (age 65±4 years) have been enrolled in the study since July 2001. Principal inclusion criteria were 1, Acute myocardial infarction >10 days and <3 months ago; 2, Presence of a distinct area of infarcted myocardium not amenable to surgical or interventional revascularization; 3, Elective CABG operation indicated to treat ischemia of other areas of myocardium; 4, Patient consent and approval by the internal review board. Autologous, pluripotent stem cells were isolated from bone marrow aspirate using antibody against the stem cell-specific surface marker AC133. The following day, CABG surgery was performed and up to 1.5x10E6 AC133+ cells were injected directly into the infarct border zone.
Results: All patients survived the operation without major complications and have been discharged with significantly improved NYHA class. Follow-up currently ranges between 6 weeks and 18 months. Echocardiography at follow-up demonstrated significantly improved global left ventricular function (LVEF, LVEDD, LVESD), while contractility in the center of the preoperatively akinetic infarct zone remained disturbed. Myocardial perfusion was assessed by scintigraphic imaging, and we saw markedly improved perfusion of the previously hypo- or non-perfused infarct zone, as well as evidence of improved tissue viability. To date, there is no evidence of new ventricular arrhythmia, neoplasia, or other treatment-related complications.
Conclusions: Transplantation of autologous, adult stem cells for myocardial regeneration can be safely performed in humans. There is evidence of neoangiogenesis in infarct tissue, associated with improved left ventricular contractility. However, controlled studies are needed to determine the true efficacy.

PANCREAS TRANSPLANTATION

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Vascularized pancreas transplantation (PTx) has been considered an experimental treatment option for patients with type I diabetes mellitus for more than two decades since its first description in 1967. This negative image was predominantly based on the fact that graft survival rates were inferior as compared to other organs due to immunological and technique related complications. Successful PTx currently is the only known therapy that establishes an insulin-independent euglycemic state with complete normalization of glycosylated hemoglobin levels.
As of the end of 2002, more than 18,000 pancreas transplants worldwide have been reported to the International Pancreas Transplant Registry (IPTR) with a one -year patient, pancreas and kidney graft survival for combined kidney-pancreas cases (SPK) of 95%, 82% and 92%, respectively. This remarkable improvement in graft outcome was mainly achieved by a reduction of technical failures and more powerful immunosuppression. Various maintenance immunosuppressive regimens including the new substances Tacrolimus (FK-506), MMF (CellCept) and Sirolimus resulted in dramatic improvement of pancreas graft survival in solitary pancreas transplant recipients. The majority of transplant centers use enteric drainage (ED) with either systemic or portal venous drainage for diversion of the pancreatic juice.
The following review summarizes currently employed techniques for pancreas transplantation, results and trends focussing on our own experience with more than 300 pancreas transplants.

HOW ASSAYS, DRUG METABOLISM AND GENES INFLUENCE CYCLOSPORINE A WHOLE BLOOD CONCENTRATIONS IN PATIENTS AFTER ORGAN TRANSPLANTATION

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Cyclosporine is a widely used and potent immunosuppressive drug with a narrow therapeutic index. Therefore cyclosporine concentrations need to be closely monitored. Various automated immunologic assays for cyclosporine whole blood monitoring are available. In the routine laboratory these assays have to be accurate and have to have a short turn around time. Therefore monitoring of cyclosporine levels is performed in the routine laboratories mainly by nonisotopic automated methods using specific antibodies. Although the reference method for cyclosporine measurements is high performance liquid chromatography (HPLC), due to the difficult handling procedure HPLC is not often used in the daily routine. However, immunological methods cross react with various metabolites of cyclosporine, such as mono- or dihydroxylated (AM1, AM9, AM19) or demethylated (AM4n) metabolites. Due to different cross reactivity patterns immunological methods yield different results for cyclosporine whole blood concentrations. Moreover different metabolizing patterns of cyclosporine in different patient groups over the time have to be considered when interpreting results obtained by immunological tests.

We have therefore compared various immunological tests for cyclosporine with the reference method (HPLC) and found significant differences regarding their respective cross reactivity with cyclosporine metabolites. In particular the metabolite AM9 yielded cross reactivities between 9,2 (FPIA, AxSYM) and 23 (CEDIA) %; AM1 cross reacted in the FPIA, TDx with 7.1 % whereas in the EMIT assay this reaction was below 1 %.

In addition, immunologically measured cyclosporine concentrations differed significantly immediately after transplantation and during the late post-transplantation periods. Metabolism was also different when various transplant groups (i.e. kidney, bone marrow, heart-lung, liver transplantation) were compared with each other. This caused significantly different metabolite concentration. Depending on the cross reactivities, different results for whole blood concentration, when measured by immunological methods, were determined. Our results indicate that cross-reactivities might therefore cause overestimation of cyclosporine concentrations. In addition different whole blood concentrations can be expected depending on the duration of the post transplant period and on the type of transplantation performed.

In a second set of experiments pharmacogenetic testing was performed on kidney transplanted patients. Cyclosporine whole blood concentrations immediately after transplantation were compared with the genetic informations. We determined the C3435T polymorphism in the multidrug resistance gene and compared the genetic findings with initial plasma levels of cyclosporine A. In intermediate and high expressing patients (wild type and heterozygote mutants) plasma levels were slightly, but significantly lower than in low expressing homozygote mutants. However, these differences can later be easily adjusted, indicating a limited value of pharmacogenetic testing before cyclosporine treatment.

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INFECTION AND REJECTION - THE ROLE OF CYTOMEGALOVIRUS INFECTION

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After renal transplantation, episodes of Cytomegalovirus (CMV) infection and acute rejection (AR) play an important role in the pathogenesis of chronic rejection (CR).
Other independant risk factors are: older donor age, conservation injury, hypertension and metabolic disorders.
In view of the fact, that the long term graft survival outcome after transplantation with living donors is significantly superior compared to cadaveric donors, our study was established to investigate the role of acute rejection and Cytomegalovirus infection within the first 90 days after kidney transplantation.
Two different groups of dialysis patients, the first with grafts from cadaveric donors (n = 216, mean age 46,5 yrs) and the second with kidneys from living donors (n = 50, related (24) and unrelated (26), mean age 49,2 yrs) were compared with the special aspect of the incidence of acute rejection and / or Cytomegalovirus infection episodes.
Rejection episodes were elaborated by core biopsy, Cytomegalovirus infection episodes were diagnosed by detection of pp 65 antigenemia in leucocytes without (asymptomatic infection) or with clinical symptoms (symptomatic infection / disease).
In the group of cadaveric donors, the incidence of acute rejection was 33%, the incidence of viral infections was 24 % , in 6 % rejection and virus infection were observed in a close coincidence, while 49 % of the patients presented neither a rejection nor a viral infection episode.
In the group of living donors, the incidence of acute rejection was 56 %, viral infections were seen in 6 %, and the coincidence of rejection and viral infection in 2 %, while neither rejection- nor infection episodes were observed in 36 %.
The clinical outcome of graft function after 90 days resulted in plasma creatinine values of 1,46 mg/dl in the first and 1,22 mg/dl in the second group respectivly.
Taking in account, that rejection and Cytomegalovirus infection are strong independent risk factors of chronic rejection, the early appearence of Cytomegalovirus infection episodes is fourfold higher and the coincidence of rejection and CMV infection is three times higher in patients with grafts of cadaveric orign, while rejection episodes alone are observed more frequently after living donation (dependent on the number of unrelated donors).
It seems to be obvious, that the early damage of the graft (long cold ischemia period,perfusion/reperfusion damage local inflammation,TNF alpha generation and vascular endothelial injury) results in a significantly higher rate of infection and /or rejection episodes instead of regeneration of the vascular integrity.
Close monitoring of the posttransplantation course is necessary for early detection of clinically subacute rejection - and / or silent Cytomegalovirus reactivation, when the development of chronic rejection should be slowed down successfully.

ACTUAL STATE AND PROBLEMS IN XENOTRANSPLANTATION

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Transplantation of animal organs into patients appears to be an option to overcome the worldwide shortage of allo-grafts. Due to several reasons (litter-size, growth, body weight etc.) the pig seems to be the species of choice to become an organ donor for man. However, preformed, xenoreactive antibodies present in the blood of man initiate a very aggressive type of rejection by binding to epitopes of a carbohydrate (gal-α 1,3-galactose) expressed on the porcine endothelium. Binding of the antibodies leads to the activation of the complement system as well as of white blood cells and thrombocytes. The endothelium changes its state from being anti-coagulatory to being pro-coagulatory. The microcirculation of the xeno-graft becomes disturbed by clots occluding vessels thereby causing hypoxia and finally necrosis of the tissue. These processes start with the very first contact of the human blood with the endothelium and lead to the hyperacute rejection of the graft within minutes to a few hours.
The use of organs from pigs transgenic for human complement factors such as CD59 or human Decay Accelerating Factor (hDAF) in combination with different immunosuppressive regimens has shown that hyperacute xeno-graft rejection can be overcome depending on the kind of organ transplanted. Non-human primates survived for up to 39 and 78 days respectively after orthotopic xenotransplantation of porcine hearts or kidneys. In contrast to this only one baboon survived orthotopic porcine liver transplantation for 8 days and none survived lung xenotransplantation for more than 24 hours.
When all rejection types (hyperacute, delayed and chronic xeno-graft rejection) are overcome, the question arises to which extent a porcine organ is able to fulfil the physiological requirements of the human metabolism. It is known from experiments and a clinical case that for example the liver of one species does not completely adapt to the new host after transplantation into another species. Starzl (1) and Ramirez (2) reported the molecular structure of coagulation and complement factors to stay donor specific upon xenotransplantation. Cynomolgus monkeys receiving porcine kidneys in a life-supporting model developed anaemia although the kidneys produced porcine erythropoetin and the animals were given human recombinant erythropoetin daily. The authors suggest that this finding was due to a physiological incompatibility of the different erythropoetins and the receptors on the target cells of the monkeys (3). However, the question whether physiological incompatibility will prevent successful xenotransplantation can only be investigated when long-time survival after xenotransplantation is reality. Another field which necessitates long-time survival to allow satisfactory investigation is microbiology. The hazard of infecting the patient with endogenous porcine germs together with the xeno-graft is nowadays of minor relevance because on the one hand porcine breads exist which are free from porcine endogenous retro viruses (PERV) on the other hands no reports of infections with PERV after clinical transplantation of porcine islets, skin or valves exist. However up to now nothing is know about the consequences of an infection with porcine germs after successful xenotransplantation. The patient will than harbour a porcine organ which will probably offer porcine germs - which can infect man but are not able to persist - the opportunity to persist in man. In the worst case porcine viruses could mutate into new germs possibly hazardous to the patient and - when excreted - hazardous to the patient's environment as well. There has been immense progress in the field of xenotransplantation during the past decades. Still, a lot remains to be done before xenotransplantation becomes an option for the clinic.

References

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ASSOCIATION BETWEEN NEOPTERIN PRODUCTION AND INDOLEAMINE (2,3)-DIOXYGENASE ACTIVITY

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Th1-type cytokine interferon-(IFN)-γ induces neopterin production in human monocyte-derived macrophages. In parallel, IFN-γ stimulates indoleamine (2,3)-dioxygenase (IDO) in various cells resulting in the formation of kynurenine and other products at the expense of tryptophan. The kynurenine per tryptophan ratio (kyn/trp) allows to estimate IDO activity. Likewise, in diseases linked with Th1-type immune activation, increased neopterin concentrations usually correlate well with kyn/trp. However, the same tryptophan degradation pathway is also taking place in the liver by tryptophan pyrrolase which is independent from cytokine stimuli. Thus, whenever increased kyn/trp is detected in the circulation, a possible involvement of activated IDO can be confirmed when elevated kyn/trp coincides with increased concentrations of markers of immune activation such as neopterin.
Due the immunobiological link, increased neopterin concentrations and kyn/trp in patients with, e.g., virus infections, autoimmune diseases or cancer are associated with decreased tryptophan concentrations. Insufficient availability of essential amino acid tryptophan may influence various biochemical pathways in which tryptophan is involved, e.g. protein biosynthesis and thus cell growth and proliferation, and activated IDO appears also to be involved not only in the development of T-cell unresponsiveness, but also in the pathogenesis of cachexia and weight loss as well as anemia in such patients. In parallel, the production of neurotransmitter serotonin, which is bionsynthesised from tryptophan, will be affected. Indeed in several clinical conditions and during cytokine treatment, increased neopterin concentrations coinciding with accelerated tryptophan degradation were found to correlate with the development of neuropsychiatric symptoms. Data indicate a possible role of immune activation and tryptophan depletion especially in the pathogenesis of specific symptoms such as memory loss, dementia and depressive mood.