Immunotherapy and beta-cell replacement in type I diabetes mellitus

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Chapter 1

General introduction and outline of the thesis

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TYPE 1 DIABETES MELLITUS

Type 1 diabetes mellitus (T1D) results from an auto-immune mediated destruction of the pancreatic beta-cells in genetically predisposed individuals. Auto-reactive T-cells are considered to play the key role in the development of the disease (1-7). The devastating complications of hyperglycemia including nephropathy, neuropathy, retinopathy and cardiovascular disease can diminish both quality of life and life expectancy. Exogenous insulin administration is the therapy used in T1D patients. The Diabetes Control and Complications Trial has demonstrated that intensive insulin therapy results in significantly lowered glycosylated hemoglobin (HbA1c) levels and prevent or delay the progression of these secondary complications. However, intensive insulin therapy could not halt the development of secondary complications in all patients, and significantly increased the risk of severe hypoglycemic episodes leading to seizure or coma (8). Although insulin therapy is the therapeutic option of choice, it remains a symptomatic approach and more physiological regulation is required. Since auto-reactive T-cells play a crucial role in the pathogenesis of T1D, T-cell immunomodulation might be an attractive strategy in the near future to ultimately cure T1D.

BETA-CELL REPLACEMENT THERAPY

At present beta-cell replacement is the only available therapy to cure patients from T1D. Pancreas transplantation has been performed since 1966. Due to increasing experience with technical aspects of the transplantation and the development of better immunosuppressive drugs solid-organ pancreas transplantation has been the most consistent method of beta-cell replacement. These developments resulted in sustained insulin independence, and normalization of HbA1c levels. Pancreas graft survival rates currently exceed 80% three year after transplantation (9-12). Controlling alloreactivity is thought to be of grave importance for successful pancreas transplantation outcomes. Current immunosuppressive regimes are mainly based on this perception. In diabetic pancreas transplant recipients recurrence of autoimmunity is of some concern. Recurrence of autoimmunity was described previously in pancreas grafts (7).

The invasive nature of the surgery and long-term complications of immunosuppressive agents limited this procedure to patients with end-stage renal failure, hypoglycemic unawareness or metabolic lability despite intensive insulin therapy. However, a relevant proportion of potential recipients for whole pancreas transplantation are not able to undergo the stress of the operative procedure because of advanced cardiovascular disease. Transplantation of pancreatic islets provides an attractive and less invasive alternative to whole-organ pancreas transplantation. After procurement the islets are transplanted

into the portal vein and get stuck in the vascular system of the liver. The primary aim of islet transplantation is to provide a safer technique to justify earlier intervention prior to the development of secondary complications. Secondly, it gives the possibility of beta-cell replacement for patients with life-threatening diabetes who cannot handle the physical stress of whole-organ pancreas transplantation. In addition to avoiding surgical complications such as arterial or venous graft thrombosis and problems related to the exocrine duct management, pancreatic islet transplantation provides the opportunity to manipulate the islets prior to transplantation in order to decrease immunogenicity of the donor pancreas graft (13,14). Other sources, such as stem cell, precursor cell derived beta-cells or xenotransplantation (15) are necessary to answer the increasing demand for beta-cell replacement therapy. Such cells might have the potential to provide an unlimited source of beta-cells independent of the limited donor pool. Allogeneic islet transplantation into a T1D recipient is also complicated by the necessity for preventing destruction of beta-cells in the islets of Langerhans. A previous study in patients receiving islet transplantation demonstrated no signs of alloreactivity, and only a marginal increase in autoreactivity to islet autoantigens in three cases that remained C-peptide-positive for more than one year. In contrast, rapid failure (<3 weeks) in three other cases was accompanied by increases in precursor frequencies of graft-specific alloreactive T-cells. One recipient had a delayed loss of islet graft function (33 weeks); he did not exhibit signs of graft-specific alloimmunity, but developed a delayed increase in autoreactivity (5).

CURRENT IMMUNOSUPPRESSIVE DRUGS

Currently used immunosuppressive drugs elicit different spectra of adverse effects. Major concerns are dealing with infectious complications, cardiovascular problems and the development of immunosuppressive related malignancies (37,38). The urge for mild immunosuppressive regimes becomes more important with the increasing patient and graft survival rates in solid-organ transplant programs and in case of considering the use of immunosuppression to treat auto-immune diseases. Knowledge of the mechanism of action and side-effects is crucial in developing new and mild therapeutics. Currently used immunosuppressive drugs are discussed below. Corticosteroids, calcineurin inhibitors, antiproliferative agents and antibody therapeutics will be discussed briefly (39) (Table 1 and Figure 1).

Table 1.

Immunosuppressive agents
Pharmacologics:
- Corticosteroids
Prednisolone
- Immunophilin binding drugs
Calcineurin inhibitors (cyclosporin, tacrolimus)
Calcineurin independent (Sirolimus)
- Inhibitors of cell division/nucleotide metabolism
Non-selective antiproliferative/cytotoxic drugs (Azathioprine)
Lymphocyte selective drugs (Mycophenolate mofetil)
Biological agents:
- Polyclonal antibodies
ATG
- Monoclonal antibodies
anti-CD3, anti-CD25

Corticosteroids

Corticosteroids are nonspecific anti-inflammatory agents. They reversibly block cytokine and cytokine-receptor expression due to activation of both T-cells and APC's (40,41). Corticosteroids are hydrophobic molecules, which makes them easily diffuse into cells and bind to cytoplasmic corticosteroid-receptors (42). The corticosteroid-receptor complexes translocate to the nucleus and attach to glucocorticoid response elements in the promoter regions of cytokine genes, thereby inhibiting the transcription of certain cytokine genes (42,43). Cytokine production of macrophages is also inhibited. This leads to inhibition of chemotaxis and phagocytosis. As a result of these actions steroids affect the whole range of steps of the T-cell activation process. Due to their non-specific immunosuppressive character and hormonal actions on different tissues steroids have many side effects: in addition to resulting in cushing syndrome, weight gain with central obesity, hypertension, dyslipidemia, peptic ulcer formation and gastrointestinal bleeding, pancreatitis, personality changes, cataract formation, and osteoporosis with avascular necrosis of bone, steroids also lead to hyperglycemia progressing to steroid diabetes (39). The latter phenomenon is especially important in the pancreas and islet transplantation setting.

This wide range of side effects makes immunosuppressive regimes without corticosteroids desirable.

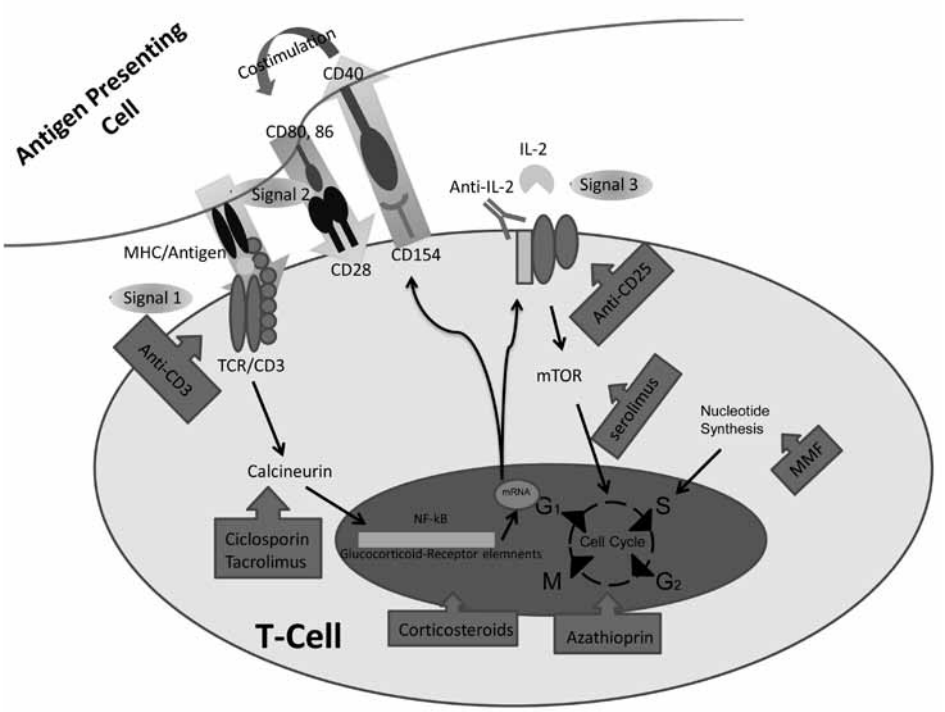

Figure 1.

Immunophilin binding drugs

Calcineurin inhibitors

Cyclosporine, a small fungal cyclic peptide, and tacrolimus-FK506, a macrolid antibiotic, have contributed enormously towards the improved results in solid organ transplantation. Both cyclosporine and tacrolimus form complexes with proteins called immunophilins. Cyclosporine binds to the so-called cyclophilins, which are present in all tissues, and tacrolimus complex with FK506 binding proteins (FKBP) (44). These complexes bind to calcineurin, and thereby inhibiting IL-2 production of CD4-cells. This leads to the inhibition of CD4-T-cell proliferation (44). Lymphocytes show greater sensitivity to cyclosporine and tacrolimus compared to other tissues. This might be caused by the limiting amounts of calcineurin in T-cells compared to other tissue (39).

Due to their non tissue-specific characteristics cyclosporine has many side-effects including acute and chronic nephrotoxicity, hypertension, increased incidence of deep venous thrombosis, tremor, headache, convulsions, and paresthesias of the limbs, hyperkaliemia and dyslipidemia. Important in T1D patients is the effect of cyclosporine on the glucose metabolism causing hyperglycemia, which is reversible. Also gingival hyper-

plasia and hypertrichosis are cyclosporine associated side effects, which are reversible by conversion to tacrolimus (39,45).

Tacrolimus is a more potent drug compared to cyclosporine (46). Tacrolimus induces a higher incidence of diabetes mellitus and neurotoxic reaction. The incidence of hypertension, hyperlipidemia, hirsutism, and gingival hyperplasia is lower compared to cyclosporine (47). Influencing the IL-2 pathways important in (re)activation of the immune system remains an interesting goal in the development of adequate immunotherapeutic strategies for transplantation and recent-onset T1D patients.

Calcineurin independent

Sirolimus, a macrocyclic antibiotic, is a highly potent immunosuppressive agent. Although it is structurally related to tacrolimus, the mechanism of action is different. Sirolimus prevents progression of T-cell from the G1 to the S phase by blocking signalling downstream IL-2R (56). Sirolimus inhibits T-cell response to cytokines and blocks lymphocytes proliferation at a point upstream from the antiproliferative agents. Therefore sirolimus should be synergistic with antiproliferative agents and calcineurin inhibitors. Side effects include thrombocytopenia, and leukopenia and hyperlipidaemia (39). In the successful Edmonton protocol for islet transplantation the immunosuppressive protocol consisted of daclizumab, tacrolimus and sirolimus (14).

Inhibitors of cell division/nucleotide metabolism

This category includes drugs interfering with the proliferative machinery of proliferating cells including auto- and allo-reactive T-cells. Although 6-mercaptopurine, cyclophosphamide and metotrexate effectively inhibit lymfocyte proliferation, their use is limited because of their non-selective mechanism of action leading to toxicity to many tissue types. Azathioprine , mycophenolate mofetil and sirolimus are the currently used antiproliferative agents. Both Azathioprine and mycophenolate mofetil interfere with the purine synthesis. Sirolimus blocks signalling downstream IL-2 receptor (39).

Non-selective antiproliferative/cytotoxic drugs

Azathioprine was used together with corticosteroids in the pre-cyclosporin era. Azathioprine can be incorporated into nucleic acids where it causes feedback inhibition of purine metabolism. In addition several enzymes are inhibited leading to reduction in the synthesis of cellular DNA, RNA and proteins. Through these actions azathioprine blocks most T-cell functions, inhibits primary antibody synthesis, and decreases the numbers of circulating monocytes and granulocytes (44,48). Azathioprine is effective in the prevention but not the treatment of acute rejection, because it has little effect on established immune responses (49).

The main side effect of azathioprine is bone marrow depression, leading to leukopenia. Anemia and thrombocytopenia may also occur (44,48). Pancreatitis is the most important gastrointestinal side effect of azathioprine (50). Due to immunosuppression and diminished immunosurveillance an increased incidence of neoplasia is associated with the use of azathioprine (44). Since mycophenolate mofetil (discussed below) does not seems to be associated with an increased risk of malignancy and is more effective in preventing allograft rejection, mycophenolate mofetil is currently favoured above the use of azathioprine in transplantation settings (38).

Lymphocyte selective drugs

Mycophenolate mofetil (MMF) is a selective inhibitor of the de novo purine synthesis, providing inhibition of T-cell and B-cell proliferation. Activated lymphocytes depend more on both salvage and de novo synthesis of guanosine nucleotides than other cell types. For this reason MMF has both a relative selectivity for activated lymphocytes and a down-regulating effect on antibody synthesis (44,51-53).

MMF elicit several side effects including gastrointestinal symptoms and viral infections. Leukopenia and thrombocytopenia are haematological side effects (44,51,54). Its long-term safety in humans has already been demonstrated when used for psoriasis as leukopenia was a very rare event with doses similar to those used in transplantation (54,55). Since MMF is currently investigated in combination with daclizumab (discussed later in the monoclonal antibody paragraph) for the treatment of recent onset T1D (www.diabetestrialnet.org), this drug is of great interest for the development of immunotherapeutic strategies.

Biological agents

Polyclonal antibodies

Polyclonal antilymphocyte globulins are gamma-globulin fractions of serum derived from animals inoculated with human lymphocytes, thymocytes or cultured lymphoblasts: namely rabbit antibodies to human lymphocytes (antilymphocyte globulin) and horse or rabbit antibodies to human thymocytes (horse antithymocytes globulin, rabbit antithymocyte globulin, and rabbit antithymocytes sera) (57,58). Polyclonal antibodies has several mechanisms of action. Polyclonal antibodies bind to T-cell antigens, which results to depletion of circulating T-cells. Complement-dependent cytolysis and cell-mediated opsonisation are proposed mechanisms resulting in this depletion. ATG Fresenius (anti-T-cell globulins) is expected (according to the manufacturer) to be more selective for activated T-cells compared to ATG Merieux (anti-thymocyt globulins). In a retrospective study ATG Fresenius was suggested to have lower long-term toxicity (59).

Polyclonal agents have several drawbacks. Their production technique leads to variable constituent of polyclonal antibodies. This unpredictability is associated with variable efficacy and side effects: increased risk of infection and lymphoproliferative disease due to overimmunosuppression, toxicity of heterologous serum prepared against human tissue, the presence of antibodies specific for nonlymphocyte antigen leading to thrombocytopenia, leukopenia, or anemia (39,60,61). Polyclonal antibody therapy is historically used in pancreas and kidney transplantation as induction therapy. Other induction protocols (e.g. monoclonal antibodies) should therefore preferably be compared to polyclonal induction protocols.

Monoclonal antibodies

The first monoclonal antibody (Moab) available for therapeutic use in the treatment and prevention of allograft rejection was OKT3 (Orthoclone OKT3; Janssen, Titusville, NJ), a murine Moab directed against the CD3 molecule, which is part of the TCR complex and functions to modulate the receptor and inactivate T-cell function. By binding to the TCR complex OKT3 blocks not only the function of naive T-cells but also the function of established cytotoxic T-cells (62). Adverse effects include a cytokine release syndrome (occurring in almost all patients and may be life-threatening) fever, chills, general weakness, hypotension and gastrointestinal side effects (63). Opportunistic infections like cytomegalovirus and the Epstein-Barr virus-associated posttransplant lymphoproliferative disorder have been reported after treatment with OKT3 (64). Because OKT3 is a murine antibody that is highly immunogeneic it commonly induces a human antimurine antibody response. Human antimurine antibodies can rapidly inactivate and eliminate mouse Moabs by neutralization (65). Given the central role of the T-cell in allograft rejection most new immunosuppressive strategies have sought to inhibit T-cell activation. Several ligand/receptor interactions occur between the T-cell and the APC during antigen presentation. A range of Moabs has been developed to block these interactions. IL-2R blockers are the most popular, because of their theoretically specificity for activated T-cells. Recently, humanized anti-CD3 antibodies (hOKT3g1(Ala-Ala) and ChAglyCD3) have been developed and used in anti-diabetic immunotherapeutic protocols (22,66,67).

The most recent generation of IL-2R blockers are chimeric or humanized antibody structures that contain both human and murine components reducing their immunogenicity (68,69).

The α-chain of IL-2R is not expressed on resting T-cells but is induced after activation and is necessary signal transducing (70). Because only activated T-cells express the IL-2R α-chain (CD25 antigen), anti- IL-2R Moabs are expected to target graft-reactive lymphocytes immediately after transplantation during induction therapy. An important characteristic of anti-IL-2R Moabs is that it does not inhibit IL-2 binding to the

IL-2R β-chain. The β- and γ-chains can be linked by the IL-2 without the participation of the a-chain if sufficiently high concentrations of IL-2 are present for long enough. The consequence is that anti-IL-2R Moabs are designed to be used in combination with a calcineurin-blockers (39). Although anti-CD25 Moabs induction therapy has been reported to reduce the number of acute rejections after transplantation it does not prevent T-cell reactivity completely because some of the treated allograft recipients still reject their graft (71-74). Daclizumab is an almost completely humanized anti-CD25 Moab. This antibody is well tolerated and reduces the incidence of infection or other adverse events (75).

Since OKT3 is a murine antibody that is highly immunogeneic it commonly induces a human anti-murine antibody response. Recent developments of humanized anti-CD3 antibodies are causing a revival of anti-CD3 therapeutic strategy. Treatment with the humanized anti-CD3 monoclonal hOKT3gamma1(Ala-Ala) delayed the deterioration in insulin production and improved metabolic control during the first year of type 1 diabetes mellitus in a pilot clinical trial. Recently, a phase 2 placebo-controlled trial with a humanized antibody, an aglycosylated human IgG1 antibody directed against CD3 (ChAglyCD3), involving 80 newly diagnosed patients, was conducted (67). Patients receiving ChAglyCD3 continued to produce their own insulin and needed less supplemental insulin to maintain normal blood glucose levels compared with patients who received a placebo. This benefit was apparent up through 18 months after the treatment, suggesting the protective effect is lasting, although for how long is not yet known. Moreover, side effects were minor and short-lived, including flu-like symptoms. A European multicenter randomized placebo-controlled trial testing ChAglyCD3 at clinical onset of T1D to preserve beta-cell function has recently been completed. Evidence of clinical benefit of hOKT3gamma1 (Ala-Ala) (anti-CD3) and ChAglyCD3 monoclonal antibody therapy in recent onset type 1 diabetes has already been reported in phase I studies.

Monoclonal antibody therapeutics are of particular interest due to their expected specificity compared to polyclonal antibody therapeutics.

The knowledge of the mechanisms of action of these drugs is often lacking, but is of grave importance for future developments of mild immunosuppressive regimes for the treatment and prevention of acute rejection in pancreas/islet transplantation or treatment of recent-onset T1D patients.

Both pancreas and islet transplantations provide us with unique possibilities for testing specificity of immunesuppressive regimens or tolerogenic strategies. Trials in both pancreas- and islet transplantation are exceedingly interesting for the development of specific immune-suppressive protocols or tolerance induction strategies applicable for recent onset T1D, because one has to deal with recurrent auto-reactivity too. During the last decades increasing experience has been obtained with immunosuppressive therapies. Successful clinical outcome and milder side effects due to more selective agents

could make the current immunotherapy used in transplantation settings more feasible in recent-onset T1D.

Control of allo- and auto-immunity is important for both pancreas and islet transplantations in order to succeed. The current immunotherapeutics used in pancreas and islet transplantation settings are effective in reducing the number of acute rejections episodes, but are largely based on prevention of rejection, rather than being anti-diabetogenic. To develop more selective and mild immunosuppressive agents to prevent both allo- and auto-immunity after pancreas and islet transplantation and for future immunotherapy in recent-onset T1D immunological monitoring of the effect of these agents is essential.

IMMUNOLOGICAL MONITORING

Immunological monitoring of trials dealing with immunotherapy in T1D and pancreas/islet transplantation is of grave importance to evaluate the reason for success or failure. This emphasizes the importance of searching for tools to unravel the immunological pathogenesis of T1D, determine disease activity and clinical efficacy of immune therapy in T1D and pancreas or islet transplantation.

The development of robust and reliable T-cell assays to detect auto-immunity in T1D has shown to be a difficult achievement (16,17). Since our T-cell assays pointed out to correlate very well with clinical outcome, important tools have become available to unravel the pathogenesis, determine disease activity and clinical efficacy of immune therapy in T1D and beta-cell replacement programs in T1D patients.

Suitable T-cell assays are necessary to test the capability of inducing specific suppression or even tolerance induction of different immunosuppressive therapies in recent onset T1D and after beta-cell replacement. To study immunological efficacy of immunotherapy in T1D and after beta-cell replacement surrogate markers for insulitis and disease recurrence or beta-cell graft function are needed.

Surrogate markers for insulitis in T1D

Antibodies: Islet auto-antibodies have been used to predict individuals at high risk for T1D (18). Although we reported that T1D could develop in a patient with X-linked agammaglobulinemia, implying that neither auto-antibodies nor B-cell function is critically involved in the pathogenesis of T1D (19), auto-antibodies provided tools to identify the candidate targets on the beta-cells. With the identification of candidate islet autoantigens recognized by auto-antibodies, the search for disease-associated T-cells was greatly influenced. Although the pre-onset predictive value of auto-antibodies is clear,

titres decline variably after onset of T1D. More importantly, islet auto-antibodies have been found not to change with interventions that have shown clinical efficacy (1,18,20-24). Consequently, auto-antibodies do not qualify as surrogate markers of either disease recurrence or preservation of beta-cell function in immune intervention studies in T1D.

T-cells: Although a recent T-cell workshop highlighted difficulties in reliability and reproducibility of cellular assays for proliferative responses to islet auto-antigens (16,17), T-cell assays have been developed for the detection of T1D disease activity and transplantation related immunity that correlate with clinical outcome (4,5). Auto-reactive T-cell reactivity against islet granules was associated with presence of insulitis in recent-onset diabetes, while established T1D and healthy controls showed intermediate and low reactivity respectively. Reactivity against the non-diabetic recall antigen Tetanus Toxoid was comparable for the three groups. This T-cell assay was able to distinguish between control and T1D patient groups (4). However, on the individual basis, T-cell proliferation assays did not qualify as diagnostic, due to the lack of reactivity in a considerable proportion of recent-onset patients (i.e., poor sensitivity), and increased responses to islet auto-antigen in non-diabetic subjects (i.e., insufficient specificity) that are inherent to this type of technology. Nonetheless, due to the limited inter-assay variation of the assay in a given individual, this methodology was shown to provide useful and interpretable information when applied longitudinally in the context of islet reconstitution therapy.

Surrogate markers for disease recurrence or beta-cell graft function

Autoimmunity

Antibodies: The usefulness of auto-antibodies in islet or pancreas transplantation setting is still controversial. A correlation between rising of GAD65 and IA-2 auto-antibody titres and graft loss due to recurrence of autoimmunity has been reported in pancreas transplantation (25,26). Earlier progressive islet graft failure has been observed in auto-antibody-positive compared to auto-antibody-negative recipients of islet allografts (27,28). Existence of auto-antibodies in our islet transplantation recipients did not correlate with rejection episodes or graft function (29). Further studies are necessary to establish the predictive value of these antibodies in islet and pancreas transplantation settings.

T-cells: With our auto-reactive T-cell assay recurrent auto-immunity after islet transplantation was associated with progressive loss of beta-cell function. Successful restoration of beta-cell function was accompanied by a lack of both allo-and auto-reactivity in our assays (5).

Alloimmunity

Antibodies: A correlation between islet allograft failure and increased titres of allo-antibodies has been reported (30). Nonetheless, existence of allo-antibodies in our islet

transplantation recipients is rare, despite implantation of islet allografts from up to 12 different donors.

T-cells: We have developed a limiting dilution analysis-assay that makes the determination of both helper and cytotoxic T lymphocyte precursor (CTLp) frequencies possible (31,32). This assay serves as excellent monitor of allograft rejection and immunotherapy in kidney, heart, cornea, liver and islets transplantation (33-36). Experimental conditions for the combined assay have been optimized to obtain cytotoxic T lymphocyte precursor frequencies that are comparable to those determined in the assay routinely used in our laboratory.

Allogeneic islet transplantation can restore an insulin-independent state in C-peptide-negative T1D patients. We reported three cases of surviving islet allografts that were implanted in T1D patients under maintenance immune suppression for a previous kidney graft (5,29). This study compared islet graft-specific cellular auto- and allo-reactivity in peripheral blood from those three recipients and from four patients with failing islet allografts measured over a period of 6 months after portal islet implantation. Successful restoration of insulin secretion was accompanied by the absence of allo-reactivity, and only marginally increased auto-reactivity to islet auto-antigens. In contrast, rapid failure was accompanied by increases in precursor frequencies of graft-specific allo-reactive T-cells; one recipient had a delayed loss of islet graft function (33 weeks). He did not exhibit signs of graft-specific allo-immunity, but developed a delayed increase in auto-reactivity. The parallel between metabolic outcome of human beta-cell allografts and cellular auto- and allo-reactivity in peripheral blood suggests a causal relationship. This study therefore demonstrates that T-cell reactivities in peripheral blood can be used to monitor immune mechanisms, which influence survival of beta-cell allografts in T1D patients. The initial study has since been expanded, and the results corroborate with our previous conclusions.

OUTLINE OF THE THESIS

Knowledge of the mechanism of action of immunotherapeutic agents is important for the development of selective and mild immunosuppressive drugs. Although the last decades taught us much about these mechanisms of different therapeutic agents like corticosteroids, calcineurin inhibitors, antiproliferative agents and polyclonal antibodies (39), there is still a lack of knowledge of the mechanisms of newer monoclonal antibody therapeutics. In chapter 2 we describe a study of the mechanism of action of anti-IL-2 receptor blocker antibodies and anti-CD3 antibodies in comparison to the well known mechanism of ATG. All of which are of great interest in the battle against allo-and autoimmunity in both pancreas/islet transplantation and immunomodulation strategy developments for T1D.

Simultaneous pancreas-kidney transplant recipients experience renal allograft rejection more frequently than non-diabetic patients referred for kidney transplantation alone. The consequences of acute rejection include prolonged initial or repeated hospitalization, as well as result in impaired graft function and reduced long-term graft survival (76-78). Induction therapy with ATG has proved to be an effective polyclonal antibody therapy to reduce the number of rejection episodes in pancreas and kidney transplantation programs. Although ATG is a powerful prophylactic therapy, it has many side effects (79-82). The efficacy of daclizumab to prevent acute rejection has been established in kidney, heart and pancreas transplantation (71,74,83,84). Both allo- and autoreactive T-cells are believed to be activated during pancreas transplantation due to the introduction of the pancreas and kidney allograft and new beta-cells, respectively. In the setting of a combined pancreas and kidney transplantation the non-selective nature of ATG may have the additional benefit to prevent recurrent destruction of pancreatic beta-cells. Due to the characteristics of daclizumab this drug might be able to prevent recurrent autoimmunity as well. We hypothesized that daclizumab and ATG would be equally effective to prevent acute rejection and restore serum glucose regulation in simultaneous pancreas-kidney transplant recipients. This study is presented in chapter 3.

In chapter 4, T-cell assays were used to study the effect on recurrent islet autoimmunity and recall-immunity in pancreas transplant recipients after different antibody induction protocols with ATG and daclizumab. Both allo- and autoreactivity are present after simultaneous pancreas-kidney transplantation. The recall-immunity, on the other hand, is supposed to be at a resting memory state during the transplant period. ATG is a non-specific immunotherapy with many side-effects (79-82). To overcome this disadvantage, monoclonal immunoglobulins directed against specific T-cell subsets have been developed. Daclizumab is a monoclonal antibody directed against the low affinity interleukin-2 receptor alpha chain (CD25) on activated lymphocytes (85,86). As a consequence daclizumab targets the activated lymphocytes and should therefore relatively spare the recall immunity after daclizumab induction therapy. Daclizumab theoretically could serve as a good alternative for the polyclonal induction therapy with ATG, provided that it affects recurrent islet autoimmunity. We tested the effect of daclizumab induction therapy on autoreactive lymphocytes and recall immunity compared to ATG induction therapy in a cross-sectional study in T1D patients successfully transplanted with a combined kidney and pancreas.

Transplantation of pancreatic islets provides an attractive and less invasive alternative to whole-organ pancreas transplantation. Survival of the grafts will depend on several variables, among which the quality and quantity of the beta-cells in the donor tissue, as well as allo and/or auto-immune reactivity of the recipient are important (87). In a limited study on islet cell after kidney transplants, it has been shown that cultured human beta-cells can survive for more than 1 year in T1D patients on maintenance im-

munosuppressive therapy for a prior kidney graft without the need for an increased immunosuppression at time of their implantation (29). Cultured islet cell grafts did induce allo- and autoimmune responses of variable intensity (5) whereby low responses were associated with long-term islet graft survival. A striking observation was the fact that in all cases low alloreactivity was found against HLA mismatches present on the islets shared with the kidney that was transplanted years before (repeated mismatch), even in case of a high T-cell alloreactivity against novel HLA mismatches only present on the islets (88). This pattern was found even when these novel mismatch and repeated mismatch were present on the same islet allograft. In islet transplantation programs sequential transplantations are often used to reach the necessary amount of beta-cells to become insulin independent. For this reason it is valuable to know if islets rather than kidney allografts can induce a similar effect on alloreactivity against HLA mismatches shared between sequential islet implantations in non-uremic T1D patients. If this were the case, it might open selection windows for subsequent islet cell grafts. In chapter 5 the results of a pilot study on three patients each of whom received three consecutive beta cell implants from different donors within an interval of 2 to 6 months is described to address this gap in knowledge.

Although it may appear that the next chapter of this thesis is somewhat of a different subject, one should consider the following. In order to appreciate the advantages of beta-cell replacement therapy early in the course of diabetes mellitus it is of the utmost importance to know the short term results and morbidity of pancreas transplantation. In the next chapter the surgical aspects of pancreas transplantation are discussed. In the early years drainage of the exocrine part was mainly achieved by bladder drainage techniques (89). The duodenum segment is anastomosed to the recipients' bladder. Urological complications, reflux pancreatitis and hypotensive periods due to the excretion of large amounts of sodiumbicarbonate occur (90-92). From the late 90's onwards a shift to enteric drainage has been observed (89). Previous studies already described the therapeutic relevance and possible disadvantages of enteric conversion after primary bladder drainage. Anastomotic leakage leading to intra-abdominal infections was the main problem of this drainage technique (11,93-96). Historically, in our institution bladder drainage was the procedure of choice. With this technique, excellent survival rates were obtained (97,98). With a two-step approach of primary bladder drainage, followed by an enteric conversion short-term disadvantages of primary enteric drainage and the long-term bladder drainage related complications may be prevented. The effectivity and safety of enteric conversion still required validation and will be evaluated in this chapter. The complication rate, graft function, rejection rates and survival rates in patients with simultaneous pancreas kidney transplantation with primary bladder drainage after enteric conversion in the current immunosuppressive era were studied.

REFERENCES

1. Bougneres PF, Carel JC, Castano L et al. Factors associated with early remission of type I diabetes in children treated with cyclosporine. N Engl J Med 1988:318: 663.
2. Lampeter EF, Homberg M, Quabeck K et al. Transfer of insulin-dependent diabetes between HLA-identical siblings by bone marrow transplantation. Lancet 1993:341: 1243.
3. Roep BO, Arden SD, de Vries RR, et al. T-cell clones from a type-1 diabetes patient respond to insulin secretory granule proteins. Nature 1990:345: 632.
4. Roep BO, Kallan AA, Duinkerken G et al. T-cell reactivity to beta-cell membrane antigens associated with beta-cell destruction in IDDM. Diabetes 1995:44: 278.
5. Roep BO, Stobbe I, Duinkerken G et al. Auto- and alloimmune reactivity to human islet allografts transplanted into type 1 diabetic patients. Diabetes 1999:48: 484.
6. Roep BO. The role of T-cells in the pathogenesis of Type 1 diabetes: from cause to cure. Diabetologia 2003:46: 305.
7. Sibley RK, Sutherland DE, Goetz F, et al. Recurrent diabetes mellitus in the pancreas iso- and allograft. A light and electron microscopic and immunohistochemical analysis of four cases. Lab Invest 1985:53: 132.
8. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med 1993:329: 977.
9. Pancreas transplantation for patients with type 1 diabetes: American Diabetes Association. Diabetes Care 2000:23: 117.
10. Smets YF, Westendorp RG, van der Pijl JW et al. Effect of simultaneous pancreas-kidney transplantation on mortality of patients with type-1 diabetes mellitus and end-stage renal failure. Lancet 1999:353: 1915.
11. Sutherland DE, Gruessner RW, Dunn DL et al. Lessons learned from more than 1,000 pancreas transplants at a single institution. Ann Surg 2001:233: 463.
12. Sutherland DE, Gruessner RW, Gruessner AC. Pancreas transplantation for treatment of diabetes mellitus. World J Surg 2001:25: 487.
13. Kim KW. Islet transplantation: a realistic alternative for the treatment of insulin deficient diabetes mellitus. Diabetes Res Clin Pract 2004:66 Suppl 1: S11.
14. Shapiro AM, Lakey JR, Ryan EA et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000:343: 230.
15. Cardona K, Milas Z, Strobert E et al. Engraftments of adult porcine islet xenografts in diabetic nonhuman primates through targeting of costimulation pathways. Am.J.Transplant 2007: 7: 2260.
16. Peakman M, Tree TI, Endl J, van Endert P, et al. Characterization of preparations of GAD65, proinsulin, and the islet tyrosine phosphatase IA-2 for use in detection of autoreactive T-cells in type 1 diabetes: report of phase II of the Second International Immunology of Diabetes Society Workshop for Standardization of T-cell assays in type 1 diabetes. Diabetes 2001:50: 1749.
17. Roep BO, Atkinson MA, van Endert PM, et al. Autoreactive T-cell responses in insulin-dependent (Type 1) diabetes mellitus. Report of the first international workshop for standardization of T-cell assays. J Autoimmun 1999:13: 267.
18. Palmer JP, Fleming GA, Greenbaum CJ et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21-22 October 2001. Diabetes 2004:53: 250.

19. Martin S, Wolf-Eichbaum D, Duinkerken G et al. Development of type 1 diabetes despite severe hereditary B-lymphocyte deficiency. N Engl J Med 2001:345: 1036.
20. Dupre J, Mahon JL. Diabetes-related autoantibodies and the selection of subjects for trials of therapies to preserve pancreatic beta-cell function in recent-onset type 1 diabetes. Diabetes Care 2000:23: 1057.
21. Fuchtenbusch M, Rabl W, Grassl B, et al. Delay of type I diabetes in high risk, first degree relatives by parenteral antigen administration: the Schwabing Insulin Prophylaxis Pilot Trial. Diabetologia 1998:41: 536.
22. Herold KC, Hagopian W, Auger JA et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J Med 2002:346: 1692.
23. Petersen JS, Dyrberg T, Karlsen AE et al. Glutamic acid decarboxylase (GAD65) autoantibodies in prediction of beta-cell function and remission in recent-onset IDDM after cyclosporin treatment. The Canadian-European Randomized Control Trial Group. Diabetes 1994:43: 1291.
24. Silverstein J, Maclaren N, Riley W, et al. Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. N Engl J Med 1988:319: 599.
25. Bosi E, Bottazzo GF, Secchi A et al. Islet cell autoimmunity in type I diabetic patients after HLA-mismatched pancreas transplantation. Diabetes 1989:38 Suppl 1: 82.
26. Braghi S, Bonifacio E, Secchi A, et al. Modulation of humoral islet autoimmunity by pancreas allo-transplantation influences allograft outcome in patients with type 1 diabetes. Diabetes 2000:49: 218.
27. Jaeger C, Brendel MD, Hering BJ, et al. Progressive islet graft failure occurs significantly earlier in autoantibody-positive than in autoantibody-negative IDDM recipients of intrahepatic islet allografts. Diabetes 1997:46: 1907.
28. Jaeger C, Brendel MD, Eckhard M, et al. Islet autoantibodies as potential markers for disease recurrence in clinical islet transplantation. Exp Clin Endocrinol Diabetes 2000:108: 328.
29. Keymeulen B, Ling Z, Gorus FK et al. Implantation of standardized beta-cell grafts in a liver segment of IDDM patients: graft and recipients characteristics in two cases of insulin-independence under maintenance immunosuppression for prior kidney graft. Diabetologia 1998:41: 452.
30. Olack BJ, Swanson CJ, Flavin KS et al. Sensitization to HLA antigens in islet recipients with failing transplants. Transplant Proc 1997:29: 2268.
31. Bouma GJ, Schanz U, Oudshoorn M et al. A cell-saving non-radioactive limiting dilution analysis-assay for the combined determination of helper and cytotoxic T lymphocyte precursor frequencies. Bone Marrow Transplant 1996:17: 19.
32. Schanz U, Roelen DL, Bruning JW, et al. The relative radioresistance of interleukin-2 production by human peripheral blood lymphocytes: consequences for the development of a new limiting dilution assay for the enumeration of helper T lymphocyte precursor frequencies. J Immunol Methods 1994:169: 221.
33. Ouwehand AJ, Baan CC, Roelen DL et al. The detection of cytotoxic T-cells with high-affinity receptors for donor antigens in the transplanted heart as a prognostic factor for graft rejection. Transplantation 1993:56: 1223.
34. Roelen D, Datema G, van Bree S, et al. Evidence that antibody formation against a certain HLA alloantigen is associated not with a quantitative but with a qualitative change in the cytotoxic T-cells recognizing the same antigen. Transplantation 1992:53: 899.

35. Roelen DL, van Bree FP, Schanz U, et al. Differential inhibition of primed alloreactive CTLs in vitro by clinically used concentrations of cyclosporine and FK506. Transplantation 1993:56: 190.
36. Roelen DL, van Beelen E, van Bree SP, et al. The presence of activated donor HLA class I-reactive T lymphocytes is associated with rejection of corneal grafts. Transplantation 1995:59: 1039.
37. Dantal J, Pohanka E. Malignancies in renal transplantation: an unmet medical need. Nephrol Dial Transplant 2007:22 Suppl 1: 4.
38. Gutierrez-Dalmau A, Campistol JM. Immunosuppressive therapy and malignancy in organ transplant recipients: a systematic review. Drugs 2007:67: 1167.
39. Mueller XM. Drug immunosuppression therapy for adult heart transplantation. Part 1: immune response to allograft and mechanism of action of immunosuppressants. Ann Thorac Surg 2004:77: 354.
40. Auphan N, DiDonato JA, Rosette C, et al. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. Science 1995:270: 286.
41. Hricik DE, Almawi WY, Strom TB. Trends in the use of glucocorticoids in renal transplantation. Transplantation 1994:57: 979.
42. Hollenberg SM, Giguere V, Segui P, et al. Colocalization of DNA-binding and transcriptional activation functions in the human glucocorticoid receptor. Cell 1987:49: 39.
43. Scheinman RI, Cogswell PC, Lofquist AK, et al. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. Science 1995:270: 283.
44. Lu CY, Sicher SC, Vazquez MA. Prevention and treatment of renal allograft rejection: new therapeutic approaches and new insights into established therapies. J Am Soc Nephrol 1993:4: 1239.
45. Satterthwaite R, Aswad S, Sunga V et al. Incidence of new-onset hypercholesterolemia in renal transplant patients treated with FK506 or cyclosporine. Transplantation 1998:65: 446.
46. Spencer CM, Goa KL, Gillis JC. Tacrolimus. An update of its pharmacology and clinical efficacy in the management of organ transplantation. Drugs 1997:54: 925.
47. Pirsch JD, Miller J, Deierhoi MH, et al. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. FK506 Kidney Transplant Study Group. Transplantation 1997:63: 977.
48. Krensky AM, Clayberger C. Transplantation immunology. Pediatr Clin North Am 1994:41: 819.
49. Crabtree GR, Clipstone NA. Signal transmission between the plasma membrane and nucleus of T lymphocytes. Annu Rev Biochem 1994:63: 1045.
50. Kirklin JK, Holm A, Aldrete JS, et al. Gastrointestinal complications after cardiac transplantation. Potential benefit of early diagnoses and prompt surgical intervention. Ann Surg 1990:211: 538.
51. Taylor DO, Ensley RD, Olsen SL, et al. Mycophenolate mofetil (RS-61443): preclinical, clinical, and three-year experience in heart transplantation. J Heart Lung Transplant 1994:13: 571.
52. Smith KG, Isbel NM, Catton MG et al. Suppression of the humoral response by mycophenolate mofetil. Nephrol Dial Transplant 1998: 13: 160.
53 Oliveira JGG, Monteiro MS, Teixeira JF et al. Humoral immune response after kidney transplantation is enhanced by acute rejection and urological obstruction and is down-regulated by mycophenolate mofetil treatment. Transpl Int 2005: 18: 1286.
54. Sollinger HW. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. U.S. Renal Transplant Mycophenolate Mofetil Study Group. Transplantation 1995:60: 225.
55. Epinette WW, Parker CM, Jones EL, et al. Mycophenolic acid for psoriasis. A review of pharmacology, long-term efficacy, and safety. J Am Acad Dermatol 1987:17: 962.

56. Kelly PA, Gruber SA, Behbod F, et al. Sirolimus, a new, potent immunosuppressive agent. Pharmacotherapy 1997:17: 1148.
57. Bonnefoy-Berard N, Verrier B, Vincent C, et al. Inhibition of CD25 (IL-2R alpha) expression and T-cell proliferation by polyclonal anti-thymocyte globulins. Immunology 1992:77: 61.
58. Johnson K, Niblack G, Richie R et al. Multicenter comparison of rejection reversal: rabbit anti-human lymphocyte serum (ATS) versus horse anti-human lymphocyte globulin (ATGAM). Transplant Proc 1989:21: 1734.
59. Ducloux D, Kazory A, Challier B et al. Long-term toxicity of antithymocyte globulin induction may vary with choice of agent: a single-center retrospective study. Transplantation 2004:77: 1029.
60. Tatum AH, Bollinger RR, Sanfilippo F. Rapid serologic diagnosis of serum sickness from antithymocyte globulin therapy using enzyme immunoassay. Transplantation 1984:38: 582.
61. Steinmuller DR, Hodge E, Boshkos C, et al. Prophylaxis and treatment of post-renal transplant rejection. Cleve Clin J Med 1991:58: 125.
62. Transy C, Moingeon PE, Marshall B, et al. Most anti-human CD3 monoclonal antibodies are directed to the CD3 epsilon subunit. Eur J Immunol 1989:19: 947
63. Bristow MR, Gilbert EM, O'Connell JB et al. OKT3 monoclonal antibody in heart transplantation. Am J Kidney Dis 1988:11: 135
64. Swinnen LJ, Costanzo-Nordin MR, Fisher SG et al. Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. N Engl J Med 1990:323: 1723.
65. Lazarovits AI, Rochon J, Banks L et al. Human mouse chimeric CD7 monoclonal antibody (SDZCHH380) for the prophylaxis of kidney transplant rejection. J Immunol 1993:150: 5163.
66. Herold KC, Gitelman SE, Masharani U et al. A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. Diabetes 2005:54: 1763.
67. Keymeulen B, Vandemeulebroucke E, Ziegler AG et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. N Engl J Med 2005:352: 2598.
68. Riechmann L, Clark M, Waldmann H, et al. Reshaping human antibodies for therapy. Nature 1988:332: 323.
69. Jones PT, Dear PH, Foote J, et al. Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature 1986:321: 522.
70. Karnitz LM, Abraham RT. Cytokine receptor signaling mechanisms. Curr Opin Immunol 1995:7: 320.
71. Vincenti F, Kirkman R, Light S et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. N Engl J Med 1998:338: 161.
72. Light JA, Sasaki TM, Ghasemian R, et al. Daclizumab induction/tacrolimus sparing: a randomized prospective trial in renal transplantation. Clin Transplant 2002:16 Suppl 7: 30.
73. Kahan BD, Rajagopalan PR, Hall M. Reduction of the occurrence of acute cellular rejection among renal allograft recipients treated with basiliximab, a chimeric anti-interleukin-2-receptor monoclonal antibody. United States Simulect Renal Study Group. Transplantation 1999:67: 276.
74. Vincenti F, Nashan B, Light S. Daclizumab: outcome of phase III trials and mechanism of action. Double Therapy and the Triple Therapy Study Groups. Transplant Proc 1998:30: 2155.
75. Webster AC, Playford EG, Higgins G, et al. Interleukin 2 receptor antagonists for renal transplant recipients: a meta-analysis of randomized trials. Transplantation 2004:77: 166.
76. Banasik M, Klinger M. Chronic allograft nephropathy--immunologic and nonimmunologic factors. Ann Transplant 2006:11: 7.

77. Nankivell BJ, Borrows RJ, Fung CL, et al. The natural history of chronic allograft nephropathy. N Engl J Med 2003:349: 2326.
78. Tesi RJ, Henry ML, Elkhammas EA, et al. The frequency of rejection episodes after combined kidney-pancreas transplant--the impact on graft survival. Transplantation 1994:58: 424.
79. Cantarovich D, Karam G, Giral-Classe M et al. Randomized comparison of triple therapy and antithymocyte globulin induction treatment after simultaneous pancreas-kidney transplantation. Kidney Int 1998:54: 1351.
80. Hesse UJ, Troisi R, Jacobs B et al. A single center's clinical experience with quadruple immunosuppression including ATG or IL2 antibodies and mycophenolate mofetil in simultaneous pancreas-kidney transplants. Clin Transplant 2000:14: 340.
81. Mestre M, Bas J, Alsina J, et al. Depleting effect of antithymocyte globulin on T-lymphocyte subsets in kidney transplantation. Transplant Proc 1999:31: 2254.
82. Bourdage JS, Hamlin DM. Comparative polyclonal antithymocyte globulin and antilymphocyte/antilymphoblast globulin anti-CD antigen analysis by flow cytometry. Transplantation 1995:59: 1194.
83. Chin C, Pittson S, Luikart H et al. Induction therapy for pediatric and adult heart transplantation: comparison between OKT3 and daclizumab. Transplantation 2005:80: 477.
84. Stratta RJ, Alloway RR, Lo A, et al. A prospective, randomized, multicenter study evaluating the safety and efficacy of two dosing regimens of daclizumab compared to no antibody induction in simultaneous kidney-pancreas transplantation: results at 3 years. Transplant Proc 2005:37: 3531.
85. Waldmann TA, O'Shea J. The use of antibodies against the IL-2 receptor in transplantation. Curr Opin Immunol 1998:10: 507.
86. Wiseman LR, Faulds D. Daclizumab: a review of its use in the prevention of acute rejection in renal transplant recipients. Drugs 1999:58: 1029.
87. Merani S, Shapiro AM. Current status of pancreatic islet transplantation. Clin Sci (Lond) 2006:110: 611.
88. Stobbe I, Duinkerken G, van Rood JJ et al. Tolerance to kidney allograft transplanted into Type I diabetic patients persists after in vivo challenge with pancreatic islet allografts that express repeated mismatches. Diabetologia 1999:42: 1379.
89. Gruessner AC, Sutherland DE. Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of October 2002. Clin Transpl 2002: 41.
90. Sollinger HW, Odorico JS, Knechtle SJ, et al. Experience with 500 simultaneous pancreas-kidney transplants. Ann Surg 1998:228: 284.
91. West M, Gruessner AC, Metrakos P, et al. Conversion from bladder to enteric drainage after pancreaticoduodenal transplantations. Surgery 1998:124: 883.
92. Sollinger HW, Sasaki TM, D'Alessandro AM et al. Indications for enteric conversion after pancreas transplantation with bladder drainage. Surgery 1992:112: 842.
93. Gruessner RW, Sutherland DE, Troppmann C et al. The surgical risk of pancreas transplantation in the cyclosporine era: an overview. J Am Coll Surg 1997:185: 128.
94. Steurer W, Bonatti H, Obrist P et al. Incidence of intraabdominal infection in a consecutive series of 40 enteric-drained pancreas transplants with FK506 and MMF immunosuppression. Transpl Int 2000:13 Suppl 1: S19.
95. Bonatti H, Berger N, Kafka R et al. Experience with ATG short course high dose induction therapy in a series of 112 enteric drained pancreatic transplants. Ann Transplant 2002:7: 22.
96. Pirsch JD, Odorico JS, D'Alessandro AM, et al. Posttransplant infection in enteric versus bladder-drained simultaneous pancreas-kidney transplant recipients. Transplantation 1998:66: 1746.

97. Smets YF, van der Pijl JW, Ringers J, et al. Mortality of cadaveric kidney transplantation versus combined kidney-pancreas transplantation in diabetic patients. Lancet 1996:347: 826.
98. Smets YF, Westendorp RG, van der Pijl JW et al. Effect of simultaneous pancreas-kidney transplantation on mortality of patients with type-1 diabetes mellitus and end-stage renal failure. Lancet 1999:353: 1915.