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Immunotherapy and beta-cell replacement in type I diabetes mellitus

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Chapter 3

Single high dose ATG-Fresenius equally reduces acute rejection episodes, but may be preferable to five doses daclizumab in pre-transplant GAD-autoantibody seropositive Simultaneous Pancreas and Kidney Transplant recipients

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Submitted.

ABSTRACT

Daclizumab and anti-thymocyte globulin (ATG) have been shown to reduce allograft rejection. We assessed the safety and efficacy of daclizumab or ATG prophylaxis in combination with triple immunotherapy in simultaneous pancreas and kidney transplant (SPKT) recipients.

Thirty-nine type 1 diabetic patients scheduled for primary SPKT were randomised to receive prophylactic therapy with either daclizumab in a total of five doses or a single high dose of ATG before reperfusion. A group of 27 patients without prophylactic antibodies was used for retrospective comparison. All patients received CsA, MMF and gradually tapered prednisone.

Baseline and transplant characteristics were comparable among groups. Both daclizumab and ATG therapy resulted in a significant reduction in acute rejection episodes within 6 months after transplantation. The incidence of rejection episodes was significantly higher in pre-transplantation GAD autoantibody positive daclizumab treated recipients compared to GAD autoantibody-negative or ATG treated recipients. IA-2 islet autoantibodies showed no effect on rejection. There were no significant differences between the groups for in-vitro autoreactivity, clinical outcome or functional parameters.

Daclizumab or ATG combined with a maintenance immunosuppressive regime consisting of cyclosporine, MMF and prednisolone were well tolerated and equally effective in reducing the incidence of acute rejection episodes in simultaneous pancreas kidney transplant recipients. Up to three years no adverse sequelae of the immunoprophylaxis or clinical and ex-vivo recurrent autoimmunity was observed. We propose that the pre-transplantation existence of GAD65 autoantibodies might serve as a marker guiding the choice for prophylactic therapy in pancreas transplantation.

INTRODUCTION

After several decades of controversy regarding its therapeutic validity, simultaneous pancreas kidney transplantation (SPKT) has become the recommended treatment for patients with type 1 diabetes mellitus and advanced or established end-stage renal failure. A vascularized pancreatic graft is currently the only option to reliably achieve a long-term, autoregulated, euglycemic state (1-4). The documented benefits of SPKT include improved life-expectancy, quality of life, freedom from insulin injections and the tedium of dialysis treatment, and stabilization or improvement of neuropathy, retinopathy and vasculopathy and prevention of recurrent diabetic nephropathy (2,5-8).

SPKT recipients experience renal allograft rejection more frequently than do non-diabetic patients referred for kidney transplantation alone. The consequences of acute rejection include not only prolonged initial or repeated hospitalization, but may also result in impaired graft function and reduced long-term graft survival (9-11). Induction therapy with antibodies are increasingly used in simultaneous pancreas-kidney transplantations (12-15). Induction therapy with polyclonal Anti-Thymocyte Globulines (ATG) has proved to be an effective therapy to reduce the number and severity of rejection episodes after pancreas and kidney transplantations. Although ATG is a powerful prophylactic therapy, it has many side effects. Daclizumab is a genetically engineered human IgG1 monoclonal antibody that specifically binds to the α-subunit (CD25) of the interleukin-2 (IL-2) receptor on the surface of activated lymphocytes. Daclizumab has been developed to overcome the side effects of polyclonal antibody therapeutics. The efficacy of daclizumab to prevent acute rejection has been established after kidney and combined pancreas-kidney transplantations (14,16-22).

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. Donor and recipient of a pancreatic graft do not have to share HLA antigens for recurrent autoimmune destruction of the graft (23,24). Immunotherapy directed against T-cells has been shown to delay disease progression in patients with type 1 diabetes of recent onset, but did not prevent beta-cell dysfunction (25). This delay was not accompanied by changes in autoantibody levels, providing evidence of the immunological memory of islet-specific T-cells (26). 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.

Both five doses of daclizumab and a single high dose of ATG-Fresenius, in combination with a cyclosporine-based maintenance regimen, have been shown to reduce the incidence of acute rejection after kidney transplantation by approximately 50% (16,27). We hypothesized that daclizumab and ATG would also be equally effective to prevent

acute rejection in de novo SPKT recipients, while ATG could probably be more effective in prevention of recurrent autoimmunity.

MATERIALS AND METHODS

Study design

This was a prospective, open-label, randomized evaluation of daclizumab (1 mg/kg for 5 doses) and a single high dose of Anti-Thymocyte Globulin (rATG-Fresenius: 9 mg/kg) in combination with cyclosporine micro-emulsion (CsA), mycophenolate mofetil (MMF), and prednisolone in de novo simultaneous pancreas-kidney transplant (SPKT) recipients. Organs for SPKT were allocated according to the Eurotransplant Kidney Allocation System without prospective matching for HLA antigens. Formal approval from the institutional ethics committee was obtained, and written informed consent was given before enrolment.

Study population and immunosuppression

Thirty-nine consecutive (C-peptide negative) patients with type 1 diabetes and (or approaching) end-stage renal failure scheduled to receive a primary SPKT (bladder-drained, systemic venous anastomosis) were randomised to receive prophylactic therapy with either daclizumab (n=20) (1 mg/kg starting before surgery and every other week for a total of five doses) or a single bolus dose of ATG-F (=19) (9 mg/kg intra-operatively before reperfusion of the first organ). Eligible patients were recipients >18 yr of age; sensitized patients (defined by panel reactive antibodies >50%) were excluded. CsA-me was given in a twice daily schedule starting 3 hours before surgery (starting dose 4 mg/kg). The target trough levels were aimed at 250 ng/ml (range 200-300) during the first 12 post-operative weeks and 150 ng/ml (range 100-200) thereafter. Additional immunosuppression included corticosteroids (methylprednisolon 500 mg pre-operatively and prednisolone: 100 mg days 1-3, 50 mg days 4-6, 20 mg month 1, 15 mg in month 2 and 10 mg thereafter) and fixed dose mycophenolate mofetil (MMF; 1000 mg b.i.d.). All patients received prophylaxis with oral gancyclovir (4 months) and trimethoprim-sulfamethoxazol (12 months). Drugs known to alter concentrations of calcineurin-inhibitors were prohibited.

We hypothesized that daclizumab and ATG-F would also be equally effective to prevent acute rejection in de novo SPKT recipients, while ATG-F could probably be more effective in prevention of recurrent autoimmunity. Previous studies with ATG-F and Daclizumab have demonstrated approximately 50% reduction in acute rejection episodes

after kidney transplantation (21,27). Sample size calculation indicated that sample sizes of at least 16 per group would allow detecting a 30% reduction in acute rejection episodes with two-tailed significance of 0.05 and 90% power. A treatment group without some form of prophylactic treatment was considered no longer ethically acceptable. For comparison, a previous cohort of 27 consecutive SPKT patients, who received the same triple maintenance immunosuppressive regimen without prophylactic antibodies was used.

Treatment of acute rejection episode(s): The diagnosis acute rejection was based on clinical criteria and/or renal or pancreatic allograft dysfunction (unexplained increase in: serum creatinine >20% from baseline; serum diastase >50%; reduced 24-hour urinary diastase excretion and/or unexplained hyperglycemia. An acute rejection episode was confirmed by a percutaneous kidney graft biopsy unless contraindicated and the severity was graded according to the Banff classification (28). Rejection was treated with bolus Solumedrol (1 g/day intravenous for three consecutive days) or Anti-Thymocyte Globulin (rATG, Merieux) was given for steroid-resistant rejection episodes and second acute rejection episodes. An acute rejection was considered steroid-resistant if no stabilization or improvement occurred within 5 days after the treatment with Solumedrol. In case, despite Solumedrol, progressive loss of graft function occurred and/or severe rejection (Banff grade ≥IIA) was found in the renal biopsy, treatment with anti-T-cell antibodies could be started earlier.

Efficacy parameters and side effects

The primary objective was to compare the incidence of early (<6 months) and steroid-resistant acute rejection episodes and time to first rejection after induction therapy with either five infusions of daclizumab or single dose ATG-F. Renal allograft function was measured by repeated 24-hour urinary creatinine excretion; pancreas function was assessed by fasting glucose and HbA1c values at month 6, 12, 24 and 36. Graft loss was defined by return to dialysis or reinstitution of exogenous insulin therapy. Standard safety evaluation included physical examination, serial blood counts, and blood chemistry studies. Evidence for recurrent autoimmunity was tested for presence of islet autoantibodies, serum glucose levels and HbA1c values, as well as cellular islet autoreactivity in a lymphocyte stimulation test cross-sectionally approximately 2 years after transplantation. Reporting of adverse effects, frequency or severity of infections and occurrence of malignancy was mandatory.

Autoimmunity

Detection of insulitis as a prove for recurrence of autoimmunity with histological biopsies is difficult and has some risks (29). Measuring autoantibodies and autoreactive T-lymfocyte reactivity in T-cell assays could serve as a marker for recurrence of autoimmunity. We applied both serological and T-cell assays to detect recurrence of autoimmunity.

Serology: Pre-and post-transplantation autoantibodies directed against IA-2 and GAD were measured using previously described radiobinding assays with in vitro transcribed and translated 35S-methionine-labelled recombinant antigen. (30) Samples were tested prior to transplantation and 42, 84, 182, 275 and 365 days after transplantation.

Lymphocyte proliferation test: The lymphocyte stimulation test was performed cross-sectionally after a successful combined pancreas and kidney transplantation. Heparinized blood was drawn between 10 and 50 months after transplantation (mean 25,4 months [±10] for daclizumab-treated patients, and 26,5 months [±10] for ATG-treated patients). Peripheral blood mononuclear cells (PBMC) were isolated from freshly drawn heparinized blood and tested as previously described (31). In short, 150.000 PBMC were cultured in tissue-coated, round-bottomed 96-well plates (Costar, Cambridge, MA) in Iscove's modified Dulbecco's medium with 2 mmol/l glutamine (Gibco, Paisley, Scotland) supplemented with 10% human type AB pool serum in the presence of antigen or medium alone in 150 ml at 37 °C, 5% CO2. After 5 days, RPMI 1640 (Dutch Modification; Gibco) containing 0,5 mCi [^{3}H] thymidine per well was added, and incubation was continued for 16 h. Cultures were then harvested on glass-fiber filters, and [^{3}H] thymidine incorporation was measured by liquid scintillation counting. The results are expressed as stimulation indexes (SI), that is, the median of triplicates in the presence of stimulus divided by the median of triplicates with medium alone.

Stimuli included Insulin (Sigma), GAD65 (Diamyd Medicals, Stockholm, Sweden), recombinant human pro-insulin and IA-2602-979, human islet homogenate (kindly provided by Dr. Ezio Bonifacio, San Raffaele Institute, Milan, Italy). Recombinant human IA-2 cytoplasmic domain (residues 602-979) with N-terminal affinity tag was produced in E.coli BL21/DE3 using IPTG induction, and purified as described, including preparative electrophoresis and electroelution (32). Endotoxin levels were 0.8 EU/mg protein as determined by the Limulus Amebocyte Lysate Assay (BioWhittaker, Walkersville, MD).

Statistical analyses

All analyses were performed according to intention-to-treat principle. Results are given as mean ± standard deviation for interval and ordinal variables unless otherwise stated. Frequencies of categorical variables and survival/rejection incidences are given as per-

centages. Comparison of interval variables between two groups was performed using Student's independent samples t-test. If statistical assumptions for parametric analysis were not met and in case of ordinal data Mann-Whitney's two-independent samples test was used instead and exact p-values calculated. For comparison of nominal (categorical) variables between two groups analysis of cross-tables with Pearson Chi-Square test or, when indicated, Fisher's Exact Test, was used. Incidence of patient and organ survival and the different acute rejection incidences were estimated by Kaplan-Meier Product-Limit Method and the equality of resulting survival distributions were tested using the Mantel-Cox statistic. Two-tailed p-values less than 0.05 were considered statistically significant. All analyses were performed using SPSS statistical software package for Windows (version 14.0.02; SPSS Inc. Chicago Ill.). All plots were drawn using GraphPad Prism version 4.02 for Windows (GraphPad Software, San Diego California USA, www.Graphpad.com).

RESULTS

Patient population

The mean recipient age was 40 ± 7 years and 60% were male. Pre-emptive transplantation was performed in 30% of patients, mean donor age 32 ± 12 years, mean cold ischemic times 13 ± 3 hrs (pancreas) and 14 ± 4 hrs (kidney). Demographic and transplantation related characteristics between the induction and non-induction groups are summarized in Table 1. Baseline demographic and transplant characteristics were comparable among the randomized induction therapy groups and the control group except for a significant difference in recipient and donor age. As expected both recipient and donor age in either induction treatment group were older compared to the historical group.

Patient, kidney and pancreas graft survival

Overall patient and (death censored) kidney and pancreas graft survival are plotted in Figure 1a, 1b and 1c and are summarized in table I. Patient, kidney and pancreas graft suvival at 1 year were not significantly different between induction and no induction groups (97% and 93%, 97% and 96%, 92% and 96% respectively). No significant differences in survival were found between patients randomized to receive induction therapy with either daclizumab or ATG or in comparison to patients without induction (Table I).

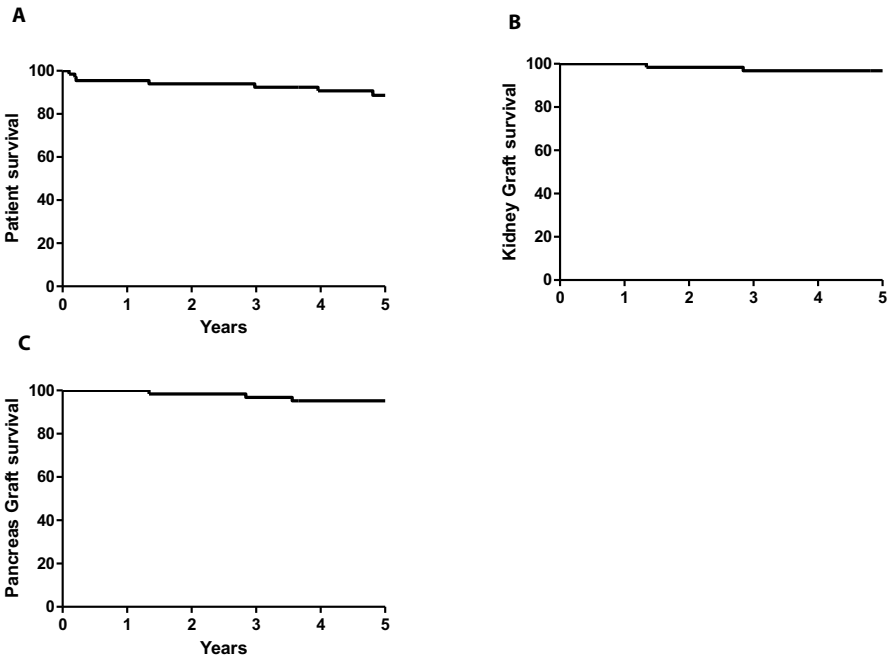


Figure 1. Overall patient (a); kidney graft (b) and pancreas graft (c) survival of patients randomized for either ATG or Daclizumab induction therapy and patients without induction therapy.

Acute rejection rates

Both daclizumab and ATG prophylaxis resulted in an approximately 50% reduction in the cumulative incidence of first acute rejection episodes at 6 months (p=0.000006). In addition time to first acute rejection was significantly later in the daclizumab group compared to the ATG treatment group (p= 0.008) (Figure 2a). Although acute rejection incidence was higher with daclizumab induction in comparison to ATG, and proportions of acute rejection requiring antibody therapy and steroid resistant rejection appeared to be higher with ATG induction in comparison to daclizumab, these differences didn't reach statistical significance. However, these parameters were significantly higher in the historic cohort in comparison to the induction groups.

Autoantibodies

Seventeen patients in the ATG group and twenty patients in the daclizumab group were tested for islet autoantibodies. No correlations of titers or seroconversion were found between post-transplantation GAD and IA-2 autoantibody titers and rejection episodes (data not shown). Twelve patients were positive for GAD autoantibodies before trans-

plantation (32%) and eight patients (22%) positive for IA-2 autoantibodies (equally distributed in both treatment groups) while five of these patients were positive for both islet autoantibodies prior to transplantation. Patients positive for GAD antibodies before transplantation showed higher acute rejection rates than GAD negative patients (58.3%

Table 1. Simultaneous pancreas kidney transplants: characteristics according to induction therapy versus non-induction therapy, and type of induction therapy

	Induction	**No Induction**		**ATG**	**Daclizumab**	
	n=39	n=27	*p-value*	n=19	n=20	*p-value*
Recipient age (yrs)	41.6 ± 7.9	37.5 ± 6.7	*0.03*	43.6 ± 8.2	39.8 ± 7.3	
Recipient sex (% male)	61.5	59.3		52.6	70.0	
Body mass index (kg/L^2)	23.6 ± 3.0	23.8 ± 2.9		24.0 ± 3.3	23.2 ± 2.7	
Diabetes duration (yrs)	28.0 ± 7.4	26.8 ± 7.3		29.2 ± 8.3	26.9 ± 6.5	
Time on dialysis (mo)	19.0 ± 12.7	21.8 ± 18.0		23.9 ± 15.2	14.8 ± 8.4	
Pre-emptive transplant (%)	28.2	25.9		31.6	25.0	
Donor age (yrs)	35.6 ± 11.2	28.1 ± 10.7	*0.008*	39.3 ± 8.4	32.1 ± 12.6	
Donor sex (% male)	48.7	59.3		42.1	55.0	
HLA-mismatch class I	3.0 ± 0.8	3.0 ± 1.0		2.8 ± 0.9	3.2 ± 0.7	
HLA-mismatch class II	1.3 ± 0.7	1.4 ± 0.6		1.4 ± 0.6	1.2 ± 0.8	
Cold Ischemia Kidney (hrs)	13.3 ± 3.7	15.5 ± 2.8	*0.012*	12.7 ± 3.9	13.8 ± 3.6	
Cold Ischemia Pancreas (hrs)	12.8 ± 3.3	13.8 ± 2.4		12.3 ± 3.3	13.3 ± 3.4	
Warm Ischemia Kidney (min)	35.9 ± 8.3	34.2 ± 8.6		36.6 ± 8.3	35.3 ± 8.5	
Warm Ischemia Pancreas (min)	31.8 ± 5.6	31.3 ± 8.3		33.4 ± 6.1	30.3 ± 4.8	
Acute Rejection incidence (6 mo) (%)	41.0 ± 7.9	88.9 ± 6.0	*0.000006*	36.8 ± 11.1	45.0 ± 11.1	
Time to first rejection (median) (days)	15 [14-16]	12 [10-14]		12 [10-14]	19 [16-22]	*0.008*
Rejection requiring antibody therapy (%)	25.6 ± 7.0	74.1 ± 8.4	*0.000036*	26.3 ± 10.1	25.0 ± 9.7	
Rejection requiring antibody therapy (%)[1]	62.5 ± 12.1	75.0 ± 8.8		71.4 ± 17.1	55.6 ± 16.6	
Steroid-resistant rejection (%)	15.4 ± 5.8	44.4 ± 9.6	*0.006*	15.8 ± 8.4	15.0 ± 8.0	
Steroid-resistant rejection (%)[1]	37.5 ± 12.1	50.0 ± 10.2		42.9 ± 18.7	33.3 ± 15.7	
Patient survival at 1 and 3 yr (%)	97.4 / 94.8	92.6 / 88.9		100 / 100	95.0 / 89.7	
Kidney graft survival 1 and 3 yr (%)	97.4 / 94.7	96.3 / 96.3		94.7 / 94.7	100.0 / 94.7	
Pancreas graft survival (1 and 3 yr) (%)	92.2 / 89.6	96.0 / 88.0		84.2 / 84.2	100.0 / 94.7	

[1] Adjusted for acute rejection incidence.
Significant p=values are mentioned.

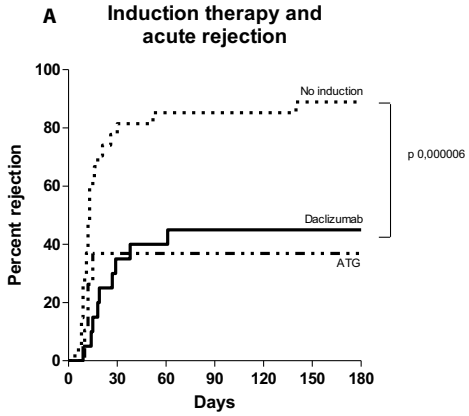
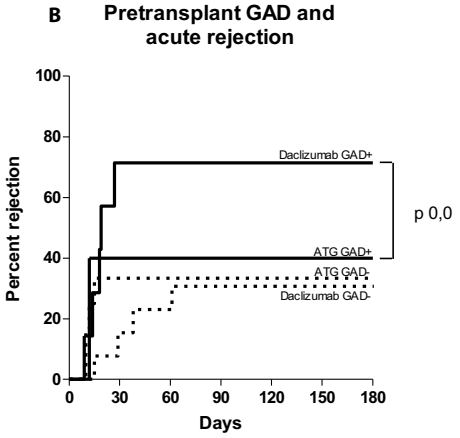


Figure 2a. Acute rejection episodes percentages for different induction regimes are depicted. Black dotted, black filled and partially dotted lines represent no-induction group, daclizumab treated patients and ATG treated patients, respectively. Significant differences between historical vs ATG or daclizumab treated patients.



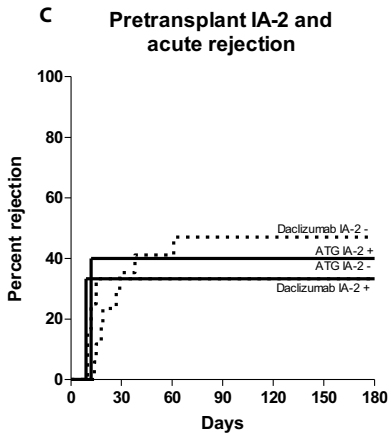


Figure 2b. Acute rejection episodes percentages for different induction regimes related to pre-transplantation GAD autoantibody status are depicted. Straight lines represent pre-transplantation GAD positive patients. Dotted lines represent pre-transplantation GAD negative patients. Significant difference between pre-tranplantation GADabs positive and negative patients treated with daclizumab.

Figure 2c. Acute rejection episodes percentages for different induction regimes related to pre-transplantation IA-2 autoantibody status are depicted. Straight lines represent pre-transplantation GAD positive patients. Dotted lines represent pre-transplantation IA-2 negative patients. No differences.

vs. 32%, p=0.09), which was not seen for IA2 autoantibodies. This difference could partly be explained by a remarkable association between GAD antibodies and the occurrence of early acute rejection in daclizumab treated patients (p = 0,02), while such an association could not be shown for ATG treated patients (fig. 2b). Pre-transplantation existence of IA-2 autoantibodies was not related to rejection episodes for both daclizumab and ATG (Figure 2c).

Recurrent autoimmunity

No statistically significant difference was found in autoreacivity (stimulation indices) against different diabetes related autoantigens (GAD, insulin, pro-insulin, IA-2, and islet homogenate) tested in a lymphocyte proliferation test 25.4 months (±10) for daclizumab-treated patients, and 26.5 months (±10) for ATG-treated patients after transplantation (Figure 3).

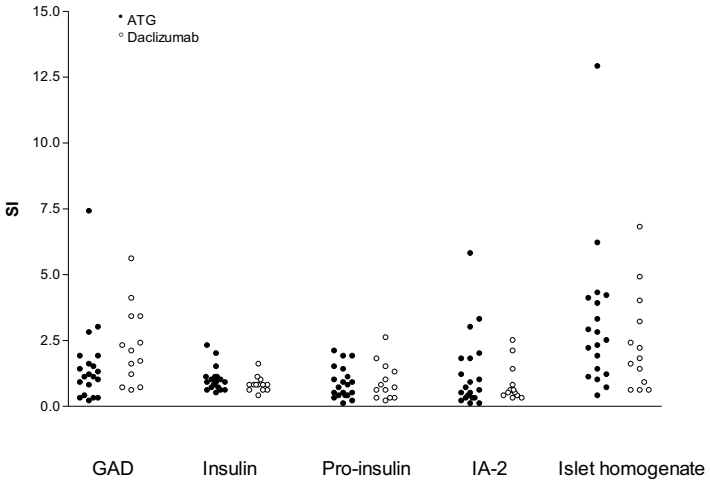


Figure 3. Stimulation indices are depicted for different autoantigens. Filled circles and open circles are ATG treated patients (including ATG induction therapy and/or ATG rejection therapy) and daclizumab treated patients, respectively. No significant difference was found.

Kidney and pancreas graft function

No significant differences between the groups for clinical outcome or functional parameters were found (Figure 4).

All patients had a follow-up of at least 3 years after implantation. At 6 and 12 months HbA1c levels were 5.3 ± 0.7 % and 5.0 ± 0.6%. The mean calculated creatinine clearance at 6 and 12 months was 54 ± 11 and 56 ± 10 ml/min, respectively.

DISCUSSION

The present study assessed the efficacy of the addition of five doses of daclizumab or a single high dose of ATG-Fresenius for the prevention of early graft rejection and steroid-

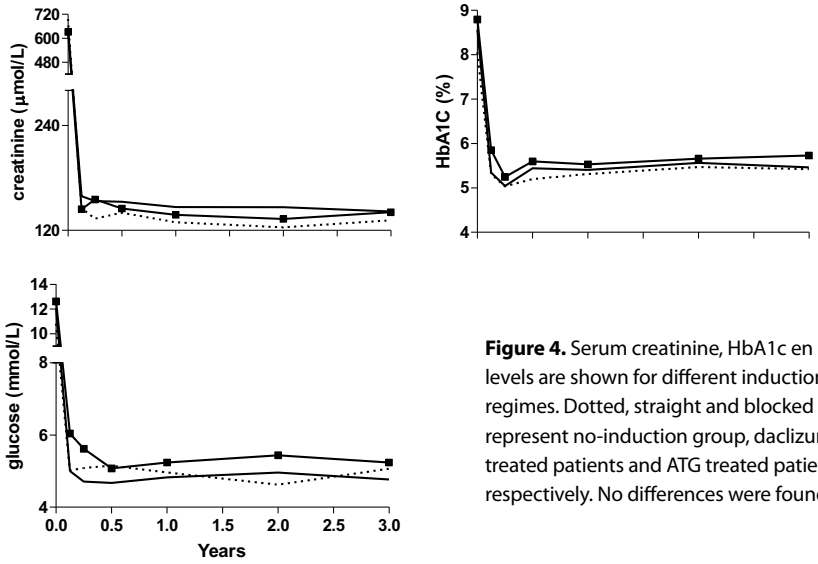


Figure 4. Serum creatinine, HbA1c en glucose levels are shown for different induction regimes. Dotted, straight and blocked lines represent no-induction group, daclizumab treated patients and ATG treated patients, respectively. No differences were found.

resistant rejection episodes and compared these results to the previous (historical) standard regime also consisting of MMF, cyclosporine micro emulsion, and corticosteroids without prophylactic therapy. Both induction strategies were well tolerated and equally effective in lowering the incidence and severity of acute rejection. Reduction of acute rejection episodes was approximately 50% in our study. In addition, no evidence of recurrent autoimmunity was observed clinically or immunologically (islet autoantibodies or autoreactive T-cells) after transplantation.

Also in kidney transplantations carrying an increased risk of delayed function or acute rejection, e.g. non-heart beating donation or highly immunized recipients, induction therapy is considered to be beneficial (33). Although the three-year patient and graft survival rates in our study population were not significantly different between the (historical) non-induction group and the patients receiving induction treatment with either ATG or daclizumab, long-term graft survival outcomes might differ. The strong association between acute rejection episodes and chronic allograft nephropathy has been consistently reported (9-11). From this point of view, especially in generally poor HLA-matched SPKT recipients, induction therapy might be preferred because of the relative high incidence of acute rejection episodes in such patients.

The dual immunological goal after SPKT is to avoid alloreactivity as well as recurrence of autoimmunity. Recurrence of autoimmunity has been described in pancreas grafts (24), but the current immunosuppressive regimes elicit an adequate protective effect on recurrent autoimmunity in the large majority of pancreas transplantation recipients (34). T-cell depleting therapy is characterized by lymphocyte loss, which results in cytokine-mediated homeostatic proliferation of T-memory cells (including autoreactive T-cell clones). Both

expansion and effector functioning of memory autoreactive T-cells should be controlled to prevent recurrent autoimmunity. In islet transplanted type 1 diabetes patients treated with depleting therapy or IL-2 blocking therapy such T-cell expansion and effector function could be blocked with MMF and rapamycin or tacrolimus, respectively (35). In our study MMF was used as maintenance immunosuppressive therapy after ATG or daclizumab induction therapy. This might explain why clinical and ex vivo T-cell autoreactivity was not observed. Islet autoantibodies can be useful to determine loss of islet graft function in some patients (36-39), but this is not consistent. An unexpected relationship between the pre-transplantation islet autoantibody GAD status and biopsy proven renal rejection episodes for the different induction protocols was found. The specificity for GAD is supported by the absence of such a relationship between the pre-transplantation IA-2 islet autoantibody status and rejection episodes. GAD and IA-2 antigens are known to be present in the pancreas and related to type 1 diabetes. GAD antigen has also been detected in renal tissue (40,41) whereas IA-2 expression has never been described to be present in renal tissue. This might explain the relationship between pre-transplantation existence of GAD autoantibodies and kidney-graft rejection episodes. Pancreas graft rejections are often preceded by kidney graft rejections and thus treated in case of anti-rejection therapy for biopsy-proven kidney rejection episodes. We speculate that ischemic and/or reperfusion injury might play a role in the presentation of cryptogenic renal antigens (including GAD) to the immune-system which might facilitate acute rejections in patients with GAD autoantibodies. From this point of view the pre-transplantation GAD autoantibody status may be of particular interest in SPKT recipients. Our speculation on the mechanism and the role of renal GAD antigens and the relationship between the presence of pre-transplantion GAD autoantibodies and rejection episodes in daclizumab treated patients has to be confirmed in larger studies.

Our study suggests that ATG, although a powerful polyclonal induction therapy with several drawbacks (42-45), might be preferable in the prevention of acute rejection episodes in GAD autoantibody positive recipients, which account for 30% of SPKT recipients (37,46). More studies have to prove the validity of this concept for tailor-made induction therapy in SPKT recipients. This study also indicates that for the majority of patients, however, daclizumab is equally effective in reducing acute rejections with less side-effects. Daclizumab has shown to be effective in reducing allograft rejection in renal transplantation without considerable side-effects (16-22). Due to the more specific effect of daclizumab and the absence of significant side-effects, this type of induction therapy supports the preferential use of daclizumab above ATG in pre-transplant GAD-antibody negative recipients. In addition, this is supported by our previous observation of a higher recurrence rate of CMV viremia in ATG treated allograft recipients, but not in daclizumab treated patients (47).

The present study has several limitations. First, the limited number of patients studied is due to the relative unique study population. Still only a minority of type 1 diabetes mellitus are included in SPKT programs, which makes studies with large population numbers

and longer follow up periods difficult to achieve. Secondly, our control group consisted of patients with the same maintenance immunosuppressive regime without induction therapy. Although a prospective control group is preferable, a study arm lacking induction therapy was considered no longer ethically justifiable. Our data showing a possible role of GAD autoantibodies to guide prophylactic therapy is an interesting observation. Further studies have to confirm this observation and clarify the underlying mechanism.

In conclusion, five doses of daclizumab or single high dose ATG-fresenius combined with a maintenance immunosuppressive regime consisting of cyclosporine, MMF and prednisolone were well tolerated and equally effective in reducing the incidence of acute rejection episodes in SPKT recipients. Up to three years no adverse sequelae of the immunoprophylaxis or clinical and ex-vivo recurrent autoimmunity was observed. We propose that the pre-transplantation existence of GAD65 autoantibodies could serve as a marker to guide prophylactic therapy in the context of simultaneous pancreas and kidney transplantation, and this may lead to a more tailor-made type of induction therapy.

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