HLA-A*24 Carrier Status and Autoantibody Surges Posttransplantation Associate With Poor Functional Outcome in Recipients of an Islet Allograft

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OBJECTIVE

We investigated whether changes in islet autoantibody profile and presence of HLA risk markers, reported to predict rapid β-cell loss in pre–type 1 diabetes, associate with poor functional outcome in islet allograft recipients.

RESEARCH DESIGN AND METHODS

Forty-one patients received ≥2.3 million β-cells/kg body wt in one to two intraportal implantations. Outcome after 6–18 months was assessed by C-peptide (random and stimulated), insulin dose, and HbA$_{1c}$.

RESULTS

Patients carrying HLA-A*24-positive or experiencing a significant autoantibody surge within 6 months after the first transplantation ($n = 19$) had lower C-peptide levels ($P ≤ 0.003$) and higher insulin needs ($P < 0.001$) despite higher HbA$_{1c}$ levels ($P ≤ 0.018$). They became less often insulin independent (16% vs. 68%, $P = 0.002$) and remained less often C-peptide positive (47% vs. 100%, $P < 0.001$) than recipients lacking both risk factors. HLA-A*24 positivity or an autoantibody surge predicted insulin dependence ($P = 0.007$).

CONCLUSIONS

HLA-A*24 and early autoantibody surge after islet implantation associate with poor functional graft outcome.

The success rate of an intraportal islet graft for achieving insulin independence critically hinges on the amount of donor tissue implanted (1). However, a positive outcome can be counteracted by other factors, including preexisting autoreactive T cells, HLA alloantibodies, or high lymphocyte counts (2–4). In recipients of a sufficiently large intraportal islet allograft (5,6), we investigated whether the presence of HLA-A*24, HLA-DQ2/DQ8, or rising islet autoantibody levels, previously shown to independently predict rapid β-cell loss in prediabetes (7,8), associates with poor functional graft outcome.

RESEARCH DESIGN AND METHODS

Transplant Protocol

Forty-one patients with C-peptide–negative type 1 diabetes (median age 45 years) with a history of recurrent hypoglycemia and negative for preexisting HLA class I and class II antibodies (4) received ≥2.3 million β-cells (~4,600 islet equivalents)/kg

body wt in one to two intraportal islet implantations (5,6) (clinical trial reg. nos. NCT00798785 and NCT00623610) under antithymocyte globulin-mycophenolate mofetil-tacrolimus therapy. After standardized islet isolation and culture, the cellular composition of the grafts was analyzed before infusion.

Assays and Outcome Measures

Plasma C-peptide and proinsulin were measured with a time-resolved fluorescence immunoassay (AutoDELFIA; PerkinElmer, Turku, Finland) (9), HbA_{1c} levels with high-performance liquid chromatography (Tosoh Bioscience, Tessenderlo, Belgium), and lymphocyte subclasses

with an EPICS XL flow cytometer (Beckman Coulter, Miami, FL). Insulin independence was defined as insulin dose ≤ 8 units $\cdot$ kg^{-1} $\cdot$ day^{-1} and C-peptide negativity as two consecutive random C-peptide values ≤ 0.05 ng/mL. Autoantibodies against GAD (GADA), against IA-2 (IA-2A), and against ZnT8 (ZnT8A) were determined by liquid phase radiobinding assays (10) before the first implantation, 6 weeks and 6 months thereafter, and 6 weeks after the second transplantation, using the 99th percentile of controls as cutoff (10). Significant autoantibody surges were defined according to Decochez et al. (11). Assays were validated by

participation in successive Diabetes Antibody Standardization Programs (10). *HLA-DQ2/DQ8* and *-A*24* were determined by allele-specific oligotyping (8). The hyperglycemic clamp procedure was performed as previously described (6). C-peptide measurements at 120, 135, and 150 min of hyperglycemia (180 mg/dL) were used to calculate the C-peptide area under the curve (AUC).

Statistical Analysis

The data were analyzed with Mann-Whitney U, χ^2, or Fisher exact tests. Kaplan-Meier analysis with log-rank test and Cox proportional hazards model, performed by forward stepwise method, were
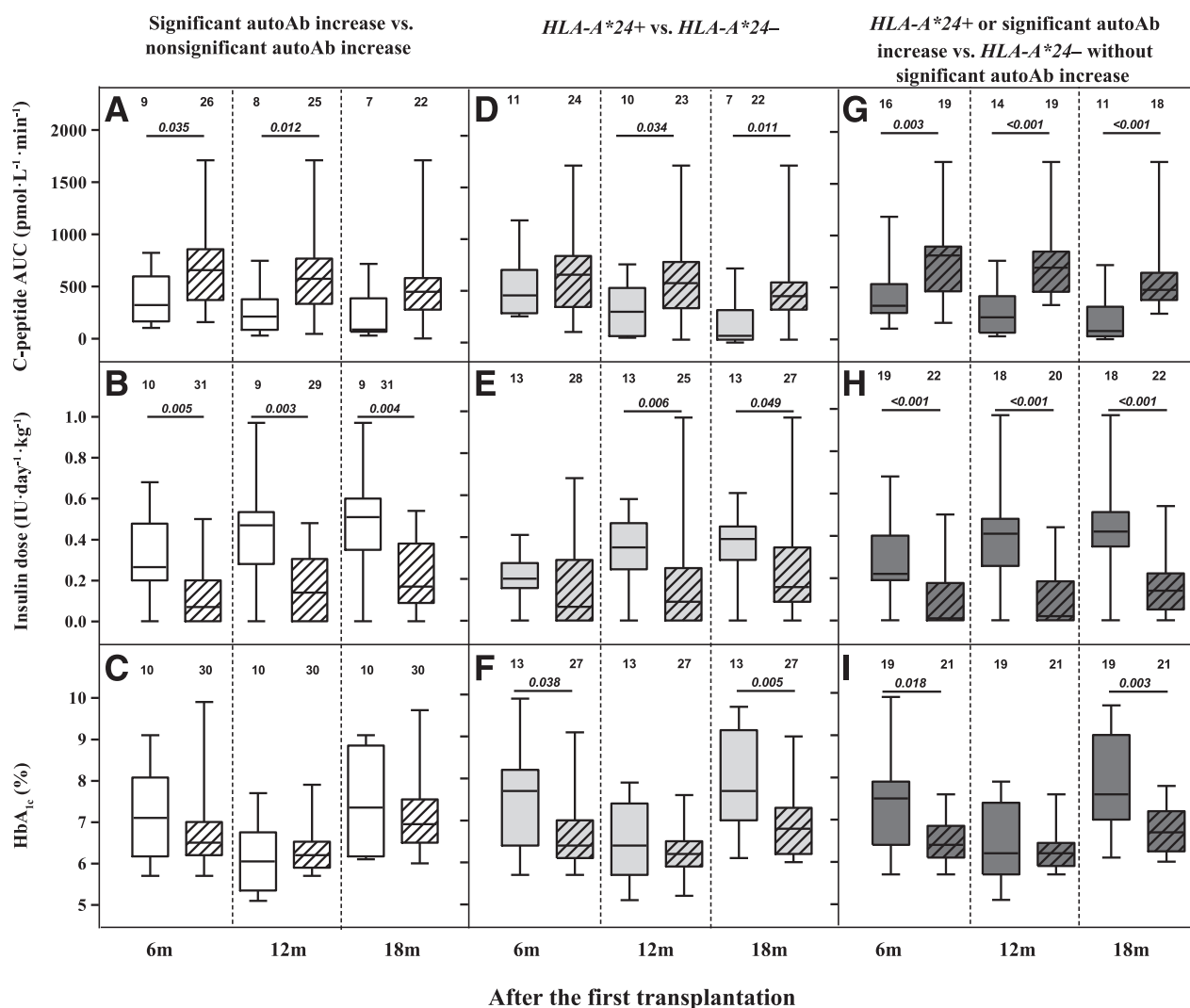

Figure 1—AUC C-peptide release measured during hyperglycemic clamp test, insulin dose, and HbA_{1c} at 6, 12, and 18 months after the first transplantation. *A–C*: Patients with (open bars) or without significant autoantibody (autoAb) increase (hatched bars). *D–F*: Patients who were *HLA-A*24* positive (solid bars) vs. *HLA-A*24* negative (hatched bars). *G–I*: Patients who were *HLA-A*24* positive or with a significant autoAb increase (solid bars) vs. those who were *HLA-A*24* negative and without a significant autoAb increase (hatched bars). Boxes represent median (interquartile range) and whiskers, minimum and maximum. Threshold for significance $P < 0.05/27$ or $P < 0.0019$ (Bonferroni correction for 27 comparisons in Fig. 1). In recipients who were *HLA-A*24* positive, outcome was not influenced by the presence or absence of *HLA-DQ2* or *-DQ8*, hence providing no evidence that the results could be explained by linkage disequilibrium with class II risk haplotypes (data not shown). IU, international units; m, months.

used to assess the development of insulin independence or C-peptide negativity. All tests were performed two sided with SPSS 20 for Windows (IBM Corporation, Chicago, IL). $P < 0.05$ was considered significant.

RESULTS

Immunogenetic Markers of Recipients
Two patients had positive tests for all three autoantibodies, 6 for two (GADA and IA-2A), 18 for one (GADA $n = 11$, IA-2A $n = 4$, ZnT8A $n = 3$), and 15 for none. Ten patients experienced a significant increase in autoantibody level, including seven in whom an additional autoantibody developed within 6 months (GADA in five and IA-2A in two) and three with increases of preexisting GADA.

Table 1—Graft and recipient characteristics of the subgroups according to *HLA-A*24* status in combination with autoantibody increase

	HLA-A*24 positive or significant autoantibody increase	*HLA-A*24* negative without significant autoantibody increase
Recipients		
n	19	22
Age* (years)	41 (33–45)	50 (42–54)†
Sex		
Male	10	13
Female	9	9
BMI* (kg/m^2)	24.1 (22.9–26.1)	23.4 (20.9–25.7)
Duration of disease (years)	22 (15–31)	27 (23–37)
Age at onset (years)	14 (9–26)	19 (13–26)
HbA$_{1c}$ level*		
%	8.0 (7.5–8.7)	7.4 (7.0–8.1)
mmol/mol	63.9 (58.8–71.6)	57.4 (52.7–65.3)
Insulin dose* (international units/kg/day)	0.53 (0.40–0.75)	0.51 (0.42–0.68)
HLA-DQ2	8 (42)	11 (50)
HLA-DQ8	12 (63)	15 (68)
HLA-DQ2/DQ8	1 (5)	6 (27)
HLA-A*24	13 (68)	0 (0)†
HLA-B*18	1 (5)	3 (14)
HLA-B*39	2 (11)	0 (0)
Immune status before ATG		
T-lymphocyte count (CD3$^+$ cells/mm^3)	1,318 (1,101–1,617)	1,234 (1,052–1,814)
B-lymphocyte count (CD19$^+$ cells/mm^3)	252 (175–324)	237 (144–281)
Autoantibody positivity		
0 antibodies	5 (26)	10 (45)
1 antibody	11 (58)	7 (32)
2 antibodies	2 (11)	4 (18)
3 antibodies	1 (5)	1 (5)
IAA	18 (95)	21 (95)
GADA	7 (37)	12 (55)
IA-2A	7 (37)	5 (23)
ZnT8A	4 (22)	1 (5)
Autoantibody levels		
GADA (WHO units/mL)	16.8 (5.3–39.9)	23.9 (5.8–617.0)
IA-2A (WHO units/mL)	1.0 (0.3–8.7)	0.3 (0.3–7.1)
ZnT8A (10^{-3} · index)	9.7 (4.8–23.5)	6.4 (1.2–11.9)
Significant autoantibody increase within 6 months	10 (53)	0 (0)†
Grafts		
Donor pancreata	6 (5–9)	8 (4–10)
Number of β-cells ($\times 10^6$/kg)		
First graft	2.9 (2.6–3.9)	2.6 (2.2–3.6)
Total	4.8 (3.7–6.4)	4.7 (3.7–5.7)
Cellular composition (%)		
β-Cells	28 (24–33)	26 (20–33)
Acinar cells	<10	<10
Nongranulated cells	49 (43–59)	52 (45–61)
Immune suppression		
Total ATG dose (mg/kg)	24 (21–24)	21 (19–24)
Tacrolimus level (AUC · day^{-1} over 6 months) (ng/mL)	9.3 (8.7–9.6)	9.0 (8.5–9.4)
Total MMF dose over 6 months (g)	311 (278–362)	317 (289–361)

Data are *n*, median (interquartile range), or *n* (%). Threshold for significance $P < 0.05/35$ or $P < 0.0014$ (Bonferroni correction for 35 comparisons in Table 1). ATG, antithymocyte globulin; IAA, insulin autoantibody; MMF, mycophenolate mofetil; WHO, World Health Organization. *Determined before the start of induction therapy with ATG (500 β-cells correspond roughly to 1 islet equivalent, but correlation is poor in cultured islet preparation). †$P \leq 0.001$ vs. *HLA-A*24* positivity or significant autoantibody increase.

Thirteen patients carried *HLA-A*24* and seven *HLA-DQ2/DQ8*.

Association With Functional Outcome

Pretransplant autoantibody levels did not correlate with AUC C-peptide or insulin dose (data not shown). However, patients with an autoantibody surge ≤6 months after the first implantation ($n = 10$) tended to have lower C-peptide levels and higher insulin doses despite a trend toward higher HbA_{1c} during 18 months posttransplantation than patients without such a rise ($n = 31$) (Fig. 1*A–C*). Likewise, patients positive for *HLA-A*24* ($n = 13$) experienced lower C-peptide levels and higher insulin doses and HbA_{1c} during follow-up than those negative for *HLA-A*24* ($n = 28$) (Fig. 1*D–F*). Both groups did not differ in the frequency of receiving a graft containing one or more *HLA-A*24*–positive donor or in the number of *HLA-A*24*–positive donors per graft (data not shown). In patients who were positive for *HLA-A*24* or who experienced an early autoantibody surge ($n = 19$) compared with patients lacking both risk factors, differences in C-peptide level ($P \leq 0.003$), insulin dose ($P \leq 0.001$), and HbA_{1c} ($P \leq 0.018$) were strengthened (Fig. 1*G–I*). Outcome was unaltered by the presence or absence of *HLA-DQ2*, *-DQ8*, or *-DQ2/DQ8* (data not shown).

Progression to Insulin Independence and C-Peptide Negativity

Patients without an autoantibody surge more often tended to become insulin independent and to remain C-peptide positive 18 months posttransplantation than those with such a surge (Supplementary Fig. 1*A* and *B*); the same applied for patients negative for *HLA-A*24* versus those positive for *HLA-A*24* (Supplementary Fig. 1*C* and *D*). All recipients lacking both risk factors ($n = 22$) remained C-peptide positive after 18 months ($P < 0.001$ vs. 47% if one or more risk factors), and 68% of them achieved insulin independence ($P = 0.002$ vs. 16% if one or more risk factors) (Supplementary Fig. 1*E* and *F*).

In the poor response group ($n = 19$) only four patients presented both risk factors (Supplementary Fig. 2); hence, the presence of either one explains a larger fraction of recipients with poor outcome. Progression rates to insulin independence or C-peptide negativity were not different according to the number of risk factors present (data not shown).

The subgroup with *HLA-A*24* or autoantibody surge ($n = 19$) did not differ from the rest in patient or graft characteristics except for a lower age and, logically, a higher prevalence of *HLA-A*24* and autoantibody surges (Table 1). To identify predictors of insulin independence (not performed for the development of C-peptide negativity due to low event numbers) all variables from Table 1 were tested in univariate Cox regression. Those with $P < 0.1$ (tacrolimus level over first 6 months, baseline HbA_{1c}) were entered in a multivariate model together with *HLA-A*24*/autoantibody surge. Only the latter remained significant ($P = 0.007$; 95% CI for hazard ratio 0.053–0.635).

CONCLUSIONS

Recipient *HLA-A*24* carrier status and autoantibody surges, mostly GADA, within 6 months after the first islet implantation associate with poor functional outcome. The joint absence of both risk factors identified individuals with higher and more persistent C-peptide release, lower insulin dose and HbA_{1c}, and higher probability of insulin independence during 18 months than in participants presenting at least one marker. This different outcome could not be explained by differences in recipient age, immune status, graft composition, or immune suppression.

The relatively large size and homogeneity of the cohort is a strength of this study. Nevertheless, our observations could have benefited from larger patient numbers in the subgroups that turned out relevant. All patients received sufficient β-cells to generate plasma C-peptide levels at posttransplantation month 2 in C-peptide–negative recipients (2,5) under similar immunosuppression.

The current results indicate that similar to observations in pre–type 1 diabetes, the presence of *HLA-A*24* or autoantibody surges predicts β-cell loss during an inflammatory and/or immune process in islet allograft recipients (7,8). The association of early autoantibody rises with poor graft outcome confirms previous observations and has been explained as the consequence of autoantigen release after β-cell death (12). The negative influence of *HLA-A*24* is, to the best of our knowledge, novel

and consistent with its association with accelerated progression to clinical onset in prediabetes (8). Efficient *HLA-A*24*–restricted presentation of autoantigen fragments to cytotoxic T cells (13) may play a role in the accelerated functional loss posttransplantation, but *HLA-A*24* expression on non–β-cells is likely to be involved because it is the recipients' *HLA-A*24* that affects outcome. The combination of *HLA-A*24* and autoantibody surges is consistent with reports that *HLA-A*24* associates with attenuated humoral responses to islet autoantigens in prediabetes and diabetes (14). The presence of *HLA-DQ2/DQ8*, another independent predictor of diabetes in risk groups (8), was not associated with poor graft outcome.

In conclusion, in recipients of an intraportal islet allograft, the presence of *HLA-A*24* or an early autoantibody surge associates with poor metabolic outcome. Autoantibody monitoring creates a time window during which immunosuppression could be changed or another therapeutic intervention planned. When comparing efficacy of transplantation protocols or prognostic value of novel biomarkers, results should be adjusted for *HLA-A*24* carrier status.

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