Autoantibody Response to Islet Transplantation in Type 1 Diabetes

Emanuele Bosi, Simona Braghi, Paola Maffi, Miriam Scirpoli, Federico Bertuzzi, Guido Pozza, Antonio Secchi, and Ezio Bonifacio

Islet allotransplantation into patients with autoimmune type 1 diabetes represents a reexposure to autoantigen. Here, measurement of antibodies to GAD and IA-2 autoantigens before and after islet transplantation in 36 patients (33 receiving islet plus kidney grafts with cyclosporin and steroid-based immunosuppression, and 3 receiving solitary islet transplants with mycophenolate but cyclosporin-free immunosuppression) demonstrated marked rises in GAD antibodies within 7 days posttransplantation in 5 patients (3 receiving islet after kidney transplants, and 2 receiving solitary islet transplants) and within 30 days in the third patient receiving solitary islet transplantation. GAD antibodies were of the IgG1 subclass, against major autoantigenic epitopes, and in cases of islet after kidney transplants, the responses were short-lived and not accompanied by HLA antibodies. Two of these patients had subsequent marked rises of IA-2 antibodies, and an additional patient had a marked rise in IgM-GAD antibodies 3 years after transplantation. Insulin independence was not achieved in patients with autoantibody elevations and was significantly less frequent in these patients. These data are consistent with a reactivation of autoimmunity that may be dependent on immunosuppression therapy and is associated with impaired graft function. *Diabetes* 50:2464–2471, 2001

β-Cell replacement through transplantation of pancreas, islets, or even genetically engineered non–β-cells remains an attractive cure for type 1 diabetes (1,2). Allotransplantation per se is associated with direct and indirect presentation of alloantigen (3). Both of these modes of antigen presentation can result in allograft rejection, and successful transplantation requires their avoidance through the use of immunosuppressive therapies. The continuing need to treat patients who have received a transplant with immunosuppression has been one factor that has limited the application of transplantation in type 1 diabetes. Recently, however, successful human islet transplantation was reported with relatively low levels of immunosuppression, thereby increasing its applicability (4). This success will undoubtedly increase interest in modifying therapies to further reduce the levels of immunosuppression and to induce tolerance so as to avoid long-term immunosuppression (5).

Transplantation in type 1 diabetes is performed in the presence of an active or memory autoimmune response to islet autoantigens (6). That this autoimmune background also potentially contributes to allograft rejection and therefore also needs to be controlled by immunosuppression is suggested by *1*) reports of inflammatory islet infiltration and selective β-cell destruction after human pancreas and islet transplantation (7–9), *2*) a profoundly lower success rate of islet allotransplantation in autoimmune diabetes models than in nonautoimmune models (10–12), *3*) a lower success rate of islet transplantation in patients with type 1 diabetes than in nonautoimmune patients (1,13,14), *4*) associations of graft failure with the presence of islet autoantibodies pretransplantation (15,16), and *5*) rises in autoantibody titers and T-cell responses posttransplantation that correlate with subsequent graft failure (16–21). The relative contribution of islet autoimmunity to graft survival, however, remains unclear.

Type 1 diabetes–associated autoimmunity is readily measurable at the humoral level with standardized assays to the three major diabetes-related autoantigens: insulin, GAD, and IA-2 (22). We have shown that despite being under immunosuppression, a minority of patients who received pancreas plus kidney allografts showed a marked rise in antibodies to GAD (GADA) and/or protein tyrosine phosphatase IA-2 (IA-2A) from 1 to 3 years posttransplantation, and that the rise was associated with subsequent pancreas but not kidney graft failure (19). We studied 36 patients who received islet plus kidney or islet-only allografts, and remarkably, despite antilymphocyte (ALG)/antithymocyte (ATG) globulin and immunosuppressive treatment, we observed subjects with major rises in IgG1 GADA within 7 days posttransplantation, indicating indirect or direct presentation of autoantigen. Some but not all of these cases were accompanied by the appearance of HLA class I– or class II–specific antibodies, suggesting that autoreactivity may be independent of allorejection. There was a relationship between response characteristics and both the immunosuppressive treatment received by the patients and graft function. These data indicate that autoimmunity in patients with type 1 diabetes is likely to contribute significantly to islet allo-

From the Departments of Medicine and Surgery, San Raffaele Hospital Scientific Institute, Milan, Italy; the University of Milan, Milan, Italy; and the University San Raffaele Vita-Salute, Milan, Italy.

Address correspondence and reprint requests to Emanuele Bosi, MD, Department of Medicine, San Raffaele Hospital Scientific Institute, Via Olgettina, 60, 20132 Milan, Italy. E-mail: bosi.emanuele@hsr.it.

Received for publication 21 May 2001 and accepted in revised form 31 July 2001.

ALG, antilymphocyte; ATG, antithymocyte; GADA, antibodies to GAD; IA, antibodies to insulin; IA-2A, antibodies to IA-2; IEQ, islet equivalents.

graft survival and should be considered in patient management and when designing therapeutic regimens.

RESEARCH DESIGN AND METHODS

Islet and kidney transplants. Between December 1989 and October 2000, 37 islet transplantations were performed either simultaneously with or after kidney graft in 30 patients with long-term type 1 diabetes and renal failure at the San Raffaele Hospital Scientific Institute, Milan. In 33 of these cases performed in 27 patients (median age 40 years [range 32–61]; median duration of diabetes 22 years [9–47]) a pretransplantation serum sample and at least three posttransplantation serum samples (median sample number 6 [3–26]) were available for autoantibody measurements and were therefore included in this study.

In five patients, islets were transplanted simultaneously with kidney graft. The remaining 28 transplantations were performed after kidney graft alone or after a simultaneous pancreas and kidney graft in which pancreas failed mainly for thrombosis (median time after kidney transplantation, 21 months [1–126]).

In the five patients who received simultaneous islet and kidney transplants, islets were always from the same donor of the kidney, and in two of these patients, they were also from two additional donors; in the 28 islet transplants performed after kidney transplant, islets were transplanted from a single donor in 11, from two donors in 14, from three donors in 2, and from four donors in 1 case.

Donors were heart-beating cadavers selected for ABO blood compatibility and negative cross-match. HLA type was not considered as a criterion for the donor-recipient matching. Human islets were isolated and purified from pancreata of cadaver donors using an automated procedure as previously described (23), with subsequent modifications (24). In all cases, islets were injected into the liver. In the first seven patients of this series, islets were injected into a branch of a mesenteric vein reached through a small midline laparotomy under general anesthesia; the remainder received islets injected directly into the portal vein, using a percutaneous transhepatic approach, under local anesthesia with continuous portal pressure monitoring. In 25 cases, patients received >6,000 islet equivalents (IEQ)/kg body wt.

Immunosuppression was based on a combination of cyclosporin and either azathioprine (18 cases) or mycophenolate (15 cases), with an additional course of 4–10 days of ALG or ATG globulin beginning immediately before the transplant procedure. A single shot of 500 mg of methylprednisolone was administered on the day of islet transplantation in 31 cases, and in 27 cases, the patient also received prednisone as part of the chronic immunosuppressive treatment. All transplantation protocols were approved by the institutional ethical committee, and all patients gave informed consent to the procedure.

Solitary islet transplants. In addition to the 33 patients who received islet and kidney allografts, another 3 patients (aged 22, 37, and 49 years; duration of diabetes, 14, 21, and 19 years, respectively) without diabetic nephropathy received solitary islet allografts; the transplant procedure was the same as in the kidney transplant cases, and the 3 patients received >6,000 IEQ/kg body wt from two donors in two cases and from one donor in the other. Immunosuppression was based on an experimental protocol without cyclosporin, including a single shot of 500 mg of methylprednisolone on the day of islet transplantation, ATG for 9 days in one subject (patient 1) or anti-IL2R antibodies (Simulect) on the day of islet transplantation in the other two patients, with an additional dose on day 4 in one of these; all three patients received mycophenolate and, in addition, vitamin D3 and metformin as adjuvants. The protocol was approved by the institutional ethical committee, and all patients gave informed consent for the procedure.

Posttransplantation monitoring, blood glucose management, and assessment of islet function. During the first 10 days after transplantation, the blood glucose level was kept at 5.5 to 8.2 mmol/l by continuous intravenous insulin administration and subsequently by intensified subcutaneous insulin therapy (3–4 injections/day). Insulin doses were progressively tapered according to blood glucose levels and discontinued when fasting and postprandial blood glucose levels were <6.6 and <8.8 mmol/l, respectively (25). Patients were considered insulin-independent when these blood glucose levels were maintained without exogenous insulin for at least 1 week. In insulin-treated transplantation patients, the target level of glycated hemoglobin was <7.0%. An assessment of islet function measurement of fasting serum C-peptide was performed by radioimmunoassay (Diagnostic Products, Los Angeles, CA) daily during the first month, as long as patients remained hospitalized, and at least once 3, 6, and 12 months and every subsequent year after transplantation. The intra- and interassay coefficients of variation of C-peptide measurement were 3 and 5%, respectively.

Measurement of GADA, IA-2A, and antibodies to insulin. GADA and IA-2A measurements were performed by radiobinding assay with in vitro translated ^{35}S-methionine–labeled GAD_{65} or IA-2, as previously described (26,27). Results were converted into arbitrary units by extrapolation from a standard curve with a local standard, designated as 100 units. The thresholds for positivity were determined from the 99th centile of control subjects and corresponded to 3 units for GADA and 1 unit for IA-2A. These GADA: and IA-2A assays obtained the and following performances at the First Combined Islet Autoantibody Workshop: GADA: 88% sensitivity, 98% specificity, 100% reproducibility; and IA-2A: 70% sensitivity, 99% specificity, and 100% reproducibility (22). Antibodies to insulin (IAs) were measured using a competitive protein A/G insulin radiobinding microassay (28).

GAD epitope antibody reactivity. GAD epitope antibody reactivity was measured against GAD65, GAD67, and GAD65/67 chimeras by radiobinding assay as previously described (29). The following GAD constructs were used to measure antibody binding: full-length GAD65 and GAD67, the $GAD65_{1–95}$/$GAD67_{102–593}$ (amino-terminal GAD65 epitopes), the $GAD67_{1–101}$/$GAD65_{96–234}$/$GAD67_{244–593}$ (amino-central GAD65 epitopes), the $GAD67_{1–101}$/$GAD65_{235–444}$/$GAD67_{453–593}$ (carboxy-central GAD65 epitopes), and the $GAD67_{1–452}$/$GAD65_{445–585}$ (carboxy-terminal GAD65 epitopes) chimeras. Radiobinding assays were carried out as for GADA.

GADA and IA-2A IgG subclass and isotype. For GADA and IA-2A IgG subclass (IgG1, IgG2, IgG3, and IgG4) and isotype (IgM, IgA, and IgE) antibody analysis, the protein A/G radiobinding assays were used, substituting the addition of the protein A/G Sepharose with IgG subclass or isotype-specific antibody-bound Sepharose beads, as previously described (30). Results were expressed as SD scores calculated from the mean ± SD of results obtained after subtraction of nonspecific binding to beads coated with anti-rat IgM for control subjects. The mean + 3 SD was used as the threshold for detection.

HLA class I and class II antibodies. Antibodies to HLA class I and class II antigens were measured by flow cytofluorimetry using a commercial kit (One Lambda, Canoga Park, CA).

Statistical analyses. Insulin independence was defined as a condition of normoglycemia in the absence of administration of exogenous insulin. The achievement of insulin independence and 95% CI were calculated by Kaplan-Meier analysis, and comparison between groups was performed using the log-rank test. For all statistical methods, the Statistical Package for Social Sciences (SPSS, Chicago, IL) was used.

RESULTS

Increased islet autoimmunity after islet/kidney transplantation. GADA and IA-2A were negative (n = 22) or at low titer (n = 10) in all but 1 of the 33 patients who underwent islet and kidney transplantation. No relationship between the detection of GADA or IA-2A at the time of transplantation was found with either achievement of insulin independence or a reduction of insulin requirement 12 months posttransplantation (data not shown).

In three patients who received islet after kidney transplantation (patients A, B, and C), marked rises in GADA were seen within 5 days posttransplantation (Figs. 1 and 2*A*). These reached a peak 8–12 days posttransplantation and subsequently declined. GADA were of the IgG1 subclass in all three patients (Fig. 2*B*) and were directed against the major carboxy central 235–444 and carboxy terminal 445–585 epitopes and showed no evidence of spreading (Fig. 2*C*). Patient A also had lesser concomitant rises in IgG2 and IgG3-GADA. No IgG4-GADA were detected in any patients. In patient A, additional IgG1 IA-2 antibody levels rose gradually from ~6 months posttransplantation to very high levels almost 2 years posttransplantation. A second rise in GADA directed against the same carboxy central 235–444 and carboxy terminal 445–585 epitopes was also seen 2 years posttransplantation in this patient. In a fourth patient (D), a rise in GADA was seen 3.2 years posttransplantation. This was of an IgM and not IgG isotype (Fig. 2*B*) and was directed against the carboxy central 235–444 epitope (Fig. 2*C*). Significant changes in

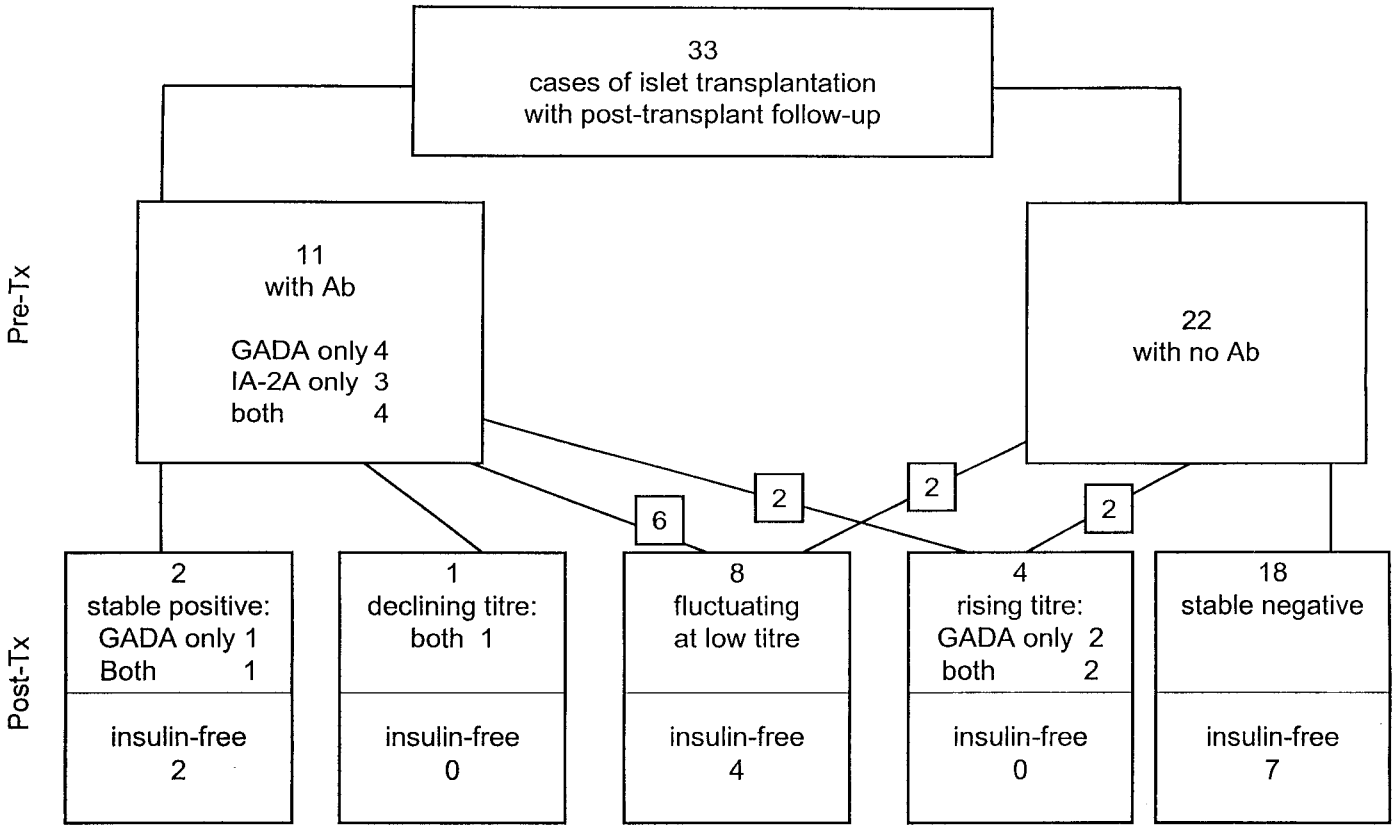

FIG. 1. Schematic representation of islet autoantibodies before and after islet transplantation in 33 cases of islet plus kidney transplantation.

IA titers were not found in these four patients, and in none of these four patients could antibodies to HLA class I or class II molecules be demonstrated posttransplantation (data not shown). All four patients received >6,000 IEQ/kg body wt from a single donor. Three (patients A, B, and C) previously had received a pancreas allograft.

No major rises in GADA or IA-2A were observed in the remaining 29 patients. GADA and/or IA-2A had minor increases with fluctuations at low titer in eight patients, they remained stable in an additional two patients, they declined in one patient, and they remained negative in the remaining 18 patients throughout follow-up (Fig. 1).

Increased islet autoimmunity is associated with decreased graft function. Insulin independence was achieved in 13 of the 33 patients for periods of 1–48 months. All 13 were in the group of 25 patients who had received >6,000 IEQ/kg body wt. None of the four patients with marked rises in islet autoantibodies achieved insulin independence during follow-up ($P = 0.04$ vs. remainder receiving >6,000 IEQ/kg body wt) (Fig. 3). Nevertheless, all four had prolonged periods (>4 months) of C-peptide levels of >1 ng/ml, and insulin requirement was reduced by >50% in all four patients, suggesting partial islet function. A marked reduction in C-peptide coincided with the rise in IA-2A and the second rise in GADA in patient A (Fig. 2*A*).

Increased islet autoimmunity may be a marker of insufficient immunosuppression during islet transplantation. In the three patients who received solitary islet allografts under the experimental immunosuppressive protocol including ATG or anti-IL2R globulin, plus mycophenolate and vitamin D3, complete or partial islet function was not achieved, suggesting that immunosuppression was inadequate for islet survival. The antibody profiles of these are shown in Fig. 4. Patient 1, who received ATG induction, showed a marked rise in IgG1 GADA commencing at day 5 posttransplantation to a peak at day 9. They were directed against all GAD epitopes and were particularly high against the minor NH_2-terminal epitope. IA-2A remained negative, and IA did not change. HLA class I antibodies appeared already at day 8, and by day 14 posttransplantation, strong HLA class II antibodies were also present. C-peptide was detected until day 8. These findings are consistent with rapid complete rejection of transplanted islets. Patient 2 received anti-IL2R instead of ATG. This patient developed strong IgG1 antibodies against the major carboxy central $GAD65_{235-444}$ epitope at day 5 posttransplantation. No HLA antibodies were detected until removal of the immunosuppressive treatment (because of islet graft failure) and were against both class I and class II antigens 6 months posttransplantation. IA-2As were also markedly elevated at 6 months. C-peptide was detectable until day 6. These findings would be consistent with a rapid autoimmune-mediated destruction of islet β-cells, which is followed by alloimmunity and IA-2 autoimmunity to the remaining graft β-cells and non–β-cells once the immunosuppression was removed. Patient 3 had no detectable GADA at the time of transplantation and showed no alloantibodies within the first 14 days post-

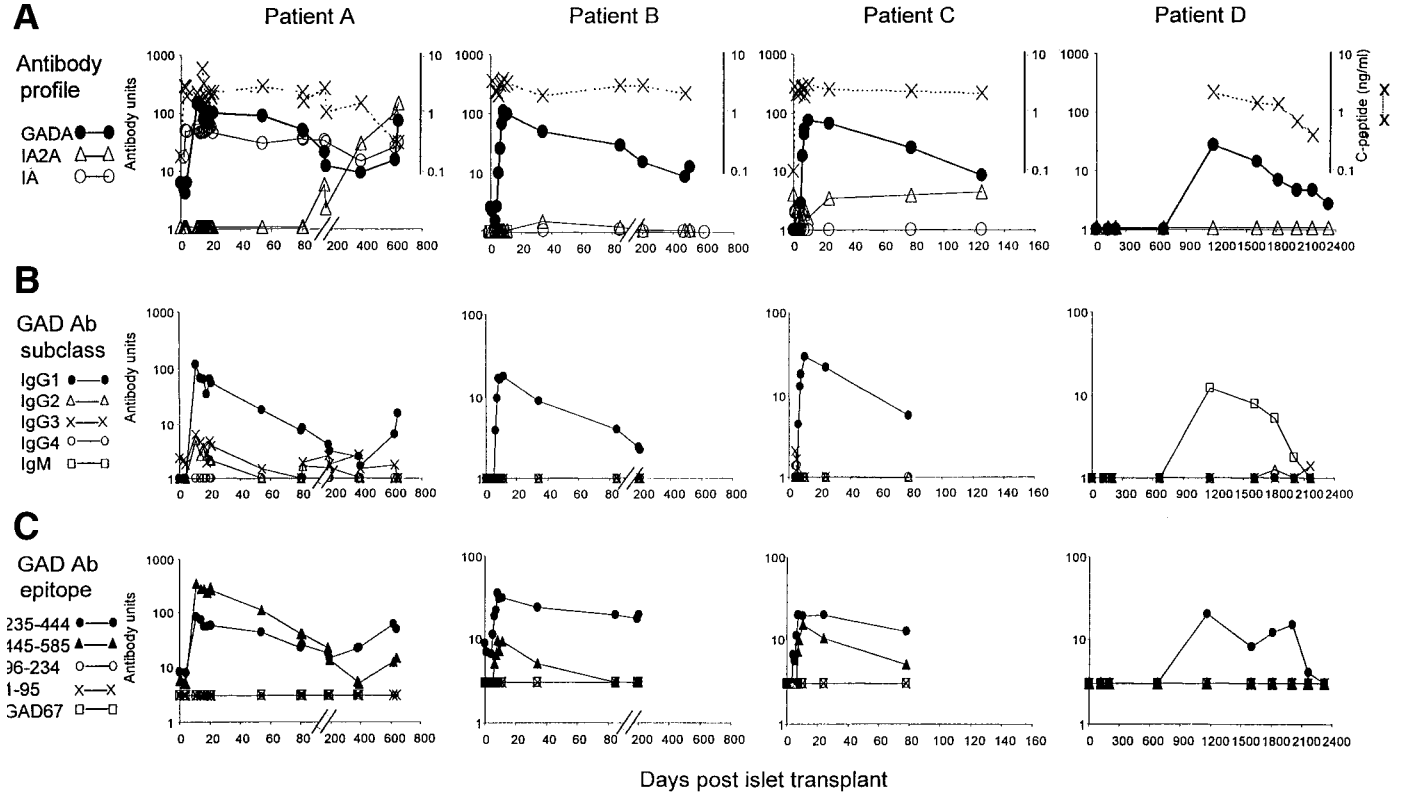

FIG. 2. Antibody changes in four patients who had major rises in GAD or IA-2 antibodies after islet/kidney transplantation. *A*: Levels of GADA (●), IA-2A (△), or IA (○) and basal C-peptide (X) are shown relative to time after islet transplantation. All four patients received one islet preparation at day 0. None became insulin-independent. *B*: GADA IgG subclass (IgG1 [●], IgG2, [△], IgG3 [X], IgG4 [○]) and IgM (□) levels identify IgG1 as the major response in patients A, B, and C and IgM in patient D. *C*: GAD epitope reactivity (carboxy central 235–444 [●], carboxy terminal 445–585 [▲], amino central 96–234 [○], NH_2-terminal 1–95 [X], and GAD67 [□]) is predominantly against the major GAD autoantigenic epitopes (carboxy central and carboxy terminal) in all four patients.

transplantation. A slight rise in GADA was detected from day 7 but was not pronounced until after day 14 posttransplantation. Both HLA alloantibodies and high-titer IgG1 and IgG3 GADA against the carboxy central 235–444 and carboxy terminal 445–585 epitopes were present 31 days after transplantation. Immunosuppression was removed 7 days later, with a subsequent rise in both HLA class I and class II antibodies but not GADA or IA-2A. C-peptide was already markedly reduced at day 14.

Increased islet autoimmunity can occur in the absence of major histocompatibility complex antigen matching. Of the three patients with immediate rises in GADA after receiving islet and kidney transplants, two were identical twins (patients A and B) who received distinct islet preparations. One of these (patient A) received islets that shared one HLA DR allele, whereas patients B and C received islets with no HLA A, B, or DR match. Patient D shared one HLA A allele (Table 1). The absence of matching in two of these cases suggests that autoantigen may be presented by recipient antigen-presenting cells.

Of the three patients who received solitary islet allografts, patient 1, who had marked early allo- and autoantibodies, shared no HLA A, B, or DR alleles with donor islets from the first preparation received and shared one HLA A and B allele with donor islets from the second preparation received 7 days later. Antibody responses were already present by the time patient 1 received the

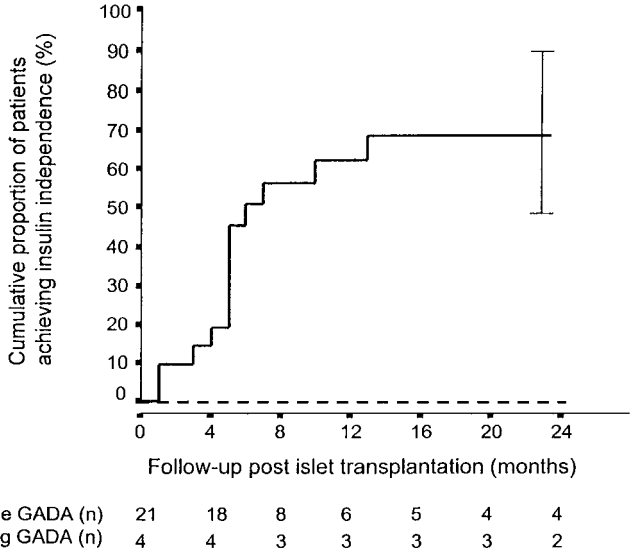

FIG. 3. Achievement of insulin independence after islet transplantation in 25 patients who received >6,000 IEQ/kg body wt. The data are shown as a cumulative frequency calculated using Kaplan-Meyer analysis. Insulin independence was achieved in a total of 13 patients; 8 of these were no longer insulin-independent at the end of follow-up. The number of cases that remained at follow-up intervals of 4 months are shown for those who had marked rises in GADA (broken line; $n = 4$) and those who had relatively stable GADA and IA-2A posttransplantation (solid line; $n = 21$). Patients who had marked rises in GADA achieved insulin independence significantly less frequently than those who had stable antibody titers ($P = 0.04$). Error bars indicate the 95% CI at 24 months after islet transplantation.

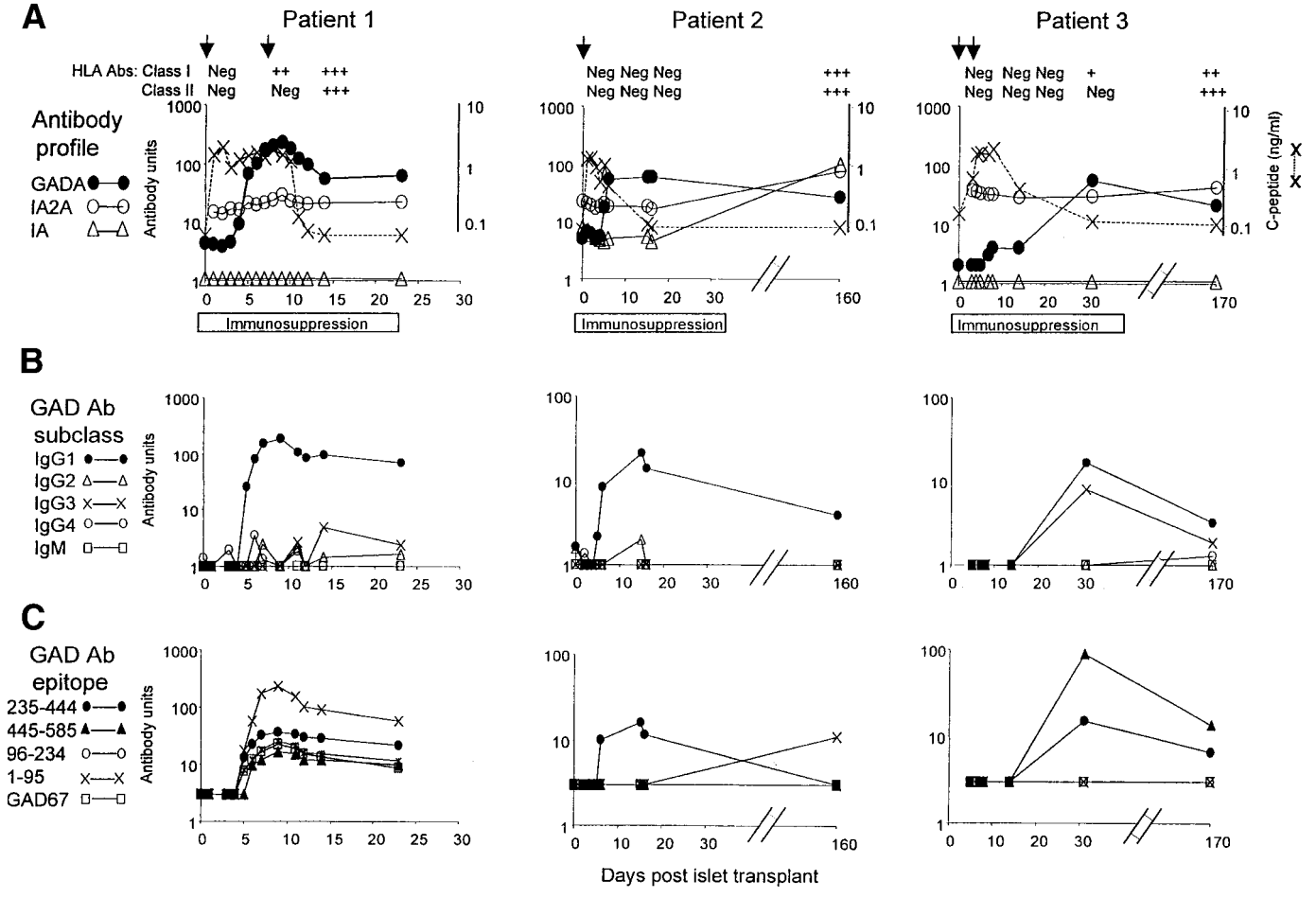

FIG. 4. Antibody changes in three patients who received solitary islet transplantation. *A*: Levels of GADA (●), IA-2A (△), or IA (○) and basal C-peptide (X) are shown relative to time after islet transplantation. Arrows indicate the time of receiving islet preparations. The period of treatment with immunosuppression is shown underneath the abscissa. No patient became insulin-independent. *B*: GADA IgG subclass (IgG1 [●], IgG2 [△], IgG3 [X], IgG4 [○]) and IgM (□) levels identify IgG1 as the major response in all three patients. *C*: GAD epitope reactivity (carboxy central 235–444 [●], carboxy terminal 445–585 [▲], amino central 96–234 [○], NH_2-terminal 1–95 [X], GAD67 [□]).

second islet preparation. Patient 2, who had marked early autoantibodies, shared one HLA A and one HLA DR allele with donor islets. Patient 3 shared a single HLA A allele with both donor islet preparations.

DISCUSSION

Islet transplantation in patients with autoimmune type 1 diabetes represents a reexposure of patient immune system to autoantigen in the context of allo–major histocompatibility complex. In this study, we showed that such exposure can, even in the presence of active immunosuppression, result in both immediate marked elevation of type 1 diabetes–associated autoimmunity to GAD and, as previously reported after pancreas transplantation (19), late autoimmune responses in a minority of patients. The occurrence of autoimmunity was independent of donor-recipient HLA matching and autoantibody titer at the time of transplantation, also occurred in the absence of alloimmunity, and was associated with reduced posttransplantation islet function.

The only unequivocal example of rapid transplant-induced reactivation of β-cell–specific autoimmunity reported so far in human type 1 diabetes is the recurrence of insulitis and clinical disease weeks after technically successful segmental pancreatic transplantation between identical and nonimmunosuppressed twins (7). In those studies, a recurrent T-cell (mainly $CD8^+$)–mediated autoimmunity was not accompanied by a reactivation of humoral autoimmunity as assessed by immunohistochemistry of graft biopsies.

Unlike the twin-to-twin transplant model, our cases are HLA-mismatched and immunosuppressed. Under these conditions, an immediate elevation of GADA after islet transplantation was seen in five patients, four of whom had low GADA titer before transplantation. Evidence that this was a recurrence of a quiescent but already mature autoimmune memory is seen in the rapidity and intensity of the IgG response and the absence of a detectable IgM response to autoantigens in these patients. HLA antibodies were readily detectable in one patient who received solitary islet allografts (patient 1), indicating that the accompanying autoantibody response may have arisen indirectly from islet damage and inflammation associated with acute rejection in this case. Although assays used for the measurement of alloantibodies may be less sensitive than the autoantibody radiobinding assays, we found no evidence of an accompanying alloimmune response at the humoral level in the remaining four patients with immediate GAD

TABLE 1
HLA alleles of donors and recipients in cases of markedly elevated islet autoantibodies

Patients	Recipient HLA			Islet donor HLA		
	A	B	DR	A	B	DR
Islet kidney transplants						
Patient A	3, 29	44, 62	4, **11**	24	35, 61	**11**, 16
Patient B	3, 29	44, 62	4, 11	1, 30	8, 13	3, 13
Patient C	3, 24	18, 44	3, 4	1, 11	52, 55	7, 14
Patient D	**1**, 9	18	11, 7	**1**, 32	27, 35	NT
Solitary islet transplants						
Patient 1	**24**, 31	**35**, 52	4	2, 10	18, 38	3, 11
				2, **24**	44, **35**	11
Patient 2	**2**, 29	8, 18	3, **11**	**2**	51, 50	7, **11**
Patient 3	**1**, 2	8, 52	3, 16	**1**, 24	51, 57	7, 11
				1, 68	14, 44	11, 13

Alleles in bold are shared between recipient and donor. NT, not tested.

antibody responses, suggesting that these patients had a direct and selective recurrence of autoimmunity. T-cell studies were not performed, but it should be noted that previous studies of T-cell response to alloantigen after islet transplantation could also demonstrate autoreactivity without accompanying alloresponses (21). Recently, a rise in GADA was also reported after the transplantation of fetal islets in nonimmunosuppressed patients with type 1 diabetes, further indicating the ability of islets to recall autoimmune memory (31). Of potential interest was the observation that two of the patients who showed immediate GADA rises were identical twins, suggesting genetic influence.

The immediate GADA responses in patients A, B, and C were short-lived. The responses reached peak levels within 2 weeks posttransplantation and subsequently declined at rates not largely divergent from the half-life of circulating IgG1 (32). A short-lived response could be explained by direct presentation of antigen by donor antigen-presenting cells, T-cell–independent B-cell stimulation by soluble antigen, clearance of immunogenic autoantigen, and active immune regulation or immunosuppression. Complete mismatch of genotyped HLA loci was observed in two of the patients with GAD responses, but direct presentation via HLA-DP or cross-presentation is possible in these cases. An alternative scenario is that soluble autoantigen is released from damaged islets and either taken up by recipient antigen-presenting cells relocated in the inflammatory response or reaches immune cell sites containing autoreactive memory lymphocytes, such as pancreatic draining lymph nodes. Resolution of the autoimmune response and stable C-peptide levels after this immediate autoantibody response suggest that it is not associated with an ongoing active immune-mediated β-cell destruction if immunosuppression is adequate. Indeed, such antibody responses may not necessarily be accompanied by T-cell–mediated islet destruction. Inadequate immunosuppression resulting in allorejection (patient 1) or unsuppressed autoimmunity (patient 2) was, however, associated with rapid islet allograft failure.

Although numbers remain small, the ability to achieve insulin independence in patients who received sufficient islet numbers seemed to be markedly decreased in patients with autoantibody elevations posttransplantation. None of these patients became insulin-independent, whereas in the series of patients who received similar islet/kidney transplantation, insulin independence within 12 months posttransplantation was achieved in >60% of patients who did not show elevations in autoantibodies. It may be argued, therefore, that recurrent autoimmunity is a major cause for the decreased function of islet allografts in patients with type 1 diabetes compared with islets autotransplanted (33,34) or transplanted into patients with type 2 diabetes (14) or without diabetes (13). Our data also suggest that some therapies may be better suited for the prevention of this recurrence than others and may therefore be more effective in maintaining islet allograft function in patients with type 1 diabetes. Immediate GAD antibody responses were observed in two of the three patients who received solitary islet transplants; this was significantly more frequent than in patients receiving islet/kidney transplants ($P < 0.05$). Although there were several differences between these patients and those who received islet/kidney transplants, it is noteworthy that these three patients did not receive cyclosporin or other calcineurin inhibitors. Also noteworthy is that the recently reported successful solitary islet transplantation using a combination of the calcineurin inhibitor FK506, anti–IL-2 receptor, and rapamycin did not show rises in islet autoantibodies (4).

This study identified GAD as a key autoantigen in the reexposure of patients with autoimmune diabetes to β-cells. Responses in all but one case were against the major autoantibody epitopes (29). It is interesting that epitope recognition differed in the one patient who had concurrent strong alloreactivity (patient 1), in whom the dominant response was against the minor NH_2-terminal epitope. This anecdotal finding may indicate diversity when autoimmunity accompanies allorejection. Studies of the early response to native β-cell autoantigens suggest insulin autoantibodies as the first to appear (35). Although IAs were not studied systematically in all patients because of a confounding humoral response to exogenously administered insulin in these patients, we were unable to demonstrate a rise in IA in any of the patients who exhibited rises in GADA, and indeed patients B, C, and 2 had undetectable levels of IA throughout follow-up. Several reasons could be postulated for the relative absence of insulin reactivity, including the possibility that treatment with exogenous insulin induces tolerance. IA-2A responses were rare, but in the one patient in whom IA-2A did rise while on immunosuppressive therapy, the antibody response occurred several months after GADA, was sustained, and was followed by a second rise in GADA and a concomitant fall in C-peptide levels. Late responses to autoantigen while receiving immunosuppression were found in one other patient (patient D). In this case, the response was atypical, with relatively strong IgM antibodies against the major GAD epitope, persisting for >12 months without a switch to IgG, and not accompanied by obvious decreased graft function. The late rise in autoantibodies seen in patient A is similar to what we have previously observed in a minority of patients with type 1 diabetes who underwent pancreas transplantation (19). Patients who under-

went pancreas transplantation did not, however, show an immediate rise in islet autoantibodies, suggesting an overall lower capacity to stimulate the memory autoimmune response.

In conclusion, this study demonstrated that autoimmunity after islet transplantation is a clinically relevant occurrence in patients with type 1 diabetes. Its avoidance is likely to be of benefit for islet allograft survival and function, and therapies that can prevent the recurrence of memory autoimmune responses are therefore advocated for successful islet transplantation.

ACKNOWLEDGMENTS

This study was supported in part by the European Union shared cost project DIABMARKER, Juvenile Diabetes Research Foundation International Grant 5-2001-424, and the University of Milan.

We are indebted to Dr. Elena Bazzigaluppi and Cristina Belloni for help in autoantibody measurements and epitope characterization; Dr. Lucilla Monti for C-peptide measurement; Dr. Elena Benazzi for help in HLA antibody measurements; Dr. Eliana Porta and the Nord Italia Transplant team for providing HLA typing data; Dr. Luciano Adorini and Dr. Alberto Davalli for collaboration in the solitary islet transplant program; and Professor Valerio Di Carlo, Dr. Luca Aldrighetti, Dr. Carlo Socci, and the transplant team for collaboration and support.

REFERENCES

1. Robertson RP, Davis C, Larsen J, Stratta R, Sutherland DER: Pancreas and islet transplantation for patients with diabetes. *Diabetes Care* 23:112–116, 2000
2. Lee HC, Kim SJ, Kim KS, Shin HC, Yoon JW: Remission in models of type 1 diabetes by gene therapy using a single-chain insulin analogue. *Nature* 408:483–488, 2000
3. Waldmann H: Transplantation tolerance: where do we stand? *Nat Med* 5:1245–1248, 1999
4. Shapiro AMJ, Lakey JRT, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238, 2000
5. Rossini AA, Greiner DL, Mordes JP: Induction of immunologic tolerance for transplantation. *Physiol Rev* 79:99–141, 1999
6. Slover RH, Eisenbarth GS: Prevention of type I diabetes and recurrent β-cell destruction of transplanted islets. *Endocr Rev* 18:241–258, 1997
7. Sibley RK, Sutherland DER, Goetz F, Michael AF: Recurrent diabetes mellitus in the pancreas iso- and allograft: a light and electron microscopic and immunohistochemical analysis of four cases. *Lab Invest* 53:132–144, 1985
8. Tyden G, Reinholt FP, Sundkvist G, Bolinder J: Recurrence of autoimmune diabetes mellitus in recipients of cadaveric pancreatic grafts. *N Engl J Med* 335:860–863, 1996
9. Stegall MD, Lafferty KJ, Kam I, Gill RG: Evidence of recurrent autoimmunity in human allogeneic islet transplantation. *Transplantation* 61:1272–1274, 1996
10. Prowse SJ, Bellgrau D, Lafferty KJ: Islet allografts are destroyed by disease occurrence in the spontaneously diabetic BB rat. *Diabetes* 35:110–114, 1986
11. Weringer EJ, Like AA: Immune attack on pancreatic islet transplants in the spontaneously diabetic BioBreeding/Worcester (BB/W) rat is not MHC restricted. *J Immunol* 134:2383–2386, 1985
12. Markees TG, Serreze DV, Phillips NE, Sorli CH, Gordon EJ, Shultz LD, Noelle RJ, Woda BA, Greiner DL, Mordes JP, Rossini AA: NOD mice have a generalized defect in their response to transplantation tolerance induction. *Diabetes* 48:967–974, 1999
13. Tzakis AG, Ricordi C, Alejandro R, Zeng Y, Fung JJ, Todo S, Demetris AJ, Mintz DH, Starzl TE: Pancreatic islet transplantation after upper abdominal exenteration and liver replacement. *Lancet* 336:402–405, 1990
14. Ricordi C, Alejandro R, Angelico CA, Fernandez LA, Nery J, Webb M, Bottino R, Selvaggi G, Khan FA, Karatzas T, Olson L, Mintz DH, Tzakis AG: Human islet allografts in patients with type 2 diabetes undergoing liver transplantation. *Transplantation* 63:473–475, 1997
15. Bosi E, Bottazzo GF, Secchi A, Pozza G, Shattock M, Saunders A, Gelet A, Touraine JL, Traeger J, Dubernard JM: Islet cell autoimmunity in type I diabetic patients after HLA-mismatched pancreas transplantation. *Diabetes* 38 (Suppl. 1):82–84, 1989
16. Jaeger C, Brendel MD, Hering BJ, Eckhard M, Bretzel RG: Progressive islet graft failure occurs significantly earlier in autoantibody-positive than in autoantibody-negative IDDM recipients of intrahepatic islet allografts. *Diabetes* 46:1097–1910, 1997
17. Esmatjes E, Rodriguez-Villar C, Ricart MJ, Casamitjana R, Martorell J, Sabater L, Astudillo E, Fernandez-Cruz L: Recurrence of immunological markers for type 1 (insulin-dependent) diabetes mellitus in immunosuppressed patients after pancreas transplantation. *Transplantation* 66:128–131, 1998
18. Sundkvist G, Tyden G, Karlsson FA, Bolinder J: Islet autoimmunity before and after pancreas transplantation in patients with type 1 diabetes mellitus. *Diabetologia* 41:1532–1533, 1998
19. Braghi S, Bonifacio E, Secchi A, Di Carlo V, Pozza G, Bosi E: Modulation of humoral islet autoimmunity by pancreas allotransplantation influences allograft outcome in patients with type 1 diabetes. *Diabetes* 49:218–224, 2000
20. Thivolet C, Abou-Amara S, Martin X, Lefrancois N, Petruzzo P, McGregor B, Bosshard S, Dubernard JM: Serological markers of recurrent beta cell destruction in diabetic patients undergoing pancreatic transplantation. *Transplantation* 69:99–103, 2000
21. Roep BO, Stobbe I, Duinkerken G, Van Rood JJ, Lernmark A, Keymeulen B, Pipeleers D, Class FHJ, De Vries RRP: Auto- and alloimmune reactivity to human islet allografts transplanted into type 1 diabetic patients. *Diabetes* 48:484–490, 1999
22. Verge CF, Stenger D, Bonifacio E, Colman PG, Pilcher C, Bingley PJ, Eisenbarth GS: Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. *Diabetes* 47:1857–1866, 1998
23. Ricordi C, Lacy PE, Finke EH, Olack BJ, Sharp DW: Automated method for isolation of human pancreatic islets. *Diabetes* 37:413–420, 1988
24. Socci C, Falqui L, Davalli AM, Ricordi C, Braghi S, Bertuzzi F, Maffi P, Secchi A, Gavazzi F, Freschi M, Magistretti P, Socci S, Vignali A, Di Carlo V, Pozza G: Fresh human islet transplantation to replace pancreatic endocrine function in type 1 diabetic patients. *Acta Diabetol* 28:151–157, 1991
25. Secchi A, Socci C, Maffi P, Taglietti MV, Falqui L, Bertuzzi F, De Nittis P, Piemonti L, Scopsi L, Di Carlo V, Pozza G: Islet transplantation in IDDM patients. *Diabetologia* 40:225–231, 1997
26. Bonifacio E, Genovese S, Braghi S, Bazzigaluppi E, Lampasona V, Bingley PJ, Rogge L, Pastore MR, Bognetti E, Bottazzo GF, Gale AEM, Bosi E: Islet autoantibody markers in insulin-dependent diabetes: risk assessment strategies yielding high sensitivity. *Diabetologia* 38:816–822, 1995
27. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E: Identification of protein tyrosine phosphatase-like IA-2 (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. *J Immunol* 155:5419–5426, 1995
28. Naserke HE, Dozio N, Ziegler AG, Bonifacio E: Comparison of a novel microassay for insulin autoantibodies with the conventional radiobinding assay. *Diabetologia* 41:681–683, 1998
29. Bonifacio E, Lampasona V, Bernasconi L, Ziegler AG: Maturation of the humoral autoimmune response to epitopes of GAD in preclinical childhood type 1 diabetes. *Diabetes* 49:202–208, 2000
30. Bonifacio E, Scirpoli M, Kredel K, Fuchtenbusch M, Ziegler AG: Early autoantibody responses in pre-diabetes are IgG1 dominated and suggest antigen-specific regulation. *J Immunol* 163:525–532, 1999
31. Brooks-Worrell BM, Peterson KP, Peterson CM, Palmer JP, Jovanovic L: Reactivation of type 1 diabetes in patients receiving human fetal pancreatic tissue transplants without immunosuppression. *Transplantation* 69:1824–1829, 2000
32. Janeway AC, Traver P: *Immunobiology: The Immune System in Health and Disease.* 3rd ed. London, Current Biology, 1997, p 3–22
33. Pyzdrowski KL, Kendall DM, Halter JB, Nakhleh RF, Sutherland DER, Robertson RP: Preserved insulin secretion and insulin independence in recipients of islet autografts. *N Engl J Med* 327:220–226, 1992

34. Teuscher AU, Kendall DM, Smets YFC, Leone JP, Sutherland DER, Robertson RP: Successful islet autotransplantation in humans. Functional insulin secretory reserve as an estimate of surviving islet cell mass. *Diabetes* 47:324–330, 1998

35. Ziegler AG, Hummel M, Schenker M, Bonifacio E: Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. *Diabetes* 48:460–468, 1999