Kinetics of diabetes-associated autoantibodies after sequential intraportal islet allograft associated with kidney transplantation in type 1 diabetes

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Objective: Presence or occurrence of pancreas auto-antibodies (aAb) has been shown to be of poor prognosis for islet cell transplantation. The aim of the study was to monitor the kinetics of these aAb after sequential intra-portal islet plus kidney transplantation with pre-Edmonton immunosuppressive regimen in order to determine whether the sequential protocol of transplantation was involved in the occurrence of the immune response.

Patients and methods: Three patients with IDDM and a previous (IAK) or simultaneous (SIK) kidney transplantation received 3 or 4 ABO compatible islet preparations. Islets (> 8 000 IEQ/kg post culture) were sequentially transplanted within a 12 day period via a percutaneous catheter. Immunosuppressive treatment included cyclosporine, steroids and mycophenolate. Plasma ICAs, GAD 65, IA2 and C peptide (C-p) levels were monitored. Type II HLA phenotype was determined in donors and recipients.

Results: Patient #1 had high anti-GAD levels (26.5 UI/I) before the IAK, while anti-IA2 and ICA levels were low. After the transplantation, C-p levels increased to 4.9 ng/ml at one month before becoming undetectable at 2 months. GAD levels remained high, ICA and IA2 aAb were undetectable. Patients #2 and #3 did not have significant levels of aAb before the islet transplantation. A slight increase in GAD was observed with each islet transplantation, followed by an overt but transient increase in ICA. IA2 levels remained undetectable. Three months after the transplantation and 2 weeks after the increase of ICA, C-p levels, that were >3.4 ng/ml at one month, fell below 0.2 (N: 0.5-2).

Conclusion: The immunosuppressive regimen used in kidney transplantation is unable to control perfectly anti-pancreas aAb production. Moreover, these results seem to indicate that the benefits of sequential islet transplantation lie more in the increased islet mass they provide than in potential immune benefit.

Key-words: Islet transplantation - Pancreas autoantibodies - Type 1 diabetes.

RÉSUMÉ
Cinétique des auto-anticorps associés au diabète après allogreffe séquentielle intraportale associée à une transplantation rénale dans le diabète de type 1

Objectif : La présence ou la survenue d’auto-anticorps (aAc) anti-pancréas est de mauvais pronostic dans la greffe d’îlots. Le but de ce travail était de suivre l’évolution de ces aAc après greffe séquentielle intraportale d’îlots associée à une transplantation rénale selon un protocole d’immunosuppression classique (pré-Edmonton) afin de déterminer si le caractère séquentiel de la transplantation était impliqué dans la survenue de la réponse immune.

Patients et méthodes : Trois patients présentant un diabète de type 1 et ayant bénéficié d’une transplantation rénale antérieure (IAR) ou simultanée (ISR) à la greffe d’îlots ont reçu 3 à 4 préparations insulinaires selon une compatibilité ABO. Les îlots (> 8 000 IEQ post-culture) ont été transplantés séquentiellement sur une période de 12 jours par cathéter percutané. Le traitement immunosuppresseur comportait de la cyclosporine, des stéroïdes et du mycophénolate. Les taux plasmatiques d’aAc ICA, GAD 65 et IA2 ainsi que les taux de C- peptide (C-p) ont été suivis. Le phénotype HLA de classe II a été déterminé chez les donneurs et les receveurs.

Résultats : Le patient #1 avait des taux élevés d’aAc antiGAD (26.5 UI/I) avant la greffe rénale (IAR), tandis que les taux d’IA2 et d’ICA étaient bas. Après la transplantation, les concentrations de C-p se sont élevées à 4.9 ng/ml avant de devenir indétectables 2 mois après la greffe. Les taux de GAD ont légèrement augmenté et les taux d’IA2 et d’IA2 indétectables. Les patients #2 et #3 n’avaient pas de taux élevés d’aAc avant la greffe d’îlots. Une légère augmentation des anti-GAD a été observée après chaque transplantation insulaire, suivie par une augmentation plus fruste mais transitoire des ICA. Les taux d’IA2 sont restés indétectables. Trois mois après la transplantation et 2 mois après l’augmentation des ICA, le taux de C-p, qui atteignait 3,4 ng/ml un mois après la greffe, s’est effondré à moins de 0,2 ng/ml (N : 0.5-2).

Conclusion : Le traitement immunosupresseur utilisé dans la greffe rénale ne permet pas de maîtriser totalement la production des aAc anti-pancréas. De plus, ces résultats suggèrent que les bénéfices de la greffe d’îlots séquentielle tiennent plus à la quantité d’îlots transplantés qu’à un hypothétique bénéfice immunologique.

Mots-clés : Greffe d’îlots - Anticorps anti-pancréas - Diabète de type 1.
The Edmonton team recently showed that the association of sequential islet allograft with a steroid-free immunosuppressive regimen improves the prognosis of islet transplantation [1]. Previously, immune reactions such as rejection and recurrence of autoimmune, and nonimmune causes such as limited islet mass, coagulation activation and toxicity of immunosuppressive drugs accounted for the short-term impairment of islet transplantation function. Among immune issues, a number of case reports have provided clear evidence of recurrent autoimmune insulitis despite sustained immunosuppression in both pancreatic and islet allografts [2-8]. The relationship between progressive islet failure and autoantibody (aAb)-positive IDDM recipients of intra-hepatic islet allografts (IIA) has been demonstrated [4, 8]. Among non-immune issues, the problem of islet mass was partly resolved by resorting to multidonor sequential allograft, a procedure that may positively affect the immune status [9]. In their assay, Shapiro et al. [1] overcame both immune and non-immune issues by modifying two points of the previous islet transplantation procedure: they adopted a multidonor sequential protocol and a steroid-free immunosuppressive regimen. Because they simultaneously modified two aspects of the procedure, it proved difficult to assess what exactly was behind improved results, especially behind the better-checked immune response. The object of this study was to monitor the kinetics of aAb in three recipients of sequential multidonor IIA and kidney transplantation with pre-Edmonton immunosuppressive regimen to determine which of either the immunosuppressive drugs or the sequential protocol of transplantation was involved in controlling the autoimmune response.

Patients and methods

Patients

Sequential iterative human IIA were performed in three C-peptide negative diabetic male patients included in the Lille islet transplantation project. The protocol was approved by the Lille University ethics committee, and all subjects gave their written informed consent before inclusion. Patient characteristics are given in Table I.

Islet transplantation

The procedure for islet transplantation has been described previously [10]. Briefly, once an islet preparation isolated from a local donor was deemed suitable for transplantation, the recipient was admitted for surgical placement of a multidonor sequential protocol and a steroid-free immunosuppressive regimen. Because they simultaneously modified two aspects of the procedure, it proved difficult to assess what exactly was behind improved results, especially behind the better-checked immune response. The object of this study was to monitor the kinetics of aAb in three recipients of sequential multidonor IIA and kidney transplantation with pre-Edmonton immunosuppressive regimen to determine which of either the immunosuppressive drugs or the sequential protocol of transplantation was involved in controlling the autoimmune response.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Clinical and biological data for three engrafted male patients (N: normal range).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient #1</td>
<td>Patient #2</td>
</tr>
<tr>
<td>Age at the time of islet transplantation (years)</td>
<td>43</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62</td>
</tr>
<tr>
<td>Diabetes duration before transplantation (years)</td>
<td>32</td>
</tr>
<tr>
<td>Duration of previous kidney graft (years)</td>
<td>6</td>
</tr>
<tr>
<td>HLA-A</td>
<td>A3, X</td>
</tr>
<tr>
<td>HLA-B</td>
<td>B5, Y</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>DR4, DR4</td>
</tr>
<tr>
<td>Blood group</td>
<td>A</td>
</tr>
<tr>
<td>Pre-graft antiGAD65 plasma levels (UI/l; N &lt; 1)</td>
<td>26.5</td>
</tr>
</tbody>
</table>

Islets were isolated with a slightly modified standard automated method [11] using purified collagenase (Liberase), before undergoing isopycnic purification in Histopaque gradients with a cell separator (Cobe 2991). Iset preparations were then cultured for one night in serum free medium so that they could undergo microbiological and functional characterization before being transplanted. Successive islet preparations were injected when available through an intra-portal percutaneous catheter as a bedside procedure until at least 8,000 islets (post-culture) per kilogram of body mass were transplanted. Immunosuppressive treatment was reinforced within the few hours before the first islet injection by an intravenous bolus of steroids and anti-lymphocyte globulins (ATG, Fresenius), followed by a triple regimen with cyclosporine, corticoids, mycophenolate mofetil and, when needed, an additional bolus of ATG before each islet injection to maintain lymphopenia. Instead of ATG, the third patient received anti-interleukin receptor type 2 (daclizumab) because he had exhibited cutaneous symptoms after the injection of antilymphocyte globulins for his kidney graft. The post transplantation regimen also included the strict control of blood glucose (intravenous insulin for two weeks after transplantation), and administration of pentoxifylline and nicotineamide. The characteristics of islet preparations and HLA donors are detailed respectively in Tables II and III. All donors had normal levels of glycated hemoglobin.

Biological data

Serum samples

In all cases, at least one pretransplant serum sample was available for assessment of ICAs, GAD 65, IA-2 aAB. Anti-
insulin aAb were not investigated because the patients had been treated with exogenous insulin for many years. All patients regularly attended the follow-up visits after the islet transplantation for evaluation of islet graft function: every day during the first two weeks, every two days during the second two weeks, twice a week during the second month, then once a week. Aab were monitored twice a week during the first month, and once a week thereafter.

Endogenous insulin secretion

Fasting serum C-peptide concentrations were measured with a commercial radioimmunoassay kit and expressed in ng/ml (RIA-coat C-peptide, Mallinckrodt, France). The detection threshold was 0.2 ng/ml.

Detection of cytoplasmic cell antibodies (ICA)

ICAs were determined by indirect immunofluorescence using sections of frozen human group O pancreas. Results are expressed in Juvenile Diabetes Foundation (JDF) units using a JDF standard reference serum. Antibody titers were determined by serial dilutions to end-point; measurements were performed according to the protocol from the International Workshop for the standardization of the ICA assay. Technical validity was assessed in the “13th ICA Proficiency Test” with a diagnostic sensitivity of 83% and a specificity of 75% for type 1 diabetes. The cut-off value of 1U/ml represents the mean ± 3SD of the GAD aAb values in 100 healthy school children (age: 11 ± 2 years). Regarding IA-2 aAb the cut-off of 1U/ml was used according to manufacturer’s instructions. Threshold for aAb positivity was 1 UI/ml.

Results

Patient #1

Patient #1 was A3/X; B5/Y; DR4/DR4 and had high anti GAD levels (26.5 UI/ml) before the IAK transplantation, while anti IA2 and ICA levels were undetectable. After the transplantation, plasma C peptide levels increased to 4.9 ng/ml on day 30 before decreasing to undetectable values on day 60. Anti GAD 65 aAb levels did not vary significantly. ICA and anti IA2 aAb remained undetectable during all the follow-up. The kinetics of C peptide and antibodies plasma levels are given in Figure 2A.

A liver biopsy showed the presence of islet cells already surrounded with lymphocytes (Fig 1).

Patient #2

Patient #2 was A3/A24; B62/B-; DR4/DR13 and did not have significant levels of antiGAD65, ICA or IA2 before the simultaneous islet-kidney transplantation. A slight but pro-

Table II

Characteristics of the islet preparation engrafted in the three patients.

<table>
<thead>
<tr>
<th>Patient #1</th>
<th>Patient #2</th>
<th>Patient #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of donor</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Timing of islet grafts (day)</td>
<td>0-2-6-8</td>
<td>0-8-10</td>
</tr>
<tr>
<td>IEQ pre-culture</td>
<td>1 092 000</td>
<td>1 156 000</td>
</tr>
<tr>
<td>IE post-culture/kg</td>
<td>10 072</td>
<td>8 009</td>
</tr>
</tbody>
</table>

Table III

Characteristics of HLA donors. The genotype combinations of donor #4 (patient #1) and donor #3 (patient #3) were susceptible for type 1 diabetes.

<table>
<thead>
<tr>
<th>Patient #1</th>
<th>Patient #2</th>
<th>Patient #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral to susceptibility</td>
<td>Protective</td>
<td>Protective</td>
</tr>
<tr>
<td>Neutral</td>
<td>Protective</td>
<td>Protective to neutral</td>
</tr>
<tr>
<td>Neutral</td>
<td>Neutral</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td></td>
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</tbody>
</table>
gressive increase in antiGAD65 aAb was observed with each islet graft: 0.22 UI/ml before transplantation, 0.35 after the first, 0.88 after the second, 1.12 five days after the third before falling back to values which were nevertheless higher than pre-transplantation values (0.5 to 0.6 UI/ml) with a slight and transient increase (0.95 U/ml) when C-peptide levels became undetectable at week 16. A peak of ICA (7 UJDF) occurred between weeks 8 and 13. IA2 remained below 0.5 UI/ml, except at week 14 (0.52 UI/ml), the cut-off limit for aAb positivity being at 1 UI/ml. Three months after the transplantation and two weeks after the normalization of ICA levels, the plasma C-peptide level, previously at 3.8 ng/ml on day 30, fell below 0.2 ng/ml (Fig 2B).

Patient #3

Patient #3 was A11/A24; B8/B55; DR1/DR3 and did not have significant levels of antiGAD65, ICA or IA2 before the IAK transplantation. A slight but progressive increase in antiGAD65 was observed with each islet transplantation, (0.24 UI/ml before graft, 0.36 after the first, 0.45 five days and 0.75 nine days after the fourth and last injection before falling back to values which were nevertheless higher than pre-transplantation levels (0.3 to 0.4 UI/ml). An overt but transient increase in ICA to 26 UJDF occurred between weeks 5 and 10. Anti IA2 aAb levels remained below the cut-off limit for aAb positivity (1 UI/ml) during the entire follow-up. Nevertheless, the plasma level of these IA2 aAb, which was undetectable before transplantation, increased slightly to 0.5 at week 1 after the first injection, 0.6 at week 10, and 0.95 at week 16. The plasma C-peptide level, having reached 3.4 ng/ml on day 30, fell below 0.2 ng/ml two weeks after ICA increased (Fig 2C).

Discussion

The object of this study was to monitor the kinetics of aAb in three recipients of sequential multidonor IIA and kidney transplantation with pre-Edmonton immunosuppressive regimen in order to determine which of either the immunosuppressive drugs or the sequential protocol of transplantation was involved in the occurrence of the autoimmune response. A number of case reports has provided clear evidence of recurrent autoimmune insulitis despite sustained immuno-suppression in both pancreatic and islet allografts [2-8]. Also, according to literature, there is a definite relationship between progressive islet transplantation failure and auto-antibody (aAb)-positive IDDM recipients of IIA [4, 8]. In our three recipients of sequential multidonor IIA and kidney transplantation with pre-Edmonton immunosuppressive regimen, islet function failure occurred within 4 months of the first graft, while diabetes-associated auto-antibodies plasma levels simultaneously increased. Our follow-up of GAD 65 aAb showed that they very slightly increased in patients with low pretransplantation GAD aAb levels (patients #2 and #3), rising with each antigenic stimulation, i.e. with each islet injection, though they never reached the positive threshold. The remaining patient (patient #1) had high pre-transplantation levels of anti-GAD aAb and these levels did not change. The slight increase in GAD aAb in patients # 2 and #3 contrasts with the marked but transient rise of ICA aAb we observed 5 to 8 weeks after the initial transplantation in these patients. Moreover, the levels of IA2 aAb, heretofore undetectable in all three patients, increased very slightly in patients #2 and 3 at the end of the peak of ICA aAb. Once again, it did not reach the positive threshold, but two weeks later, plasma C-peptide levels were found to be undetectable.

Figure 1
Liver biopsy in patient #1, performed 10 days after the first islet injection. Two endocrine islets immunostained with an anti-insulin antibody are visible in brown, already surrounded with lymphocytes (Magnification x100).
Follow-up of plasma C-peptide and pancreas autoantibodies in the 3 patients after sequential intraportal islet graft.

A) Patient #1: sequential intraportal islet graft (day 0, 2, 6, 8). ICA and IA2 antibodies remain undetectable during all the follow-up.

B) Patient #2: sequential intraportal islet graft (day 0, 8, 10). ICA increased during 7 weeks (from week 7 to 14) and plasma C-peptide fell down two weeks later.

C) Patient #3: sequential intraportal islet graft (day 0, 9, 10, 12). ICA levels increased during 6 weeks (from week 3 to week 9) and C-peptide fell to undetectable values a fortnight after the peak at week 5.
levels — having reached 4.9, 3.8 and 3.4 ng/ml respectively after one month — became undetectable. Despite the small number of patients recruited for our study, results are concordant with literature: the presence of pancreas-associated auto-antibodies at any moment of an islet transplantation means poor prognosis of the islet transplantation.

Previous studies of the autoimmune response during islet transplantation dealt with recipients of kidney and a single islet transplantation treated with an immunosuppressive regimen including steroid/cyclosporine. The single islet injection is the main difference with our protocol, which featured a multi-donor sequential procedure. While the deleterious consequences of aAb, whether anti-GAD or ICA, are well demonstrated in islet graft regardless of their timing, the question of the sequential pattern of the graft on the genesis of the autoimmune response may be raised. In our 3 patients, the only type of antibodies that varied during the period of sequential islet transplantation were anti-GAD aAb in the 2 patients whose pre-transplantation anti-GAD levels were low. Their levels rose with each antigenic stimulation, i.e. with each islet injection. However they never reached the positive threshold, therefore their prognostic value is difficult to assess. The overt peak of ICA aAb was delayed after the two weeks of sequential islet injections and was associated with islet function failure. These results do not differ from the only other study devoted to kinetics of aAb found in literature [8]. However their protocol differed from ours in that they resorted to a single islet transplantation.

Sequential islet transplantation is synonymous of multi-donor strategy. The poor influence of HLA mismatch induced by this strategy may counterbalance the improved initial quality of the transplantation. However, in rodents, there is evidence that multiple donor allo-transplantations are immunologically advantageous [9, 12]. And according to the islet transplantation register, 54% (27/50) of islet-engrafted patients with functional islets after one year were transplanted with at least two donors [13]. Last, the Edmonton group has shown that multidonor sequential transplantation can be a successful approach if a suitable immunosuppressive regimen is used [1]. Thus, neither the multi-donor strategy nor the sequential pattern of the transplantation seem to overtly influence the recurrence of the autoimmune response.

Actually it is still unclear whether increased aAb are the cause or the consequence of islet destruction, the later being due to either the auto-immune recurrence of the disease or the alloimmune rejection process. The dissociated and/or transient increase of anti-pancreas aAb is not exactly suggestive of diabetes-associated aAb kinetics at the onset of type 1 diabetes since most often, at least a double positivity of aAb (GAD and IA2) is found while the increase of ICA is demonstrated during a longer period [14-16]. Different explanations are suggested. Lack of sensitivity of the commercial kits used to measure the aAb was of low probability since our technique gave satisfying performance in the XIth International Workshop conducted by S Caillat in 1998 in recently-diagnosed type 1 diabetes. Transfer of auto-antibodies from the donors is also unlikely, since ICA levels were not detectable immediately but several weeks after islet infusions in recipients. Non specific immune reaction related to cell lysis after infusion, inducing antigenic stimulation could be suggested. Nevertheless, cellular reaction around the transplanted islet was visible, arguing for a double humoral and cellular immune reaction, as described in autoimmune process [17]. However, specific reactive T cells were not studied. The quick disappearance of ICA could also be related to the relatively low islet mass that was transplanted compared to a whole pancreas. The quick destruction of the source of the antigenic stimulation would lead to a rapid extinction of the humoral reaction. Last, the role of immunosuppressive regimen may be raised. It would prevent the expression of the classical humoral auto-immune response encountered in recent-onset type 1 diabetes [18-20].

However the preferential rise of ICA in our study may reflect a more severe process of β-cell destruction similar to the one observed in young children with more severe metabolic disorders and lower serum C-peptide levels [21]. Reactivation of type 1 diabetes aAb in patients receiving human fetal pancreatic tissue transplants without immunosuppression has already been demonstrated [22]. Moreover, it has recently been shown that combination therapy with low dose sirolimus and tacrolimus is synergistic in preventing spontaneous and recurrent autoimmune diabetes in non-obese diabetic mice, a fact that may explain the success of the edmontonian immunosuppressive regimen [23]. Nevertheless, a small mass of islets conjugated with the powerful antigenic stimulation provided by the islet allograft may accelerate the destruction of transplanted β-cells. These results confirm the pejorative value of increased ICA or GAD after an islet transplantation. Their assay could be used as an early prognosis marker after islet transplantation. But even if the peak of ICA occurs before the C-peptide level drops, it is probably already too late to prevent transplantation failure [24].

As for genetic predisposition, our three C-peptide-negative diabetic patients harbored HLA DR3 and/or DR4 genotypes conferring genetic susceptibility to auto-immune type 1 diabetes. This fact was concordant with their history of type 1 diabetes and the onset of disease-associated aAb, as in the Milanese study in which 6 of the 7 patients who experimented posttransplantation markedly elevated islet aAb were also DR3 or DR4 [8]. Moreover, patients #2 and 3 were HLA-A24, an allele that may modulate the swiftness of β-cell destruction [25]. This allele was also present in 2 of the 6 recipients who were DR3 or DR4 and had markedly elevated islet auto-antibodies after islet transplantation, leading to graft failure in the Milanese series; the only patient in
the Milanese study who was not DR3 or DR4 displayed the most delayed rise in anti-GAD aAb, suggesting a genetic influence on aAb production [8]. Indeed, among ICA-positive kindred of a diabetic referent, allele A-24 is more often found in those who are going to develop diabetes; A24 is also associated with earlier onset of the disease and a lower frequency of spontaneous remission [25].

To conclude, in this study, the kinetics of pancreas aAb after IIA in kidney-transplanted patients were characterized by the persistence or the transient occurrence of only one type of aAb followed by a drop in plasma C peptide. Therefore it suggests the persistence or recurrence of a humoral immune process uncompletedly checked by the immunosuppressive regimen used in kidney transplantation. These results seem to indicate that the benefits of sequential islet transplantation lie more in the increased islet mass they provide than in potential immune benefit. Nevertheless, these results need to be confirmed on larger series.

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