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Immunological monitoring in beta cell replacement: towards a pathophysiology-guided implementation of biomarkers

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Abstract (153 words)

Purpose of review: Grafted beta cells are lost because of recurrence of T1D and/or allograft rejection, two conditions diagnosed with pancreas graft biopsy, which is invasive and impossible in case of islet transplantation. This review synthesizes the current pathophysiological knowledges and discuss the interest of available immune biomarkers.

Recent findings: Despite the central role of auto-(recurrence of T1D) and allo-(T-cell mediated rejection) immune cellular responses, the latter are not directly monitored in routine. In striking contrast, there have been undisputable progresses in monitoring of auto and alloantibodies.

Summary: Except for pancreas recipients in whom anti-donor HLA antibodies can be directly responsible for antibody-mediated rejection, autoantibodies (and alloantibodies in islet recipients) have no direct pathogenic effect. However, their fluctuation offers a surrogate marker for the activation status of T cells (because antibody generation depends on T cells). This illustrates the necessity to understand the pathophysiology when interpreting a biomarker and selecting the appropriate treatment.

Keywords (4 to 6)

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1 Introduction

Beta cell replacement (through pancreas or islet transplantation), is the only available treatment that allows restoring endogenous insulin production, thereby providing an optimized glucose metabolism for patients with type 1 diabetes (T1D). Beta cell replacement is indicated for T1D patients with severe chronic complications and end-stage renal disease [simultaneous pancreas kidney transplantation (SPK) or less frequently simultaneous islet and kidney transplantation (SIK)]; for T1D patients after kidney transplantation [pancreas after kidney (PAK) or islet after kidney (IAK)] or for T1D patients without kidney disease but with glycemic instability and severe hypoglycemia [pancreas transplant alone (PTA) or islet transplant alone (ITA)] [1]. Beta cell replacement allows halting and even sometimes partially reversing diabetes chronic complications [2-8] and increases patient survival [9, 10] and quality of life [11, 12].

Five-year pancreas graft survival after SPK is between 70 and 75% with a median pancreas graft survival of ~15 years [[13] and <https://rams.agence-biomedecine.fr/greffe-pancreatique>]. Five-year insulin independence after ITA is 30 to 40% [[14] and https://citregistry.org/system/files/10th_AR.pdf], with five-year prevalence of patients with hemoglobin A1C (HbA1c) <7.0% and absence of severe hypoglycemic events estimated ~50% (https://citregistry.org/system/files/10th_AR.pdf). Recently, a consensus on the definition of function and failure of beta cell replacement therapy on behalf of The International Pancreas & Islet Transplant Association (IPITA) and European Pancreas & Islet Transplantation Association (EPITA) has been published [15].

Besides early failure, the two main causes responsible for the loss of pancreas or islet grafts are immunological: allograft rejection and recurrence of T1D. Biopsies are the gold standard for the diagnosis of pancreas graft rejection, with an available international standardized rejection-grading schema [16]. However, pancreas graft biopsies are invasive and not performed in every center.

Furthermore, pancreas graft biopsies are less commonly used for the diagnosis of the recurrence of T1D since: i) the presence of islets in pancreas biopsy is not guarantee and ii) there is significant heterogeneity in the way islets from the same pancreas are affected by the recurrence of autoimmune disease. For islet transplantation, the situation is even worse since grafted islets are disseminated in recipient's liver and cannot be biopsied, which makes mandatory the development of other tools for the diagnosis of immune-mediated attacks.

Several biomarkers are routinely used to follow pancreas or islet transplant recipients. Metabolic biomarkers (HbA1C, glycemia, C-peptide and insulin, fasting or following stimulation...etc) reflect beta cell function. They are unspecific of the cause of beta cell dysfunction and they may be influenced by confounding factors such as lifestyle and insulin-resistance. Furthermore, their alteration arrives late in the course of disease: i.e. when most beta cells have already disappeared, which is too late for therapeutic intervention. Lipaseemia is an exocrine marker, useful for the follow-up of pancreas transplant recipients as it reflects inflammation of the graft and in most cases rejection. This marker is however useless in case of autoimmune recurrence (that do not affect exocrine tissue) and for islet transplant recipients. Recently, another type of biomarkers has been developed to monitor with a very high sensitivity beta cell death. The detection in the circulation of insulin deoxyribonucleic acid (DNA) with unmethylated CpG sites [17] or of microRNAs selectively expressed in beta cells (such as miRNA-375[18]) have both been shown to provide sensitive and accurate quantification of islet damage after beta cell replacement [19]. However, as for metabolic biomarkers, biomarkers of beta cell death are not specific of the etiology.

In this regard immunologic biomarkers appear as highly desirable because, since they directly reflect the immunopathological mechanisms involved in beta cell destruction (recurrence of T1D versus rejection), they could guide the choice of the best therapeutic option and allow evaluating its efficacy.

The aim of this review is to synthesize the current knowledge regarding the pathophysiology of T1D, of recurrence of the autoimmune disease, and of rejection, and to set the place of each available immunological biomarkers for the follow-up of pancreas or islet transplant recipients.

2 Immunological monitoring of T1D recurrence after beta cell replacement

2.1 Magnitude of the problem

Recurrence of autoimmune disease was first described in 1989 by Sutherland et al in eight recipients of pancreas transplant from identical twin or HLA-identical sibling donors, with non or minimal immunosuppression [20]. Pathologic examination (biopsy or explanted transplant) showed selective loss of beta cells, others endocrine cell types appearing normal, and inflammation of islets with residual beta cells [20]. Histological features compatible with recurrence of autoimmune disease were subsequently described in recipients of cadaveric pancreas transplant [21, 22]. In a large cohort of 223 SPK recipients with a mean follow-up of 6.2 years, the team from Miami estimated that 7.6% of patients developed recurrence of autoimmune disease, defined as presence of classical diabetes symptoms (i.e. hyperglycemia requiring insulin therapy, severe loss of C-peptide) in the absence of rejection. The diagnosis was confirmed by biopsy in 5.8% of the recipients (loss of insulin staining and insulinitis) and accounted for 36.2% of graft loss [23].

One of the first report of autoimmune recurrence after islet transplant was published by Stegall et al in 1996. They showed a dense mononuclear cell infiltrate with a preferential loss of insulin-staining cells relative to glucagon-staining cells in the islets transplanted in the forearm of the recipient [24]. In contrast to pancreas transplantation, islet allograft is not easily accessible for histological evaluation. There is only one case report of biopsy-proven autoimmune recurrence of an intra-hepatic islet allograft (sparse T-cell lymphoid infiltrate surrounded the islet and no insulin-containing cells but many glucagon-containing cells) [25].

2.2 Immunological monitoring of T1D and its recurrence

2.2.1 Immunogenetic biomarkers

Immunogenetic biomarkers allow predicting the risk for the loss of tolerance responsible for T1D. There are over 50 regions of the genome that harbor T1D susceptibility genes [26]. Gene polymorphisms associated with the risk of T1D are mainly in the HLA class II region, the best-known being HLA DR4-DQ8 and HLA DR3-DQ2. They affect T cell recognition and tolerance to foreign and autologous molecules [27]. They may contribute to suboptimal thymic presentation of autoantigens and circulation of autoreactive T cells. Multiple genes outside HLA are also associated with the risk of T1D, the strongest effects coming from polymorphisms of the insulin and PTPN22 (which encodes a lymphocyte protein tyrosine phosphatase) genes. These genes affect features of specific immune responses and modify the vulnerability of beta cells to inflammatory mediators. For example, the insulin gene T1D risk-associated polymorphisms affect central tolerance induction against proinsulin due to low expression in the thymus [27].

In the context of beta cell replacement, donor HLA typing has also informative values. It has been showed that SPK recipients with T1D recurrence carried the high-risk HLA-DR3/DR4 heterozygous genotype twice as often as SPK recipients that were normoglycemic [23]. The risk of T1D recurrence was also increased when recipients of pancreas graft shared HLA-DR alleles with their donors [23]. These two features were also associated with autoantibody conversion [23]. Similarly, in islet transplantation, HLA class I and II mismatching is a protective factor against autoantibody increase [28] and the recurrence of CD8+ T-cell autoreactivity [29].

2.2.2 Autoimmune response in T1D

Many of the antigenic targets of the autoimmune response responsible for T1D are present within secretory granules. Some are specific of beta cells [insulin, islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), zinc transporter (ZnT)8], while others are expressed in other cells and tissues, including neuroendocrine cells [glutamic acid decarboxylase (GAD)65, a tyrosine

phosphatase like protein insulinoma-associated protein (IA2) [30]. In a context of stress, target cells also express neoepitopes that are not available for negative thymic selection. Neoepitopes may be generated from different ways: post-translational modification of native antigens (citrullination, transglutamination), fusion of peptides derived from different proteins, alternative splicing, and defective ribosomal intermediate products [30, 31]. The cellular degradation machinery has also a role in the generation of neoantigens [32].

Inflammation is a key condition in the pathophysiology of T1D. In genetically predisposed patients, environmental exposures (such like infection by enterovirus, which have a tropism for beta cells) may promote loss of tolerance via presentation of self-antigen in an immunogenic context and/or by molecular mimicry [27, 30]. Inflammation not only increases the expression of HLA-I/peptide complexes on beta cell surface but also triggers post-transcriptional and post-translational modifications involved in the generation neoantigens. These two mechanisms unmask beta cells, which become visible to auto-reactive T cells. Furthermore, inflammation also promote the acquisition of effector functions by autoreactive T cells.

Importantly both beta cell replacement strategies are associated with inflammation. After pancreas transplantation, ischemia-reperfusion creates an inflammatory environment. The injection of purified islets in the portal vein of the recipient promotes an innate immunity-dependent inflammatory response named instant blood-mediated inflammatory response (IBMIR). Islet isolation procedure induce islets to produce danger signals like tissue factor, a mediator of platelet activation and aggregation and cytokines and chemokines, that recruit cells such as monocytes/ macrophages and neutrophils. IBMIR consists of a thrombotic reaction characterized by activation of the coagulation and complement cascades, cloth formation and leukocytes recruitment into the islets. It is estimated that 60-80% of transplanted islets can be destroyed by IBMIR within days when islets are transplanted into the liver [33]. The introduction of anti-inflammatory treatment after islet transplantation led to better results [34].

2.2.3 Monitoring of humoral autoimmune response

After interaction with activated autoreactive T cells in lymph nodes, autoreactive B cells differentiate into plasma cells and release autoantibodies (**Figure 1**). Immunoglobulins are massive proteins, which are largely sequestered in the circulation due to their size [35]. Because autoantibodies specific for beta cells cannot freely access their antigenic targets *in vivo*, they are devoid of pathogenic effect in T1D [36]. Although these autoantibodies have never been proven directly pathogenic for patient's own beta cells, they are routinely used as biomarkers in T1D. The islet-specific autoantibodies, which are routinely monitored in the clinic are: anti-insulin, anti-GAD65, anti-IA2 and anti-ZnT8. Many assays for the measurement of autoantibodies exist. The Islet Autoantibody Standardization Program (IASP), supervised by the Immunology of Diabetes Society (IDS) aims at the standardization of islet autoantibody assays and the evaluation of laboratory performance [37]. Autoantibodies are used in relatives of T1D patients as biomarkers of pre-symptomatic T1D. Their characteristics allowing for stratification of diabetes risk include age at seroconversion, antibody number, titer, affinity, antigen specificity and epitope binding [38]. Autoantibodies are useful for prediction and diagnosis of T1D but useless for the follow-up of T1D patients or for the monitoring of disease progression in immunotherapy trials.

In line with this theory, the presence of autoantibodies before pancreas transplantation does not correlate with transplantation outcome and recurrence of the autoimmune disease on pancreas graft sometimes occurs in the absence of autoantibodies [39]. Yet, in apparent contradiction with this theory, several studies have reported that changes in the repertoire of autoantibodies (i.e. appearance, spreading from one to multiple specificities, or increase of the titer), particularly for anti-ZnT8 antibodies, predict subsequent pancreas [40, 41, 23] and islet [42, 28] graft loss. These findings are not contradictory. Instead, they suggest that monitoring the fluctuations of ("per se" non-deleterious) autoantibodies offers an indirect but convenient way to monitor the cellular arm of the autoreactive immune response. Of note, while the mere presence of autoantibodies in the

circulation of a candidate to a pancreas transplantation doesn't seem to be a problem (see above), the situation could be different in case of islet transplantation. Purified islets are indeed directly injected in the portal vein of recipient, allowing circulating autoantibodies to bind to the islets before their integration in the hepatic parenchyma [35]. This difference could account for the observation that presence of autoantibodies before islet transplantation could be associated with a reduced chance for insulin independence [35].

2.2.4 Monitoring of cellular autoimmune response

Autoreactive T cells acquire an effector phenotype in lymph nodes and then migrate through the circulation to the targeted tissue: pancreas islets. Autoreactive T cells can only be monitored in the circulation, where their frequency (often below 10 per million T cells) and affinity (typically 10–100-fold lower than pathogen-specific T cells) are low, requiring in vitro amplification for analysis. Different assays are used to monitor autoreactive T cells. Peripheral blood mononuclear cells (PBMC) are incubated with islet antigens and proliferation (dye-dilution assay), cytokine production (ELISPOT assay) or upregulation markers associated with cellular activation (antigen specific activation assay) can be measured. Another way to measure the frequency and phenotypic characteristics of CD4 or CD8 T cells is the use of flow cytometry with soluble, multimeric class I or class II MHC loaded with islet peptide. That requires the identification of MHC class I and MHC class II restricted epitopes of beta cell antigens [43]. These assays are not standardized and not routinely used (**Table 1**).

Although naive T cells autoreactive against beta cell antigen may be detected in the circulation of healthy subjects, they display an antigen-experienced phenotype only in T1D patients [38, 33, 30]. T1D patient autoreactive T cells express the effector and memory marker CD45RO, whereas almost all autoreactive T cells from healthy individuals are CD45RO negative [33, 30]. In T1D patients, autoreactive T cells proliferate in response to beta cell antigens in the absence of costimulatory signals [33, 30]. Autoreactive T cells from T1D patients have shorter telomere length than

autoreactive T cells from healthy controls [32]. Autoreactive T cells exhibit proinflammatory cytokine profiles in T1D patients and regulatory profiles in healthy subjects [30].

Regulatory T cell profile is also modified in T1D patients. The peripheral frequency of a regulatory T cell population, characterized by the coexpression of CD3 and CD56 molecules, is reduced in individuals with new-onset T1D [44]. T1D patients exhibit impaired regulatory T cell function and effector T cell resistance to regulatory T cell suppression [30].

The monitoring of autoreactive T cells in T1D patients is useful to understand the pathogeny of the disease but also for the follow-up of patients after therapeutic intervention [45] (**Table 1**).

Direct monitoring of the cellular effectors of the autoimmune response is difficult and currently relies on time consuming and non-standardized techniques (ex-vivo antigen-specific activation/proliferation; ELISPOT...etc). Some authors have suggested that it was possible to identify autoreactive T (CD4+ and CD8+) cells using HLA multimers loaded with relevant antigenic peptide [46-48]. Although relatively straightforward, this technique can only monitor the cellular reactivity against a limited number of autoantigens and only in the recipients with compatible HLA typing (**Table 1**). Comparisons of TCR clonotypes of tetramer binding T cells revealed that identical clones are present for long time intervals in the circulation of pancreas recipients [46] and that some T cells clones are present both in the circulation and the pancreas transplant draining lymph nodes [49]. These data, together with the phenotype (CD45RO+ CCR7-) of autoreactive T cells found in biopsies and the draining lymph node of pancreas graft suggest that autoreactive T cell population is expanded from long-lived effector memory precursors [50, 47, 48] (**Figure 1**). HLA multimer technology has also been used to demonstrate the presence of autoreactive CD4+ and CD8+ T cells in the peripheral blood of pancreas recipient before symptoms of T1D recurrence [46-48] and some authors reported that the reappearance of circulating autoreactive T cell populations after depleting induction correlated with pancreas graft loss [48]. Finally, autoreactive T cells have been identified in

pancreas biopsies of recipients with T1D recurrence, in association with insulinitis and loss of insulin staining [48].

In islet transplantation, insulin B10-18-specific CD8 T cells have been evidenced using HLA A2 tetramers in recipients with autoimmune recurrence and islet allograft loss, but not in patients with persistent beta cell function or patients diagnosed with allograft rejection [51]. An association between the presence of cellular autoimmunity assessed by lymphocyte stimulation tests performed the first year after transplantation on one hand and delayed insulin-independence and lower circulating C-peptide levels on the other [52]. Finally, the emergence of GAD65-specific T-cells (using overlapping peptide library, which permits simultaneous detection of both CD4+ and CD8+ antigen-specific T-cell responses in cultures) preceded the development of chronic graft dysfunction in 5 islet-transplanted patients [53] (**Table 1**).

3 Alloimmune response after beta cell replacement

3.1 Immunopathology of graft rejection

Direct allorecognition of donor-specific HLA molecules as intact complexes on the surface of passenger antigen-presenting cells (APC) activates 1 to 10% of a recipient's T cells [54]. The latter infiltrate the graft and destroy all types of allogenic cells (not only beta cells as in the case of recurrence of T1D), a process known as T-cell mediated rejection (TCMR). The progressive disappearance of passenger APC (which cannot be replenished) leads to the fading of this direct allorecognition pathway, which explains why the incidence of TCMR gradually declines with time post-transplantation [55]. Recipient's immune system can however also recognize graft alloantigens through another mechanism: the "indirect" pathway. The latter consists in the recognition of allogeneic peptides generated by the processing of donor-specific HLA molecules (and many other polymorphic non-HLA alloantigens) by recipient's APC and presented within MHC-II molecules on their surface. The indirect pathway activates much fewer CD4+ T cells (~1:10000), but the latter are

critically important for the differentiation of allospecific B cells into DSA-producing plasma cells [56]. **(Figure 1)**. Alloantibodies (like autoantibodies, see paragraph above) are largely retained in the circulation due to their size. Their binding to the only accessible alloantigens, those expressed by pancreas graft vasculature leads to the activation of the classical complement pathway and/or the recruitment of Fc γ receptor-expressing cytotoxic innate immune effectors [56]. Immunosuppressive drugs given to prevent rejection episodes mainly acts through the blockade of allospecific T cells [57] but recent data suggest that up to 20% of patients exhibited residual activatability of T cells involved in providing help to B cells [58].

The gold standard to confirm the diagnosis of rejection after solid organ transplantation is the graft biopsy. An international consensual classification (Banff grading schema) is available to grade pancreas rejection [16]. TCMR is defined by active septal and acinar inflammation (T cell infiltrate) with arteritis as severity criteria. Antibody-mediated rejection (AMR) definition combines histologic evidence of acute tissue injury, C4d positivity in interacinar capillaries and the presence of donor-specific antibodies in recipient's circulation. A key problem is to determine when pancreas graft biopsy shall be performed. In contrast with most type of transplanted organ, the presence of a dysfunction of the graft (i.e. rise in fasting glycemia) is already an indication of very advanced (and usually not reversible) rejection episode. Biomarkers are therefore needed to guide biopsy and allow diagnosing rejection at an earlier stage. This need is even more pressing for islet graft recipients in whom biopsy cannot be performed at all. Indeed, only 5 to 10 g of purified pancreatic islets are injected into the portal vein of the recipient, leading to their dissemination within the 1.5 kg of liver parenchyma. Consequently, the chance for a percutaneous needle biopsy to sample an islet is estimated below 5/1000.

3.2 Immunogenetic biomarkers

In pancreas transplantation, the risk of rejection increases with the number of HLA mismatches between donor and recipient [13]. Similarly, in islet transplantation, HLA class I and II mismatching is

both a risk factor for donor specific antibody generation [28, 59] and lower graft survival [60] (although it is protective against autoantibody increase [28] and the recurrence of CD8+ T-cell autoreactivity [29]). Islet graft sharing the same HLA class I molecules of a previous tolerated graft (kidney or islets) seems to be protected against rejection [61].

In fact, rather than the mere count of HLA mismatches, recent findings in the field of renal transplantation suggest that the “quantification of the immunogenicity” of the graft should rather rely on the “epitope load” [62]. HLA molecules indeed consist of a set of polymorphic amino acid configurations, which are often referred to as epitopes [63]. Although individual epitopes can be shared between several HLA alleles, each individual HLA allele carries a unique set of epitopes. Several publications have reported that a low number of epitope mismatches (or load) is associated with a reduced incidence of DSA formation, and a lower incidence of AMR in renal transplantation [64]. Although the value of epitope load has not been thoroughly investigated yet in beta cell replacement, this epitope load seems to inversely correlate with the risk to develop DSA and/or a wider DSA repertoire after pancreas and islet transplantation [65, 59].

3.3 Monitoring of humoral alloimmune response after beta cell replacement

Although many minor histocompatibility antigens have been evidenced [66, 67], only anti-HLA alloantibodies are routinely monitored after transplantation, thanks to the development of sensitive and reliable solid phase assays [68]. Presence of donor specific antibodies (DSA) has been associated in most studies with worse pancreas graft survival [69-72]. The few studies that did not observe this association had enrolled much less patients [65] and/or have followed them for shorter periods (usually <24 months) [73]. The routine follow-up of DSA in pancreas transplant recipients is largely inspired from what is done in kidney transplantation. Appearance of *de novo* DSA is generally regarded as an indication for pancreas biopsy to diagnose AMR. However, the presence of histological lesions in this circumstance is inconstant (~ 25-50% in kidney transplantation; [74]) and how these patients should be monitored is unclear. A non-invasive biomarker indicating that DSA are

actively creating damages to the graft would be extremely useful to guide the re-biopsy. Although some preliminary data exist in kidney transplantation [75], there is no such biomarker available for pancreas recipients.

De novo DSA appear in around one third of islet recipients [76, 77, 65], very often after immunosuppressive drug withdrawal (and therefore after the loss of islet allograft) [59]. The impact of *de novo* DSA appearance on islet graft outcome is most controversial. Some authors found an impact of *de novo* DSA appearance on graft survival [76, 78, 28, 79] whereas others did not [80, 65, 59]. These contradictory results may be explained by the low number of patients in every study, different immunosuppressive protocols, different assays for DSA monitoring and different definitions of graft function. Another explanation could be the possible resistance of islet graft to AMR due to endothelial chimerism and vascular sequestration of DSA [35]. In contrast with pancreas transplantation, where vessels are from donor origin, reestablishment of islet blood flow occurs through sprouting of capillaries of recipient origin. This vascular chimerism, together with the sequestration of DSA in the circulation, could protect islet grafts from AMR [35]. As explain above, B cells need to receive the help of follicular helper T cells to differentiate into DSA-producing plasma cells. Change in DSA titers may therefore be the reflect of an insufficient blockade of alloreactive T cells by immunosuppressive drugs, which could explain the association found by some authors between DSA appearance (or change in titer) and islet graft outcome [76, 78, 28, 79].

3.4 Monitoring of cellular alloimmune response after beta cell replacement

In case of SPK transplantation, because both organs are from the same donor, the kidney has been treated traditionally as the "sentinel" organ to biopsy, presumably representing the status of both allografts. However, several studies have reported the lack of concordance between the 2 organs, the discrepancies in grade and type of rejection, and the tendency for higher rejection grades in concurrent or pancreas only rejections [81], thus supporting the rationale for realization of pancreas biopsies. Furthermore, pancreas graft biopsy is the only option to diagnose pancreas graft rejection

in PAK and PTA. Pancreas graft biopsies are currently guided by non-immune biomarkers, in particular rising lipasemia. Indeed, in contrast with recurrence of T1D, TCMR indeed induces lesions not only of endocrine but also of exocrine tissue [81]. Lipasemia however lacks specificity and direct monitoring of alloreactive T cells appears highly desirable. However, monitoring of alloreactive T cells relies on the same labor-intensive, non-standardized techniques used for the monitoring of autoreactive T cells (see paragraph above) that are not currently available in the clinic. To the best of our knowledge there is no data available in the literature regarding the value of the monitoring of alloreactive T cell after pancreas transplantation (**Table 1**).

There is a little more data available in the field of islet transplantation, but unfortunately these evidences are scarce and conflicting. A correlation between the number circulating alloreactive T cells quantified by ELISPOT and graft failure has been reported in 7 islet recipients [78], whereas no such association was observed in a larger study of 25 recipients using the same technique [82]. Huurman et al also found no association between cellular alloreactivity (assessed by mixed lymphocyte cultures) and short-term outcome in 21 islet recipients [52]. The same group however reported an increase of highly avid allospecific cytotoxic T cells during tapering of immunosuppressive drugs in a pilot study of 5 islet recipients [83] (**Table 1**).

Finally, in the absence of a better option, regular screening of the serum for DSA (the generation of which depends upon a T-dependent humoral response), can be considered as an easy and convenient way to indirectly monitor alloreactive T cells (as discussed in the above paragraph).

5. Conclusion

Recurrence of the autoimmune disease and allograft rejection are the two main causes of late loss for grafted beta cell. Because immune biomarkers directly reflect the different steps of these two conditions, they are expected to allow both diagnosing the cause of beta cell destruction and

evaluating the efficacy of therapeutic interventions, two goals out of the reach of currently used metabolic biomarkers or recently developed markers of beta cell death.

Despite the central role of auto- (in recurrence of T1D) and allo- (in T-cell mediated rejection) immune cellular responses in the destruction of allogeneic beta cells, the immune biomarkers allowing their monitoring (ELISPOT and other ex-vivo assays) are rarely used in the clinical practice, mostly because they are labor intensive and very difficult to standardize.

In striking contrast, there have been undisputable progresses in monitoring of auto and alloimmune humoral responses. Except for pancreas recipients in whom anti-donor HLA antibodies can be directly responsible for antibody-mediated rejection, autoantibodies (and alloantibodies in islet recipients) have no direct pathogenic effect. However, the fluctuation of their titers can be easily monitored and offer an interesting surrogate for the activation status of T cells (because the generation of antibodies depends on T cells). Therefore, although contra-intuitive, raise in autoantibodies titer should lead to anti-T cell treatment. This perfectly illustrates the necessity to have a good understanding of the pathophysiology when interpreting a biomarker.

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This review explains the benefit / risk ratios of each beta cell replacement therapy and proposes a decision tree for transplantation indications.

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Table 1. Value of immune biomarkers in the follow-up of type 1 diabetes, pancreas and islet transplantations

Biomarker	Assay	Use	Type 1 diabetes			Pancreas transplantation			Islet transplantation		
			Pathogeny	Disease prediction	Follow-up of therapeutic intervention	Pathogeny	Disease prediction	Follow-up of therapeutic intervention	Pathogeny	Disease prediction	Follow-up of therapeutic intervention
Genetic biomarkers ¹	- HLA typing - Eplet load	routine research	yes -	yes -	no -	yes yes	yes yes	no no	yes yes	yes yes	no no
Autoantibodies	- immunoassays ²	routine	no	yes	no	no	yes	yes	no	yes	no data
Autoreactive T cells	- antigen specific activation assay - dye-dilution assay - ELISPOT assay - MHC multimer assay	research	yes	yes	yes	yes	yes	yes	yes	yes	no data
Alloantibodies	- flow cytometry	routine	-	-	-	yes	yes	no data	no	discordant data	no data
Alloreactive T cells	- ELISPOT assay - mixed lymphocyte cultures	research	-	-	-	yes	no data	no data	yes	discordant data	no data

¹ Genetic biomarker useful after beta cell replacement is donor HLA typing.

² Islet autoantibody assays are standardized by the Islet Autoantibody Standardization Program, supervised by the Immunology of Diabetes Society.

Figure legend

Figure 1. Pathophysiology of type 1 diabetes, autoimmune recurrence and allograft rejection

Elements from recipient origin are in blue shades, those from donor are in orange shades.

Schematic representation of the different steps of the autoimmune response (involving cellular and humoral effectors) directed against beta cells from native pancreas (upper row) in case of T1D, or against allogenic beta cells from pancreas (middle row) or islets (lower row) grafts in case of T1D recurrence.

Schematic representation of the different steps of the alloimmune response (involving cellular and humoral effectors) directed against donor-specific HLA molecules expressed by all (including beta) cells of pancreas (middle row) or islets (lower row) grafts.

Abbreviations are: autoantibodies, AutoAbs; alloantibodies, AlloAbs; antibody-mediated rejection, AMR; antigen-presenting cell, APC; Type-1 diabetes, T1D.

Figure 1

