REVIEW

Immunological aspects of allogeneic pancreatic islet transplantation: a comparison between mouse and human

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SUMMARY

Pancreatic islet allotransplantation is a treatment for patients with severe forms of type 1 diabetes. As long-term graft function and survival are not yet optimal, additional studies are warranted in order to continue improving transplant outcomes. The mechanisms of islet graft loss and tolerance induction are often studied in murine diabetes models. Despite numerous islet transplantation studies successfully performed over recent years, translation from experimental mouse models to human clinical application remains elusive. This review aims at critically discussing the strengths and limitations of current mouse models of diabetes and experimental islet transplantation. In particular, we will analyze the causes leading to diabetes and compare the immunological mechanisms responsible for rejection between mouse and human. A better understanding of the experimental mouse models should facilitate translation to human clinical application.

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Introduction

Islet allotransplantation is a promising therapy to treat severe type 1 diabetes (T1D) patients with the goal to achieve independence of exogenous insulin administration. However, transplanted islets succumb to an early phase of rejection and to a progressive loss of graft function because of inflammatory and immune reactions. Therefore, only 10% of patients remain insulin independent 5 years post-transplant [1]. This progressive islet graft loss together with the limited access to donor organs for transplantation call for improvements in the field. For several years, clinical studies have sought to determine more efficient transplantation conditions, identifying the limits of current techniques [2]. On the other hand, several experimental animal models are available. Nonhuman primate models are regarded as highly representative for clinical translation [3], however, studies are tightly regulated, require very specialized expertise, and they are banned in several areas of the world. From physiologic and metabolic points of view nonhuman primates still substantially differ from humans [4] and currently there is no autoimmune diabetes model available. In addition, despite a number of rat and other non-mouse rodent models of diabetes and islet transplantation, ongoing experimental research mainly depends on the widespread use of mouse models, which form the basis for

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this review. However, there are important differences in the regulation of the immune system between humans and mice that still need better understanding. Mice and humans share about 90% of their genes [5], but there are 300 unique genes in each species [6]. In addition, recovery from injury in mice compared with humans is faster. Indeed, in mice, increased gene expression following injury is switched off after a few days, whereas genes remain active for weeks or even months in humans [7]. This may help to reduce inflammation detrimental to the transplanted islets, in addition, mice have a different response to endotoxin compared with humans, an aspect that is related to a decreased upregulation of toll-like receptor (TLR) in mice compared with humans [8]. According to these observations, mice exhibit, to some extent, a faster but milder immune reaction compared with humans.

The aim of this review is to explore and compare cellular aspects occurring during immune rejection of islet allografts. To better understand the differences in islet graft rejection in humans and mice, it is helpful to focus on differences in their immune system, as well as to assess the mechanisms of autoimmune reaction that lead to human T1D and to diabetes in the currently used mouse models. Knowledge of these immunological aspects is fundamental for choosing wisely the most appropriate experimental model and for translation of the experimental results into clinical applications.

Comparison of the immune system between mouse and human

The human and mouse innate and adaptive immune systems are composed of the same immune cells, such as macrophages, dendritic cells (DC), natural killer (NK) cells, B cells, and T cells. However, their proportion in the blood differs between humans and mice. Although neutrophils (50–70%) followed by lymphocytes (30–50%) are the most abundant white blood cells in humans, mouse blood is composed of 75–90% lymphocytes and only 10–25% neutrophils [9]. The physiological consequences of this striking difference are not well understood. These cells present many similarities; nevertheless, some differences in terms of phenotype and reactivity have been observed and will be addressed below.

Macrophages are divided into subtypes: those presenting the classical phenotypes ($CD14^{hi}$ $CD16^{-}$ in humans and $Gr1(Ly6C)^{hi}$ in mice) and those presenting the non-classical phenotype ($CD14^{+}$ $CD16^{+}$ in humans and $Gr1(Ly6C)^{low}$ in mice). Although the phenotype is different, macrophages from both species share the same development pathway [10,11]. Murine and human macrophage populations are similar with respect to the different subpopulations, adhesion molecule, and chemokine expression and function. However, genomic analyses reveal that $CD14^{+}$ $CD16^{+}$ double-positive human macrophages exert more phagocytic activity compared with their murine homolog $Ly\text{-}6C^{low}$ [12]. Indeed, human macrophages and mouse macrophages are different in size (21.2 μm vs. 13.1 μm) [13], which may be important because phagocytosis depends on the size of the phagocytozing cell and the size of the particle to be phagocytozed [14]. Further, human and murine macrophages react differently to lipopolysaccharide (LPS). LPS induces massive production of reactive oxygen species by murine macrophages; however, in humans, the induction of reactive oxygen species by LPS is arduous [15]. It appears that murine macrophages are more reactive to LPS and act immediately after a minimal stimulus to delete foreign material through reactive oxygen species [6,16], further suggesting that murine macrophages respond faster in the presence of allograft material.

Dendritic cells are the most specialized antigen presenting cells, classified into two subsets: lymphoid tissue resident and nonlymphoid tissue resident. Both are present in humans and mice. Furthermore, DCs share the same function in humans and mice, and transcriptional analysis shows that the two genotypes share homology [17]. However, differences exist at the level of surface antigen expression. Indeed, human DCs express CD1a and CD1c, which are not present in murine DCs. Moreover, CD4 is present in all human DCs but only in the splenic DCs from mice [17].

NK cells are part of the innate immune system and known for their capacity to destroy virally infected or cancerous cells, but also able to shape adaptive immune responses mainly by the release of pro-inflammatory cytokines such as IFNγ. They are characterized by a $CD56^{+}/CD3^{-}$ phenotype in humans and CD27 expression in mice. NK cytotoxicity is tightly regulated by the fine-tuning of activating and inhibitory receptors including killer cell Ig-like receptors (KIRs) in humans and the C-type lectin-like family receptors Ly49s in mice [18,19]. These receptors differ in protein composition and consequently in their binding capacities but share common signaling pathways in mice and humans [6,19]. Comparative studies showed that murine NK cells have a lower cytotoxic capacity than

human NK cells following *in vitro* culture. This observation might in part be explained by the constitutive strong expression of perforin and granzyme B in humans, which is only weakly expressed in mice [19] and suggests that human NK cells are more reactive than murine NK cells.

B lymphocytes as well as plasmocytes are principally similar in mice and humans. In addition, their capacity for hypermutation and class-switch during antibody production is maintained in both humans and mice [20]. Nevertheless, the immunoglobulin (Ig) subtypes are different in the two species. Humans produce IgG_1, IgG_2, IgG_3, IgG_4, IgM, IgE, IgA_1, IgA_2, and IgD; mice produce IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgM, IgE, IgA, and IgD. In addition, each mouse strain produces typical IgG subclasses [6,21].

T lymphocytes in mice and humans share the T-cell receptor (TCR), its function and T-cell subtypes such as T helper (Th) 1, Th2, Th17, and T regulatory cells (Tregs). Significant differences exist in the proportion of T lymphocytes in human and mouse blood. Human blood is composed of 30–50% lymphocytes, whereas mouse blood contains as much as 75–90% lymphocytes [9]. Moreover, the costimulatory receptor CD28 is expressed in 100% of murine CD4 and CD8 lymphocytes, whereas in humans only 80% of CD4 and in 50% of CD8 lymphocytes express CD28 [6,21]. Furthermore, in the mouse thymic T cells express Thy-1, which is widely used for T cell recognition. In contrast, human T cells do not express Thy-1 [6]. On the other hand, activated human T cells express major histocompatibility complex (MHC) class II molecules, whereas mice do not [6]. Memory T cells in mice represent 5–10% of the total peripheral blood mononuclear cells, whereas memory T cells represent 50% in humans [22]. This discrepancy in memory T- and B-cell percentages is proposed to originate from antigen exposure during lifespan (which is considerably longer for human than for mice) and this phenomenon is defined as heterologous immunity [22]. The encounter with an antigen at any time activates T and B cells that persist during the whole life in form of memory cells to protect the organism from future antigen expositions. Recent findings show that memory cells not only exhibit faster reactivation after antigen encounter, but can also cross-react with related antigens [23]. Analysis of CD8 memory T-cell density in adult human blood, laboratory mice blood and neonatal cord blood revealed their almost complete absence in neonatal humans and laboratory mice. The CD8 memory T-cell subset was also compared between nonlymphoid tissues in adult and neonatal humans and laboratory and feral house mice. The results highlight the similarity among feral house mice and human adults in that they both expressed high levels of memory cells compared to laboratory mice and neonatal humans, where memory T cells were almost absent [24]. These findings strongly suggest that the adult human immune system includes an increased immune memory repertoire compared with laboratory mice, and that this feature is a consequence of the specific pathogen-free (SPF) housing of laboratory mice. Indeed, SPF housing protects mice from pathogens and avoids the encounter with exogenous antigens, thus resulting in a decreased memory T-cell repertoire. As a consequence, the lower number of memory T cells may have an impact on the acute and chronic alloimmune reaction toward the graft, decreasing the intensity and reactivity of immune cells [25].

The described immunological characteristics give some indications to explain the different outcomes and kinetics of immune destruction between allogeneic experimental mouse and clinical islet transplantation.

Clinical islet allotransplantation has initially resulted in poor graft survival, with loss of function within the first weeks after transplantation. These results have progressively improved since the Edmonton protocol in 2000. Currently, the introduction of new immunosuppressive protocols including the use of novel cell depletion agents (e.g., Alemtuzumab) has resulted in a 5-year insulin independence rates higher than 50% [26]. In parallel, targeting pro-inflammatory pathways (such as by use of anti-TNF-alpha antibodies) has further curtailed early islet loss.

In contrast to what is observed in clinical situation, 3–4 weeks are required prior to graft rejection after allogeneic islet transplantation in non-immunosuppressed immunocompetent mouse recipients, and some recipients are able to maintain islet graft function for longer periods [27].

These different outcomes might be explained by the following mechanisms: NK cells are more cytotoxic in humans compared with mice. The reduced capacity of murine macrophages to present antigens may help to explain why mice preserve allografts longer than humans [6]. In addition, an increased number of memory T and B cells are present in humans, suggesting that the adaptive immune reaction in humans might be increased [25].

Autoimmune-component in Type 1 diabetes in humans, in nonobese diabetic mice and in streptozotocin-treated mice

Similarities

T1D in humans and the nonobese diabetic (NOD) mouse model represent an autoimmune disease caused by the same genetic modification that impairs T-cell maturation. The genes involved in the pathogenesis in humans are located in the human leukocyte antigen (HLA) locus, in particular *HLA-DQA1, HLA-DQB1, HLA-DRB1, HLA-DR3, and 4* [28–30]. These genes encode for proteins involved in the extracellular presentation of antigens, including the autoantigens present in T1D [31]. Furthermore, non-HLA genes, such as *INS, CTLA4, IFIH1, PTPN2, PTPN22, CLEC16A, CAPSLIL7R*, and *IL2RA* are also risk factors for the development of T1D [32]. The ortholog of the human HLA-DQ gene in mice is the I-Ag7 gene. In HLA-DQ8 and I-Ag7 a single amino acid modification at position 57 in the β chain was identified, which confers susceptibility for binding to an insulin peptide [33] showing that structural polymorphism in HLA has a pronounced effect on the peptides that these molecules bind [34]. Indeed, T1D in humans and NOD mice are both characterized by the presence of islet autoantigens, which are recognized by autoreactive immune cells [35], in particular CD4 T cells, CD8 T cells, macrophages, and DCs, and, furthermore, by impaired Treg immunomodulatory functions (Fig. 1a). Autoreactive T-cell-mediated β-cell destruction begins early in life and leads to the onset of the pathology at young age. In humans and mice, when 80% of the β-cell mass is destroyed, hyperglycemic events commence, and diabetes is manifest [31]. The autoimmune origin of the T1D is corroborated by the persistence of autoreactive memory T cells, which are reactivated by islet grafts [35]. Indeed, NOD mice and humans dispose of a large pool of memory effector T cells that are ready to be reactivated [36]. Accordingly, infiltrated T cells express the same TCR repertoire as T cells involved in the onset of T1D. Further, polymorphism of TCR on memory T cells has been associated with the autoimmune onset of diabetes in humans [30].

Differences

Even though NOD mice develop T1D in a manner similar to humans, the role of autoantibodies in the development of T1D differs. In humans, the development of the disease does not necessarily involve autoantibodies and may occur with the presence of T-cell subsets exclusively [37]. While a large percentage of T1D patients (85%) present islet cell autoantibodies in blood sera [38], T1D can develop in the absence of B cells and autoantibodies and may occur exclusively with the presence of T-cell subsets [37]. However, it has been suggested that B-cell population facilitates the evolution of the disease [34]. Furthermore, in humans autoantibodies against GAD65 and IA-2 are considered highly predictive for the development of diabetes [39]. Despite this inconsistency of the presence of autoantibodies, the level of certain types of autoantibodies correlates with accelerated islet cell destruction and onset of T1D (Fig. 1a) [40]. In NOD mice, however, the presence of autoantibodies is essential for the development of T1D, and their absence protects against T1D. This has been demonstrated in a model of B-cell-deficient and immunoglobulin transgenic NOD mothers. In these animals, the absence of the transmission of maternal immunoglobulins protects their progeny from the onset of spontaneous diabetes. A similar effect was also observed for NOD embryos implanted into a non-autoimmune mother strain, suggesting that transmission of antibodies to the progeny might be involved in the autoimmune disease [41]. Further, B-cell-deficient NOD mice rarely develop diabetes [42] as well as the depletion of B cells using an anti-CD20 monoclonal antibody reverse the onset of hyperglycemia in NOD mice [43,44]. Phase 2 studies in patients with T1D, treated with rituximab to selectively deplete B cells, were, however, not successful at long term. One-year follow-up of the patients showed that rituximab treatment only partially preserved beta-cell function highlighting, however, that B cells also contribute to the pathogenesis of T1D in humans [45,46].

Although the NOD mouse model provides helpful insights on the development of autoimmune diabetes, its value in islet transplantation is limited. In human T1D, the islet antigens that are the target of autoreactive T cells remain for the most part unknown, and presumably different from those in the NOD mouse. Therefore, only little information can be gained from using the NOD mouse to study recurrence of autoimmunity translatable to the clinical setting.

The induction of diabetes by streptozotocin (STZ), a broad-spectrum antibiotic that specifically destroys islet β-cells by necrosis, represents another commonly studied experimental mouse model [47]. STZ selectively enters cells through the glucose transporter GLUT2 present in the cell membrane of β-cells (Fig. 1b). GLUT2 is

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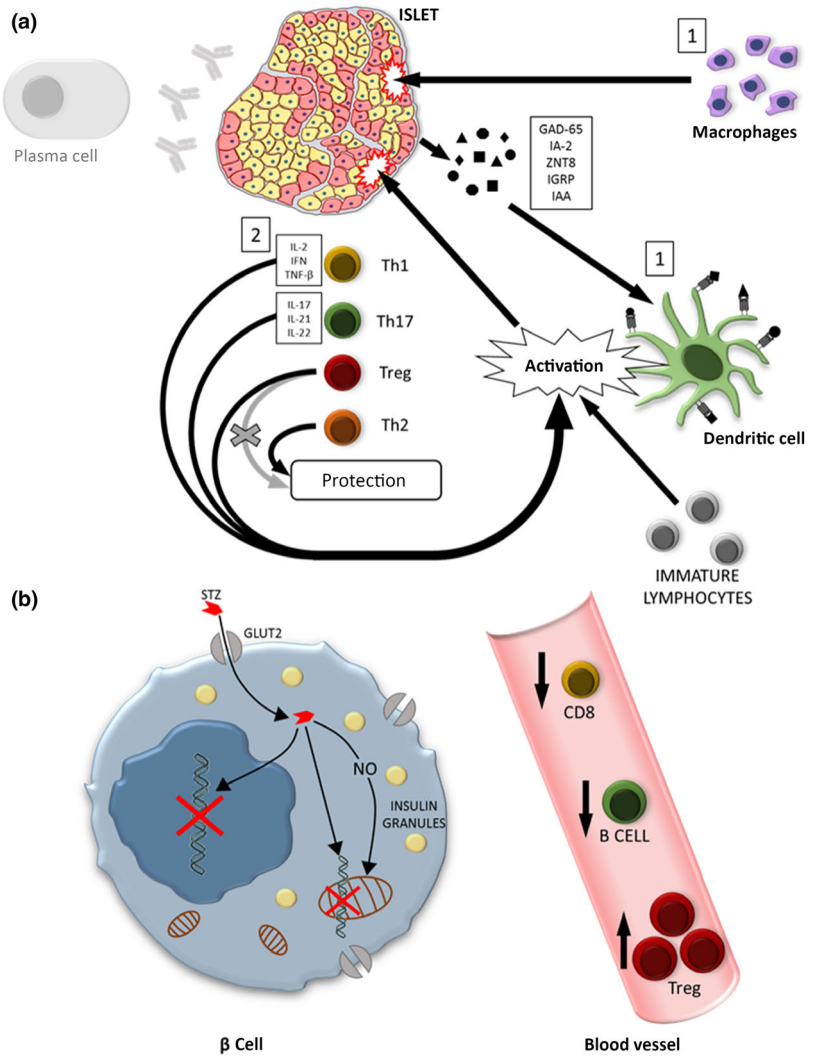

Figure 1 Mechanisms leading to T1D. (a) Autoimmune islet destruction occurring in humans and NOD mice: islets secrete antigens (GAD-65, IA-2, ZNT8, IGRP, and IAA) that are recognized by DCs and macrophages, which start to destroy islets. 2: Immature lymphocytes are activated through antigen presentation by DCs in Th1, Th2, and Th17, which secrete cytokines that increase T-cell activation and expansion and subsequently destroy islet cells. Plasma cells and autoantibodies are represented in gray given that their involvement is not clearly demonstrated. (b) Diabetes induction by streptozotocin (STZ). STZ enters through GLUT2 receptors expressed on the surface of β-cells and destroys nuclear and mitochondrial DNA. STZ induces the production of reactive oxygen species (NO), which induces mitochondrial dysfunction, leading to β-cell death. CD8 T cells and B cells are decreased and Tregs are increased in blood vessels after STZ injection.

further expressed in the kidney and liver; known side effects in this model include kidney and liver damage, reduced animal body weight, and increased risk of developing tumors [48,49]. STZ also affects the immune system, leading to early lymphopenia *in vitro* and *in vivo* with an unexpected toxicity against CD8 T cells and B cells. Furthermore, STZ increases the level of TGF-β and Treg proliferation in the blood (Fig. 1b) [50]. These properties of STZ may even exert a protective effect against the onset of diabetes in young NOD mice, potentially by inducing a regulatory T-cell population [51]. Further, since STZ exhibits immunosuppressive effects, islet grafts may survive longer in STZ-induced diabetic mice compared with mice injected with other diabetes-inducing drugs [49]. STZ-induced diabetic mice represent an economic strategy to induce diabetes, since the maintenance of NOD mice under SPF conditions is associated with relatively high costs. Furthermore, the injection of STZ allows a controlled T1D onset, contrarily to NOD mice where the onset of the pathology remains unpredictable [52]. However, results obtained with STZ-induced diabetic mouse

models do not reflect human T1D because of the absence of autoimmunity. The STZ model is therefore more suitable for metabolic studies related to islet transplantation than to assess the impact of immunity. Chemical induction of diabetes with STZ is commonly used to study diabetes and islet transplantation, however, it is important to note that toxic side effects such as lymphopenia complicate interpretation of the results [53].

These limitations need to be taken into account in translating experimental results obtained in mice to clinical studies. Finally, ongoing research with humanized NOD mouse models may provide more relevant results [54].

Immune reactions toward pancreatic islet grafts in human and mice

Similarities

The immune reactions toward islet allografts highlight several important similarities between T1D in humans and the NOD mouse model.

First, in humans and NOD mice, an autoimmune reaction against islet grafts occurs and participates in islet cell destruction. The onset of islet cell destruction is caused by autoreactive immune cells in T1D patients and NOD mice; autoimmunity recurrence remains a major concern in islet graft destruction. Humans transplanted with islets require immunosuppression, which also has the effect of suppressing autoimmunity at same time. The recurrence of autoimmunity involves the reactivation of memory CD4 and CD8 T cells. These CD4 and CD8 T cells are orchestrated by antigen presenting cells (APCs), such as DCs, which have been educated to destroy islet antigens [55]. Memory cells are rapidly reactivated and expanded [56] and migrate into the islet grafts in order to selectively destroy β-cells (Fig. 2) [56]. Therefore, islet transplantation in patients with autoimmune T1D compared with those with non-autoimmune T1D has a lower rate of success because of the autoimmune reaction. In addition, NOD mice exhibit autoimmunity to islets after transplantation, as demonstrated in mice treated with a tolerance inductive therapy, which did not display any tolerogenic effects [57].

Second, the alloimmune reaction is comparable in both species given that the same immune cells, such as DCs, T cells, and macrophages, are involved in islet allograft destruction. Islets express antigens such as insulin, IA-2, GAD-65, and ZnT8 that are highly antigenic and activate T- and B-cell responses [58]. These antigens are recognized by the host immune system through direct or indirect presentation. Direct presentation involves the immediate recognition of APCs derived from the graft and activation of the host T lymphocytes. Indirect presentation involves the presentation of antigens of the graft by host APCs, leading to the activation of the immune system [59]. As in humans, donor and host DCs play an essential role in islet graft rejection in mice. The selective depletion of DCs through cytochrome c treatment significantly increases graft survival in mice up to 100 days, and islets maintain high viability with low inflammation, demonstrating their involvement in the graft rejection (Fig. 2) [60]. Once the immune system is activated, macrophages, neutrophils, NK cells, granulocytes, DCs, B cells, CD4, and CD8 T cells migrate around the graft, drive a pro-inflammatory cascade and graft destruction [61,62]. CD8 T cells of the host can directly destroy islet cells [63] and are considered together with CD4 T cells as the most important cell types to measure in order to predict graft outcome after islet transplantation [58]. Mouse studies reveal that treatment with an anti-CD3 antibody decreases the numbers of CD4 and CD8 T cells in the graft. However, the interruption of the anti-CD3 treatment allowed a complete recovery of the CD4 T cell level and included a high number of Tregs, whereas the level of CD8 T cells recovered only partially [64]. These results suggest that anti-CD3 treatment could be a promising immunosuppressive therapy (Fig. 2). Moreover, CD4 T cells play a major role in islet graft rejection, as confirmed in a study of islet transplantation in CD4 ko mice where graft survival was prolonged and cell infiltration reduced. In contrast, transplantation of islets in CD8 ko mice results in efficient graft destruction and a massive presence of mononuclear cell infiltration [65].

To allow diabetes reversal in patients, generally the infusion of multiple doses of islets is required. These multiple infusions increase the risk of rejection because of HLA mismatches or to donor-specific alloantibodies [66]. Indeed, the HLA typing, that is essential prior to kidney transplantation, is not compulsory in islet cell transplantation. However, the presence of pre-existing HLA antibodies, formed after previous blood transfusion or organ transplantation or pregnancy significantly reduce islet graft survival [67,68]. In mice, MHC is generally not taken into account and syngeneic and allogeneic combinations of islet transplantation are usually performed [69].

These considerations/studies suggest that the mechanisms of islet graft rejection in human and mice involve

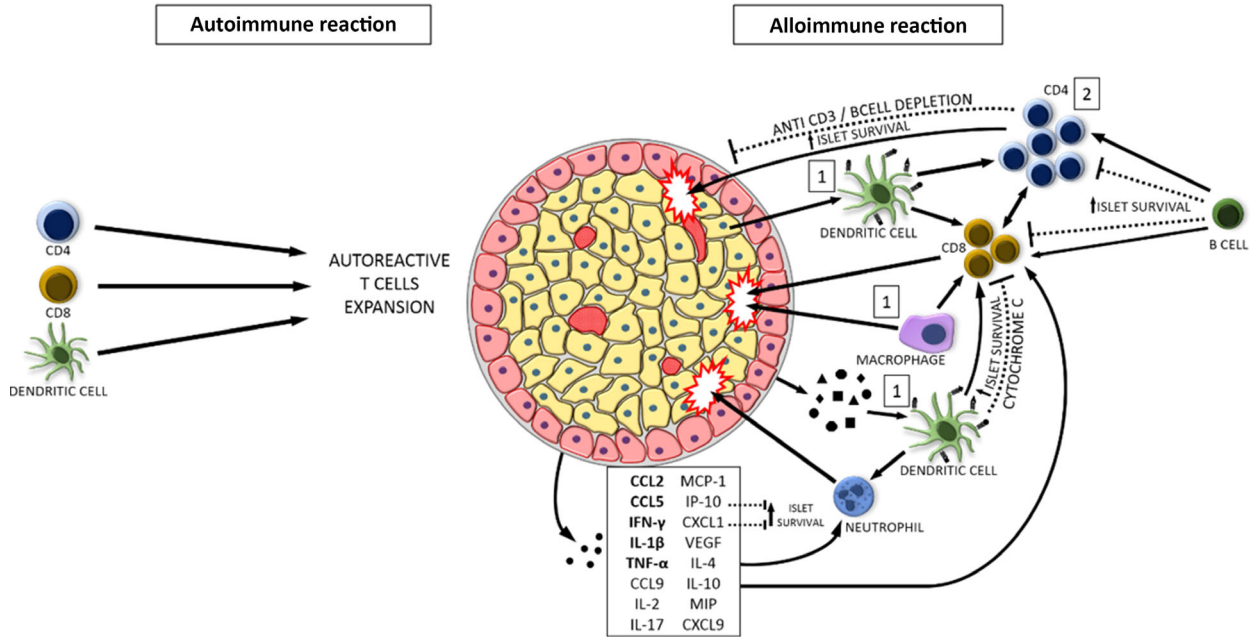

Figure 2 Immune reaction toward islet grafts in humans. Autoimmune reaction: Memory autoimmune cells (CD4 T cells, CD8 T cells, and DC) are rapidly reactivated after islet transplantation, expand, and destroy the islet graft. Alloimmune reaction: At first, antigens derived from the graft activate DC and macrophages that subsequently activate CD8 T cells, CD4 T cells, macrophages, and neutrophils. Blocking DCs by Cytochrome C increases islet graft survival in mice. CD4 and CD8 T cells are also activated by DCs derived from the islet graft and by B cells. In mice, blocking of B cells and the use of anti-CD3 inhibit CD4 T-cell activity and increase islet graft survival.

similar immune cells in the context of auto and allo immunity.

Although very little is known on the role of NK cells in human islet transplantation, their role in murine models remains controversial. Human β cells express an unknown ligand for the activating NK receptor NKp46 and are killed *in vitro* in an NKp46-dependent manner. Moreover, NK cells are absent from the vicinity of islets of healthy mice but are detected *in situ* in proximity with β cells in NOD mice [70]. NK cytotoxicity against islets plays a role in early islet graft loss after intraportal islet transplantation [71] and improved graft survival and function was observed in NK cell-depleted CD1d$^{-/-}$ diabetic mice [72]. In contrast, Beilke *et al.* [73] demonstrated that NK cells promote islet costimulation blockade–mediated islet allograft tolerance via a perforin-dependent mechanism.

Recent studies in mice, have shown that regulatory B cells promote transplantation tolerance [74], and a role for regulatory B cells in tolerance induction in an allogenic islet transplantation setting has been demonstrated recently [75]. Interestingly the regulatory function of B cells in this model of graft tolerance is dependent on NK cells. Whether these results are relevant to human immunology remains however unclear and needs further investigations.

Differences in terms of transplantation sites

The immune response may be dependent upon the site chosen for islet transplantation. The preferential site for transplantation in humans is the liver, where islets are infused through the portal vein. Islets are, thus, placed into immediate contact with the blood flow, causing IBMIR that, together with the activation of the innate immune system, leads to an immediate destruction of approximately 50–75% of infused islets [76]. Intraportal islet transplantation is usually not performed in mice given the smaller size of the liver vessels in which islets would cause major liver embolism. In mice, islets are commonly transplanted under the kidney capsule [77], resulting in mild inflammation immediately after transplantation [1]. Indeed, early after transplantation, islets seem to be protected against the immune system given the lack of vascularization [78].

Investigators have looked at the kidney capsule, omental pouch, gastric submucosa, peritoneal space, spleen, bone, and muscle among other potential sites for islet transplantation, some of which have been tested in humans [79,80]. The advantages and disadvantages of several islet transplantation sites have been cataloged and compared between humans and other animal

species and efforts to improve engraftment in easily accessible islet graft sites are warranted [78,81].

Some of the problems associated with non-highly vascularized sites, e.g., poor oxygen supply, are currently being addressed with the use of scaffolds and extracellular matrix support to the islets, with promising results [82,83].

Experiments in the mouse to determine the best site for islet transplantation may be useful to provide background for further investigations, however, large mammals are more informative to assess the site-specific challenges relevant to the clinic.

Conclusions

In conclusion, the NOD mouse model exhibits many similarities with human T1D and provides valuable insights in terms of pancreatic histopathology, islet inflammation, and the generation of specific autoantibodies against β-cell antigens as well as impairment of β-cell function. However, there are also important differences precluding generalization of the obtained results into clinical relevance. One should keep in mind that the different animal models can be used to study a specific aspect of T1D, but do not reflect the clinical disease. The mouse model of STZ-induced diabetes is only suitable for immunological studies with appropriate precautions. Careful selection of the models to reflect the conditions of human allotransplantation and critical interpretation of the results are warranted to improve translation to clinical procedures and benefits, including transplantation site and immunosuppressive regimens.

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Conflicts of interest

The authors have declared no conflicts of interest.

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