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Hematopoietic stem cell therapy for type 1 diabetes: induction of tolerance and islet cell neogenesis

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Abstract

Diabetes is a chronic disease with significant morbidity and mortality. Pancreas or islet cell transplantation is limited by a shortage of donors and chronic immune suppression to prevent allograft rejection. Consequently, interest exists in islet cell neogenesis from embryonic or mesenchymal stem cell as a possible cure for diabetes. However, unless tolerance to islet cells is re-established, diabetes treated by islet cell transplantation would remain a chronic disease secondary to immune suppression related morbidity. If islet cell tolerance could be re-induced, a major clinical hurdle to curing diabetes by islet cell neogenesis may be overcome. Recent studies suggest that adult hematopoietic stem cells (HSC) can reintroduce tolerance to auto-antigens. It is possible that HSC may also be able to switch lineage and, therefore, be a convenient source of stem cells for both inducing tolerance and islet cell regeneration.

Keywords: Stem cell therapy; Islet cell neogenesis; Type 1 diabetes

1. Type 1 diabetes: an autoimmune disease

Evidence that diabetes is an autoimmune disease comes from: animal models such as non-obese diabetic (NOD) mice [1], protective and susceptibility HLA associations [2], cytotoxic T cells and auto-antibodies against islet cell antigens such as glutamic acid decarboxylase (GAD) [3,4], rare case reports of disease transfer from donor to recipient following HSC transplant [5], and, response to immune suppression [6].

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2. Morbidity and mortality of diabetes

Insulin, while prolonging life, is not a cure. Morbidity and mortality is usually a result of infections or diabetic vasculopathy (Table 1). Vascular complications result from hypertension, hyperlipidemia, and advanced glycosylated end products (AGEs) [7]. AGEs arise from hyperglycemic-related post-translational glycosylation of intra and extra-cellular proteins. These glycosylated proteins are thought to increase free radical injury to endothelium resulting in accelerated atherosclerosis [8]. Clinically, this translates to correlation of disease-related mortality with mean blood glucose levels. In fact, for every 1% increase

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Table 1
Morbidity and mortality of diabetes

1	300–600% higher mortality than the general population
2	Sixth leading cause of death in the America
3	Most common cause for blindness in America
4	Most common cause for dialysis in America
5	Death under age 20 generally from hypoglycemia, ketoacidosis, or infection
6	Death above age 20 generally from renal and/or vascular disease
7	Age-adjusted incidence of myocardial infarction is 4–6 times higher, and survival following myocardial infarction is worse, compared to non-diabetics
8	Cerebrovascular accidents (CVA) are more common in diabetics
9	Peripheral vascular disease with claudification common
10	Minor trauma may precipitate gangrene, ulcer and lower extremity amputation

in HgbA1c, mortality increases approximately 11% [9–12].

3. Insulin treatment for type 1 diabetes

Diabetes is treated with either conventional insulin therapy or intensive insulin therapy (IIT). Since the risk of cardiovascular complications correlates with level of hyperglycemia (HgbA1c), the goal of IIT is tight control of blood sugar. While IIT is known to slow disease (e.g. retinopathy, nephropathy) progression by 35–70% [10–12], it does not prevent eventual development of these complications [10–13].

IIT has a higher incidence of hypoglycemic reactions (more than three times higher than conventional insulin treatment) that may cause seizures and or death [14]. IIT requires meticulous monitoring of blood sugar (4–6 times a day), frequent insulin injections (more than 3 times per day or an insulin pump), close control of diet, and is practical only in highly motivated groups. As quoted from the literature 'Achieving optimal blood glucose control, without an unacceptable rate of hypoglycaemia or unacceptable restrictions on lifestyle, is not simple with presently available insulin preparations and monitoring tools' [14]. 'Accordingly the appropriate use of insulin to obtain good metabolic control requires the continued and informed expertise of both patient and advising professional, but also attention from both to self-motivation in order to make the desired lifestyle changes possible' [14]. The incremental cost per year of life gained by IIT has been estimated at US$28, 661 [15]. Access to medical care, education, and motivation for IIT is influenced by socioeconomic status. This results in a disproportionate percentage of conventional insulin therapy in lower socioeconomic groups.

In practice, only 20–30% of type 1 diabetics are on IIT [16], approximately 10% on an insulin pump and 20% on multiple injections. Therefore, in clinical practice, therapy associated with higher mortality, i.e. conventional insulin therapy, remains the ongoing standard of care. Finally, the requirement for insulin injections, whether conventional or IIT, often has negative psychological and social implications for the patient [17].

4. Autologous HSCT: induction of tolerance

4.1. Rationale

The rationale for autologous HSCT is to give a short pulse of intense immune ablative therapy (usually over 4–7 days) followed by infusion of HSC to minimize the duration of myelosuppression (usually 7–11 days following HSC). HSCs are progenitor cells for all immune cells including T and B lymphocytes, macrophages, dendritic cells, neutrophils, and NK cells. In numerous animal models, HSCs depending upon conditions may cause, prevent or cure autoimmune diseases. In general, spontaneous onset animal autoimmune diseases require an allogeneic HSCT to be cured. Environmentally induced animal autoimmune diseases may be cured by a syngeneic or pseudoautologous HSC.

In the early 1980s, lupus prone mice with an intact thymus underwent allogeneic HSC trans-

plantation from normal donors resulting in amelioration or cure of the lupus-like manifestations [18]. Similarly, transplant of HSCs from diabetes prone mice into a normal strain caused diabetes while diabetes in NOD mice may be prevented by allogeneic HSC transplant from a non-disease prone strain [19]. This model system demonstrated that allogeneic HSC depending on source may cause or prevent autoimmune disease. While feasible with animals, allogeneic HSC transplantation was considered to morbid a therapy for patients with chronic autoimmune diseases.

In the 1990s, it was demonstrated that environmentally induced animal autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) and adjuvant arthritis (AA) could be cured by syngeneic, autologous or pseudo-autologous (using syngeneic animals in the same stage of disease as the recipient) HSC transplants [20–23]. Since autologous HSC transplants, in contrast to allogeneic HSC transplants, are relatively safe, these animal trials provided a rationale to treat human autoimmune diseases with autologous HSC. Although not yet used to treat diabetes, phase I (safety) autologous HSC transplant clinical trials began 5 years ago for several autoimmune diseases [24]. Phase III (efficacy) trials are now being designed for some human autoimmune diseases [24].

For diabetes, the majority of identical twins are discordant for disease, although the number of diabetes susceptible genes shared between twins may correlate with concordance [25]. This suggests an important causative role for environmental exposure [26] and indicates that autologous HSC transplantation, similar to induced animal autoimmune diseases, may reintroduce tolerance to islet cells in at least a subset of type 1 diabetics.

4.2. Risk/benefit

The risk of toxicity from HSC transplant varies by patient selection, conditioning regimen, stem cell source, and supportive care. The regimen of cyclophosphamide (200 mg/kg) and rabbit antithymocyte globulin (ATG) (6.0 mg/kg) with autologous HSC has virtually no mucositis or organ toxicity. The major risk arises from 7–11 days of neutropenia which when treated by pre-emptive antibiotics should result in few serious infectious. At our institution, a cyclophosphamide/ATG regimen has been used safely in 15 patients with systemic lupus erythematosus, all of whom have had astonishing improvements. The anticipated mortality for this regimen in otherwise healthy new onset diabetes could be anticipated to be 1% or less. The only anticipated long term toxicity is age dependent sterility. This may be avoid in males by sperm bank. From experience using the same regimen in aplastic anemia, normal ovarian function and fertility return in all females under 26 years of age but in only 33% over 26 years old [27].

Table 2
Possible criteria for hematopoietic stem cell transplantation of diabetes

1	Type 1 diabetes
2	Within 3 months of onset
3	C-peptide within normal range
4	No other co-morbid diseases
5	Ability to sperm bank and understand risk of infertility
6	Certified by a 3rd party physician to be ineligible or unlikely to be compliant with intensive insulin therapy

4.3. Eligibility

Candidates for an initial protocol of autologous HSC transplant should have new onset type 1 diabetes with detectable C-peptide and determined by a third party physician to be ineligible or unlikely to be compliant with IIT (Table 2). Besides duration of insulin independence, precursor frequency of GAD cytotoxic T cells, and titer of ICA may be followed. If immunologic parameters such as ICA titer normalize but the patient still becomes insulin dependent, the patient may be tolerant to islet cells but have insufficient surviving islet cell mass. In this scenario, islet cell neogenesis may be attempted by a variety of methods (Fig. 1).

5. Islet cell regeneration

Stem cell sources for islet cell neogenesis are shown in Fig. 1. Generation from autologous

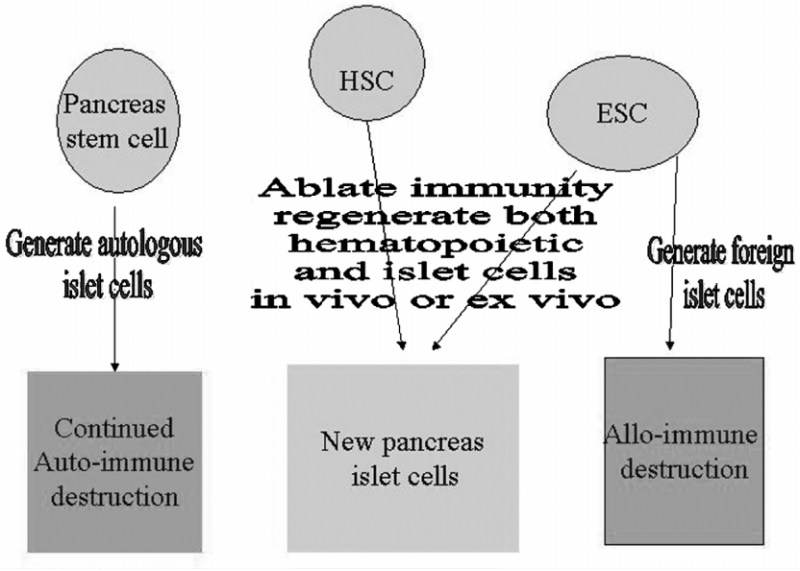

Fig. 1. Stem cell sources for islet cell neogenesis. HSC, hematopoietic stem cell; ESC, embryonic stem cell.

mesenchymal pancreatic stem cells will result in continued autoimmune destruction unless tolerance is induced first. If HSC transplantation can reintroduce islet cell tolerance, islet cell growth factors such as islet neogenesis associated protein (INGAP) may help re-establish normogylcemia without exogenous insulin [28].

Embryonic stem (ES) cells may be used to regenerate both marrow and islet cells. The ES cell derived immune cells should then be tolerant to the genetically identical ES cell derived islet cells. Alternatively, adult HSC may conceivably be a source of both marrow and islet cells. In murine models, it now appears that HSC can change lineage. Under appropriate conditions, murine HSC can be converted into neurons, cardiac myocytes, or hepatocytes [29–32]. It remains unclear if human HSC have the plasticity to switch tissue lineage commitment. Recently, human livers from female recipients of male bone marrow transplant donors were analyzed and Y chromosome specific DNA was detected in a small percentage of hepatocytes [33]. This implies that the male hepatocytes originated from the donor HSC.

The ability of HSC to induce tolerance and possibly repair damaged tissue has practical significance because hundreds of millions of HSC can be easily and repeatedly collected from the peripheral blood by apheresis with little risk to the donor. Autologous HSC are not encumbered by the ethical and immunologic issues associated with embryonic stem cells. Stem cell therapy and specifically HSC therapy to reintroduce tolerance and conceivably repair damaged tissue may become an important new weapon in the therapeutic armamentarium against diabetes.

Take-home messages

- Type 1 diabetes is an autoimmune disease.
- Cure will require induction of tolerance to islet cells and depending on timing of intervention possibly islet cell regeneration.
- Either adult marrow stem cells or embryonic stem cells may be capable of inducing both tolerance and islet cell regeneration through differentiation into both hematopoietic and islet cells.

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The World of Autoimmunity; Literature Synopsis

Graft-versus-host disease, interferon-gamma and autoimmunity

Graft-versus-host disease (GVHD) is a known complication of bone-marrow transplantation. Whereas acute GVHD develops in B6D2F1 hybrid recipients of wild-type C57BL/6 parental strain grafts, use interferon-gamma gene knockout donors results in prolongation of the disease in the recipients and a higher level of engraftment, particularly of T cells. Ellison et al. (Immunology 2002;105:63) studied the nature of the latter kind of GVHD characterized by lesions containing large, mixed cellular infiltrates in the skin, liver, pancreas, salivary gland, lung and kidney. They found that spleen cells from these recipients produce IL-4, IL-5 and IL-13 in culture, but respond poorly to concavalin A and lipopolysaccharide. The recipients' sera contain ANA, with specificity in some for dsDNA. There were also eosinophilic infiltrates developing within the target organs. This developed syndrome in the absence of interferon-gamma resembles in certain aspects both chronic GVHD and SLE. It is possible that the absence of interferon-gamma favors the development of an autoimmune-like syndrome in the mice. Of note that chronic GVHD in human subjects also have many features resembling some autoimmune diseases such as systemic sclerosis and Sjogren's syndrome.

Autoantibodies in Porphyria Cutanea Tarda

Porphyria cutanea tarda (PCT) is characterized by a deficiency of the cytosolic enzyme uroporphyrinogen decarboxylase which is involved in the synthesis of haem. Whereas this enzyme is deficient in all tissues in familial PCT, its deficiency is confined only to the liver in sporadic PCT. As sporadic PCT is frequently associated with hepatitis C virus (HCV) infection, and HCV infection is associated with autoimmune manifestations, Ma et al. (Clin Exp Immunol 2001;126;47) investigated whether autoimmune reactions are also involved in the pathogenesis of PCT. The authors compared autoantibodies to human cytosolic and microsomal liver fractions between patients with PCT, patients with other liver disorders and healthy subjects. Anti-cytosolic antibodies were more frequent in PCT patients (46%) than in the other groups, and within the PCT group they were more frequent in HCV-positive than-negative patients (57% versus 11%, respectively). Moreover, reactivity towards a 40-kDa cytosolic polypeptide was present in 20 PCT patients, of whom 19 were HCV-positive, more frequent than in any other group. The severity of liver damage and anti-HCV antibodies were also associated with anti-cytosolic antibodies. In contrast, no difference was found in the frequency of anti-liver microsomal antibodies between groups. These results provide another evidence for the contribution of infectious agents in general (and HCV in particular) to autoimmunity, and suggest that sporadic PCT might have an autoimmune component.