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Research Article

Advancement in Therapeutic Efforts and Tools for Prevention and Treatment of Insulin Dependent Diabetes Mellitis

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Abstract

Insulin Dependent Diabetes Mellitus (IDDM) is an autoimmune disease and is also called type 1diabetes. It occurs mostly due to CD8+ and CD4+ T lymphocytes which are actually the T-helper cells and very rarely by some virus and drugs which take the β-cells, insulin and proinsulin as its target. Th-cells may also affect by other ways i.e. by secreting the CXCL10 which are also reactive against the β-cells. In these patients there is low level of insulin causing high level of glucose also called hyperglycemia. To treat this autoimmune disorder we take some important steps either against the autoimmune responses or in the favor of proliferation of the β-cells or may take steps for the production of the insulin by either ways. There is possibility to give the patients monoclonal antibodies against the causative agents. We can also use the stem cells from pancreas that can grow to the new matured functional islet of langerhan. Moreover replacement of beta cell mass, non-endocrine cell production, antigen-based therapy, pancreatic transplantation, use of immunosuppressive monocyte and control of IDDM by using naturally found CD4 + CD25+, regulator of T lymphocytes, are other approaches. If we come to know the exact mechanism of CXCL10/CXCR3 system it may also be useful for us to treat the disease.

Keywords: Insulin Dependent Diabetes Mellitus, IDDM, Islet Transplantation, Antigen-Based Therapy

INTRODUCTION

Insulin dependent diabetes mellitus is an autoimmune disease. Which come about especially, due to abnormal activation of T lymphocytes against the auto antigens that are insulin-producing β-cells (Daneman *et al.*, 2006). The β-cells, and other cells, are found in small area of endocrine organ called the pancreatic islets of langerhans (Mannering *et al.*, 2010; Kaufman 2003). Type 1 diabetes causes hyperglycemia and many other symptoms e.g. polyuria (hypermicturation), polydipsia (frequent thirst), polyphagia (increased appetite), and loss in weight (Cooke *et al.*, 2008). Czech children also suffer from the short breast feeding which is also associated with the harms of IDDM (Malcova *et al.*, 2006), but it is reduced in later life (Borch-Johnsen *et al.*, 1984; Shehadeh *et al.*, 2001).

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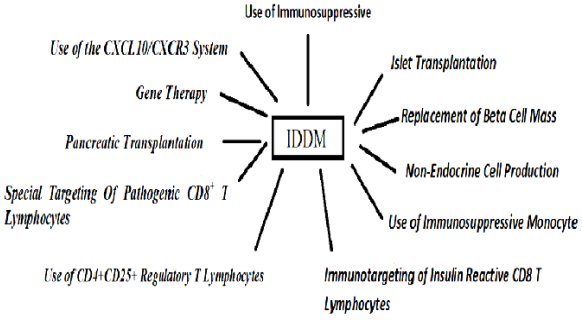

Figure 1: Targeting areas for IDDM

AUTOIMMUNE INSULITIS

Autoimmune insulitis, results in type 1 diabetes. In this disease there is β-cell death through many stages which lead to the progress of autoimmune insulitis (Lehmann *et al.*, 1998; Tian *et al.*, 2001). First stage takes place in the local lymph nodes, where the antigenic epitopes of the peptides specific to the β-cells are recognized and processed by the antigen processing cells (APCs) and many lymphocytes are proliferated and developed. Subsequently, many cytokines are secreted (Rabinovitch and Skyler 1998) and cell mediated response takes place against the β-cells, which causes

sucidal or apoptotic cell death of the β-cells, ultimately results in type 1 diabetes (Kaminitz *et al.,* 2007). These patients require insulin injections daily to control the hyperglycemia (The DCCRT Group, 1993)

ANTIGENIC INSULIN EPITOPE

Regarding the pathogenesis there are many islets molecules which act as the target side for immune cells. Actually the amino acid sequence of proinsulin/insulin is the target sides for the immune cells. If we prepare two insulin genes i.e. 1 and 2 which competitively combine with the proinsulin gene, having 16^{th} amino acid sequence changed to alanine. This change then becomes a preventive measure for IDDM in mice. The mice that have altered insulin never suffer from type 1 diabetes. But even if a single original insulin gene is present it can cause diabetes (Nakayama *et al.,* 2005). So it is proved that the original insulin or proinsulin have antigenic properties.

TREATMENT OF IDDM

Use of Immunosuppressive Agents (non cell based approach)

In the late 1970s there were many approaches to treat the autoimmune disease. The physicians treated the patients with immunosuppressive agents. In 1981, Eliot and colleagues (Elliott *et al.,* 1981) gave prednisone, in those patients who were recently been diagnosed for this disease, to prevent β-cell damage by the autoreactive immune system. But there is considerably high level of C-peptide in urine rather the normal value one year after their use. Different studies were conducted on the use of azathioprine (Cook *et al.,* 1989), azathioprine plus prednisone (Silverstein *et al.,* 1988), cyclosporine and consequently demonstrated the lower level of C-peptide in plasma. During the study, few people experienced small time span, less than a year when they need no insulin therapy for the cure of disease. Many toxic effects during and after the withdrawal of immunosuppressive therapy reduced their use (Barra *et al.,* 2009).

Cell Based Approaches

1. Islet Transplantation

For therapy of Type 1 diabetes (T1D) we have to give insulin exogenously throughout the life (Stress *et al.,* 2004). Even with careful glucose control, such treatment carries the danger of hypoglycemic attacks that can direct towards anxiety, seizures, coma and death (Shapiro *et al.,* 2000). There is also an alternative, cell replacement therapy which can be helpful in severely ill patients. In this transplantation of the pancreas or the insulin producing part (Trucco, 2005; Shapiro *et al.,* 2000) is done in the patients. Cell-based therapies have two approaches, substitution of islet cells (Shapira *et al.,* 1999; Rayhill *et al.,* 2000; Shapiro *et al.,* 2000; Sutherland *et al.,* 2001; Ryan *et al.,* 2001; Ryan *et al.,* 2002; Robertson, 2004; Hering *et al.,* 2005) by cells similar to those of islet cells, origin of which may be an embryo (Fändrich and Ungefroren., 2010) or the adult stem cells, but this approach is still ineffective because of less human donors for the transplantation (Danovitch *et al.,* 2005; Wynn *et al.,* 2004; Ojo *et al.,* 2004). So less than 1 % of the patients receive are let transplantation therapies each year (Lechner, Habener, 2003; Lacy, 1982). The main hurdle in this transplantation is immune barrier (Ogata, Platt, 2004). But the use of embryonic tissues gives small immune response (Foglia *et al.,* 1986; Statter *et al.,* 1988; Deke *et al.,* 1997; Eventov-Friedman *et al.,* 2006) and it may be the advantage in ameliorating

rejection using pig embryo pancreas. Moreover the patients also require the immunosuppressive schedule (Liao *et al.*, 2007). These two therapies cause the clinical success (Kabelitz 2008).

Current successes in treating type 1 diabetic patient with islet transplantation lead to clinical success. Ductal structure of the adult pancreas have numerous stem cells that can both be self-regenerated and matured to functionally adult islets of Langerhans if we grow them in-vitro conditions. In vitro-generated islets demonstrate sequential alteration in mRNA transcript against islet-associated indicators and also as controlled insulin reaction following glucose challenges. When entrenched into diabetic mice, in vitro-generated islets induce neovascularization and reverse insulin-dependent diabetes. The possibility of growing functional endocrine pancreas from stem cells provides new opportunities to produce many islets, for implantation (Peck *et al.,* 2002; Brendel *et al.,* 1999). But by the islet transplantation, chances of survival of the transplanted islets are not more than 5 years in most cases (Shapiro *et al.,* 2006).

2. Replacement of Beta Cell Mass

Insulin-secreting pancreatic beta-cells multiplication increases due to gradually increasing need for insulin and also after physiological injury (Bonner-Weir *et al.,* 1989; Donath *et al.,* 2008; Dor *et al.,* 2004; Sorenson & Brelje, 1997; Teta *et al.,* 2005; Withers *et al.,* 1998; Meier *et al.,* 2000). It is commonly recognized that beta-cells have limited life duration and that dead beta-cells are usually removed from the body (Dor *et al.,* 2004; Bonner-Weir *et al.,* 2004; Finegood *et al.,* 1995; Pick 1998; Montanya *et al.,* 2000; Grossman *et al.,* 2010). Since the beta cells are the actual target sides of the T lymphocytes so their replacement can also reverse the type 1 diabetes (Peck *et al.,* 2004).

3. Non-Endocrine Cell Production

The production of alternate cells by genetically adapted non-endocrine cells to secrete insulin in response to glucose imbalancement is also another approach to treat IDDM (Peck *et al.,* 2004).

4. Use of Immunosuppressive Monocyte

Fändrich F and Ungefroren H said that we will establish a new kind of adapted monocytes which will have the ability to suppress immune system and also some anti-inflammatory properties. Such type of immunosuppressive monocytes supply the possible choice that they may be used as autologous cellular transplants to play hazardous role for autoimmune reactions and defend the other viable β-cells (Fändrich & Ungefroren, 2010).

5. Immunotargeting of Insulin Reactive CD8 T Lymphocytes

Insulin is mainly attacked by CD4 and CD8 T cells in IDDM (Mannering *et al.,* 2010; Anderson *et al.,* 1999; Santamaria *et al.,* 1995; Benoist, Mathis, 1999). If we use non obese diabetic (NOD) T cells which contain transgenes produced for expression of MHC class 1 insulin peptide complexes joined to a factor (inscd3-ζ) which is a T cell activator, to shift towards insulin-killer CD8 T cells. These activated, inscd3-ζ CD8 T cells target the insulin killer CD8 T cells (Scott *et al.,* 2010). New approaches are established that inhibit progression of IDDM development and also stop more β-cell damage of transplanted islet. Self-reactive T lymphocytes act to be precious agents for studying the pathogenicity and other diabetic procedures. T-lymphocyte autoreactivity also provides the information about the

future of transplanted allografts of the islet in the patients with IDDM. Moreover, such type of studies also give evidence about operated tolerance against the immune system of islet homoplastic grafts along with precious information about the better modified therapies against the immune system. Now, approaches are made to identify the T-cell over-reactivity against the self and alloantigens in IDDM patients receiving homoplastic grafts of islet are used to check islet homoplastic grafts continued existence in relation with a variety of immunosuppressive treatments and to direct reduction of these treatments after victorious reinstatement of insulin formation. The immune system of our body is multifaceted; this is the reason that it may hinder the experimental procedures, carried out on the immunity against the self and alloantigens. So our valuable mission is to hinder the part of T-lymphocytes in the progression of the IDDM and also the degradation of the islet homoplastic grafts (Van de Linde & Roep, 2005).

6. Use of CD4+CD25+ Regulatory T Lymphocytes

There are the cells that regulate the T-lymphocytes called as (T (reg). The lack of these cells may encourage, establish and employ the T-cell auto reactivity hence the occurrence of IDDM. CD4 (+) CD25 (+) T (reg) (nT (reg)) cells, found in the body naturally, normally exhibit strong antagonizing effect on T cell function both in vitro and in vivo, might be imperfect in checking self immunity in autoimmune insulitis. Prediabetic CD25 (+)-depleted CD4 (+) T cells unlike all CD4 (+) T cells, if injected in immunocompromised patients show a strong diabetogenic effect. Simultaneous shift of CD4 (+) CD25 (+) T cells noticeably stop disease progress and β-cell lymphocytic penetration, even if T1D is induced by CD4 (+) T cells from BDC2.5 transgenic or diabetic NOD mice. At last, Mannering SI et al showed that CD4 (+) CD25 (+) T (reg) prefer to gather in swollen pancreas, where they strongly stop antigen-specificity increase and diabetogenic cytokine production. This process is very useful in control and delay of autoimmune insulitis (Piccirillo *et al.*, 2005; Mannering & Brodnicki, 2007).

7. Special Targeting Of Pathogenic $CD8^+$ T Lymphocytes

The degrees with which the peptides are presented towards the MHC by the APCs suggest the CTL (cytotocic T-lymphocytes) response. The MHC-I has L chain, which has the membrane bound β_2-microglobulin (β_2m). These have the role in the complex formation of MHC with the peptides (Margalit *et al.,* 2006). Alon Margalit et al performed different tests to check the conversion of TCR ligands to T cell activation receptors, again directing the T lymphocytes, which have been modified by gene therapy towards the autoreactive $CD8^+$ T lymphocytes. To find the results they used the affinity of the non polymorphic β_2 micro globulin light chain to form complex with all MHC-I heavy chains. They explained the shape as well as expression in a T cell hybri doma of two modalities of β_2 microglobulin polypeptides, joined to transmembrane and intracellular part of CD3ζ chain. In the absence of a particular antigenic peptide, the chimeric product associates with different endogenous MHC-I heavy chains and triggers T cell activation upon heavy chain cross linking. If the antigenic peptide bind through covalent bond attached with N terminus of chimeric polypeptide,

transfectants show increased in the membranous peptide–class I complexes as well as response towards the antibodies and target T lymphocytes in a peptide particular arrangement. Their results gave basics of the universal genetic approach aimed at antigen particular immunotargeting of autoreactive $CD8^+$ T lymphocytes (Margalit *et al.,* 2003).

8. Pancreatic Transplantation

Pancreas is usually transplanted after the transplantation of the kidney (Fioretto *et al.,* 1993; Jukema *et al.,* 2002; Gaber *et al*., 1995). This is because that introduction of a new kidney requires intake of immunosuppressive drugs such as cyclosporin. Nevertheless this allows the introduction of a new, functioning pancreas to a patient with diabetes without any additional immunosuppressive therapy. However, pancreas transplants alone can be wise in patients with extremely labile IDDM (Nathan *et al.,* 1991).

If kidney and pancreas are transplanted mutually it enhances the worth of life and also give us relieve from insulin use and kidney dialysis (Becker *et al.,* 2001; Adang *et al.,* 1996; Corry *et al.,* 1990; Kiebert *et al.,* 1994; Esmatjes *et al.,* 1994; Nathan *et al.,* 1991). As demonstrated by the Minnesota group and others, the recurrence of diabetic nephropathy is attenuated (El-Gebely *et al.,* 1995; Bilous *et al.,* 1989; Barbosa *et al.,* 1994; Wilczek *et al.,* 1995). It also has advantage in reducing the diabetic retinopathy (Fioretto *et al.,* 1998). Normal level of fasting glucose as well as glucose associated with hemoglobin leads to hindrances in developing the complications in retina, nephrones, neurons and macrovasculature (Navarro *et al.,* 1997; Koznarova *et al.,* 2000; Osei *et al.,* 1990; Wang *et al.,* 1994). Fourth it may stop or reverse the diabetic pain. This also improves the autonomic neuropathy, increase reflex functioning of heart as well as gastrointestinal movement (Navarro *et al.,* 1997). SPK mutual transplants also improve the vesicopathy in diabetic patients (Fioretto *et al.,* 1998). Lastly, diabetic cardiovascular disease is attenuated after SPK transplantation (Robertson *et al.,* 1991; Martin, 1995; Cheung *et al.,* 1994; Abendroth *et al.,* 1994; Rayhill *et al.,* 2000). But the survival cases are only for about 5 years (Shapiro *et al.,* 2006).

Response towards metabolism of lipids and glucose — if the pancreas is transplanted it shows response successfully against the glucose in the serum taken either orally or through IV route and also show response towards the stimulation of secretin and arginine given intravenously (Luzi *et al.,* 1990; Robertson *et al.,* 1991; Landgraf *et al.,* 1991; Katz *et al.,* 1991; Diem *et al.,* 1990). But it shows the 2-3 times increase in the background as well as peripheral insulin level in the venous drainage in patients who are receiving the pancreatic grafts. This increased insulin level in the blood is due to the effect that the insulin in circulation (American Diabetes Association, 2002) bypass the first pass effect by liver that otherwise clear about more than half of the insulin (Lee *et al.,* 2000; Konigsrainer *et al.,* 1994; Fioretto *et al.,* 1995).

9. Gene Therapy

A recombinant adeno-associated virus (rAAV) that expresses a single-chain insulin analogue (SIA), which possesses biologically active insulin activity without enzymatic conversion, under the control of hepatocyte-specific L-type pyruvate kinase (LPK) promoter, which regulates SIA expression in response to blood glucose

levels. Here we show that SIA produced from the gene construct rAAV-LPK-SIA caused remission of diabetes in streptozotocin-induced diabetic rats and autoimmune diabetic mice for a prolonged time without any apparent side effects. SIA gene therapeutic approach may approve to be fruitful as a curative measure of IDDM (Lee *et al.,* 2000).

10. Use of the CXCL10/CXCR3 System

Despite intervention with insulin, type 1 diabetes gradually deteriorates the patients' quality of life. Its etiology, however, remains controversial. Some studies argue that glutamic acid decarboxylase (GAD) antigen and GAD-reactive T cells are critical players in the development of diabetes by affecting the Th cell balance. A T-helper 1 (Th1)-dominant immune response is considered to be important in beta-cell failure in both human and animal models of type 1 diabetes. The Th1-type chemokine, CXCL10, and its receptor, CXCR3, are involved not only in the immune response, but also in the suppression of beta-cell proliferation. Thus, understanding the CXCL10/CXCR3 system may be important for finding a cure of type 1 diabetes. In a short review, Shimada A discussed the role of the CXCL10/CXCR3 system in type 1 diabetes and proposes relevant treatment options (Rotondi *et al.,* 2003). The T-helper (Th) cell system is critical for a healthy immune system that balances reactive and suppressive cell compartments. It has been argued that an imbalance in the Th cell system, caused by a predominance of the Th1 response, favors the development of autoimmune diabetes (Sia, 2005). CXCL10 is an interferon-gamma inducible chemokine and reacts with its receptor, CXCR3, on Th1 cells (Rotondi *et al.,* 2003). Elevated levels of CXCL10 were detected in new onset type I diabetes patients and correlated with levels of GAD-reactive CD4 T cells (Shigihara *et al.,* 2006). In addition, CXCL10 is produced by beta-cells, and suppresses beta-cell proliferation (Shigihara *et al.,* 2006). Therefore, they believed that the CXCL10/CXCR3 system plays a decisive role in the pathogenesis of type 1 diabetes. Some studies have suggested that the CXCL10/CXCR3 chemokine system plays a critical role in the autoimmune process and in beta-cell destruction in type 1 diabetes. Blocking the CXCL10 chemokine in new onset diabetes seems to be a possible approach for treatment. In combination with another regulatory intervention strategy, such as GAD autoantigen sensitization, this approach could contribute to a curative treatment for type 1 diabetes. They envisaged that further research into the CXCL10/CXCR3 system will enable to develop a new and effective therapy (Shimada *et al.,* 2009).

Antigen-Based Therapy

Antigen based therapy is also a therapeutic measure for the inhibition of IDDM by producing either active tolerance (inhibition of the response of T lymphocytes) or by passive or indirect tolerance (undoing the pathogenecity of T-cells). However ABTs is not yet used clinically and studies are still under process. Even though preliminary trials for its application as curative policy for IDDM find no courage, But medical researches and studies sure us that this process has ability to hinder the autoimmune insulitis and also protect the β-cell functioning in adults as well as in children who are recently diagnosed to be suffered from IDDM. The studies on NOD mice reveal that the role of ABTs in immunology

is very much appreciated as active and multifaceted, today than in previous days. Difference in cause and immunotherapeutic trials of IDDM among rodents and humans has been comprehensively reviewed in another place (Okubo *et al.,* 2008; Roep *et al.,* 2004; Akirav *et al.,* 2008; Staeva-Vieira *et al.,* 2007). ABTs encourage controlled response against a given selfantigen, as well as encourage dispersion of controlled response against additional target tissue selfantigens (Tian *et al.,* 1997).

Table 1: Causes of IDDM and treatment strategies

Causes	Treatment strategies
Autoimmune disorder due to reactivity of T lymphocytes	Immunosuppressive agents Use of immunosuppressive monocytes Use of CD4+CD25+ Regulatory T Lymphocytes Use of the CXCL10/CXCR3 System against proliferation of T lymphocytes Antigen-Based therapy For undoing the pathogenicity of T lymphocytes
Destruction of islets of langerhans	Transplantation of islets of langerhans
β-cell destruction	Replacement of β-cell mass Nonendocrine cell production producing insulin
CD 8 T lymphocytes against insulin	Immunotargetting of insulin reactive CD8 T lymphocytes Special targeting of Pathogenic $CD8^+$ T lymphocytes
Destruction of pancreas	Transplantation of pancreas
Genetic factor	Gene Therapy

CONCLUSION

IDDM is an autoimmune disease, it occurs mostly due to CD8+ and CD4+ T lymphocytes and very rarely by some virus and drugs which take the β-cells, insulin and proinsulin as its target. To treat this autoimmune disorder there must be two approaches either we suppress the autoimmune response or we make the way for the β cell proliferation. There is possibility to give the patients monoclonal antibodies against these causative agents. We can also use the stem cells from pancreas that can grow to the new matured functional islet of langerhan. Moreover replacement of beta cell mass, non-endocrine cell production, antigen-based therapy, pancreatic transplantation, use of immunosuppressive monocyte is other approaches. Understanding the CXCL10/CXCR3 system and its opposal may lead to clinical success for type 1 diabetes. Furthermore, the GAD autoantigen sensitization interference may be another approach for treatment for type 1 diabetes. Use of CD4 + CD25+, regulator of T lymphocytes, found naturally, is another approach. At the end I want to add my opinion. I think in all the methods discussed above the immune-targeting to either of lymphocytes is best method but one thing must be kept in mind that these must be highly specific.

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