ISSN: 2348-8948
Vol: 2; Issue: 6

RESEARCH ARTICLE

Novel therapeutic vaccine for Diabetes mellitus: Literature Review

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Date Received:	Date of Accepted:	Date Published:
01-Jun-2014	13-Jun-2014	26-Jun-2014

Abstract:

Diabetes mellitus is a group of metabolic disorders of carbohydrate, fat, and protein metabolism. It results from disorder of the pancreas, the gland which produces insulin or insulin action (sensitivity). This review provides the most important vaccines used against Diabetes mellitus.

Keywords: Diabetes mellitus, insulin, vaccine

Introduction

Diabetes mellitus is a group of metabolic disorders of carbohydrate, fat, and protein metabolism. It results from disorder of the pancreas, the gland which produces insulin or insulin action (sensitivity). Insulin is required to absorb sugar from the blood into the body's cells; without it, sugar levels in the blood become very high and cause serious health problems[1]. Diabetes is associated with serious complications and premature death, but timely diagnosis and treatment of diabetes can prevent or delay the onset of long-term complications (damage to the cardiovascular system, kidneys, eyes, nerves, blood vessels, skin, gums and teeth)[2].

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate due to population growth, urbanization and increasing of obesity and physical inactivity. Over the past 30 yrs., the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people. It is important to note that the rise in prevalence is seen in all six inhabited continents of the globe. Although there is an increase in the prevalence of type1diabetes, also the major driver of the epidemic is the more common form of diabetes, namely type 2 diabetes which accounts for more than 90 per cent of all diabetes cases[3,4].

Classification of diabetes mellitus

Type 1 diabetes Mellitus

Type 1 diabetes is caused by the immune-mediated destruction of islet insulin-secreting beta cells. This chronic destructive process is associated with both cellular and humoral immune changes in the peripheral blood that can be detected months or even years before the onset of clinical diabetes. Throughout this pre-diabetic period, metabolic changes, including altered glucose tolerance and reduced insulin secretion, deteriorate at variable rates and eventually result in clinical diabetes[5].

Type1 diabetes accounts for 5 to 10 percent of all diagnosed cases of diabetes, but is the leading cause of diabetes in children of all ages. Type1 diabetes accounts for almost all diabetes in children less than 10 years of age. It mostly has an acute onset, with children and adolescents usually able to pinpoint when symptoms began. Start can occur at any age, but it most often occurs in children and young adults. Children and adolescents may present with ketoacidosis as the first indication of type1 diabetes. Others may have post-meal hyperglycemia or modest fasting hyperglycemia and/or ketoacidosis in the presence of infection or other stress[6].

Treatment of Type1 Diabetes

For patients with type1 diabetes, it has been clearly demonstrated that intensive insulin therapy results in both improved glycemic control and reduction in diabetes-related complications including nephropathy, retinopathy, neuropathy, cardiovascular morbidity and mortality. Although research findings strongly support the use of intensive insulin therapy, there are associated drawbacks:

1. Greater effort is required on the part of the inmate to coordinate diet, activity, insulin administration, and glucose monitoring.
2. There is up to a three-fold increase in the incidence of hypoglycemia (a significant concern for correctional facilities).
3. Weight gain is more likely, sometimes limiting patient compliance[7].

Type 2 diabetes mellitus

Type2 diabetes is a disease resulting from a relative, rather than an absolute, insulin deficiency with an underlying insulin resistance. It is associated with obesity, age, and physical inactivity it also used to occur mainly in adults who were overweight and older than 40 years. It more often occurring in young people aged 10 or older. Type2 diabetes is more common in certain racial and ethnic groups such as African Americans, American Indians, Hispanic/Latino Americans, and some Asian and Pacific Islander Americans[2].

Treatment for Type2 Diabetes

- Insulin treatment
- Oral hypoglycemic agents
  - ❖ Biguanides: Metformin & Metformin XR (Glucophage®)
  - ❖ Sulfonylureas: Glyburide (Micronase®) and Glipizide (Glucotrol®)
  - ❖ Thiazolidinedione: Pioglitazone (Actos®) and Rosiglitazone
  - ❖ Alpha-glucosidase inhibitors: Acarbos and Maglitol[8,9].
  - ❖ Meglitinide: Nateglinide & Repaglinide
  - ❖ DPP4 inhibitors: Sitagliptin and Saxagliptin[10].

Gestational diabetes mellitus (GDM)

It is diabetes or any degree of glucose intolerance that is diagnosed during pregnancy. Importantly, women with an early diagnosis of GDM, in the first half of pregnancy, represent a high-risk subgroup with an increased incidence of obstetric complications, recurrent GDM in subsequent pregnancies and future development of Type2 diabetes. Other factors that place women with GDM at increased risk of Type2 diabetes are obesity and need for insulin for glycemic control. Furthermore, hypertensive disorders in pregnancy and afterwards may be more prevalent in women with GDM[11].

The following guidelines should be considered when managing inmates with GDM:

Close surveillance of the mother and fetus must be maintained throughout the pregnancy. Self-monitoring of blood glucose should be done on a frequent (daily) basis. Use of post-prandial monitoring is preferred. Monitoring of urinary glucose is not an adequate measure. Screening for hypertension should include measurement of blood pressure and urine protein. Clinical estimation of fetal size and asymmetric growth via serial ultrasounds, especially early in the third trimester, may identify large infants who would benefit from maternal insulin therapy. All inmates with GDM should receive dietary counseling and be provided with adequate calories and nutrients during pregnancy. If women are unable to manage their gestational diabetes with diet and activity alone, sometimes medication or insulin is needed[12].

VACCINES

Edible vaccines are those vaccines based on genetically engineered expression of an antigenic protein by an edible plant that trigger an animal's immune response. In simpler words, they are simply sub-unit vaccines that are edible in nature. Here, the gene of interest is introduced into plants and then these altered plants are induced to manufacture the corresponding proteins. This process is known as transformation and the altered plants are called transgenic plants[13].

Edible vaccines hold great promise as a cost-effective, easy-to-administer, easy-to-store, safe and socio-culturally readily acceptable vaccine delivery system, especially for the poor developing countries. It involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. A variety of delivery systems have been developed. Initially thought to be useful only for preventing infectious diseases, it has also found application in prevention of autoimmune diseases, birth control, cancer therapy, etc[14].

Edible parts of different plant species like the grains or fruits are utilized for the expression of desired antigen of interest. Cereals like rice and maize, fruits like banana, leaves of many plants (tobacco, alfalfa, peanut leaves), tubers like potatoes, tomatoes, soybean seeds, cowpea, pea, carrot, peanut and lettuce have been extensively used for high levels of antigenic protein expression[15].

Edible vaccine against diabetes mellitus

Transgenic plants expressing auto antigens are being produced in attempt to cure diseases in which the immune system recognizes the body's own proteins as foreign. The rational is that an appropriate oral dose of a plant-derived auto antigen will inhibit the development of the autoimmune disease[16,17]. In the case of type1 diabetes, lymphocytes infiltrate the pancreatic islets and selectively destroy the insulin secreting beta cells. One strategy for vaccine development is to reduce the pathological lymphocytic infiltration by tolerisation[18]. Tolerization is defined as any mechanism by which a potentially injurious immune response is prevented, suppressed, or shifted to a non-injurious class of immune response. This process may involve clonal deletion, anergy (unresponsiveness), or active suppression of T cells by regulatory cytokines. Antigen dose and exposure frequency are primary factors that determine which of the suppression mechanisms are induced[19].

Low doses of antigen tend to generate active suppression by TH2- produced regulatory cytokines, primarily TGF-, IL-4, and IL-10. High antigen doses may trigger an anergic response or result in clonal deletion of the auto reactive lymphocytes. Auto antigens given orally have been shown to induce tolerance in animal and human studies with promising results for type1 diabetes[20].

In the earliest experiments fro Higgins and Weiner, 1988 [20] investigating suppression of insulin-dependent diabetes mellitus (IDDM—Type1 diabetes) auto antigen alone was given orally and found to moderately suppress or delay disease onset. Large (mg) quantities of auto antigen were usually required to induce significant oral tolerance, presumably because the protein is partially degraded in the stomach and is not highly immunogenic. While digestion usually disrupts the metabolic activity of an antigen, oral tolerance is unaffected and may even be enhanced via association of the gut-associated lymphoid tissue (GALT) with the remnant protein fragments. To resolve the problem of extracting large quantities of auto antigens from animals, edible transgenic plants were utilized for synthesis and delivery of oral auto antigens for suppression of type1 diabetes[21].

In type1 insulin dependent diabetes mellitus (T1DM) which affects 0.2-0.3% of the population, the smaller isoform of glutamic acid decarboxylase of 65KDa (GAD65) is a major auto antigen. Oral administration of disease specific auto antigens can prevent or delay the onset of the autoimmune disease symptoms. It is expressed properly folded, fully active and immuno-chemically competent GAD65 in tobacco and potato[22].

Transgenic potato plants expressed human insulin (another major IDDM auto antigen) at levels up to 0.05% of teaspoon. To direct delivery of plant-synthesized insulin to gut associated lymphoid tissues, insulin was linked to the C terminus of the cholera toxin B subunit (CTB). Non-obese diabetic mice were fed which showed substantial reduction in pancreatic islet inflammation (insulitis) and a delay in the progression of clinical diabetes[23].

Oral administration of CTB subunit, when coupled with an auto antigen, was shown to induce a state of immunological tolerance. Oral delivery of CTB conjugated to specific auto antigens was shown to enhance auto antigen mediated protection of mice against type1 autoimmune diabetes in non-obese diabetic (NOD) mice. The results of the diabetes studies indicated that CTB-auto antigen conjugates reduced IFN-γ production and the migration of regulatory T cells into pancreatic islets. Linkage of CTB to an auto antigen was shown to provide up to a 10,000 fold reduction in the amount of auto antigen required for generating immune-tolerance[24].

Mechanisms underlying CTB-auto antigen activated immunological tolerance were shown to include inhibition of dendritic cell maturation, auto reactive T cell development or stimulation of Th2 and regulatory T cell proliferation and activation, or both. Immunosuppressive cytokine secretion, including increased IL-10 secretion was observed after oral administration of CTB conjugated to insulin, resulting in suppression of diabetes onset in NOD mice[25].

How do edible vaccines work?

Plant tissues containing vaccine proteins can be directly consumed with little or no preparation. During digestion,

proteins that elicit an immune response are gradually released onto the vast surface area of the digestive tract, where antigen uptake occurs[21]. An antigen in a food vaccine gets up by M cells in the intestine and passed to various immune-system cells, which then launch a defensive attack- as if the antigen were a true infectious agent. That response leaves long-lasting memory cells able to promptly neutralize the real infectious agent if it attempts an invasion[26].

Advantages and disadvantages of edible vaccines

Advantages of edible vaccines

- Edible means of administration.
- Reduced need for medical personnel and sterile injection conditions.
- Economical in mass production and transportation.
- Storage near the site of use.
- Enhanced compliance (especially in children).
- Delivery of multiple antigens.
- Integration with other vaccine approaches.

Limitations

- Development of immunotoleance to vaccine peptide or protein.
- Stability of vaccine in fruit is not known.
- Evaluation of dosage requirement is tedious.
- Selection of best plant is difficult.
- Certain foods like potato are not eaten raw, and cooking the food might weaken the medicine present in it.
- Acidic stomach may hart protein[24,27].
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HEAT SHOCK PROTIEN 60(HSP60) DERIVED PEPTIDE P277 VACCINE AGAINST DIABETE MELLITUS

Heat shock proteins (HSPs) are a group of proteins inducible by heat shock; they could also be induced by other stimuli such as growth factors, inflammation, and infection. Heat shock proteins are highly conserved in all prokaryotes and eukaryotes and have an intracellular role as chaperone molecules. Human Hsp60 also has a role in the regulation of the innate immune system[28]. Heat shock protein 60 (Hsp60) is another important self-antigen in the pathogenesis of diabetes. In therapeutic applications hsp60 is not given as a whole protein but as a peptide p277 derived from the native sequence of human heat shock protein 60. The sequence of this peptide p277 was first identified in the NOD mouse with the help of diabetogenic T-cell clones responding to the mycobacterium tuberculosis hsp60[29].

Based on the protective and therapeutic effects of p277in animal models as well as the findings in the human disease, a human p277 vaccine, DiaPep277, has been developed. DiaPep27 differs from the native p277 sequence in two amino acid positions, which were introduced for stabilization purposes. Since the sequence of this peptide contained two cysteine residues a more stable form was subsequently generated in which the cysteine residues were replaced by valine. DiaPep277 was also effective in delaying T1D in NOD mice[30].

Prevention of beta cell destruction by peptide p277 vaccine

Type1 diabetes mellitus is caused by the progressive destruction of the insulin-producing beta cells through an autoimmune process. Auto immune destruction remains subclinical until the number of beta cells is insufficient to produce the amount of insulin needed to maintain glucose homeostasis; at this point, diabetes becomes apparent. Primary cure of type 1 diabetes would require stopping the autoimmune process in time to rescue the beta cells[31].

The autoimmunity that brings about type1 diabetes has been studied in NOD mice. Autoimmune T cells in NOD mice react spontaneously with many different self-antigens, one of which is the 60 kDa heat-shock protein (hsp60). It is observed that treatment of NOD mice with a peptide of hsp60, peptide p277, could save residual beta-cell function even late in the course of autoimmunity, after the onset of clinical hyperglycemia. Cessation of beta-cell destruction seemed to result from the induction by p277 of a shift in the cytokine profile of hsp60 autoimmunity from a pro-inflammatory T-helper-1 (Th1) phenotype to an anti-inflammatory T-helper-2 (Th2) phenotype[32].

The immunomodulation of hsp60 autoimmunity induced by p277 was specific; Th1 immunity to bacterial antigens was not affected by p277 treatment. In view of the observation that patients with type1 diabetes, like NOD mice, show spontaneous T-cell autoimmunity to hsp60; and then it is tested whether the p277 peptide might be used to prevent the autoimmune destruction of beta cells in human beings[33].

One of the laboratories of Research Unit of Autoimmune Disease in Israel has found that the autoimmune destruction of beta-cells and subsequent IDDM can be arrested by the administration to NOD mice of a peptidep277; comprising residues 437–460 of the 60 kDa heat shock protein. Successful treatment of IDDM with peptide p277 was associated with the production of antibodies to p277[34].

The Department of Immunology, The Weizmann Institute of Science, Rehovot 76100 which is also found in ISRAEL tested the effectiveness of peptide p277 in treating NOD mice with advanced insulitis. A variety of antigenically non-specific treatments given to very young NOD mice, 4 to 6 weeks of age, had been shown

to abort the later development of diabetes. It is found that a single subcutaneous injection of p277 was effective when given just before the onset of clinical hyperglycemia (12 weeks of age), when half the mice were already diabetic (15 weeks of age), and even when two-thirds of the mice were diabetic (17 weeks of age) .The result of this study indicate that peptide p277 was effective in arresting beta-cell destruction even late in the autoimmune disease process[33].

Study results reported from the same institute in May 2004 indicate that vaccination with p277 or with HSP-60 induced a change in the expression of the autoimmunity not only to p277 and HSP-60 but also to other antigens in the diabetes collective such as insulin and glutamic acid decarboxylase. T cell autoimmunity to these antigens switched from a damaging Th1 type of response to a protective Th2 type of response. Requisite toxicology studies and phase 1 human trials set the stage for placebo-controlled phase 2 studies in patients with new-onset type 1 diabetes, and the results indicated that vaccination with peptide p277 could successfully arrest beta-cell damage[35].

According to data presented by researchers at the American Diabetes Association's 71st Scientific Sessions(June, 2011) in phase two study, the altered heat shock protein (known as DiaPep277) – administered subcutaneously to 100 patients newly diagnosed with type1 diabetes – succeeded in protecting the beta cells, replicating in humans the findings in laboratory mice. The drug works by increasing protective T-cells that secrete cytokine, which prevents the destruction of beta cells from immune attack. Administering this vaccine also allowed beta cells to continue to secrete insulin for up to two years following a type 1 diagnosis; a promising result that points toward the potential for prevention. This shows that the vaccine protects the beta cell function over time; the drug is currently undergoing phase three trials in which beta cell function, insulin use and glucose control are being monitored as key outcomes[36].

Peptide p277 in toxin-induced type1 diabetes

A sufficiently large dose of the toxin streptozotocin quickly kills beta cells, and so causes acute, toxic diabetes in most strains of mice. It is also found that peptide p277 vaccination to be effective in a toxin-induced model of type1 diabetes. However, certain strains of mice respond to low doses of streptozotocin that cause sub-clinical damage by later developing a form of chronic autoimmune insulitis and diabetes[37]. Very low doses of streptozotocin induced type 1 diabetes in mice, and the process was accompanied by the development of autoimmunity to HSP60 and to its peptide p277 epitope. In contrast to the NOD mice that responded to a single dose of peptide p277, the toxin-induced autoimmune disease required several doses of peptide p277 in IFA. Thus, peptide p277, albeit at different dose schedules, was effective in stopping the progress of autoimmune diabetes induced by a toxin, as well as that developing spontaneously in NOD mice[38].

Mechanism of action of peptide p277 vaccine

Elias D, Meilin A, Ablamunits V et al. were studied that the effect of p277 treatment on the immunology of Type I diabetes in two anatomical sites in the body: the spleen and the islets. The splenic T cells of NOD mice treated with p277 in IFA showed four features of interest;

1. Down-regulation of the Th1 cytokines IFN and IL-2, and up-regulation of the Th2 cytokines IL-4 and IL-10 in the T-cell response to HSP60 and peptide p277;
2. Down-regulation of the T-cell proliferative responses to HSP60, p277, and GAD peptides;
3. Down-regulation of Th1-like IgG isotype antibodies to HSP60, GAD, and insulin; and
4. No modification of a spontaneous Th1 cytokine response to a Mycobacterial HSP65 peptide[39].

Several studies suggested that the mechanism of action of DiaPep277 might be similar to the ones proposed for vaccination with GAD (e.g. induction of T regulatory cells). It has become evident however that DiaPep277 (and hsp60) can also exert direct effects on the immune system. Hsp60 can activate beta-cells via the Toll like receptor 4 (TLR4), which respond by producing IL-10. Furthermore, TLR4 activation by hsp60 also occurs in macrophages and dendritic cells promoting pro-inflammatory effectors. At the same time hsp60 can also induce anti-inflammatory effects promoted through TLR2. It is reported DiaPep277 does not engage TLR 4 but only TLR2, which leads to the generation of a T-cell mediated anti-inflammatory environment[32].

DIAMYD VACCINE FOR DIABETES MELLITUS

Diamyd®, an antigen based therapeutic diabetes vaccine that will slow or in the best case, halt the immune system's destruction of the body's insulin-producing cells in what is called autoimmune diabetes[40].

Glutamic acid decarboxylase isoform 65 (GAD65) is the active substance in the Diamyd® vaccine. This is a human enzyme that plays an important role in the central nervous system (CNS). Its function is to convert glutamate to GABA, a substance that attenuates signal transmission in the CNS. A person with too little GABA may suffer from epileptic attacks or seizures. The enzyme is also present in the insulin-producing beta cells in the pancreas, although its role there is not completely understood. However, the world's leading diabetes researchers agree that GAD65 has a central role in the development of autoimmune diabetes, meaning type 1 diabetes and LADA[41].

About type 1 diabetes and the diabetes vaccine Diamyd®

Type 1 diabetes, also known as juvenile diabetes, is a lifelong and very serious disease that often affects young children. Auto immune destruction of pancreatic islet beta cells is the major cause of type1 diabetes mellitus. This destruction is associated with cellular and humoral immune response to several beta cell auto antigens, both of which can precede the clinical onset of disease[40].

Indeed, the presence of antibodies against glutamic acid decarboxylase (GAD), insulinoma-associated antigen (IA-2A), or insulin (IAA) alone or in combination has been shown to predict type1 diabetes together with islet-cell antibodies (ICA), IA-2A and GAD antibodies are present at the time of diagnosis in 80-90% of patients with type1 diabetes. These autoantibodies, especially GAD antibodies, may also occur in up to 10% of adults initially classified as type 2 diabetes, a condition referred to as Latent Auto Immune Diabetes in Adults (LADA). The disease process in LADA patient is similar to that in type1 diabetes in that they share some HLA genetic susceptibility and some type1 diabetes-associated antibodies. In type1 diabetes compared to LADA, however, insulin secretion is lower and the rate of progression to insulin dependency is higher[42].

Preclinical study results from Diamyd Medical (Diamyd and trade) in spontaneously non obese type 1 diabetic (NOD) mouse demonstrated that the destruction of pancreatic islet beta cell was associated with T cells recognizing GAD65. It has also been shown that the administration of small quantities of GAD65 effectively prevent auto immune beta cell destruction and reduce or delay the development of spontaneous diabetes. Treatment with Diamyd is intended to prevent, delay, or stop the autoimmune attack on the beta cells. The aim is to prevent the onset of autoimmune diabetes, or to preserve the body's capacity to regulate blood sugar. Studies have shown that even a very small preservation of endogenous insulin secretion and slight improvement of the blood sugar control can significantly reduce the risk of both acute and long-term diabetes complications[43].

Mechanism of action of diamyd vaccine

For several rodent models of autoimmune diseases it has been possible to target auto reactive T cells therapeutically and block their pathogenic activity by using auto antigens themselves. In this way a selective tolerization of T cells is achieved, thereby avoiding the need for general immunosuppression[44].

Several observations indicate that the active mechanism for Diamyd® begins when GAD65 is injected together cells (dendritic cells, macrophages or B cells). The peptide fragments contain portions of the GAD65 molecule called determinants. These determinants have meaning the body's own capacity for producing insulin the potential to induce tolerance, which results in up-with alum under the skin and GAD65 is broken down into small peptide fragments called antigen presenting regulation and activation of a certain group of GAD65-specific regulatory T cells. Regulatory T cells down-regulate the antigen specific killer T cells that would otherwise attack the insulin-producing beta cells. This immune-modulatory effect of the diabetes vaccine is considered to induce an immunological tolerance that can counteract the breakdown of beta cells in patients with autoimmune diabetes[38,45].

Clinical studies on diamyd vaccines

Phase 2 clinical studies

The results provide strong evidence that treatment with Diamyd® is safe and can preserve the insulin producing function. The studies conducted to date provide strong evidence that treatment with Diamyd can delay the development of the disease by preserving the beta cell function in patients with type1 diabetes. Preserving the beta cell function is important, since it helps patients to better control the disease, thus resulting in fewer long term complications[46].

In August 2006, DIAMYD MEDICAL COMPANY announced positive results from a 15-months phase2 trial in 70 children and adolescents with type 1 diabetes. GAD-antibody positive type1 diabetes patients having presented with disease within 18 months were included in the study. Significant efficacy was demonstrated in preserving beta cell function. On average, the 35 patients that received Diamyd experienced only half the decline in meal-stimulated insulin secretion, as measured by meal-stimulated C-peptide levels, compared to placebo. In patients treated within 3 months of diagnosis, the Diamyd-treated patients on average actually showed an improvement in endogenous insulin secretion[47].

Promising results are reported from a trial of GAD therapy in the New England Journal of Medicine on October 8, 2008[48]. This 30-months randomized controlled phase2 trial assessed the ability of alum-formulated GAD (Diamyd) compared with placebo to preserve residual insulin secretion and reverse recent-onset type1 diabetes. The study included 70 children and young people between the ages of 10 and 18 who all had antibodies against GAD experienced the disease for longer than 18 months. Vaccination was given with two injections at an interval of four weeks. The patients were divided into two equally large groups of 35 individuals in which one group received 20 micrograms of the active substance and the other received the placebo. The objective was to test clinically if GAD treatment could improve the patient's own insulin production in type 1 diabetics. Just over one and a half years after the study began, the first positive study data could be reported. The ability of Diamyd to preserve beta cell function, reported in the groups treated with Diamyd and in the

measured as meal-stimulated C-peptide, was shown to be significant, compared with the placebo, after a 15-month follow-up.
The group that was vaccinated lost only half as much during the period, compared with the placebo group. The statistically significant effect remained even 30 months after the first injection. It was also shown that vaccination with Diamyd® results in a lasting and specific effect on immune defenses, which may be the explanation for the preserving effect on the beta cells. Overall, no serious adverse effects were noted that could be related to treatment with Diamyd[49,50].

On the other hand outcomes from Swedish GAD-vaccination trial suggests that ten percent of patients diagnosed with non-insulin requiring diabetes are GAD antibodies positive and constitute latent autoimmune diabetes in adults (LADA). The autoantibodies in these patients are suggestive of an ongoing progression to insulin dependency. To evaluate the safety of and immunomodulation from GAD65 administration, formulated as a vaccine, 47 GAD antibodies positive non-insulin requiring diabetes patients were treated in a placebo controlled double blind phase II clinical trial using a prime-and-boost dose regimen[42].

Alum-formulated GAD65 (4, 20, 100 or 500 μg) or alum alone (placebo group) was injected subcutaneously in four separate dose groups using a staggered dose escalation. Safety evaluations are obtained from each patient during a six-month follow up period. The blood samples are analyzed for beta cell function as well as for cellular and humoral markers of type 1 diabetes autoimmunity. GAD and IA-2 autoantibody levels as well as their epitopes, and isotopes were determined. Morphometric and functional analysis of T cells and B cells were performed including those of cytokine elaboration. The results from this study provide evidence for the safety of alum-formulated GAD65 and give novel information important to the design of clinical trials aimed at inducing immunological tolerance to GAD65[43].

Phase 3 clinical studies

Diamyd has been evaluated Phase3 studies in US, comprising more than 200 children and adolescents between the ages of 10 and 20 newly diagnosed with type1 diabetes. In May 2011, the results from the US study were announced, showing that the primary efficacy endpoint of preserving beta cell function, as measured by meal-stimulated C-peptide, was not met, although a small positive effect was observed. Patients treated with Diamyd® had on average 16.4 percent more remaining C-peptide at 15 months compared to those who received placebo. The treatment was well tolerated, as demonstrated by a similar number of adverse events

placebo group[52].
The New England Journal of Medicine 2011/2012 announced the results of phase 3 trials in nine European countries (Finland, Germany, Italy, UK, Netherlands, France, Sweden, Spain and Slovenia). This study was multicenter, randomized and double-blind trial performed at 63 clinics. 320 patients with recent-onset type 1 diabetes who were 10 to 20 years of age were screened between August 2008 and November 2009. Inclusion in the trial required detectable serum GAD65 autoantibodies, a fasting C-peptide level above 0.3 ng per milliliter (0.1 nmol per liter), and a duration of type 1 diabetes of less than 3 months. The study was proceed on 3 groups with subcutaneous injections of 20 μg of GAD-alum on days 1, 30, 90, and 270 (four-dose regimen) on first group; subcutaneous injections of GAD-alum on days 1 and 30 and of placebo on days 90 and 270 (two-dose regimen) on second group; and injections of placebo on days 1, 30, 90, and 270 on third group[48].

The primary outcome was the change in the stimulated serum C-peptide level (after a mixed meal tolerance test) between the baseline visit and the 15-month visit. Secondary outcomes included the glycated hemoglobin level, mean daily insulin dose, rate of hypoglycemia, fasting and maximum stimulated C-peptide levels. The stimulated C-peptide level declined to a similar degree in all study groups, and the primary outcome at 15 months did not differ significantly between the combined active-drug groups and the placebo group. The use of GAD-alum as compared with placebo did not affect the insulin dose, glycated hemoglobin level, or hypoglycemia rate. Adverse events were infrequent and mild in the three groups, with no significant differences. In conclusion the result from phase 3 study have been less encouraging in contrast to previous phase2 study and the reason for this is stated as it may be differences in populations or the larger numbers of clinicians with possibly different approaches to conventional treatment. The other is during the phase 3 study, an influenza epidemic occurred and resulted in widespread vaccination, which also may have influenced the results[53].

Future prospective of Diamyd vaccine
A new clinical study with the diabetes vaccine Diamyd® was started in February 2013. In the study Diamyd® will be tested in a unique combination with other drugs, aiming to potentiate the effect of the diabetes vaccine.

The Diamyd Medical AB Company has entered into an agreement with Linköping University to conduct the researcher-initiated study. The study has been approved by the Swedish Medical Products Agency.
The study, which is the first study of its kind, combines the diabetes vaccine Diamyd® with relatively high doses

of vitamin D and the anti-inflammatory drug ibuprofen. The purpose of the treatment is to preserve the body's own ability to control the blood sugar level in children and adolescents newly diagnosed with type 1 diabetes.

The aim of the combination is to create favorable conditions for the diabetes vaccine Diamyd® to take effect by temporarily dampen the inflammation in the pancreas, while vitamin D is believed to strengthen the part of the immune system that Diamyd® should stimulate. The study is called DIABGAD-1 and will also evaluate the effect of a double dose of Diamyd® and the protein GAD, which is the active substance in Diamyd®. The study was included 60 children and adolescents in Sweden and it will be conducted at pediatric diabetes clinics[54].

SUMMARY

Diabetes mellitus is a group of metabolic disorders of carbohydrate, fat, and protein metabolism that results from defects in insulin secretion, insulin action (sensitivity), or both. The two major classifications of DM are type 1 (insulin deficient) and type 2 (combined insulin resistance and relative deficiency in insulin secretion).

Goals of therapy in diabetes mellitus are directed toward attaining normoglycemia, reducing the onset and progression of retinopathy, nephropathy, and neuropathy complications, intensive therapy for associated cardiovascular risk factors, and improving quality and quantity of life. Type 1 treatment necessitates insulin therapy but treatment of type 2 DM often necessitates use of multiple therapeutic agents (combination therapy), including oral and/or injected anti-hyperglycemic and insulin to obtain glycemic goals.

Now a day's many researches are underway to develop therapeutic vaccines against diabetes mellitus. Oral delivery of auto-antigens can suppress immune activity by switching on suppressor cells of the immune system, producing immunological tolerance. Auto antigens given orally have been shown to induce tolerance in animal and human studies with promising results for type1 diabetes. On the other hand heat shock protein hsp60 shows a promising effect on developing vaccine for insulin-dependent diabetes mellitus. Therapeutic vaccination with hsp60 epitope p277 can arrest the spontaneous diabetogenic process both in NOD mice and in human associated with a Th1 to Th2 cytokine shift specific for the autoimmune T cells.

The other most interesting was Diamyd® vaccine, which originate from the protein GAD (glutamic acid decarboxylase) which is active substance for the prevention and treatment of autoimmune diabetes. Treatment with Diamyd is intended to prevent, delay, or stop the autoimmune attack on the beta cells. The aim is to prevent the onset of autoimmune diabetes, or to preserve the body's capacity to regulate blood sugar. Studies have shown that even a very small preservation of endogenous insulin secretion and slight improvement of the blood sugar control can significantly reduce the risk of both acute and long-term diabetes complications.

Table1.1 oral agents for glycemic control in patients with type2 diabetes[8,10]

Generic (Brand Name)	**Strength (mg)**	**Initial Dose (mg)**	**Max Daily Dose (mg)**	**Usual Daily Dose (mg)**
Biguanide				
Metformin (Glucophage)	500, 850, 1000	500 once or 850 daily	2550	1500-2000 mg divided (BID)
Metformin extended release (Glucophage XR)	500, 750	500 daily with evening meal	2000	1500-2000 daily or divided
Sulfonylureas				
Glimepiride (Amaryl)	1, 2, 4	1-2 daily	8	4 daily
Glipizide (Glucotrol)	5, 10	2.5, 5 daily	40	10 - 20 divided (BID)
Glipizide SR (Glucotrol XL)	2.5, 5, 10	5 daily	20	5 - 20 daily or divided (BID)
Glyburide (Diabeta, Micronase)	1.25, 2.5, 5	2.5-5 daily	20	5 - 20 daily or divided (BID)
Glyburide, micronized (Glynase)	1.5, 3, 4.5, 6	0.75-3 daily	12	3 - 12 daily or divided (BID)
Thiazolidinedione				
Pioglitazone (Actos)	15, 30, 45	15-30 daily	45	15 - 45 daily
Alpha-glucosidase inhibitor				
Acarbose (Precose)	25, 50, 100	25 daily with meal	300	50 - 100 TID before meals
Miglitol (Glyset)	25, 50, 100	25 daily with meal	300	25 - 100 TID
DPP 4 Inhibitors				
Sitagliptin (Januvia)	25, 50, 100	50-100 daily d	100	100 daily
Saxagliptin (Onglyza)	2.5, 5	2.5-5 daily d	5	2.5-5 daily
Meglitinide				
Repaglinide (prandin)	0.5, 1.2	0.5 with meal	16	0.5-4 QID

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