



Treatment of Type 1 Diabetes Mellitus by Increasing Human Leukocyte Antigen-G Expression with Polymeric Nanoparticles Using Gene Therapy

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Abstract

Type 1 Diabetes Mellitus in most cases is an autoimmune disease. Insulin injection is just a symptom therapy that is bothering for the patient and usually does not correct the blood glucose level appropriately. Attempts to replace the lost pancreatic cells such as islet and stem cell transplantation were not permanent cures because the original problem which was autoimmunity still existed. On the other hand, while using allogenic cells, immune system rejects the foreign cells. We suppose an approach to use the cells that are not affected by autoimmunity and can divide and replace the pancreatic β cells. Human Leukocyte Antigen-G (HLA-G) protein suppresses the immune system by affecting the T cells and natural killer cells and some other immune system cells and is responsible for keeping the fetus from maternal immune system in pregnancy. Autologous mesenchymal stem cells and insulin producing cells are candidate cells to be transfected with HLA-G gene. Transplantation of these genetically modified autologous stem cells to the patient leads to permanent production of β cells that are out of the reach of the immune system. As they are autologous cells, there is no fear of rejection. Nanoparticle based gene delivery is the desired procedure since there is no fear of tumor genesis with this method.

Keywords

Diabetes Mellitus, HLA-G, insulin producing cells (IPCs), Polymeric Nanoparticles, Gene therapy

Introduction

a. Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) is an autoimmune disease in which the immune system invades autologous pancreatic cells which produce insulin, a hormonal peptide necessary to regulate blood sugar. The routine therapy for T1DM is longlife insulin injection and usually causes complications for the patient. Pancreatic islet transplantation is an alternative therapeutic method but the main concern is the rejection by the host immune system (1). On the other hand, by autologous stem cell therapy, the problem of autoimmunity still remains. Recently, a unique human blood-derived cell population has been identified that displays high potential for producing insulin and can be used as a source of available autologous stem cell for pancreatic islet regeneration (2). These insulin producing cells (IPCs) are easily separated from the blood by culturing the mononuclear cells in petri dishes and are good candidates for gene therapy with HLA-G gene (2).

b. Human Leukocyte Antigen-G

Among various mechanisms protecting the embryo from maternal immune system attack, the role of major histocompatibility complex (MHC) molecules expression is the most important one (3). The majority of MHC molecules are absent on trophoblast cells that reduce the availability of fetal antigens to maternal lymphocytes; however, they increase the susceptibility of trophoblast cells to the natural killer (NK) cell mediated lysis. Expression of Human leukocyte antigen-G (HLA-G) on these cells inhibits NK cells attacking the fetus. HLA-G is a non-classic MHC molecule class 1b (3). Present knowledge of the properties of the HLA-G molecule showed that it acts through blocking the immune cells to maintain the functional and structural integrity of immune-privileged tissues such as cornea (4). Therefore it is normally expressed in some healthy adult tissues like cornea and thymus without any important complication. Some studies showed that accidental ectopic expression of HLA-G in transplanted organs resulted in less rejection rates in recipients (5). While expressed, HLA-G becomes the major inhibitory ligand for NK cells through interaction with killer inhibitory receptors (6). Other studies have shown that it can directly inhibit cytotoxic T cell mediated cell lysis (7) and CD4+ T cell-mediated immune reaction (8). HLA-G modulates the immune function by interacting with inhibitory leukocyte immunoglobulin like receptors (LIRs) 1 and 2 as well as some natural killer cell receptors. LIRs 1 and 2 are expressed by monocytes, macrophages, and cytotoxic T lymphocytes (8). HLA-G can be expressed under four membrane-bound isoforms (HLA-G1, -G2, -G3, and -G4) and three soluble ones (HLA-G5, -G6, and -G7) that are generated by alternative splicing of a

primary transcript (9). It was shown that some patients who exhibit better graft acceptance expressed ectopic HLA-G (10).

Hypothesis

The hypothesis is to modify the autologous IPCs to escape from autoimmunity and use them to cure T1DM. First of all, IPCs should be separated from the patient's blood by culturing mononuclear cells in petri dishes. These cells then undergo transfection with HLA-G primary transcript in which the promoter of beta- microglobulin is inserted at the 5' end to ensure the high expression rate. There are different methods to deliver a gene to cells like viral vectors but there is usually a fear of tumorigenesis. Nowadays, using polymeric nanoparticles to deliver the desired gene has shown many advantages including low toxicity, biocompatibility, biodegradability, and not being tumorigenic. By using homologous recombination we can be sure that the desired gene has been integrated into the genome. After delivering the desired gene, the cells are again injected to the patient's blood. The cells will reproduce the pancreatic β cells and insulin will be released properly. These cells are not accessible to the immune system and will be as a pool of stem cell in the whole life to reproduce β cells. The whole process is shown in figure 1.

In the field of gene therapy in T1DM, other ideas such as insulin gene transfer into non-IPC cells are also promising. But one problem that still remains is the uncontrolled release of insulin from these cells in response to glucose plasma level. But IPC cells are under the control of normal mechanisms of the insulin secretion regulation.

There are other therapeutic methods in T1DM such as encapsulation of islets before transplant, but in spite of promising results in animal studies, a clinical product or therapeutic procedure based on encapsulated cells does not yet exist. This is because a number of barriers remain to be addressed, which include a source of functional cells, a stable, biocompatible membrane offering immune protection to the implant, a construct architecture ensuring cell viability and construct function, and the engineering of immune acceptance of the construct post-implantation (11). It seems that by using HLA-G transfected IPC cells these barriers do not exist.

Evaluation of hypothesis

a. Non-viral gene delivery systems

Non-viral Gene delivery vectors based on cationic polymers consist of several amine groups in their structure and have been used extensively as a gene carrier (12). They can provide unlimited DNA packaging capacity and well defined physicochemical properties. Ideal polymeric vectors should form a

stable polyplex with DNA to keep its stability in a biological media, hide from the host surveillance systems, and deliver the therapeutic nucleic acid to the target cell. Nowadays, chitosan have become one of the non-viral delivery systems that have gained increasing interest as a safe delivery system for gene delivery.

b. Chitosan properties as a gene delivery vector

Chitosan is a natural polymer (i.e. a co polymer of D-glucosamine and N-acetyl glucosamine) obtained by deacetylation of chitin (Fig.2). Chitin is the second most abundant polysaccharide in nature after cellulose. The main commercial sources of chitin are the shell wastes of shrimp, crab, and lobster. It is a biologically safe, non-toxic, biocompatible, and biodegradable polysaccharide. Chitosan nanoparticles have gained more attention as gene delivery carriers because of their better stability, low toxicity, and simple and mild preparation method. (13,14). Because of its positive charge, it can easily form polyelectrolyte complexes with negatively charged plasmids through electrostatic interaction.

c. Nanoparticles/DNA complex

The efficacy of chitosan as a gene delivery vector is based on its cationic properties. At acidic pH, below the pKa, the primary amines in the chitosan structure change to positively charged. These protonated amines help chitosan to bind to negatively charged DNA via an electrostatic interaction. This Interaction leads to the spontaneous formation of nano-size complexes (polyplexes) in the aqueous medium (15).

d. Nanoparticles mediated HLA-G cDNA delivery

Nanoparticles containing plasmid cDNA are formulated, by using ionic gelation technique. Pharmaceutical properties of Nanoparticles-cDNA complex such as size, morphology, zeta potential and encapsulation efficiency can be studied (16).

Measurement of mean diameter (size) and morphology observation of nanoparticles: Light scattering method is used to measure the mean diameter and polydispersity of nanoparticles. The morphological characteristics of the nanoparticles are examined using a high resolution Transmission Electron Microscope (TEM) machine (17).

Measurement of Zeta potential of nanoparticles: The zeta potential of nanoparticles is commonly used to characterize the surface charge property of nanoparticles. It reflects the electrical potential of the particles and is influenced by their composition and the medium in which it is dispersed. Measurement of zeta potential of the nanoparticles is performed using Zetasizer (17, 18).

e. Introducing nanoparticles containing cDNA to IPCs in vitro

Determination of HLA-G encapsulation efficiency of nanoparticles: Encapsulation efficiency and loading capacity of nanoparticles with different formulations are determined by ultra-centrifugation of the samples. The amount of free cDNA in clear supernatant is determined by UV spectrophotometry at 280 nm (19).

In vitro cell transfection: One day before the transfection, the IPCs are transferred to 6-well plates. As soon as the cell confluency reached 80%, cells are transfected by the polymeric nanoparticles containing HLA-G cDNA. Then, the cell culture plates are incubated for 24 h, 48 h, 72 h, and one week (20) and then the results are analyzed.

Determination of transfection efficiency by flow cytometry: To determine the transfection efficiency of polymeric nanoparticles containing HLA-G cDNA, flow cytometry quantitative measurement is used. The transfection efficiency is determined as the percentage of the transfected cells divided by total cells counted (20).

f. Other methods of induction of HLA-G expression

Eslami MB and his coworkers introduced a method to induce the HLA-G expression which is treatment of target cells in vitro such as dendritic cells by interferon beta [21].

g. In vitro study of the transfected cells

Modified IPCs can be evaluated by some in vitro studies. Static insulin secretion from the modified IPCs can be measured after exposing the cultured cells to different glucose levels. On the other hand to evaluate the inhibitory effect of HLA-G on the immune system we can co-culture the modified IPCs and peripheral blood T cells from the same person.

h. Injection of modified IPCs into the animal models and final analysis

The transfected cells must be treated with Bromodeoxyuridine (BrdU) for further detection. Two groups of type one diabetic animals can be chosen as case and control groups. Then the treated cells will be injected into the portal vein of animals in case group. On the other hand, the solvent buffer of stem cells can be injected into the portal vein of animals in control group. In addition, other routes of transplantation of the cells like intraperitoneal injection are applicable. Blood glucose and glucose tolerance test and insulin level must be determined before and after transplantation to evaluate immunotolerance and viability and functionality of the transplanted cells. Biopsies can be taken and stained by immunohistochemistry to detect the injected cells. Finally the results will be analyzed to find if there is any significant difference between case and control groups or not.

i. Autoimmune diabetes animal models and measuring the autoimmune response

Two well-established animal models have been used for autoimmune diabetes: the Biobreeding (BB) rat and the nonobese diabetic (NOD) mouse strains. In NOD mice model for evaluating the autoimmune response, T cell lymphocyte function suppression resulted in delayed Diabetes induction. Anti-islet-cell antibodies also exist in NOD mice during the active inflammatory stage of the disease. Invasive lymphocytic infiltrates associated with the islets of langerhans (insulinitis) which is the hallmark of the disease progression in NOD mouse, also exist in human insulin dependent diabetes mellitus (22). The autoimmunity response can be evaluated by taking blood samples every 1-2 weeks from animal's tail vein and detecting autoantibodies using available kits. Also as autoimmunity causes inflammatory stress especially in pancreas, biomarkers of inflammation such as TNF- α and IL-1 β and oxidative stress mediators like myeloperoxidase activity, lipid peroxidation, carbonyl and thiol content of pancreatic tissue can be evaluated (23).

Conclusion and discussion

In T1DM the immune system invades autologous pancreatic cells which results in insulin insufficiency. T1DM has many short time and long time

complications. This type of diabetes like other autoimmune diseases can be only controlled and cannot be permanently cured and also most of the complications are not preventable. One of the different ways that have been suggested for amelioration of T1DM, is transplantation of allogeneic stem cells, but the rate of rejection by this method is high. Transplantation of autologous stem cells is also suggested but the main problem, autoimmunity, still remains. To solve this problem we found a way to inhibit the immune system not to attack the autologous stem cells which is a natural mechanism in the body. Among various mechanisms protecting the embryo from maternal immune system attack, the role of MHC molecules expression, like HLA-G, is the most important mechanism (3). Expression of HLA-G, a non-classic major histocompatibility complex molecule class 1b, inhibits NK cells (3) cytotoxic T cell mediated cell lysis (7) and CD4+ T cell-mediated immune reaction (8). We suggest using polymeric nanoparticles to deliver the HLA-G gene into autologous IPCs and then injecting these cells to the patient. This method can introduce a profound effect on the approach to the autoimmune diseases such as rheumatoid arthritis and T1DM since most of the existing therapies are not disease modifier.

Overview Box

What do we already know about the subject?

Like other autoimmune diseases, autoimmune diabetes can be only controlled and cannot be permanently cured. Transplantation of the autologous stem cells for these patients might be a promising way but the main problem, autoimmunity, still remains. On the other hand transplanted allogeneic stem cells may be rejected.

What does your proposed theory add to the current knowledge available, and what benefits does it have?

Our hypothesis finds a new way in which the autologous stem cells are modified by the gene therapy so that the host immune system cannot invade the autologous cells. This can be achieved by introducing HLA-G gene into the autologous stem cells in vitro and injection of modified cells into the body. This disease modifying method also can be used for other autoimmune diseases.

Among numerous available studies, what special further study do you propose for testing the idea?

A clinical trial is needed to evaluate the idea but feasibility of this method should be primarily evaluated in animal models. It must be clarified if the modified cells are effective enough to maintain the blood glucose level in a limited range and if there is any side effects or not.

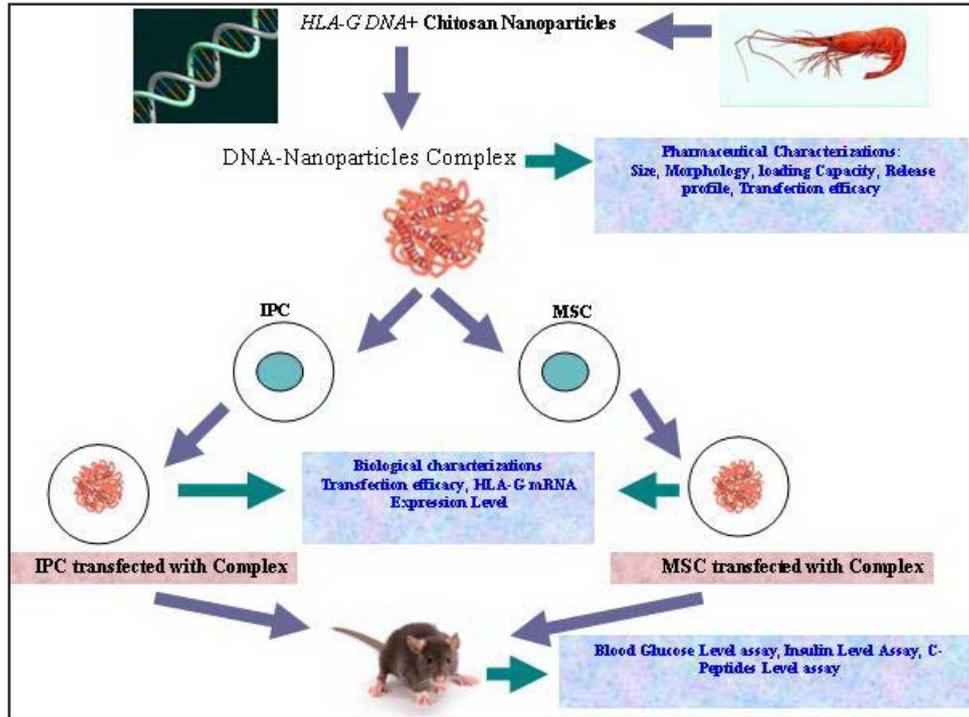


Figure 1. Evaluation of the hypothesis can be done at points that are shown in this figure.

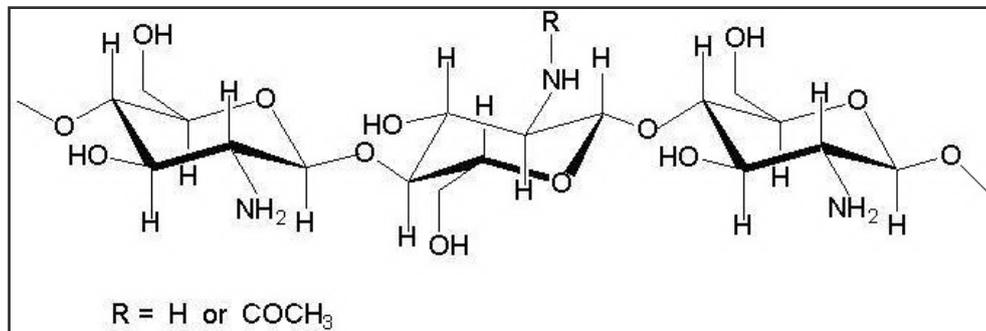


Figure 2. Chemical structure of chitosan.

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