IPS cells from type 1 diabetes mellitus for disease modeling and therapy.

-A review of the possibilities and limitations-

Master Thesis
Author: Ivan Stojanovic  s0189472  Date: November 2009
Master program:  Master of Science (Health Science)
University of Twente, Enschede, the Netherlands

Supervisors:
Prof. dr. Wiebe Kruijer
(Molecular Cell Biology, Faculty of Science and Technology, University of Twente)

Dr. Janine Post
(Molecular Cell Biology, Faculty of Science and Technology, University of Twente)

Dr. Carine Doggen
(Health Technology and Services Research, Faculty of Management and Governance, University of Twente)
Preface

Before you lies my masterthesis that I have written in order complete my Master of Science (Health Science) study at the University of Twente. In this preface I would like to give my thanks to some people that have helped me to bring this thesis to completion.

Over the past few months that I have spent making this thesis I have learned a great deal about diabetes mellitus type 1 and the promising innovation that are iPS cells. This is not only because I have read a great number of scientific papers regarding these topics but also largely because of the possibility that was given to me to attend informative and entertaining meetings of the Molecular Cell Biology workgroup at the Faculty of Science and Technology of the University of Twente. I would hereby like to thank prof. dr. Wiebe Kruijer and dr. Janine Post and the rest of their colleagues of the Molecular Cell Biology workgroup for letting me attend their meetings and enabling me to complete my masters degree with a project related to their ongoing research in the field of iPS cells. I would also like to thank prof. dr. Kruijer in particular for his extensive advise, insight and revision suggestions that he managed to provide to make this thesis as accurate as possible despite his busy schedule.

I would also like to thank dr. Carine Doggen of the Health Technology and Services Research workgroup at the Faculty of Management and Governance of the University of Twente for her advise, expertise and support she has provided me with during my project. In addition she has informed me of relevant additional information or events that could have provided me with useful insights. Her fresh perspective on this topic helped me to set the scope for the paper.

Lastly I would like to thank all of the people involved with the Health Science curriculum at the Faculty of Management and Governance of the University of Twente for the the provided services and education that I received during the time of my Master study there. Thanks to the wide scope of the Health Science curriculum (and my previous knowledge due to my Bachelor's degree in Biology and Medical Research) I was able to complete this master thesis.

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Introduction

Type 1 diabetes mellitus (IDDM) is caused by an autoimmune response inside the patient's body. However, the actual disease mechanisms and progression of the disease are poorly understood. This study will try to explore these vague aspects of IDDM by reviewing recent articles that focus on IDDM and the progression of it. Furthermore, this study will attempt to indicate how big of a problem type 1 diabetes mellitus actually is becoming and that there is a need for a permanent cure, which currently is not available.

Stem cells (like embryonic stem cells) might provide an answer in the search for the cure to IDDM. However, embryonic stem (ES) cells have not been able to become common practice in the field of regenerative medicine and the treatment of IDDM due to problems surrounding them. These problems include ethical issues regarding their controversial origin and medical application limitations due to the need of immunosuppression to prevent ES derived graft rejection.

The recent breakthrough discovery of induced pluripotent stem (iPS) cells has shown promising possibilities for regenerative medicine and disease modeling, possibly eliminating some of the problems surrounding ES cells. However, there are still a lot of questions surrounding these new iPS cells and the technology used to create them. This paper will review the current technological state of iPS cells and their future development, as well as their advantages and disadvantages. In addition, possible applications for iPS cells (in relation to IDDM) for both therapeutic as well as disease modeling purposes will be reviewed and proposed.
IPS cells from diabetes mellitus type 1 for disease modeling and therapy – a review of the possibilities

Summary

Type 1 diabetes mellitus (IDDM) is becoming an increasing problem. IDDM patients have a lacking or entirely non-operative insulin production. In IDDM patients the pancreatic beta cells (usually responsible for insulin production) have been destroyed by an autoimmune response. The actual mechanisms behind this autoimmune response are currently poorly understood. This paper has reviewed recent research that focussed on explaining the mechanisms behind autoimmune responses and progression towards IDDM.

Genetic susceptibility to IDDM was also included, reviewing a number of the best documented associated genetic loci, showing that IDDM is a polygenic disease. As IDDM is not only explicable through genetic susceptibility, this paper also focussed on serum metabolomics of IDDM patients. The metabolomics give an insight into the processes and metabolic changes within the human body preceding autoimmunity and show that metabolic changes can give early indications of progression towards clinical IDDM.

Currently there is no generally accepted cure for IDDM. Insulin injections are the most common therapy, however this does not restore patient insulin production. Insulin injections are more reminiscent of disease management instead of an actual cure. Treatment options for IDDM patients were compared to possible future treatment developments. Beta cell producing systems, based on embryonic stem cells and induced pluripotent stem cells (ES and iPS cells), can replenish insulin production in IDDM patients. IPS cells have great potential due to their similarities to ES cells and their patient and disease specificity. Currently however iPS cells are not used therapeutically due mainly to limitations inherent to their production which is based on forced expression of transformation factors traditionally using viral vectors. Through this forced expression human somatic cells can be reprogrammed into a pluripotent state after which they can be differentiated into any desired cell type. In this paper, future developments of iPS cell production and application (in relation to IDDM) were reviewed. These developments include therapeutic application and the usage of iPS cells for the generation of highly accurate IDDM disease models.
Diabetes mellitus type 1: an introduction

Diabetes mellitus type 1 (also known as “juvenile onset diabetes mellitus” and “insulin dependent diabetes mellitus, IDDM”) is a specific form of diabetes mellitus. The disease comes in several forms, the 2 main primary forms of diabetes mellitus are: type 1 insulin dependent diabetes mellitus and type 2 insulin independent diabetes mellitus. Additionally there are secondary forms of the disease like gestational diabetes mellitus and forms that are related to other medical disorders. Patients with diabetes mellitus when they are untreated show an abnormally high blood glucose level, a condition called hyperglycemia. This hyperglycemia is caused by a metabolism disorder, which disrupts the blood sugar balance in the body causing it to rise. Insulin is one of the main hormones that is involved in the regulation of blood glucose levels. It makes liver and muscle cells take up glucose from the blood after which it is stored as glycogen in liver and muscle tissue. In diabetes mellitus patients, this system is disrupted. According to the World Health Organization, approximately 171 million people worldwide suffer from diabetes. That number will only increase with the estimate being 366 million patients by the year 2030[1]. Diabetes as a whole is a steadily growing issue as is seen by the figures given. Diabetes mellitus type 1 on its own is also becoming more of an alarming situation. In Europe, Finland has one of the highest incidence rates and some studies are calling it an “accelerating epidemic”[2] . Though incidence rates vary from country to country, it is worth noting that over the past few years the incidence rates have grown the most in countries with a historically low incidence rate. This means that countries that originally did not have a high prevalence of diabetes mellitus type 1 over time in relative terms will move more closer to countries that did have high prevalence numbers and that there will be less of a difference in terms of the amount of patients in the various countries.

Patterson et al., describe in their progressive registration study that the estimated number of new cases of diabetes mellitus type 1 in the year 2005 in children under the age of 15 in Europe was 15,000. For 2020 their study shows a prediction of 24,400 annual new cases and that the prevalence in children under the age of 15 will rise by 70% if the current trends continue[3].

As described earlier there are 2 primary diabetes mellitus forms. In type 1 the insulin production is severely limited. In type 2 the patients have an insulin resistance. Insulin is produced but it is not able to function. Both of the forms express the classical diabetes mellitus phenotype: polydipsia (excessive thirst), polyphagia (excessive eating) and polyuria (excessive urination) all as a consequence of hyperglycemia. Diabetes mellitus type 1 (IDDM) differs from type 2 diabetes mellitus in that it is caused by an autoimmune response that targets and destroys the insulin-producing beta-cells of the islets of Langerhans in the pancreas.

IDDM symptoms clinically manifest themselves long after the actual autoimmune process has started to attack the beta-cells (sometimes manifestation occurs after several years). Destruction of beta cells must be high (over 90% destruction) in order for clinical manifestation of the disease. What the actual reason is behind the autoimmune attack on beta-cells is not fully understood [4]. There are various theories that discuss possible causes for the autoimmune attack on beta cells. A possible cause could be a virally triggered autoimmune response that attacks beta cells in the pancreas after the immune system responds to viral infection. However there are also theories that suggest that there are genetic factors that play a role in this destructive autoimmune response. This genetic susceptibility seems to be of a polygenic nature, meaning that there are several genes connected to IDDM expression. Recent studies have shown that monozygotic twins have a concordance rate of 30% - 50%, indicating that when one twin has the disease the other is not affected despite their identical genome [5]. This outcome suggests that environmental factors also play a large part in IDDM prevalence.
Autoimmune response and its mechanisms

The immune system
Immune responses can protect an individual from harmful external factors like microorganisms. The reaction can be overly strong however, in which case the response can become harmful to the host. The immune response is mediated by T and B cell lymphocytes. These cells have divergent antigen recognition, high antigen specificity, potent effector activity and a long lasting immunological memory. T cells mature in the thymus whereas the B cells are generated by bone marrow. Unregulated immune responses (like allergic reactions or autoimmune responses) however can become very harmful for the host[6]. It is therefore vital to have knowledge about the mechanisms within the immune system that maintain unresponsiveness to self-antigens and regulate the quality and magnitude of the immune response to non-self-antigens.

Autoimmunity
Immune self-tolerance and homeostasis are achieved through 2 types of mechanisms: cell-intrinsic “recessive” and cell-extrinsic “dominant”. In the cell-intrinsic mechanism some lymphocytes will undergo cell apoptosis when they are exposed to self-antigens while at an immature state in their generative organs. Others may undergo receptor editing, in which self-reactive T or B cell receptors are replaced by non-reactive counterparts. The lymphocytes that have evaded premature apoptosis and receptor editing may mature further. The activation threshold of these lymphocytes for cellular inactivation may be further raised by the expression of inhibitory receptors or negative signaling molecules. These cells may also not survive long due to activation induced cell death[6].

The cell-extrinsic mechanism is based on the concept that a subpopulation of T cells regulate the activation and expansion of over-active T cell lymphocytes. These regulatory T cells (Treg) are specialized in immune suppression. In every immune response not only effector T and B cells are recruited and activated but also Treg cells are dispatched. In order for immune homeostasis and self-tolerance to be achieved and maintained, a fine balance between Treg cells and other lymphocytes is crucial. Disruption of Treg function or abnormal development of these cells is the primary cause of autoimmune diseases[6].

Treg cells and regulation of the immune response
Previous studies have shown that adult and neonatal thymectomy in normal mice and rats causes autoimmune damaging of various organs. In addition tissue specific auto-antibodies appear in the blood circulation[7]. This is attributable to the fact that due to the absence of the thyroid no more Treg cells are produced. Thymectomy in adult rats also causes type 1 diabetes mellitus. This indicates that also type 1 diabetes seems to be caused by an autoimmune response due to a lack of Treg cells. When the rats were injected with T cells from normal animals (specifically T cells of the subpopulation CD4+ or CD4+CD8−) the autoimmunity development was inhibited[8, 9].

Sakaguchi et al., proposed a more specific marker that would be able to identify the specific CD4+ T cell subpopulation that “prevents” autoimmunity. Their research has shown this marker to be CD25+ (or more accurately the CD25+CD4+ T cell subpopulation)[10]. Removal or depletion of Tregs not only causes autoimmune responses but also augments immune responses to other antigens (non self-antigens). It increases the host immunity to micro-organisms leading to inflammatory bowel disease due to a non-regulated immune response aimed at the (commensal) bacterial bowel flora. It also provides host immunity to tumors. On the other hand a surplus of Treg cells suppresses allergic reactions and establishes a higher tolerance for organ grafts[11-14].
The most reliable marker for natural Tregs however currently is Foxp3 (forkhead box p3) [6]. Foxp3 is member of the forkhead transcription factors. It serves as a master regulator of Treg development and function. Wildin et al., reported in 2001 that they have found a mutation in the human FOXP3 gene in IPEX patients. IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome) is a rare autoimmune disease which has its onset at an early age causing type 1 diabetes mellitus, infections, food allergies and enlargement of secondary lymphoid organs (amongst other symptoms)[15]. Research has shown that Treg cells express Foxp3 (Foxp3+ Tregs), where as other T cells do not[16, 17]. Foxp3+ T cells begin to appear shortly after birth and depletion of these cells causes autoimmunity and inflammatory diseases[18, 19].

The CD25 marker of Tregs is part of the interleukin 2 receptor (IL-2R). IL-2 is a cytokine hormone that plays a role in the differentiation and proliferation of T cells. However, IL-2 is also involved in activation, expansion and maintenance of natural Treg cells[6]. This is further proven by numerous studies that have been performed on this subject. Experiments in mice that lack CD25 or IL-2 show that the number of Foxp3+ Tregs is lower than in normal mice[20]. Also neutralization of IL-2 by anti IL-2 monoclonal antibodies causes autoimmune symptoms as seen in Treg deficiency[21]. Since IL-2 is produced mainly by activated non-Treg cells, a type of feedback loop exists between the non-Treg and Treg cells. IL-2 secreted by activated non-Treg cells is bound by IL-2R on the Treg cells. After binding Treg cells then start to suppress the non-Treg cells and their immune response. If this feedback loop is disrupted in some way (i.e. due to a lack of CD-25 or otherwise a defective IL-2R receptor) autoimmune disease can develop[6].

Treg-mediated immunological suppression has been the subject of extensive research which has brought forward several proposals as to how this suppression is actually achieved. Sakaguchi et al., have suggested a model of Treg-mediated suppression in which antigen-specific Tregs (upon antigenic stimulation) are quickly recruited to the antigen presenting cell (APC) through migration under the influence of chemokines. Here they outcompete antigen-specific naive T cells by aggregating around the APC. Tregs which are antigen activated and are in contact with the APC downmodulate the APC function, which is dependent of CTLA-4 expression on Tregs. Further differentiation of Tregs may then follow, after which they secrete granzyme/perforin and/or other immunosuppressive cytokines (IL-10 and IL-35), this will inactivate or kill responder T cells around the APC[6]. Seeing how activation of naive T cells is a relatively long process that takes several hours of contact between an APC and a naive T cell, this quick interception of the APC by the Treg cells is sufficient to abort the activation of responder T cells, resulting in a suppression of the immune response.

Genetic polymorphisms regulating Treg function

As indicated above, the Treg cell-surface molecule CTLA-4 (cytotoxic T cell associated antigen 4) is an important determinant in immunosuppression. CTLA-4 expression is directly controlled by Foxp3 and CTLA-4 dysfunction or deficiency evokes autoimmune disease underscoring Foxp3 as a functional marker for Treg cells[6, 22-24]. Genetic susceptibility to autoimmune diseases (such as type 1 diabetes mellitus) is significantly increased by polymorphisms in various other Treg expressed genes including: Cd25, Ctna4, Il2 and Ptpn22. These observations demonstrate that genetic polymorphisms have an impact on Treg development and function[6, 25]. Since complete deficiency of the Cd25, Ctna4 and Il2 genes causes autoimmunity, (combinations of) more subtile changes of these genes may have a similar effect.

Additionally, environmental factors possibly also affect Tregs negatively. Due to their higher metabolic activity and greater proliferation compared to non Tregs, regulatory T cells are more sensitive to ionizing radiation, radiomimetic drugs and vitamine deficiency[6, 21, 26-28]. Currently little is known about how environmental factors exactly influence and potentially alter Treg function. Uncovering the underlying mechanisms seems of prime importance in order to understand the population wide onset of type 1 diabetes and autoimmune disease.
Insulin production

In order to understand the disease mechanism involved in the autoimmune response of IDDM patients, the function of the pancreatic beta cells including the regulation of insulin production and release need to be understood.

Insulin release by beta cells of the pancreas

Insulin production is confined to the beta cells of the pancreas (islets of Langerhans). In IDDM patients, insulin production is virtually non-existant due to a so-called single-cell disorder in which these cells are destroyed by an autoimmune response. Beta cells encompass a complex stimulus response system which, when functioning properly, ensures plasma glucose levels to stay within a narrow range of 5 – 8 mM/L. This system responds to several external cues of a nutrient and non-nutrient nature. It is crucially important that the beta cell insulin response is both rapid and in proportion to what is needed according to the glucose level in an individual's body. Too little insulin leads to hyperglycemia which is a potentially lethal condition. However too much insulin causes hypoglycemia which is equally dangerous. This is why therapies to replace the insulin producing beta cells are not common practice yet. It is one thing to replace the function of the beta cells by inducing non-beta cells to produce insulin, which is easily achievable through techniques like gene therapy. However, to actually create a balanced insulin response system that is needed to effectively treat patients capable of maintaining a proper glucose level has proven to be a formidable task.

In order for glucose stimulated insulin release to occur in the beta cell, glucose first needs to enter the cell. This is achieved by the transport of glucose by the GLUT 2 transporter. Enzymes then metabolise glucose into ATP (adenosine triphosphate). ATP-sensitive potassium channels respond to intracellular ATP levels causing the potassium channels to close. This results in plasma membrane depolarization and opening of calcium channels. As a result, calcium ions (Ca^{2+}) enter the beta cell triggering the activation of calcium-sensitive downstream signalling pathways. This initiates the secretion of insulin. Additionally, insulin secretion is also stimulated by specific ligands including acetylcholine, cholecystokinin, glucagon and glucose-dependent insulinotropic peptide. These ligand-receptor interactions activate intracellular signal transduction resulting in the production of second messengers that raise intracellular Ca^{2+}. This similarly activates the pathways that result in the release of insulin[29].
In order for cell replacement to be successful, implanted cells will need to have a similar stimulus response system like healthy beta-cells have for the sake of appropriate insulin secretion.
Genetic susceptibility to IDDM and genetic factors

In the past 30 years several loci have shown to be associated with IDDM. The oldest and best documented ones are the HLA class II genes in the major histocompatibility complex (MHC) located on chromosome 6p21. Five other loci have been identified over the years. These include: the CTLA4 gene located on chromosome 2q33, the interferon-induced helicase IFIH1 region located on chromosome 2q24, the interleukin-2 receptor alpha chain IL2RA (CD25) located on chromosome 10p15, the gene coding for insulin INS located on chromosome 11p15 and the PTPN22 gene located on chromosome 1p13[30].

The table summarizes the various loci found to be associated with IDDM in the studies mentioned above.

<table>
<thead>
<tr>
<th>Gene name (or gene closest located to the locus)</th>
<th>Gene map location</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC HLA class II</td>
<td>6p21</td>
</tr>
<tr>
<td>BACH2</td>
<td>6q15</td>
</tr>
<tr>
<td>CD226</td>
<td>18q22</td>
</tr>
<tr>
<td>CTLA4</td>
<td>2q33</td>
</tr>
<tr>
<td>CTSH</td>
<td>15q24</td>
</tr>
<tr>
<td>C12orf30</td>
<td>12q24</td>
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<tr>
<td>ERBB3</td>
<td>12q13</td>
</tr>
<tr>
<td>IFIH1</td>
<td>2q24</td>
</tr>
<tr>
<td>IL2RA (CD25)</td>
<td>10p15</td>
</tr>
<tr>
<td>INS</td>
<td>11p15</td>
</tr>
<tr>
<td>ITPR3</td>
<td>6p21</td>
</tr>
<tr>
<td>KIAA0350</td>
<td>16p13</td>
</tr>
<tr>
<td>PTPN2</td>
<td>18p11</td>
</tr>
<tr>
<td>PTPN22</td>
<td>1p13</td>
</tr>
<tr>
<td>SH2B3</td>
<td>12q24</td>
</tr>
<tr>
<td>TAGAP</td>
<td>6q25</td>
</tr>
<tr>
<td>Between C1QTNF6 and SSTR3</td>
<td>22q13</td>
</tr>
<tr>
<td>Near PRKCG</td>
<td>10p15</td>
</tr>
<tr>
<td>Near various genes (ADAD1, IL2, IL21 and KIAA1109)</td>
<td>4q27</td>
</tr>
<tr>
<td>Near various genes (CD69, CLEC2D, CLECL1, KLRB1, LOC374443 and OVOS)</td>
<td>12p13</td>
</tr>
</tbody>
</table>

*Table 1: Various genes associated with IDDM susceptibility.*
Allelic variation
As this section focuses on the genetics of IDDM, it is important to know the meaning behind some of the genetic terms that are used in this section.
Due to DNA research we now know that genes have various forms, these various forms are called alleles. So-called diploid organisms have their genes split into 2 alleles, one inherited from each parent respectively. If a person is heterozygous for a gene it means that the person has 2 different alleles for that particular gene. If a person is homozygous, it means that the person has 2 identical alleles for the gene. In the example below a Punnett square is illustrated, showing a monohybrid cross of 2 individuals. In this example there is an individual with genotype “Aa” that has brown eyes and an individual with genotype “aa” that has blue eyes.

Figure 2: Punnett square showing a monohybrid crossing of two individuals.

In this example the “A” allele is dominant over the “a” allele. This means that if these 2 alleles are paired, the dominant “A” allele will express itself over the recessive “a” allele. This is also relevant to IDDM, as there are various genes involved in IDDM susceptibility and they appear in various forms of zygosity.
Genetic variation of the (HLA)-DQ-beta chain
IDDM in children from the United States is 20 times more frequent in occurrence than children from China[31]. Similarly incidence of the disease in Korea is only 10% of that in the United States. According to a study by Bao et al. this difference is due to an allele that leads to aspartic acid on position 57 (asp57 or A) in the human leukocyte antigen (HLA)-DQ-beta chain. People that have this charged amino acid “A” are “protected” against IDDM, while individuals with “NA” (non charged amino acid) are more susceptible to IDDM. Bao et al. analyzed probands of the IDDM registries in Allegheny County, Pennsylvania, USA. Of them none was homozygous A, while 96% were homozygous NA and 4% were heterozygous NA. In a group of 18 Chinese IDDM patients 1 was homozygous NA and 13 were heterozygous. In a group of 25 healthy Chinese people used as a control 23 people were homozygous A. This is indicative of a connection between NA and IDDM. Furthermore, the low number of IDDM patients in China is in concordance with high number of homozygous A people in the Chinese population. Racial origin therefore seems to play a role in IDDM prevalence.
Thomson et al., however have shown that the presence or absence of asp57 in the DQ-beta chain is in itself not enough to explain inheritance of IDDM[32].

In another study, Baisch et al., examined 266 unrelated white IDDM patients. Their main findings were that the HLA-DQw1.2 allele was dominantly protective, as it was found in 2.3% of the IDDM patients and in 36.4% of the controls. Furthermore, they found that HLA-DQw8 had the effect of dominant susceptibility. People in the study that were HLA-DQw1.2/DQw8 however had a relative risk of only 0.37. This showed that HLA-DQw1.2 and its protective effect predominated over the effect of HLA-DQw8[33].

As mentioned earlier the incidence rate of IDDM in Korea is about 10% of that in the USA. Park et al. suggested that ethnic differences in allelic associations with IDDM might be explained by different linkage disequilibrium patterns of DR and DQ genes. Linkage disequilibrium is the association of alleles in a non-random way at 2 or more loci which are not necessarily located on the same chromosome. The research confirmed that the susceptibility effects of HLA DRB1 – DQB1 haplotypes were consistent in Koreans and Caucasian populations. Their research indicates consistency of HLA haplotype transmission between populations with different diabetes incidence rates. They conclude that the HLA haplotypes influence for determining susceptibility to IDDM may be universal[34].

The ITPR3 locus
Roach et al., confirmed prior studies showing association with IDDM and the MHC HLA-DR/DQ chains by performing genetic mapping on a Swedish population group. In addition, they identified another association in the region centromeric to the MHC. The association was found within the inositol 1,4,5-triphosphate receptor 3 gene (ITPR3) located on chromosome 6p21. According to Roach et al., ITPR3 can be considered as an excellent candidate gene for association with IDDM.

The ITPR3 receptor protein releases Ca\textsuperscript{2+} from intracellular stores thereby mediating second messenger signaling. The ITPR3 protein and ITPR3 mRNA are upregulated rapidly in beta cells of the pancreas after stimulation with glucose followed by protein degradation in proteasomes. Roach et al., remain cautious however in interpreting the results as evidence of causality[35].

Genome wide IDDM linkage association
The Wellcome Trust Case Control Consortium (WTCCC) performed a genome wide association scan on 7 diseases, which included IDDM. The scan showed significant association with 6 chromosome regions: 4q27, the ERBB3 gene on chromosome 12q13, the C12orf30 gene on chromosome 12q24, 12p13, the KIAA0350 gene on chromosome 16p13, and the PTPN2 gene on chromosome 18p11 [36]. Todd et al., has confirmed the following associations from the WTCCC study: 12q13, 12q24, 16p13 and 18p11. In addition they also found consistent statistical association with the T lymphocyte co-stimulation gene CD226 on chromosome 18q22[30].
In order to identify additional new genetic factors involved in increasing the risk of susceptibility for IDDM, Hakonarson et al. have performed a genome-wide association study in a pediatric cohort of European origin. A new association was identified in addition to previously known genetic associations. The newly identified association was found within a 233-kb linkage disequilibrium block on chromosome 16p13. KIAA0350 is contained within that area and its product is predicted to be a sugar-binding C-lectin. They have found 3 common non-coding variants of the gene which showed significance for association. This result shows that it is useful to take a genome-wide approach in order to identify previously unsuspected genetic determinants that are possibly associated with IDDM.[37]

In a later study Hakonarson et al. again identified a new IDDM associated locus on chromosome 12q13. Both of the newly found loci were also found in the independent study by the WTCCC[38].

Both celiac disease and IDDM are associated with HLA class II genes which suggests a common genetic origin. Smyth et al., tested for possible non-HLA loci sharing. A significant association to IDDM was found in the TAGAP gene on chromosome 6q25. They additionally confirmed the association of SH2B3 on chromosome 12q24 [39]. This shows that common biologic mechanisms may be a shared cause of the diseases.

In a meta-analysis of 3 genomewide association studies Cooper et al., confirmed a previously known locus on chromosome 4q27. Additionally, they identified 4 other previously unknown loci to be associated: a locus in intron 3 of the BACH2 gene located on chromosome 6q15, a locus near the PRKCO gene located on chromosome 10p15, a locus in intron 1 of the CTSH gene located on chromosome 15q24 and a locus located between the C1QTNF6 and SSTR3 genes located on chromosome 22q13[40].

The oldest and the best documented genetic anomalies that are related to IDDM are found in the HLA region, but as the results of the cohort studies above show, in the recent years many new associations were found that may play a part in IDDM susceptibility.

These genetic epidemiological cohort studies are capable to identify genetic factors that are associated with diseases. In the studies mentioned above statistical methods were used to identify loci that were associated with IDDM. Using these methods genetic anomalies were identified and statistically confirmed to be tied with possible IDDM susceptibility. Depending on the individual's genetic profile and previous genetic studies, it is now possible to determine if an individual is susceptible to IDDM. In the future these genetic cohort studies can provide more insight into genetic predisposition of IDDM as well as other diseases. Already known associations can be (re)confirmed and new ones identified, resulting in increased accuracy of the found genetic profile of IDDM and understanding of the factors involved in genesis of the disease.
IDDM metabolomics

Although there are clear genetic risk associations involved in the susceptibility to developing IDDM, genetic risk factors alone are not always conclusive proof if an individual actually will or will not develop IDDM. A patient can have one of the genetic susceptibility profiles, but this does not mean the disease will actually manifest itself. Serum metabolomics could provide more insight in the development of the disease.

Islet autoantibodies
Detection of islet autoantibody positivity has been the first detectable signal for the onset of autoimmunity and the progression towards manifestation of IDDM [4]. Initial detection of auto-antibodies can be months or years before any clinical symptoms manifest themselves, but detection of the disease at this stage might be too late to take preventive measures. This is where serum metabolomics might provide useful answers and detection of certain serum levels might prove to be specific and early enough to warrant successful preventive measures and avoid progression to overt IDDM.

Metabolic dysregulation
Orešič et al., have performed a longitudinal prospective cohort study in which they studied and compared serum metabolite profiles of children who progressed to IDDM (progressors) and children who remained healthy (nonprogressors). The sample cohort population was taken from the Finish Diabetes Prediction and Prevention study (DIPP) which was a genetically defined cohort population. The results were compared with an additional cohort which was not genetically defined and which was taken from the Special Turku Coronary Risk Factor Intervention Project for children (STRIP). Progressors and nonprogressors of the sample population were then matched for gender, date and place of birth and HLA genetic risk group status.

The study suggests that autoimmunity in IDDM is preceded by metabolic dysregulation. According to the results of the study, children that later developed IDDM had reduced levels of succinic acid (a citric acid cycle component) and phosphatidylcholine (a plasmamembrane component) at birth. Throughout the follow up the children had reduced levels triglycerides and anti-oxidant ether-phospholipids.

Phosphatidylcholine acts as a major source of choline in the human body. The results therefore suggest that these children are choline-deficient since birth. Choline controls the secretion of triglycerides by the liver, which explains the low triglyceride level in the progressors. In addition choline is a regulator of gene expression, linking abnormal changes in choline levels to long term changes in gene expression. Epigenetic changes can cause genes to be permanently “knocked out” without alterations to the DNA sequence. Therefore choline deficiency could possibly knock down the function of certain genes that play a part in IDDM prevention.

Choline (as well as succinic acid) metabolism is largely dependent on an individuals gut flora composition. It is possible that the gut flora composition is dependent on maternal diet and the composition of the flora during pregnancy. This could ultimately influence serum levels and the immunesystem[4].
Protection from oxidative stress
Ether phosphatidylcholines were consistently low in progressors. In normal serum plasmalogens are the most common form of ether phospholipids. Plasmalogens can protect cells from oxidative stress and damage. Deficiency of plasmalogens may indicate that individuals who are genetically susceptible to IDDM are experiencing metabolic stress that not only diminishes phospholipid levels but also (because of the lack of antioxidant capacity) increases the risk of IDDM development as the beta cells of these patients are more susceptible to oxidative damage[4].

Role of lyso-phosphatidylcholines and branched-chain aminoacids
Auto-antibody positivity was preceded by increased levels of pro-inflammatory lyso-phosphatidylcholines several months before seroconversion. These reactive lipid by-products are generated through the hydrolysis of phosphatidylcholines. Lyso-phosphatidylcholines are capable of activating various pro-inflammatory molecules which in turn stimulate the immunesystem. The increased levels of lyso-phosphatidylcholines are possibly consistent with the effects that are seen after oxidative stress that is associated with a pro-inflammatory event (like a viral infection). Diminished levels of ketoleucine and increased levels of branched chain amino acids and glutamic acid precede the appearance of glutamic acid decarboxylase- and insulin autoantibodies. Increased levels of branched chain amino acids augment the secretion of insulin. In addition increased levels of branched chain amino acids have been reported in previous studies in overt IDDM. After the appearance of glutamic acid decarboxylase- and insulin auto-antibodies, the levels of branched chain amino acids and glutamic acid returned back to normal. Orešič et al., suggest that initial autoimmune response might be of a physiological nature and that it is intended to restore the metabolic homeostasis. The disease is therefore possibly caused (or at least partially influenced) by a defective response towards beta cell auto-antigens[4].

The study also compared metabolite profiles of children at high and moderate genetic risk (based on HLA-riskfactor). No significant difference was found between the two genetic risk groups among the progressors and non progressors[4].

Orešič et al., conclude that dysregulation of metabolism precedes autoimmunity and clinical IDDM. Additionally, the authors suggest that autoimmunity in IDDM patients possibly is a relatively late response to metabolic disturbances that occur before autoantibody positivity and that metabolic or immunomodulation interventions at the time preceding autoantibody positivity might prove to be a potentially suitable strategy to prevent IDDM[4].

Although these results are providing some new insights in the metabolic aspects and stage before clinical manifestation of IDDM, they need to be verified by other studies using another well characterized population cohort.

Insulin as a trigger for IDDM
It is not fully clear what is the trigger or target is that causes the autoimmune response that destroys the insulin producing beta-islet-cells. Indications exist that insulin might actually be the target antigen causing autoimmune type 1 diabetes. Kent et al., cloned single T cells in a non biased manner from pancreatic draining lymph nodes of subjects with type 1 diabetes mellitus and from non-diabetic control subjects. Long-term diabetic patients showed a high degree of clonal T-cell expansion in their pancreatic lymph nodes, while the control subjects did not show this feature. Oligoclonally expanded T-cells from diabetes patients with the IDDM susceptibility allele DR4 recognized insulin epitope A 1-14 restricted by DR4. This research shows the existence of clonally expanded and insulin-reactive T cells from the site of autoinflammatory pancreatic drainage in IDDM patients [41]. Insulin itself therefore seems to be a major determinant in pancreatic beta cell destruction and onset of type 1 diabetes.
Treatment and future developments

Insulin injections
Currently there is no generally accepted cure for IDDM. The main treatments that are given to patients are in essence disease management which does not permanently cure the patient. They solely are treating the disease symptoms and increase the quality of life, as long as the treatment is maintained. The most common treatment for IDDM patients are insulin injections. As patients with IDDM have a diminished (or even an entirely non-existent) insulin production, injectable insulin is needed to manage the blood glucose levels. Injection of insulin is usually done by jet injectors, hypodermic needles or insulin pumps. IDDM can also be treated with a special diet regime. Diet control is not the primary IDDM treatment but can still have beneficial effects for IDDM patients in the form of blood glucose normalization. As mentioned earlier, these methods only treat the symptoms of IDDM but they do not effectively cure the patients, indicating that pancreatic insulin production is not restored.

Insulin injection can be relatively complicated for the patient who usually injects him or herself. Appropriate dosages need to be calculated after each meal in order to prevent too high or too low levels of insulin injections. Both hypo- and hyperglycemia (too low and too high blood sugar levels) can occur after injection of an inappropriate dosage of insulin.

Given the burden of insulin injections for IDDM patients, alternatives research has been conducted in several medical fields to find a way to restore insulin production, thereby effectively curing IDDM.

Pancreas and islet cell transplantation
Pancreas transplantation in theory does restore insulin production, but the actual procedure is unpractical due to limited pancreas donor supply and difficulty of the procedure. Additionally, the requirement for long-term immuno-suppressives by the patient makes pancreas transplantation an unpopular option.

Islet cell transplantation is an alternative to pancreas transplantation. Donor islets are infused in the recipient's liver where upon settling the islets will start to produce insulin. Though the procedure is more simple than a full-scale pancreas transplant, it still has its limitations due to the need of high-dose immunosuppression and limited numbers of available islet cell donors.

Beta cell producing systems: stem cell based solutions
Stem cell based technologies might be able to fill the void in the search for a cure. A study by Voltarelli et al., has shown that 14 out of 15 IDDM patients that underwent high-dose immunosuppression and autologous non-myeloablative hematopoietic stem cell transplantation exhibited an increase in beta cell function. Furthermore, a majority of the patients showed prolonged insulin independence (for various amounts of time)[42]. A limitation of the study is that there was no control group included. This approach still requires high doses of immunosuppressive drugs. However it does indicate that insulin production was “restarted” because of the regeneration of beta cells in the pancreas after immunosuppression.
Islet-like clusters

Jiang et al., have developed a reproducible method to generate islet-like clusters (ILC's) from human embryonic stem (ES) cells. Embryonic stem cells are pluripotent cells derived from the inner cell mass of preimplantation embryos. These cells can be cultured *in vitro* and differentiated into any cell type including insulin producing cells that resemble pancreatic beta cells[43]. Tateishi et al., have adopted the Jiang protocol and used it to successfully produce ILC's from induced pluripotent stem cells referred to as iPS cells[44]. IPS cells are pluripotent cells derived from normal somatic cells by genetic reprogramming that resemble embryo derived embryonic stem cells. The efficiency of the Tateishi protocol however was relatively low. Nevertheless, it is still a promising prospect that patient-specific iPS cells can be used to produce ILC's that can be implanted in patients to “replenish” the beta cell count and hence insulin production. Most importantly, these cells can be implanted in the absence of immunosuppression since iPS are self in contrast to ILC's from ES cells which are non-self.

In response to recent developments (like the one by Jiang et al. and Tateishi et al.) research has been focussing on increasing the efficiency of differentiation of pluripotent cells into mature insulin producing cells. Zhang et al., have reported a way to differentiate pluripotent cells (both ES and iPS cells) with increased efficiency [45]. The insulin producing cells (derived from ES cells) that were obtained using their protocol were shown to be similar in terms of characteristics in their final stage compared to mature pancreatic beta cells. On average the differentiated population consisted of 25% of insulin-positive cells, which is considerably higher compared to previous efforts. These cells express pancreatic beta cell markers (being: Glut2, Isl-1, MafA, NeuroD, Nkx6-1, Pdx1) and co-express C-peptide/insulin, NKX6-1 and PDX1 which is considered a specific functional characteristic of mature pancreatic beta cells. When considering that these cultures consist of 25% of insulin-positive cells, the levels of C-peptide release after glucose stimulation is comparable to the levels released by adult beta cells. C-peptide is a “by-product” protein which is released when insulin is secreted. Zhang et al. in their paper have stated that in the future these cells will be implanted into diabetic immunodeficient mice to further evaluate cell functions *in vivo*. 
Induced pluripotent stem cells (iPS), what are they and why are they needed?

In vitro models of human disease
Biomedical research is for a large portion based on studying normal and pathogenic cellular processes. Disease modeling using animal models (i.e. murine models) has its limitations in accurately mimicking human pathophysiology. This is particularly the case with human contiguous gene syndromes. A contiguous gene syndrome is a disease which is caused by anomalies in 2 or more genes located next to each other on a chromosome.

As an alternative human cells cultured in vitro can be used as disease models provided that the cultured cell type represents the in vivo counterpart regarding its pathophysiological characteristics. In vitro culturing of disease causing primary human cells does have its limitations however, including limited availability, the isolation of pure population from cell mixtures, culture conditions and (short) life span. Because of the difficulties accompanied with primary human cell cultivation there is a lack of human in vitro models models that can be used to study normal and pathogenic tissue function. Many important questions regarding normal and abnormal human development and disease pathogenesis can therefore not be answered properly. An example of such a disease is IDDM. It is known that an autoimmune response ultimately causes the disease symptoms but at present it is poorly understood what is/are the initial trigger(s) and how the onset of the disease then further develops[4]. The availability of in vitro cultured cells of IDDM patients that represent different stages of pancreatic development represents an invaluable tool to study both genetic as well as non-genetic factors that eventually lead to beta cell destruction. Obviously, these cells cannot be obtained from affected pancreatic tissue but rely on other sources capable of producing these patient-specific cells.

Human ES cells
Embryonic stem cells are embryonic cells isolated from the inner cells mass of preimplantation embryos. Under defined culture conditions, these cells can be cultivated as undifferentiated cells undergoing self-renewal while maintaining the capacity to differentiate into all cell types of the body. This property is referred to as pluripotency and is distinct from the more restrictive differentiation capacity of some adult stem cells like hematopoietic stem cells. Pluripotency is maintained by an intricate network of pluripotency regulatory factors that are organized in a so-called feed forward loop. Once a critical level of (one of) these gene products is reached the feed forward-loop is activated and pluripotency maintained. On the other hand, interference with the expression of the pluripotency factors leads to loss of pluripotency and induction of differentiation.

Human embryonic stem (ES) cells are an unlimited source of pluripotent cells. ES cells not only enable human development to be studied in vitro, they also can be used to study the cellular effects of single and multiple gene disorders [46] provided ES cells with the genetic lesion are available. Last but not least: differentiated derivatives of ES cells can be used therapeutically following transplantation to replace diseased or aged cells.

IPS cells
A recent breakthrough in generating patient specific ES cells was the finding that normal human fibroblasts can be reprogrammed to ES cells through expression of a specific set of transcription factors[47]. Forced expression of these transcription factors activates chromatin remodeling and re-establishment of the core feed-forward regulatory circuit that controls ES cell pluripotency.
The wider implication of these findings is that patient-specific primary fibroblast cell cultures can be reprogrammed to an ES cell-like or induced pluripotent (iPS) state. These cells can be propagated as human ES cells as well as induced to differentiate in vitro into a desired cell type[47]. These fibroblasts may contain any disease mutations despite the notion that the disease causing effects becomes manifest in another cell type. During iPS cell generation these mutations are maintained and reveal their phenotype after differentiation into a specific cell type that causes the disease in patients[46]. IPS cells are therefore an excellent and unique tool to study the molecular basis of disease causing mechanisms as well as to use these cells for drug screening and gene replacement therapies[48, 49].
An example to illustrate the principle is long QT syndrome. LQTS is a disease that is caused by genetic mutations in genes involved in the generation of cardiac action potentials, ultimately causing lethal arrhythmias. This disease can be modeled by generating cardiac myocytes from affected individuals using iPS cells. Candidate drugs preventing arrhythmia can then be tested in this in vitro model. Using such a model, drugs that potentially can cause arrhythmias in specific groups of sensitive patients can also be identified [50]. Similarly, iPS cells could be used in toxicology research and the identification of teratogenic and (embryo)toxic substances[51].

Patient specific iPS cells
Park et al. have described a method to produce iPS cells from either dermal fibroblast or bone marrow derived mesenchymal cells. The patients from whom the cells were taken all had genetic diseases with either Mendelian or complex inheritance. The patients had the following diseases: adenosine deaminase deficiency-related severe combined immunodeficiency (ADA-SCID), Shwachman-Bodian-Diamond syndrome (SBDS), Gaucher disease (GD) type III, Duchenne (DMD) and Becker muscular dystrophy (BMD), Parkinson disease (PD), Huntington disease (HD), juvenile-onset, type 1 diabetes mellitus (IDDM), Down syndrome (DS)/trisomy 21, and the carrier state of Lesch-Nyhan syndrome. The cells obtained from patients were reprogrammed using 4 reprogramming factors: OCT4, SOX2, KLF4 and c-MYC. After continued culturing of the reprogrammed cells, ES-like cells started to appear that could be stably propagated as iPS cell lines. DNA-based tests were performed to confirm if these iPS cell lines were indeed disease specific. Most of the cell lines exhibited the expected genotypic traits, with the exception of the diabetes type 1 cell line as the genetic basis for this disease is complex and was genotypically not fully understood at the time. The iPS cells were checked for pluripotency by examining the expression of pluripotency controlling genes and differentiated along specific lineages representative of the three embryonic germ layers (ectoderm, endoderm, mesoderm)[46]. The results indicate that patient specific iPS cells can indeed be used to generate differentiated cell types in which the effects of disease causing mutations can be studied.

IPS cell limitations and future developments
According to Yamanaka, one of the most important challenges is the development of sensitive, simple and reliable methods for the evaluation of effectiveness and safety of therapeutically used iPS cells[50]. IPS cells could eliminate several problems that are related to the use of ES cells, the main problems being: immune rejection of transplanted ES cells (as they are not patient specific) and the controversial use of embryos. However iPS cells bring their own set of unique challenges that need to be solved before they can be used for regenerative medicine.

The use of iPS and ES cells for transplantation purposes requires the generation of homogeneous populations of differentiated cells to be transplanted. Differentiation into the desired cell type should be highly specific, efficient and leave no undifferentiated cells behind as undifferentiated ES and iPS cells are tumorigenic that can result in teratoma formation[50]. In addition, iPS cell derivatives should be screened for the presence of chromosomal anomalies resulting from in vitro culturing as well as epigenetic abnormalities resulting from incomplete reprogramming that could interfere with normal cell physiology[52].

So far, no general selection protocol is available to “scan” which cells after differentiation would be suitable for therapeutic use. As mentioned earlier, undifferentiated cells in graft tissue could cause undesired effects including teratoma formation. A possible solution to this could be the treatment of graft tissue with cytotoxic antibodies that are able to identify and destroy cells that still express ES cell surface antigens[53].
Currently the production of iPS cells derived from somatic cells is based on reprogramming of these somatic cells using multiple viral vectors that act as transport vehicles used to induce expression of the reprogramming factors in the somatic cells. This can cause safety issues as viral integration can take place in endogenous genes that could result in disabling certain vital genes or activate a nearby gene which can cause cancer in case viral integration occurs in the vicinity of an oncogene. In iPS cells however these viral integration sites can be identified by sequencing[50]. Lenti- and retroviruses can also cause reactivation of the transcription factors they carry (see table 2), this too can cause tumors in particular with reactivation of c-MYC. Additionally, the continuous expression of the transcription factors might suppress iPS cell differentiation [50]. To overcome these limitations intense efforts by several research groups are ongoing to develop methods resulting in generation of virus free iPS cells. In the classical Takahashi and Yamanaka protocol four transcription factors i.e. OCT3/4, SOX2, KLF4 and c-MYC are required to reprogram fibroblasts into iPS cells[47, 54].

Additionally it is currently not possible to tell for each iPS clone that is generated if the reprogramming process was complete. This so-called aberrant reprogramming can potentially lead to impairment of differentiation which can increase the risk of immature teratoma formation[50].

The expression of OCT3/4 is highly specific for pluripotent embryonic stem cells and is the most indispensable factor in the production of iPS cells[50]. The other factors can be compensated for by using replacement factors (see table 2).

<table>
<thead>
<tr>
<th>Transcription factor</th>
<th>Expressed in</th>
<th>Can be replaced by</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT3/4</td>
<td>Pluripotent stem cells</td>
<td>Can not be replaced</td>
</tr>
<tr>
<td>SOX2</td>
<td>Neural stem and progenitor cells</td>
<td>SOX1</td>
</tr>
<tr>
<td>KLF4</td>
<td>Intestine, skin, stomach and skeletal muscle</td>
<td>KLF2 or KLF5</td>
</tr>
<tr>
<td>c-MYC</td>
<td>Ubiquitous expression</td>
<td>N-MYC or L-MYC</td>
</tr>
</tbody>
</table>

Table 2: transcription factors and their expression

Soldner et al., reported to have successfully produced iPS cells free of reprogramming factors and viral vectors with somatic cells from Parkinsons disease patients. This in theory minimizes some of the safety issues. IPS cells were obtained similarly to the previously described method but to remove the threat of the continued presence of the reprogramming factors, the viral vectors that were used were removed using the enzyme Cre-recombinase which excises the factor carrying lentiviruses out of the iPS cells. Following removal, the cells did not lose any of their pluripotency characteristics. Furthermore, they are more suitable for human disease modeling compared to their “virus-carrying” counterparts as the reprogramming factors can influence the molecular characteristics of the iPS cell[55]. Although these cells no longer harbour the viral vectors, there might still be genetic alterations within the cell due to viral integration prior to the removal.

Woltjen et al., reported an alternative way of producing iPS cells using a technique called PiggyBac (PB) transposition. Also with this system the DNA sequences carrying the reprogramming factors can be removed from the genome at the expense of a few nucleotide integration mark[56]. Kaji et al., have been able to generate iPS cells using plasmids[57], while Yu et al., have reported to have produced iPS cells by using nonintegrating episomal vectors. These vectors were then removed resulting in iPS cells completely free of transgene sequences and vectors[58]. However, small parts of the plasmid vectors integrated in the genome of the host cell are hard to identify and it needs to be seen whether the claims are justified.
IPS cells from diabetes mellitus type 1 for disease modeling and therapy – a review of the possibilities

Zhou et al., have managed to reprogram murine embryonic fibroblasts by direct delivery of recombinant reprogramming proteins, thereby creating protein-induced pluripotent stem cells. This method generates in theory safer iPS cells as it eliminates the risk of genetic modification of the somatic target cells genome as this method does not involve the use of DNA. Zhou et al. used 4 recombinant cell penetrating reprogramming proteins i.e Klf4-11R, c-Myc-11R, Oct4-11R and Sox2-11R to reprogram mouse embryonic fibroblasts. IPS cells could be obtained although with extremely low overall efficiency[59]. Most importantly however these studies show that is possible to reprogram somatic cells without the use of DNA as factor delivery agent and these cells are generally regarded as most “safe” for future therapeutic application.

Small molecules inhibiting enzymes in cellular signal transduction and/or physiology have been shown to enhance the reprogramming efficiency. Huangfu et al. have managed to produce iPS cells from primary human fibroblasts using valproic acid (VPA) and just 2 factors (OCT4 and SOX2) [60]. Some molecules therefore are capable to replace one or more factors highlighting the importance of the blocked enzyme in the reprogramming process. Extrapolating these findings, it seems likely that by blocking and/or activating key components of the reprogramming machinery iPS cells can be obtained that are safe for therapeutic application. Given the recent progress it seems reasonable to expect these cells to be available within the near future.

IPS cell applications: Gene therapy

Once iPS cell generation has progressed to a state that is regarded safe for clinical application, these cells can then be used to regenerate tissues which were affected in for instance single gene disorders. The tissue that requires replacement may be generated from iPS cells that have been corrected for a genetic defect before transplantation back into the patient. Diseases like Parkinsons disease and sickle cell anemia can benefit from this approach [55, 61]. Raya et al. have induced patient specific pluripotent cells from Fanconi anemia patients. Fanconi anaemia is a disease of an autosomal recessive nature in which the bone marrow of the patients fails to produce blood cells. Other clinical features include missing or abnormal thumbs and kidney anomalies. Currently there is no cure for Fanconi anaemia patients and treatment currently consists of bone marrow transplantation. IPS cells from Fanconi anaemia patients can be corrected from their genetic defect and then differentiated into disease-corrected haematopoietic progenitors. Once the drawbacks that currently prevents clinical use of iPS cells are overcome this strategy can be used for cell therapy applications[48]. Similarly, Ye et al. have isolated iPS cells from a homozygous beta 0 thalassemia patient, which is also an autosomal recessive disorder. In beta 0 thalassemia there is no production of beta globine chains and the excess production of alpha globine chains results in anaemia, red blood cell membrane damage and toxic aggregate formation. Treatment consists of frequent blood transfusions or in extreme cases bone marrow transplantation. IPS cells from a thalassemia patient have now been obtained offering the possibility of gene correction. Prenatal diagnosis and selective abortion have been able to effectively decrease the number of children born with this disease in the countries that have adopted screening measures. The tissue samples that are used in the prenatal screening can now be used to generate (corrected) iPS cells, which could aid in perinatal treatment, giving parents an option to keep the child in combination with a more effective treatment for the disease during the early years of the child's life[62].
IPS cell applications: \textit{In situ} transdifferentation

IPS cells can thus after differentiation be used for regenerative medicine and transplantations. However currently there are no IPS specific differentiation protocols and as such differentiation protocols need to be adapted from ES cell differentiation[52]. Though differentiation of iPS cells is usually not as efficient as differentiation of ES cells. It is currently not yet fully understood what the reason is behind the lack of efficiency, but it is believed that possibly there is an epigenetic “memory” system in cells, this memory system reminds the cells of their adult cell state even after they have been reprogrammed into a pluripotent state (making differentiation more difficult and thus less efficient)[52].

A different possibility to therapeutically apply the concept of cellular reprogramming is lineage reprogramming. In this process, somatic cells are not reprogrammed to full pluripotency but are reprogrammed into a different cell type that is related to the somatic lineage of the source cell used for the transformation. Rolletscheck and Wobus have stated that “transdifferentiation” using transcription factors might prove to be an alternative strategy to change cell fates for the sake of regenerative medicine. Transdifferentiation could potentially even provide safer graft material as the cells are not returned to full pluripotency thereby minimizing the risk of teratoma formation due to the presence of residual pluripotent cells in the tissue grafts[52]. An example of transcription factor induced lineage reprogramming is transdifferentiation of exocrine pancreatic cells into endocrine beta cells[63]. The exocrine cells were reprogrammed using adenoviral vectors harboring 3 specific reprogramming factors (MafA, Ngn3 and Pdx1). The transdifferentiated cells closely resemble pancreas beta cells and are indistinguishable in terms of morphology and ultrastructure. In addition, the transdifferentiated cells express genes that are vital for beta cell function and they are able to secrete insulin.

Replacement cells that are derived from iPS cells need to be compared to their \textit{in vivo} counterparts. They need to be similar in both function and proliferation. If the derived cells are not similar to their normal counterparts the replacement therapy might prove unsuccessful.

As mentioned earlier, Zhang et al. have successfully differentiated ES and iPS cells into mature insulin producing cells. Before clinical application of such iPS derived insulin producing cells it is important to compare these cells to healthy mature beta cells. The iPS derived cells should have a similar stimulus response system for insulin secretion like healthy beta cells have. It is important that the functioning of these cells is evaluated \textit{in vivo} (by use of an animal model for instance) before they can be used clinically. Evaluation of the iPS derived insulin producing cells is needed to verify if (and for how long) a patient with IDDM would become insulin independent after transplantation of the derived cells into the patient. Furthermore it is needed to evaluate if terratoma formation will occur.

IPS cell applications: Modeling IDDM with iPS cells

Despite many advances in the recent years when it comes to biomedical research, molecular technologies and disease modeling, there as of yet still are no models for IDDM that are capable of accurately mimicking the disease while at the same time being a representative model for a particular group of patients. In order to be able to study IDDM and its characteristics more effectively and in a controlled environment such novell models are needed. These models could play an important role in identifying and confirming new (genetic) factors that play a part in IDDM. Additionally these models could provide accurate and useful insights in terms of drug screening: f.e. which drugs and other chemical substances can either improve or worsen the symptoms of IDDM and for which group of patients would these findings apply.

IPS cells could play a crucial part in the realization of these new models as these cells have the same genetic profile of the patient they originate from. This would enable the generation of specific models which are capable to represent specific groups of patients with a defined genetic profile. These models will enable future research to accurately study IDDM and its possible causative
factors. Currently IDDM is modeled using a murine model in which mice are injected with multiple doses of streptozotocin which produces a diabetic syndrome that includes an autoimmune response against pancreatic cells[64]. Though this model offers possibilities to study some of the aspects of IDDM, using this model however it is impossible to study the onset and development of IDDM in groups of patients with a specific genetic profile.

The research community acknowledges the demand for a human derived model that is genetically identical to the patient. ES cells currently are also used to model certain rare genetic diseases. These cells are acquired by pre-implantation screening of IVF embryo's. If it turns out that one of those embryo's have a genetic defect, the ES cells can then be collected and cultivated in order to model this rare disease. IPS cells could be used similarly to model genetic diseases, the main difference to ES cell derived models is however that iPS cells can be used to model any individual on the earth, with all of the various diseases and not just the rare diseases found in pre-implantation screening.

As described earlier, such a genetically accurate disease model for IDDM currently does not exist. Various models (both in vitro and in vivo) could be created for this purpose to simulate the progression of IDDM using iPS cells from patients with specific genetic profiles that were previously associated with IDDM (see table 1). These cells will have the exact same genetic information as the patient they have been derived from, in theory they should behave the same way when used in a model as they would in the patient. For the sake of modeling in vivo diabetic immunodeficient mice could be used. After induction of iPS cells from a genetically susceptible IDDM patient, these iPS cells could then be then be differentiated into ILC's which could then be implanted into the diabetic mice. Insulin and C-peptide levels of the mice should then be monitored in order to evaluate the functioning of the implanted cells. The mice should be monitored for a considerable amount of time to note if there will be a decline in insulin production due to the failing of the beta cells/ILC's. For comparisons sake a control group of mice should be included in which the mice undergo the same procedure, only these mice will have ILC's made from iPS cells from genetically non susceptible individuals that do not have IDDM.

Another conceivable model would be the implantation of pancreatic precursor cells into mice. These precursors should (like the ILC example above) be generated from iPS cells which originate from genetically susceptible IDDM patients. After implantation of these precursors one could study the maturation of these cells into insulin producing cells in vivo, instead of differentiating them in vitro first. Similarly to the first example, insulin and C-peptide levels should be monitored for a certain period of time and an eventual decrease in function should be noted. As a control group mice should be implanted with pancreatic precursor cells derived from healthy individuals that are not genetically susceptible to IDDM.

Another possible way to model IDDM is in vitro modeling[65]. This would involve a so-called cellular interaction model. For such a model it would be required to generate at least 2 different cell types, ILC's and cell types that play a part in the immune system. The cell types should be generated from iPS cells from the same patient, so that the model is representative for that particular patient (and his or hers genetic profile). After successful generation, these cell types should then be made to interact with each other so that onset and development of IDDM can be studied in vitro. As a control a similar model should be made from an individual that does not have IDDM and is is not genetically susceptible.

An accurate IDDM model would also beneficial for drugscreening studies. The models could be used to test current and novel drug treatments for IDDM. This way drugs can be identified which would have beneficial or alternatively negative effects on the manifestation of IDDM in a patient.
Which somatic cells can be used for iPS cell generation?

Human and mouse iPS cells are most commonly generated from fibroblasts. Additionally mouse iPS cells have been described to have been generated from bone marrow cells, gastric epithelial cells, hepatocytes, B lymphocytes, neural stem cells and pancreatic cells[50]. Human iPS cells have been generated from blood progenitor cells, keratinocytes and hair follicle cells[50]. It is important to know which cells can be used to generate iPS cells as well as which cells are obtainable with relative ease and in sufficient quantity. However not all cells might be just as suitable for iPS cell generation. Some cells might be more suitable for complete reprogramming minimizing the risk of residual epigenetic controlled gene expression abnormalities in the differentiated progeny. Generation of specific cell types like pancreatic beta cells might be easier if iPS cells are generated from cells of endodermal origin as no lineage specific barriers have to be crossed. Some iPS cells have less retroviral integration sites than others, depending on which cell type they have been derived from. This can prove to be a preferable source due to their limited number of integration sites and decreased probability of insertion mutagenesis. An example of a source for these kind of iPS cells are human keratinocytes[50].

Cell age might also be a factor to keep in mind. The age of the patient that will be used as a donor to create patient specific iPS cells can be a critical factor in the production of high quality iPS cells. Older cells have accumulated more recessive mutations then younger ones as they have been exposed longer to various stress factors and reactive oxygen. If iPS cells are created from these “old” cells, the entire array of the acquired recessive mutations will be present in the resulting iPS cells. The quality of these “old” iPS cells needs to be evaluated properly to determine if these recessive mutations will give rise to undesired effects. A possible way to overcome the drawbacks of somatic cell mutation is the use of preserved cord blood and to derive iPS cells from this tissue source[52, 66].

ES and iPS cells can potentially form teratomas (when undifferentiated cells remain behind in the transplant tissue). Somatic stem cells (like hematopoietic and mesenchymal stem cells) can not. Currently it is not yet possible to successfully generate somatic stem cells or progenitor cells from a somatic source (such as a fibroblast). However if generation of somatic stem cells would be possible it would eliminate the risk of immature teratoma formation which currently exists with iPS cells[50].
Discussion

Patients that suffer from IDDM no longer have (an adequate) insulin production in their pancreatic beta cells. Currently there is no generally accepted cure. Treatment only consists of “disease management”. Stem cells could potentially provide a cure by differentiating them into mature pancreatic insulin producing cells, after which they are implanted into the patient. However the use of embryonic stem cells is considered controversial and the material that is used is not patient specific, meaning it would require immunosuppression. IPS cells which are patient specific might provide the answer in the search for a cure that would not require immunosuppression. These cells can be differentiated into insulin producing cells due to their pluripotent characteristics. Patient-specific IPS cells not only provide a cell source for potential cell replacement therapy but also provide a unique tool to generate cells in which the disease symptoms become manifest. In this way accurate disease models can be created that can be used to study disease pathophysiology and to identify drugs for therapeutic use. In addition, genetic mechanisms behind diseases like IDDM can also be accurately studied using human disease models based on IPS cells.

IPS cell production is currently most commonly performed by forced expression of reprogramming factors using lenti- and retroviral vectors. Since the viral vectors can integrate into the genome of the cell that is being reprogrammed there is a risk of insertional mutagenesis that may result in permanent genetic alteration that can cause cancer. Since 2 of the 4 reprogramming factors are onco- and/or tumor suppressor genes, reactivation of the viral transduced genes in cells derived from IPS cells may lead to malignancy. It is therefore essential to find alternative ways to generate IPS cells including excision of the reprogramming factors at the IPS cell stage or by using small molecules that can replace reprogramming factors, include kinase-, histon-, DNA methylation inhibitors and siRNAs. Recent evidence suggests that it will be possible to reprogram somatic cells into IPS cells without the use of viral vectors.

Clinical application of IPS cells is currently not possible due to limitations. The limitations being: first, use of viral vectors to induce forced expression of transformation factors (described above). Secondly, the lack of a generally accepted evaluation protocol of IPS cells, in which IPS cells are screened for aberant reprogramming and chromosomal as well as epigenetic abnormalities. Third, current differentiation protocols (when used with IPS cells) have a relatively low efficiency. If IPS cell derivatives are going to be used clinically in the future, highly efficient differentiation protocols are needed not only to boost efficiency but also to boost IPS cell derived graft tissue safety. If these limitations are overcome, regenerative medicine will be getting a tremendous boost and might become common practise instead of a future possibility.

When put into context with IDDM, IPS cells can be used to generate cells that are similar in functionality and characteristics to mature pancreatic beta cells. The cells resulting from this procedure respond to glucose stimulation and secrete insulin. Although in vivo confirmation tests of insulin independence are presently lacking, it is nevertheless a hopeful prospect that IPS cells could in the future open the door towards a real cure for IDDM patients. As the current IDDM treatments merely treat some of the symptoms of IDDM and do not permanently cure it, there is a need for a permanent cure to replace the current treatments. IPS cells could in the future potentially provide regenerative treatment options with which insulin producing cells could be replenished in IDDM patients and insulin production within the patients themselves be restarted. The main advantage of a regenerative approach as opposed to the current practise of insulin injections is that IPS cell derived replacement cells can potentially generate insulin producing cells that respond to blood sugar fluctuations and thereby release an appropriate amount of insulin, whereas the regular insulin injection treatment is not always appropriate to the blood sugar levels and correct dosage is reliant on the patient and his or her calculations.
In the more immediate future iPS cells can be used to create *in vivo* and *in vitro* IDDM disease models, this is needed seeing how currently patient specific disease models of IDDM do not exist. *In vivo* models could be used to study the onset of IDDM in specific groups of patients with a specific genetic profile. In addition *in vivo* models would enable to study cellular differentiation of iPS cells (derivatives). *In vitro* models could be used to study the pathophysiological aspects IDDM in more detail on a cellular/molecular level. These models would provide valuable new insights into how IDDM develops and would improve understanding of the disease which is currently lacking. These insights would enable the development of more effective treatments for IDDM.

It is essential that a proper evaluation protocol is developed and used to make sure that somatic cells have been fully reprogrammed, this would minimize risk of undesired teratoma formation. In order for iPS cells to become part of everyday clinical practise it is imperative that their shortcomings are overcome.

So far no research has been conducted into the public (patient) and health professionals (physicians) opinion towards iPS cell based techniques. It needs to be clear whether both public and health professionals are willing to accept iPS cells and its related technology. Without their “support” the technology will most likely not be accepted in therapeutic practise, regardless of the promising possibilities that iPS cells offer.
References

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IPS cells from diabetes mellitus type 1 for disease modeling and therapy – a review of the possibilities


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