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An Update on Etiopathogenesis and Management of Type 1 Diabetes Mellitus

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Review Article

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Abstract

The incidence of type 1 diabetes mellitus (T1DM) is increasing at a massive pace globally. It results possibly from a genetic susceptibility and exposure to an environmental trigger. What is important is to use guidelines like staging the disease before overt hyperglycaemia occurs for which one need biomarkers also called whistle blowers. We have tried to summarize the pathogenesis, including honeymoon phase and subsequently various antigen based and non antigen based interventional trials, besides therapies which help in beta cell neogenesis. Role of stem cell, modifications of umbilical cord stem cell and lastly isle cell transplantation is covered. Treatment plans can be based on C peptide levels which gives a fair bit of idea about prognosis. Despite so much research insulin in some or other form remains main stay although not curative. Emphasis should be made to diagnose before overt disease occurs when therapies like teplizumab may work.

Keywords: Type 1 DM; Autoimmunity; C peptide; Stem cells; Teplizumab; Islet cell transplantation

Introduction

The incidence of type 1 diabetes mellitus (T1DM) for people of USA between age 0-19is 1. 7/1000. T1DM usually occurs in people younger than 30and is therefore known as juvenile onset DM, even though it can occur at any age. T1DM is a chronic autoimmune disorder which precipitates in genetically susceptible individuals by environmental factors [1]. The body's own immune system attacks the islets of langerhans of the pancreas destroying or damaging them sufficiently to reduce and ultimately eliminate insulin production. The incidence has been increasing globally during the past decades by 5. 3% in a year in USA. If these trends keep persisting doubling of new cases of T1D in European children under 5 year is predicted to occur between 2005-2020 and prevalence of individuals <15years will be 70% [2], a left shift towards early age [3]. Thus whatever triggers the onset is rising and affecting individuals who have susceptibility [4,5]. There has been an increasing search for such triggering factors for many years and only indirect evidence exists regarding certain viral infections. An attempt has been made to summarize the pathogenesis and treatment of T1DM, which is increasing at such a pace.

Kettner et al. studied the association between specific types of infertility treatment and childhood type 1DM. Of a total 565,116 singleton pregnancies, 14,985 were conceived by ovulation induction(OI), intrauterine insemination(IUI) and 8490 by IVF/ICSI. During the follow up period 2011(0. 4%) children developed T1DM. The primary analysis showed no association between infertility treatment and childhood T1DM. In secondary analysis OI, or IUI with FSH was associated with an increased risk of type 1 DM(Hazard ratio3. 22;95%CI-1. 20-8.64). No clear association was seen with other types of fertility treatment or specific treatment. Thus they concluded that no association between fertility treatment

and childhood DM was found. OI or IUI with FSH may be associated with an increased risk of childhood DM. However this maybe due to confounding by indications of infertility and thus need further investigation [6].

Insel RA, et al. 2015 (for the scientific statement of JDRF, the Endocrinie Society, and the American Diabetes Association) showed that T1DM is a continuous process as per prospective, longitudinal studies which progresses sequentially at variable but predictable rates through distinct identifiable stages before the onset of symptoms. Stage 1 is defined as the presence of β cell autoimmunity as evidenced by the presence of two or more islet auto antibodies with normoglycaemia and is pre symptomatic, stage 2 is the presence of β cell autoimmunity with dysglycemia and is pre symptomatic, and stage 3 as onset of symptomatic disease . Using this classification gives a standard Taxonomy for T1DM which will help in development of therapies and designing clinical trials to prevent symptomatic disease, promote precision medicine. It also gives a framework for an optimized benefit/risk ratio which will Impact regulatory approval, reimbursement and intervention in early stage of T1DM to prevent symptomatic disease [7].

Pathophysiology

A complex interplay of genetic, environmental and autoimmune factors selectively targets insulin producing β cells in type 1 diabetes mellitus (T1DM),ultimately ending in complete β cell destruction . The role of genetic factors in T1DM, has been understood since a long period of time, which is emphasized by familial clustering with other endocrine disorders and by the concordance rate in identical twins of 30-40%. Since these concordance rates in identical twins are not as high as in T2DM (i. e>80%), environmental factors must be clearly playing a major role [8,9]. Although the presence of an environmental trigger for T1DM is very likely, even identical twins do not express identical T cell receptor and immunoglobulin genes; as a result total concordance may not be expected. Siblings who are HLA identical to the pro band have a 12-15% risk for development of DM by the age of 20years. Although many of the genes linked to T1DM have not been found till date, some are known. HLA genes, located on the short arm of chromosome 6contribute to roughly 50%of genetic susceptibility to DM. 2 HLA class II genotypes DR4-DQ8and DR3DQ2 are present in roughly 30-40% of children with T1DM. The genotype having both heliotype carries the biggest risk of DM (approximately 5%) and is most commonly seen in early disease. While DR15DQ6 heliotype is very protective, being found in only 1% of children with T1DM as compared to 20%of the general population. HLA susceptibility haplotypes are over expressed in adult onset type 1, but at lower frequency than in classic T1D in youth. Other genes are likely to contribute to the genetic susceptibility to T1DM. These include insulin gene (on chromosome 11) and a number of other loci which are associated with other autoimmune conditions, suggesting the existence of common pathways, which predispose to loss of self tolerance. Another gene interferon induced helicase (IFIH1) located on chromosome 2encodes a protein involved in innate immunity and plays a role in recognition of the RNA genomes of certain viruses. It is suggested that highIFIH1 levels might provoke exaggerated responses which predispose to autoimmunity. Many other genes have also been implicated like PTPN 22(encoding lymphoid protein tyrosine phosphates [LYP]. Interleukin -2 receptor α gene(IL2RA)region, gene encoding cytotoxic T lymphocyte associated antigen 4(CTLA4) associated protein in the IDDM 12 region [10,11], which underscores the polygenic nature of the disease. Though single gene defects like IPEX and APS can cause T1DM these are rare.

Role of Viruses

Historically environmental cause of T1DM focused on viruses, because of seasonal pandemic of infections and rarely because of isolation of specific pathogens. Epidemics of mumps, rubella and coxsackie viruses have been associated with increased frequency of T1DM. Specific and convincing rare examples of virus induced DM have been reported. But it is believed that virus mediated β cell damage is not responsible for the massive destruction of β cells but it triggers an autoimmune response in genetically predisposed individuals. Thus viruses may contain molecules that resemble a β cell protein and viral infection could thus nullify self tolerance and trigger autoimmune responses. Viruses have long been suggested as a potential environmental trigger for T1DM. Still despite decades of research, the evidence supporting a relationship between viral infections and initiation or acceleration of islet autoimmunity remains mostly circumstantial as per the review by Coppieters et al. The biggest association between viruses and T1D involves enterovirus species, of which some strains have the ability to induce or accelerate disease in animal models. Several hypotheses have been brought forward to mechanistically explain how viruses may affect autoimmunity and β cell decay. Recent observations that certain viral infections, when encountered at the right time and infectious doses can prevent autoimmune disease illustrates that the potential relationship may be more complex than previously thought. [reviewed by Coppieters [12]. Other environmental agents like cow's milk, wheat protein and Vitamin D have been implicated as causative factors but like viruses no final proof has been given.

It has long been recognized that approximately 80%of patients with new onset T1DM have antibodies directed against various islet cell proteins, which include insulin, glutamic acid decarboxylase (GAD 65/GAD 67) and the secretory granules protein islet cell Antigen 512(IA2). These antibodies as biomarkers have been important tools for studying the potential of early identification and prevention of total β cell destruction in individuals susceptible to T1DM. Until the mid 1980's it was mistakenly thought that the autoimmune destruction of β cells was mediated by those antibodies rather than their being an epiphenomenon as is now understood. Rather β cell destruction is mediated by a variety of cytokines or by direct T lymphocyte activity which causes apoptosis or cellular destruction. Both animal models and human pathological studies have established that islet targeted inflammatory cell infiltrates known as insulitis, are composed of $CD8^+$and $CD4^+$Tcells, macrophages and B cells are linked to the onset of DM [13].

Coppieters et al. studying obtained frozen pancreas from 45 cadaveric T1D donors ranging from 1weekto>50years. They reported both single and multiple $CD8^+$T cells auto reactivities were detected within individual islets in a subset of patient's upto 8years after clinical diagnosis. Pathological features such as class 1 hyper expression and insulitis were specific for T1D and persisted in a small portion of the patients with longstanding disease. Insulitic lesions consistently presented in a multifocal pattern with varying degrees of infiltration and β cell loss across affected organs. They concluded that their observation provided first direct proof of islet auto reactivity in human islets and underscores the heterogenic and chronic disease course [14].

Overtime the islets become completely devoid of β cell and inflammatory infiltrates. α, δ and pancreatic polypeptide cells are left intact ,which shows the specificity of the autoimmune attack in β cells. A critical role for T cells is suggested by studies involving pancreatic transplantation in identical twins. Monozygotic twins with DM who receive kidney and pancreatic grafts from their non diabetic genetically identical siblings required little or no therapeutic immune suppression. However these patients eventually experienced a resumption of insulitis with the subsequent recurrence of DM. Evidence indicates that T cells in DM autoimmunity also derives from clinical trials using immunosuppressive drugs like cyclosporine, or antibodies like component of Tcell receptor (anti C3) or that after antigen presentation by B cells (anti CD20) slows their progression of recent onset DM but this effect is not sustained if immune suppression is withdrawn.

Immune Events in T1DM

Before clinical symptoms of T1DM become clear a lot of silent immune changes occur. Auto antibodies are produced and self reactive lymphocytes become activated and infiltrate the pancreas to destroy the insulin producing β cells in the islets of langerhans [15]. This persistent targeted destruction may go unnoticed for many years and the initial symptoms only become clear after most of the β cells have been damaged or become dysfunctional making the individual dependent on insulin for survival (Figure 1). Hence a lot of importance is given to biomarkers, which act as whistle blowers of an ongoing autoimmune response.

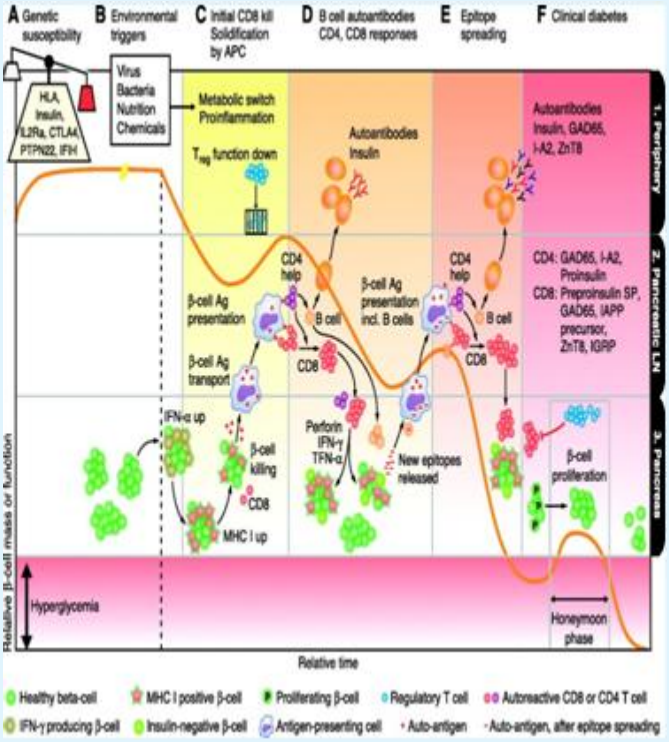

Figure 1: Courtesy ref no 13-How T1D might arise. This figure represents the beta-cell mass or function (represented by the orange line) as well as the different immunological phases (columns with alphabetized tabs on top) that occur in the relevant anatomical sites (rows with numerical tabs on the right). Specific events will be referred to via alphanumerical coordinates in the following explanation. Once the orange line of beta-cell function falls into the red zone, the individual is clinically diagnosed with type 1 diabetes. A complicated series of events precedes this and remains largely unnoticed. Initially, an unfortunate concurrence of genetic susceptibility (*a1*) and an environmental trigger (*a2*) sets an individual up for developing diabetes by causing two events. In the pancreas, beta-cells upregulate interferon

(IFN)-α (*b3*) and subsequently MHC class I (*c3*). This exposes the beta-cells to attack by autoreactive CD8 T cells with specificity for antigens in the pancreas (*c3*). Consequently, the released beta-cell antigens are picked up by resident antigen-presenting cells (APC) (*c3*) and transferred to the pancreas-draining lymph node (LN) (*c2*). Meanwhile in the periphery (*c1*), the environmental trigger has caused a metabolomic switch creating a proinflammatory environment that favors effector T-cell responses over Treg function. Beta-cell antigens presented in this proinflammatory context and with CD4 help (*c2*) initiate conversion of B cells into plasma cells (*d2*) and the appearance of insulin autoantibodies (seroconversion) (*d1*). Also, autoreactive CD8 T cells are stimulated to proliferate (*d2*) and migrate into the pancreas (*d3*). The stress induced by this second wave of beta-cell killing (*d3*), which involves perforin, IFN-γ, and tumor necrosis factor (TNF)-α, causes some beta-cells to halt insulin production (pseudoatrophy). The killing also causes the release of new beta-cell antigens that are picked up by APCs, including migrated B cells (*d3*), and get shuttled to the pancreatic LN (*d3-d2*). This engages new specificities of CD4 and CD8 T cells (*e2*) and B cells (*e1*) in a process called epitope spreading. A subsequent wave of beta-cell killing is therefore more severe and usually results in severe depletion of beta-cell function and mass (*e3*). Surprisingly, the autoimmune inflammation can also stimulate some beta-cell proliferation (*f3*), so that the beta-cell mass temporarily resurrects. Also, Tregs can sometimes overpower and dampen the effector response (*f3*). The fluctuation between destructive autoreactive responses and the alleviation by immune regulation and beta-cell proliferation possibly creates a nonstop relapse-remitting profile of beta-cell mass (orange line). Eventually, the autoreactive response wins though, and T1D is diagnosed when only 10–30% of functional beta-cells remain. The remission after clinically diagnosed diabetes is termed the honeymoon phase (*f3*), a temporary state of relative self-sufficient insulin production.

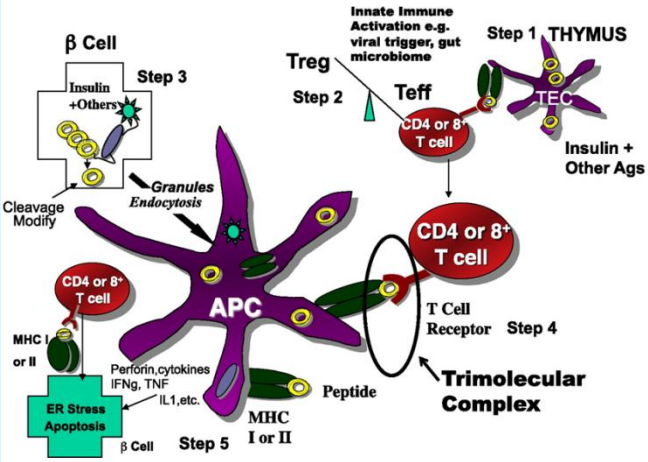

Figure 2: Courtesy ref 13Simplified model of the immune pathogenesis of type 1 diabetes. Major components include *Step 1*) The thymus where peptides of peripheral antigens are expressed and presented by HLA molecules on the surface of medullary thymic epithelial cells (mTECs cells) to T-cell receptors, leading to deletion of many but not all anti-islet autoreactive T-cells. *Step 2*) Regulatory T-cells (Treg) and effector T-cells are both produced, and their balance is crucial for maintaining tolerance. Innate immune activation can affect the balance in terms of activating autoimmunity. *Step 3*) The β-cell itself not only produces target antigens but also modifies molecules, such as chromogranin, by cleavage at critical sites, thus creating peptides recognized by pathogenic T-cells. There is evidence that processing of molecules such as insulin within the β-cell creates peptides that are then taken up by antigen-presenting cells either as whole, dead β-cells, or specifically, granules of β-cells, for eventual further processing and presentation of islet peptides to effector T-cells. *Step 4*) The trimolecular complex, involving the MHC-presenting molecule/peptide in the appropriate "register"/T-cell receptor recognizing both and, like a lock and key, is the essential recognition unit for adaptive organ-specific autoimmunity. *Step 5*) Finally, CD4 T-cells orchestrate multiple arms of the immune system (e.g., CD8 cytotoxic T-cells, pathogenic cytokine production), resulting in specific destruction of islet β-cells.

Why β cells are targeted and destroyed by the immune system? It was originally thought that molecules targeted by the immune system were only present in β cells. However studies later showed that T-cells targeting of specific auto antigens represents a much more complex process (Figure 2) than was originally thought and may depend on tissue specific localization and processing of a given target antigen. Many examples have been of epitopes of islet antigens produced by β cells which are then recognized by specific anti islet T-cells.

Tissue specific cleavage might be critical to the development of a target peptide. Eg. the neuroendocrine molecule chromogranin A is cleaved to produce a particular peptide (WE14) with in islets which is recognized by the pathogenic BDC2. 5Tcellreceptor(TCR)of NOD mice with respect to pockets 5-9 of the IA^g c MHC II molecule in the NOD mouse, the only class II presenting molecule in the NOD mouse [16].

In contrast other cells which express or are fed naked chromogranin A do not effectively produce this autogenic peptide. The tissue specific cleavage to produce the WE14 peptides removes four N terminal amino acids which are present in chromogranin A, which if present would fill pockets 1-4of IA^{g7} binding groove, which once removed finishes the binding and natural TCR stimulation. Therefore specific cleavage within beta cells of chromogranin A is essential for diabetogenicity of BDC2. 5Tcells.

Antigenic peptide may occur in islet cells which are not necessarily in APC's exposed to the target molecule. Unanue, et al. discovered that insulin peptide B:9-23is produced within NOD islet cells and that APC process the peptide and the whole insulin secretory granules. APC's belonging to islets can directly stimulate anti B:9-23T cells(without exogenous antigen)and for many antiB:9-23 cell clones, provision of insulin to APC which does not=>stimulation but the processed peptide needs to be presented. B:9-23 peptide presentation is also unusual, low affinity register to $IA^{g7}$and binding in this register is the main point for presentation to NOD and B:9-23 TCR [17].

Further for T1DM to occur it is necessary that T cells target multiple islet antigens eg CD8Tcells clone which target the islet specific glucose 6phosphatase catalytic subunit related protein (IGRP) is very diabetogenic. This only occurs if T-cell targeting insulin peptide or other class II restricted peptides are also present. While removing IGRP auto reactivity does not reduce the DM progression in the NOD mice [18]. In different animal models of DM, $CD8^{+}$Tcells specific for a single antigen can=>loss of β cells and DM [19]. Thus functional synergy between CD4 and $CD8^{+}$Tcells and their cytokine production probably due to potent pathogenic response occurs in the initial stages of disease development. But if targeting of multiple antigens and antigen spreading is required for DM, at which stage it occurs is not clear.

T1DM from β Cell Perspective

At least there are 3 ways by which β cells may cause its own destruction

1) The β cell maybe a very easy target for immune destruction. Stress induced changes which occur during local infections can => increased development of specific auto antigenic peptides recognized by pathogenic T cells.

2) It is seen that there is raised β cell sensitivity to cytokine mediated killing. β cells are specially very sensitive to the cytokine interleukin(IL) 1β (Figure 3).

3) Changes in β cell physiology which are persistent (like hyper expression of class 1 molecules) once autoimmunity has been initiated enhance their sensitivity to immune destruction. Hence it is to be expected that β cells within islets are mainly destroyed even though other cells types like islet cells producing glucagon express many similar antigens and survive.

4) Islet β cells are very prone to cellular destruction which is self directed since they are very sensitive to different forms of endoplasmic reticulum(ER) stress, as shown by mutations which affect insulin protein folding [20].

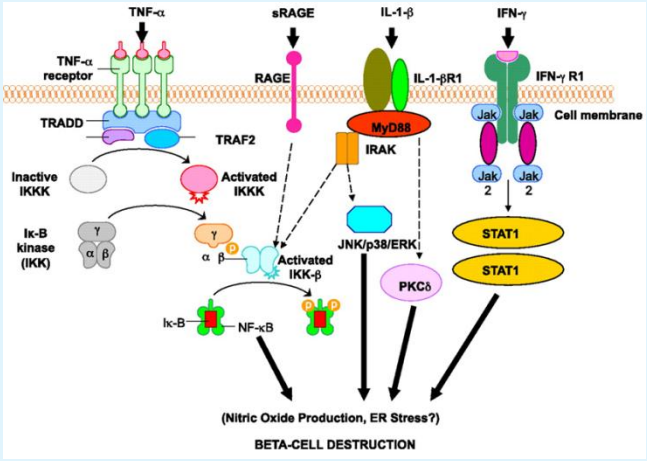

Figure 3: Courtesy ref no 20-Activation of inflammatory mediators in pancreatic β-cells in type 1 diabetes. Tumor necrosis factor-α (TNF-α), IL-1β, and interferon-γ (IFN-γ) are the most likely cytokines acting in synergy during inflammation of pancreatic β-cells, leading to the activation of a final common pathway, such as nuclear factor-κB (NF-κB) and, ultimately, to β-cell destruction. NF-κB can be activated by a variety of stimuli, including TNF-α, IL-1, receptor for advanced glycation end products (RAGE), and Toll-like receptors (TLRs). IL-1β is an inflammatory cytokine that plays a major role in immune-mediated β-cell destruction. Interestingly, in patients with type 2 diabetes, the IL-1 pathway blockade with an IL-1 receptor antagonist (Anakinra) improved glycemic control and β-cell secretory function and resulted in a significant reduction marker of systemic inflammation, namely, C-reactive protein and IL-6 [56]. A recent clinical study indicated that the blockade of the IL-1β pathway in type 1 diabetes resulted in the reduced ability of mononuclear cells to traffic to sites of inflammation [57]. The latter observations provide evidence for a possible mechanistic link between type 1 and type 2 diabetes, and additional studies are necessary to unravel the common inflammatory pathways involved in the pathologic etiology of these two diseases. Compelling evidence indicates that cytokines influence the expression of inducible NO synthase (iNOS) leading to NO production. IL-1β and IFNγ, by NO synthesis, were reported to markedly decrease sarco(endo)plasmic reticulum Ca2+ ATPase 2b (SERCA2b) protein expression, deplete Ca2+

stores, and activate ER stress pathway, which is a potential contributing mechanism to β-cell death. Furthermore, cytokine-induced (IL-1β + IFN-γ) apoptosis of INS-1 cells appears to depend on NO production, as demonstrated by the use of the NO dioxygenase blocker *N*G-methyl-l-arginine. NO also contributes to cytokine-induced apoptosis through potentiation of Jun NH2-terminal kinase (JNK) activity and suppression of Akt/protein kinase B. Although whether oxidative stress plays a key role in the pathogenesis of type 1 diabetes is still being discussed, a reduced antioxidant capacity has been demonstrated in patients with type 1 diabetes compared with healthy control subjects. To summarize the cytokine signaling, TNF-α signals through trimerized p60 receptors that interact with the TNF receptor type 1–associated death domain protein (TRADD). Fas-associated protein with death domain (FADD) is then recruited by TRADD, thus allowing binding of receptor-interacting protein (RIP) and TNF receptor–associated factor 2 (TRAF2) to the receptor complex. TRAF2 activates NF-κB through NF-κB–inducing kinase (NIK)–inhibitor of κB kinase (IKK) and activates the JNK/p38 pathways. TNF-α is an inflammatory cytokine that appears to be associated with a number of autoimmune disorders, including type 1 diabetes. TNF-α may activate intraislet resident macrophages, resulting in the release of IL-1β, which generates iNOS expression and the overproduction of NO in β-cells. Alterations in the number and function of CD4+CD25+ T-cells may be an additional mechanism by which TNF-α may cause type 1 diabetes in NOD mice. The role of RAGE mediated by NF-κB has not been entirely elucidated, although RAGE may be an important intermediary in causing monocyte production of inflammatory mediators such as TNF-α. It is possible that increased expression of RAGE in response to hyperglycemia may lead to activation of innate and even adaptive immune responses and enhance β-cell destruction. After IL-1β binding to IL-1βR1, MyD88 is recruited to the receptor complex. MyD88 interacts with IL-1 receptor–associated kinase (IRAK), allowing the binding of TRAF6 to IRAK. TRAF6 causes activation of mitogen-activated protein kinase/stress-activated protein kinase and activation of the NF-κB pathway by transforming growth factor-β–activated kinase 1 (TAK1)–mediated activation of IKK. IL-1β also stimulates activation of protein kinase C-δ (PKC-δ), possibly through phospholipase C generation of diacylglycerol. ERK, extracellular signal–regulated kinase; Jak, Janus kinase; STAT1, signal transducer and activator of transcription-1.

Metabolic Changes

Right now seroconversion to autoantibody positivity is the first change linking it to an ongoing autoimmune response. As per Oresic, et al. [21] metabolic deregulation occurs before autoimmunity in T1DM. Increased serum concentration of lysophosphatidylcholine (lyso PC) precedes the occurrence of each islet autoantibody. In samples from the Finnish DIPP study cohort [22] characteristic changes in serum metabolites were found only in the children who later developed T1D. These changes included reduced succinate, PC, phospholipids and ketoleucine as well as raised glutamic acid. Pro inflammatory molecules can be activated by the reactive lipid by products [23] which function as a natural adjuvant of the immune system [24]. It remains unresolved if these metabolic events trigger the initiation of the autoimmune period or are just easier to detect.

Atkinson et al using the model of β cell homicide/suicide stated that hyperglycemiamay lead to acceleration of type 1 DM through enhanced antigen exposure by β cells or through other mechanisms such as enhancing their suicide [20]. As per the National Institute of Health Diabetes Care and Complications Trial (CDCCT) the reduction in β cell function was decreased in participants in the group which received intensive insulin treatment versus the control group, which received standard insulin treatment [25]. Even mild persistent hyperglycemia leads to marked depletion of insulin stores, which affects insulin release from the remaining β cells leading to a vicious cycle with ultimate deterioration of glycaemic control [26,27]. These findings provide a motivation underlying metabolic therapies which provide β cells rest to allow for insulin repletion and future secretory response [28]. Subtly islet secretory defects occurring early are now an accepted part, in at least a subset of patients who develop T1DM [29].

Role of B Cells in DM Associated Anti Islet Antibodies

In T1DM important auto antibodies are reactive to islet auto antigens (islet cell auto antibodies or ICA), Insulinoma associated antigen 2(I-A2,ICA512), insulin(micro IAA/m IAA,GAD65,and zinc transporter 8(Zn 8)[30]. The early presence of auto antibodies implies a role for antibody producing plasma B cells in the initial immunological events. B cells definitely contribute to Pathogenesis of human T1D [31]. In the NOD model B cells infiltrate the pancreas during early stages of insulitis and genetic or antibody mediated ablation of B cell in NOD mice is protective [32,33]. B cells are likely active participants in the immune response of their capacity to present antigen to diabetogenic CD4and CD8 T cells.

Besides having histo compatibility complex polymorphisms, β cell auto antibodies and auto reactive cells, T1D, it has become clear that innate cells may also

play critical roles. Thus Valle et al aimed to monitor peripheral immune cells in early stages of T1D (i. e. in healthy autoantibody positive subjects) and in more advanced phases of the disease (i. e. at disease onset and years of diagnosis). They found a mild but significant and reproducible peripheral neutropenia which both precedes and accompanies T1DM. This decrease was not due to peripheral neutrophil cell death, impaired differentiation, or due to presence of anti neutrophil antibodies. Neutrophils were observed by electron microscopy and immunehistochemical analysis in the exocrine pancreas of multi organ donors with T1D (both at onset and later stages of disease) and not in that of multi organ donors with T2DM. These pancreatic infiltrating neutrophils were mainly localized at the level of very small vessels. Thus their findings suggested the existence of a currently unrecognized clinical phenotype, which might reflect unexplored pathogenic pathways underlying T1DM [34].

Role of Islet Specific T cells

T-cells are considered to be the final executors of β cell destruction. Proof for this is the precipitation or prevention of DM by transfer or removal of CD4 or CD8T cells respectively. CD8Tcells mediated β cell killing is likely a major mechanism of β cell destruction. CD8T cells present in insulitic areas in NOD mice as well as human (Figure 4) can destroy β cells upon activation through MHC class 1expressed in β cells. If there is MHC class I deficiency secondary to lack of β2 microglobulin or there is β cell restricted MHC class 1 deficiency this is enough to stop development of DM, prevent β cell destruction in NOD [35]. Mechanistically this β cell destruction can involve the release through CD8T cells of cytolytic granules, which contains perforin and granyzyme, or by Fas and Fas lig and dependent interaction. CD4 T-cells mostly help both Bcells along with CD8Tcells by giving cytokines like IL-21 as well as a positive feedback loop by CD40L-CD40 interactions to APC which ends in an active auto reactive CD8T cell response. Presence of these cells in insulitic lesions suggests a sign and direct inflammatory qualities. Looking for auto reactive T-cell epitopes has been centered around the four proteins, which are also the major autoantibody targets; pro insulin (PI), GAD65; IA2 and Zn T8 [36].

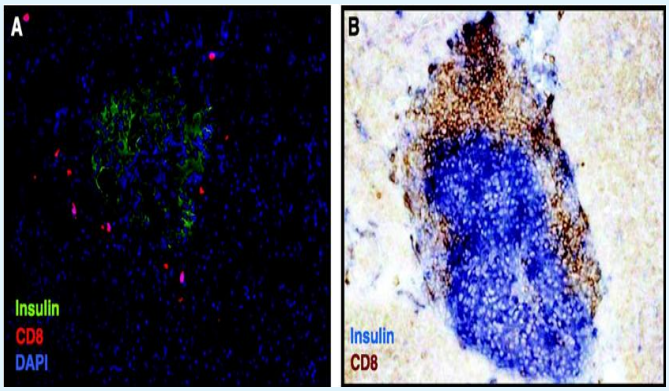

Figure 4 : Courtesy ref no13-The degree of pancreatic infiltration in T1D patients is limited compared with the insulitis in NOD mice around diabetes onset. Infiltration in or around the islets of Langerhans in the pancreas comprises CD8 T cells, CD4 T cells, but also B cells, macrophages and very few dendritic cells. In this respect, i.e., the type of cell infiltrating the islets, pancreatic section from human and NOD mice correspond. But the degree to which the pancreas and islets are inflamed, i.e., the number of infiltrating cells that can be found, is much more limited in humans than in NOD mice. *A*: typical degree of infiltration in recent-onset type 1 diabetic pancreas in humans. Staining of human pancreas sections for insulin (in green) and CD8 T cells (in red) indicates that only low levels of CD8 T cells can be seen in the proximity of the islets. A similar low degree of infiltration is observed for CD4 T cells and B cells in pancreatic sections of T1D patients (not shown). More information can be found in Reference 99. *B*: typical level of inflammation around diabetes onset in female NOD mice. Staining for insulin (in blue) and CD8 (in brown-red) shows a severe degree of infiltration by CD8 T cells in the islets. The type and degree of infiltration of CD4 T cells at this time point are usually similar (not shown).

Antigen specific Intervention Trials

Basically the idea behind anti gen (Ag) based therapeuticsis to induce Treg responses (active tolerance), anergizing/deleting pathogenic T-cells (passive tolerance), without having side effects of long term immune suppression. Tolerizing against insulin or GAD 65has been effective in experimental setups [37,38]. This approach has led to success in T1D patients [39-41]. Future trials should also target other peptides [36]. Some investigations in DM prone mice suggest that ignored determinants of β cell Ag's are a more optimal choice to inhibit late stage optimal disease [42]. Various clinical intervention trials use insulin for targeting as it is the antigen which initiates in the NOD mice, in addition to being a major auto Ag in human T1D. A phase I trial has

confirmed the data in animal models that incomplete Freunds Antigen(IFA) enhanced human insulin B chain vaccination is not only safe, but also can induce insulin specific Treg for upto 2years after vaccination[43]. The effects on glycaemic control will be revealed by a follow up trial [44]. ACpG –free pro insulin based DNA –plasmid vaccine BHT-3021from Bayhill Therapeutics is a 2nd approach. This vaccine is designed to tolerize the immune system to pro insulin by combining DNA codons for an immunodominant peptide of insulin [45] and immunomodulatory CpG oligonucleotides [46]. Data on recent onset diabetics, NOD mice suggested that BHT 3021 induces proinsulin –specifc Treg which can act as bystander suppressors. In new cases of DM, the vaccine can preserve C peptides and reduce HbA1C [47]. This shows raised efficiency of Bayhill plasmid as compared with the first generation of insulin B expressing pCMV Plasmids [48].

GAD65 is other target for Ag specific therapy. GAD Alum is an aluminium hydroxide (Alum) formulation of full length recombinant human GAD 65(Diamyd Therapeutics). This was found to be safe and to preserve insulin secretion in patients with late onset autommune Diabetes of adult hood (LADA) [49]. A phase II trial later in recent onset T1D showed marked preservation of residual insulin secretion and a GAD specific immune response, both humoral and cell mediated [50]. Phase III studies are on way in Europe and USA. Selection of patients in these trials was on criteria of increased GAD65 auto antibodies. The formulation is causal to Dynamid's GAD Drug as i) adjuvant decreases the amount of Antigen required and ii) aluminium salts preferentially induces humoral rather than cellular immune response [51]. Immune readouts show an increase in FoxP3 and TGFβ in cells from GAD-Alum treated patients as compared to placebo after 15mths [23]. Still it is very early to conclude whether GAD 65and/or insulin are optimal target Antigen's to induce Treg which can modulate the course of human T1D,Combination therapies with a short term course of a suitable immune modulator are considered to increase efficiency in recent onset patients.

Intervention Trials-Non Ag Specific

As autoimmunity is the main effectors mechanism in T1D, many trials have used drug regimens to silence and or modulate the immune response preferably without negative effects on Tregs. These immunosuppressive regimens will also prove valuable, if not critical for the success of islets transplants or/ β cell regenerative therapy.

First immunosuppressive agent used in placebo controlled double blind clinical trials for T1D was cyclosporine A. Cyclosporine inhibits calcineurin which is responsible for the activation of IL-2 transcription. Lack of IL-2 and other cytokines decrease the function of effectors T cells, but also of Tregs. Although cyclosporine treatment induced remission of T1DM, its chronic use had to be dropped because of unacceptable side effects [52,53]. However the limited success indicated that immunosuppressant can decrease the autoimmune inflammation in T1D.

Dia pep 277 was initially used in an Antigen specific therapy. The idea was that this peptide from HSP60 becomes an auto Antigen in T1D because of cross reactivity [54,55]. Insights got from later studies show that it is a systemic modulator, HSP60 causes Treg induction through TLR2 [56,57]. In a phase II trial it was shown that DiaPep277 preserved C Peptide upto 18mthsin adult new onset T1DM patients [58]. IL-10 production accompanied β cell preservation before treatment and decrease in auto antigenic specific T cell proliferation after treatment [59]. But till now no Dia Pep 277 specific Treg have been characterized in mice or humans. However while a phase III study is ongoing in adults, no treatment effect was observed in children with T1DM [60]. One important class is biological modulators, which comprises of Antibodies' (Ab) with target receptors on T cells, e.g is FcR nonbinding anti C3 monoclonal antibody (mAb) have shown the most promising results thus far in T1D therapy. Anti CD3 mAb acts at various levels. It causes a short term internalization of the TCR – CD3 complex which makes the cell blind to Ag [88]. Also it alters TCR mediated signal transduction so that energy or apoptosis is induced preferentially in activated Th1 cells [62]. The apoptosis gets mediated partly by CD95-CD95L interaction with neighbouring cells. This may explain why effecter T cell death is most dramatic where the T cell density is highest at the site of inflammation. Moreover antiCD3 treatment also results in Treg development [63]. It is thought that the Treg may protect against damage by effectors T cells long after the drug has been eliminated from the body. To get these entire effects optimal dose is essential. If mAb is too low it causes insufficient modulation and Treg generation while too much mAb could=>stimulation of the effector Tcells and cytokine release. Multiple clinical trials have been initiated and were based on 2 different Ab's both fully humanized CD3; Teplizumab (US Trials) TRX4; Oleplizumab (European Trials). With teplizumab progression of recent onset T1D got halted for>1yearin most patients (phase11)but 3yearfollowing therapy the patients continued to have better preservation of C peptide levels and a lower use of insulin as compared to controls[64,65] . TRX4/Otelixizumab also preserved β cell function very efficiently and decreased the insulin requirement drastically even 18mths after single course therapy [66].

Yet none of these treatments achieved euglycaemia. The European study also revealed 2 side effects i) anti idiotypic Ab's were detected 2-3 weeks after injection of the drug. This should only become a problem when repeated treatment is needed. . ii) There was reactivation of Epstein Barr virus but this was temporary self limiting and isolated [67]. Herold, et al. studied if 2 courses of teplizu mab, decreases the decline in C peptide levels in patients with T1D 2years after disease onset. They treated 52 patients with new onsetT1D with teplizumab for 2weeks at diagnosis and after 1 year in an open label, randomized controlled trial. In the intent to treat analysis of the primary end point, patients treated with teplizumab had a decreased decline in C peptide at 2 weeks (mean-0. 28nmol/L (95%CI-0. 36to-0. 20) versus control (mean-0. 46nmol/L (95%CI-0. 57 to -0. 35;p=0. 002), a 75%improvement. In a post hoc analysis they characterized clinical responders and found that metabolic (HbA1c and insulin use) and immunologic feautures distinguished this group from those who did not respond. Hence they concluded that teplizumab treatment preserved insulin production and reduces the use of exogenous insulin in some patients with new onset T1D. Metabolic and immunologic features at baseline can identify a subgroup with robust responses to immune therapy [68].

Other approaches using polyclonal anti T-cell Ab and ALS [69] and anti thymocyte globulin (ATG) might be able to temporarily eliminate a larger proportion of T-cells from the blood stream. Murine ATG can prevent DM in a late stage in NOD mice and can induce Treg [70]. It is not clear whether [ATG-Fresenius] prolonged the honeymoon period and improved stimulated C peptide levels upto 12mths into the study [71]. Hence ATG mono therapy is now tested in a phase II trial, the study of Thyroglobulin to arrest T1D (START). Yet ATG can lead to development of cytokine release syndrome and maybe even a lymphopenia induced outgrowth of auto reactive T-cells as was shown for other depletion based immune suppression [72,73]. Combination treatment with equine ATG and prednisone, a steroid which can counter act the cytokine release syndrome=>prolonged honeymoon phase in new onset T1D [74]. But most promising in the ATG trials was that some subjects went into complete remission and were insulin independent for at least 1mth.

CD20 specific mAb rituximab (Rituxane) is an immunemodulatory drug borrowed from the translational arena. The aim of rituximab is to erase potentially potent Ag presenting population of B-cells without affecting long lived Ab producing plasma cells [32]. B-cells are needed for T1D is supported by work in NOD mice [75]. In a phase II clinical trial some preservation of C peptide levels for 3-6mths was seen [31], which confirms that B cells also are involved in pathogenesis in T1D. But the efficiency is small as compared to anti CD3 treatment [65,66]. Supposedly Ocrelizumab, a humanized anti CD20 Ab successfully used in RA [76] may increase efficiency of β cell depletion in T1D.

Basiliximab an anti CD 25mAb (chimeric mouse human mono clonal Ab) and daclizumab (humanized IgG1 mAb) do not cause a cytokine release syndrome). Hence they are being used much regularly in place of ATG as an induction therapy. However in T1D antiCD3 mAb are only used in combination therapies.

Costimulatory molecules CTLA-Ig (Abatacept) belongs to another class of target molecules. CTLA4 fused to an immunoglobulin chain interferes with co stimulation of T-cells. CD28/B7interactions mediate costimulation and markedly increase peripheral T cell response. Conversely CTLA4 interacting with the same B7 molecules dampen T cell activity. Hence CTLA4Ig mediates its marked effects by preventing positive costimulation of CD28 by B7during activation. This leads to limited clonal expansion, induction of passive cell death and IDO (reviewed in 77,78]. Safey profile of CTLA4 by treatment might be better than other immunosuppressive agents. CTLA-Ig does not deplete T cells. However because of the role of CD28 in Treg development and survival [78], CTLA4 Ig may negatively affect Treg. But CTLA4ig therapy did not affect Treg in renal transplantation [79]. CTLA4-Ig monotherapy is currently in a phase II clinical trial. A high affinity variant of CTLA4Ig (LEA29Y,betacept),is being tested in islet transplantation in a phase I/II trial(NCT00468409)and the LEA29YEmory Edmonton Protocol (LEEP-NCT00468403)phase II clinical trial combines CTLA4Ig with daclizumab or basiliximab (against active transplant rejection)and mycophenolate mifelib (maintenance immunosuppressive therapy [80].
Cytokine manipulation has been revived currently. Cytokines which affect IL-2,IL-15 and those Which have a role in inflammation and β cell death are considered as targets. IL1 is selectively cytotoxic to rodents and human β cells in vitro. Anti IC1 therapies can decrease diabetes incidence in animal prevention models [81-83]. Just like in RA, several trials assess if anti IL1 therapy may be used in the treatment of T1D. Results from a completed phase I/II trial using IL 1RA (anakira, Kineret by Amgen [84] in new T1D cases have not been released [85]. In an ongoing phase II/III pts will inject themselves Anakira once dailyx2years, which requires big commitment on their part. Also 3 phase II trials are pending. One will test Canakinumab, a fully human anti IL1β monoclonal Ab, in new onset T1D patients. Another will test the anti IL1 mAb Roma 52 in established (>2yrs) well controlled T1D. The RID study will test Ritonacept, a dimeric fusion

protein as compared to cytokine trap for IL-1β after satisfactory safety data in gouty arthritis [86].

The cytokine tumour necrosis factor (TNF) α is a master regulator of the inflammatory response in many organ systems [87]. TNF α antagonists like etanercept (a soluble TNF receptor) and infliximab (an Ab)are being used successfully in RA. In T1D, phase II trials data showed that treatment with etanercept reduced Hb A1C and increased endogenous insulin production which indicated that it =>preservation of β cell function [88]. However it is not that simple. TNFα may play a double role in T1D. eg TNF and TNFR2 agonists can selectively kill human auto reactive CD8Tcells [89]. In animal models, TNF α propels or decreases the diabetogenic response early or late in the T1D process respectively [48,90,91]. Further confusion is created by some clinical case reports regarding development of T1D in arthritis (JIA or RA) pts following etanercept therapy[92,93], but also the resolution of T1D in patients needing anti TNFα therapy for RA [94]. This controversy needs to be resolved. Importantly Bacille Calmette Guerin (BCG) vaccination increases systemic levels of TNFα and was used in an unsuccessful interventional trial. A Follow up study will show better timing and dosage [95,96].

Different hypothesis is that autoimmunity is due to lack of type 1 IFN. Type 1 IFNs can counteract type II IFN, which is most probably a central factor in autoimmune inflammation [97]. Clinical trials have so far shown that low dose ingested rh IFNα is safe and more effective at preserving C peptide levels as compared to high doses [98,99]. Mechanistically ingested rhIFNα de creased TNFα levels in MS patients which indicated link with TNFα blockade therapy [100]. But this hypothesis is controversial. These suggestions that activation of TLR s by double stranded RNA or poly 1 c viral mimic through induction of IFNα may activate or accelerate immune mediated β cell destruction [101].

Granulocyte colony stimulating factor (GCSF), a neutrophil mobilizing agent prevents DM in NOD mice by induction of both tolerogenic dendritic cells and Tregs [102,103]. Whether GCSF (Neutralata) is safe and preserves C peptide is under phase I/II trial. Also a combination trial along with ATG is on way.

Another form of treatment tries to delay the β cell destruction by decreasing the amount of insulin they secrete. This is thought to decrease β cell stress associated with the diabetic state and may also decrease the presented auto antigen like proinsulin. Diaz oxide An ATP sensitive K channel opener showed preservation of insulin production in recent onsetT1D patients but also substantial side effects are also not effective in preserving β cell function [104].

Cell based Therapy

From animal models, cell based tolerogenic immunotherapy is getting popular. Basic idea is to compensate for deficiency by transferring cell types which have immunomodulatory capacity. It was first indicated by immune tolerance getting reestablished after adoptive transfer of autoantigenic specific Treg or Tr into NOD mice [105,106]. Clinical trials needed to expands outside and reinfusion of larger numbers [107].

Various technical problems may be faced for a bonafide set of markers for pure human Treg (recently set at $CD4^{+}CD127^{low/minus}$ $CD25^{+}$[108-110], a low frequency of transfers, in vitro expansion methods, survival of these cells in vivo, corrected homing to the target tissue, the inability to eliminate the transferred cells and instability of the regulatory function [111,112]. Therefore the field is divided into believers and nonbelievers. Some cell based tolerogenic therapy, a viable routine clinical approach. Others prefer to target β cell Ag's in conjunction with small molecules or mAb to augment islet specific immune regulatory cells directly into immunoregulatory dendritic cells [IDC]. This can also prevent DM in NOD mice [113,114]. In current clinical trials in Pittsburgh autologous monocyte derived DC's are treated exvivo with antisense phosphothionate modified CD 86 costimulatory molecules. One problem is that instability of antisense knockout mice allow reexpression of targeted molecules. Also the therapy rationale promotes the production of IL7 BY Idc as survival factor for Treg. But IL7 is also important for naïve and memory cells [113,115], so presumably for autoreactive T-cells as well. B cells with a regulatory phenotype were augmented in some of the patients who received the immunomodulatory DC's.

Replacing Cell Shortage

Aim of some treatment is to directly compensate for the β cell loss as simple used form, which is done by insulin injections. Other treatments increase β cell display in response to autoimmune attack [116] or non immune stimulus [117,118].

These include

I) Stimulation of insulin secretion ii)islet neogenesis from existing β cells mass.

iii) Islet neogenesis from progenitor cells iv)islet transplantation v)islet transplantation of stem cells

i) Stimulation ofinsulin secretion

Borrowed from T2DM therapy GLP1 agonists exenatide and liraglutide have long half life, which binds to albumin for slow release [119,120]. GLP1 receptor activation modestly delay DM onset in NOD mice [121]. Mechanistically exenatide not only stimulates insulin secretion but might also increase β cell replication and increase Treg frequency in NOD mice. These effects on β cell mass and the immune system are controversial. Exenatide monotherapy is in phase IV trial and a combination trial of exenatide and daclizumab gave disappointing results [122]. While liraglutide supports the engraftment and function of syngeneic transplants in NOD mice[123],this has =>phase II/III trial testing liraglutide monotherapy.

One can try to slow down the physiological degradation of GLP1. S itagliptin can prolong islet graft survival [124,125] and can partially reverse DM in NOD mice [126].

Fujita T et al in concert with Taito Co(T; Mitsui Sugar Tokyo Japan and Yoshitomi Pharmaceuticals Industries Ltd (Y. Mitsubishi Tanabe Pharma corporation, Osaka Japan)developed an immunomodulator F7Y720 (fingolimod), which is a synthetic analog of myriocin (ISP1),a metabolite of isaria cinclariiin Japan[127,128].

This FTY720 besides acting in preclinical transplantation models, also acted in various models of immunological diseases like rheumatoid arthritis [129], myasthenia gravis [130], multiple sclerosis [131], type 1 diabetes [132] and atopic dermatitis[133].

FTY720 has different mechanism of action from other immune suppressants like sacrolimus hydrate and cyclosporine. Fingalimod effects SIP signaling by inducing partial degradation of the receptors [134-138]. Hence it suppresses immune response by cornering circulating mature lymphocytes from blood and peripheral tissues to the secretory lymphoid tissues and thymus [137,138].

Sitagliptin a dipeptidyl peptidase 1V (DPP4) inhibitors is a new approach in therapeutic arena of type 2 diabetes mellitus. DPP-4 metabolises incretin hormones like glucagon like peptide1 (GLP1) and glucose dependent insulitropic polypeptides (GIP) and hence sitagliptin causes an increase in the amount of these hormones. Active GLP1 and GIP activates glucose dependent insulin biosynthesis and release, and GIP also reduces glucagon release, delaying gastric emptying and improves satiety. DPP4 inhibitors enhance glycaemic control, insulin secretion and β cell function in rodents [139]. In non obese diabetic (NOD) mouse, a very good animal model of spontaneous type 1 diabetes mellitus [140], autorective Tcells attack islet β cells=>depletion of insulin secretion in these mice.

Usually type1 diabetes mellitus occurs during childhood and treatment comprises of intensive insulin therapy, which consists of four or more insulin injections daily. This insulin self injection puts a big burden on patients and there is difficulty in getting an acceptable blood glucose control, mainly in a period when secondary sexual characteristics are developing. Tsuji et al had earlier reported the effectiveness of FTY720 in combination with once daily insulin glargine injection [132]. In this study they examined the effect of FTY720 in combination with sitagliptin and examined it in NOD mouse. They divide NOD mice who had developed type 1 DM, spontaneously into four groups based on the therapy they received i) FTY720 (0.1mg/kg orally six times a week plus sitagliptin1mg/kg orally six times a weekii)FTY720 (0.1mg/kg orally six times a week iii)Sitagliptin1mg/kg orally six times a week and iv)the vehicle(water alone). Therapeutic efficacy was examined in terms of survival rate, ratio of insulin positive β cells /total islet area, extent of islet inflammation, insulin score)and blood glucose levels; The administration of FTY720- plus sitagliptin markedly improved survival (83% at 70 days after onset $p<0.05$)compared with sitagliptin alone(17%)or vehicle alone(. 0%). The fasting blood glucose level, the ratio of insulin positive β cells /total islet area and the insulin score in the surviving mice, which had been treated with FTY720 plus sitagliptin were improved to the normal levels as in age matched NOD mice with normoglycaemia. Thus they concluded that combination therapy with FTY720 and sitagliptin is a promising candidate for type1 DM treatment, and might allow the treatment of type1 DM with oral agents only [140].

B Cell Neogenesis

Gastrin stimulates β cells neogenesis without increasing proliferation and hypertrophy and without decreasing β cell death [96,141]. Also β cell mitogenic qualities of EGF analog E1-INT (Transfer therapeutics) decreased daytime insulin usage by 35-75% and helped maintain stable glucose control as measured by HbA1C in the T1D patients. As per the company, results were same in NOD mice. But others showed that gastrin and EGF are required to be used together to raise β cell mass and correct hyperglycaemia in recent onset NOD mice [142,143]. A phase I study (E1+GII NT, NCT00853151) is ongoing.

Islet Cell Regeneration

Islet neogenesis associated protein (INGAP) peptide therapy induces islet cell regeneration from progenitor cells which are resident in pancreas in a manner which recalls islet development during normal embryogenesis [144]. INGAP peptide can increase β cell mass and reverses hyperglycaemia in animal models [111,145]. In phase I/IITRIALS INGAP peptide injections are safe and can increase C peptide secretion but hardly decrease Hb A1clevels[146]. A trial to optimize dose is on.

Islet Transplantation

Edmonton case series show that roughly 2/3rd of recipients remained insulin independent for 1 year following their final islet infusion [147]. Long term results are unfortunately not encouraging. Islet function decreases over time and 5years post transplantation <10%of the recipients remain insulin independent [148,149]. Allosensitization against transplant from multiple donors can be controlled with immune suppression, but the regimens being used these days may propel auto reactivity in long term along with acting negatively to affect β cell function. In Edmonton protocol, patients infused with pancreatic islets from multiple cadaveric donors simultaneously got immune suppression in form of a humanized CD25 mAb (daclizumab)and continuously administration of low dose rapamycin (sirolimus),which inhibits the response to IL-2 and FK506 (tacrilimus), a calcineurin inhibitor blocking IL2 production . Yet the regimens causes lymphopenia and a rise of levels of homeostatic cytokines which drive the expansion of autoreactive CD8Tcells [150,151]. Hence Edmonton protocol has been modified in several ways e. g ATG+ Etanercept and maintenance immunosuppression using cyclosporine and everolimus (a sirolimus derivative) made 5/6recipients insulin independent at 1 year and 4/6for additional 3years[76,147]. A recent phase I/II trial will assess if treatment with anti CD3mAb sirolimus and low dose tacrolimus can prevent islet transplant rejection. But animal studies regarding antiCD3 have shown that they do not induce tolerance when tacrolimus was given together even though it continues immune suppression [152]. Another phase II trial will test efficacy of a steroid free calcineurin inhibitor free immunosuppression protocol for islet transplantation (NCT00315627) based on sirolimus ,MMF and compath. T1D pts getting intraoral islet cells under ATG –tacrolimus –MMF therapy have lower graft function if auto reactive T cells were detected before transplantation [153]. Current immune suppressive drugs can also interfere with β cell function [112,154]. Rapamycin impairs engraftment [155], with angiogenesis [156], causes insulin resistance [157] and inhibits β cell replication [158]. Rapamycin also like corticosteroids, tacrolimus and MMF decreases insulin transcription [154]. A study also suggested that MMF also inhibits β cell neogenesis [159].

Human islet cell isolation techniques are still unsatisfactory [161] to yield 12,000 islet equivalent /kg body weight required to restore insulin independent normoglycemia in recipients [162].

Stem Cells

Beta cell production can be done by differentiation of embryonic stem (ES) cells or [234] induced pluripotent cells (IPS) [163] or the reprogramming of cells from their insulin phenotype into β like cells [164]. Stem cells can regenerate the β cell mass in vivo as shown for bone marrow derived stem cell transfers in immunodeficient mice, which chemically induced pancreatic damage [109]. But in a phase I/II trial stem cells from umbilical cord blood assisted the preservation of C peptide levels poorly [165]. Zhao et al hypothesized that human cord blood derived multipotent stem cells (CBSC's) can control autoimmune responses by altering regulatory T cells and human islet β cell specific T cell clones offered a new approach to overcome autoimmunity underlying T1D. They developed a procedure for Stem cell educator therapy in which a patient's blood is circulated through a closed loop system which separates lymphocytes from the whole blood. It briefly cocultures them with adherent CBSC's before returning them to the patients circulation in an open label phase I/II study, patients(n=15)with T1D received one treatment with the Stem cell Educator. Median age Was 29years (range 15-41)and median diabetic history was 8years(Range 1-21). Stem cell educator therapy can markedly improve C peptide levels, decrease the median glycated haemoglobin A1c values and decrease the median daily doses of insulin in patients with some residual cell function(n=6)and patients with no residual pancreatic islet β cell function(n=6). Treatment also produced an increase in basal and glucose stimulated C peptide levels through 40weeks. However participants in the Control group (n=3)did not establish significant changes at any follow up. Individuals who received Stem cell Educator therapy exhibited raised expression of co stimulating molecules, specifically CD28 and ICOS)increases the number of $CD4^+CD25^+FoxP3^+$Tregs and restoration of Th1/Th2/Th3 cytokine balance. Thus they concluded Stem cell Educator therapy is safe and in individuals with moderate or severe T1D, a single treatment produces lasting improvement in metabolic control. Initial results indicate Stem Cell Educator Therapy, reverse autoimmunity and promote regeneration of islet β cells. Successful immune

modulation by CB-SC's and the resulting clinical improvement in patient status may have important implications for other autoimmune and inflammation related disease without the safety and ethical concerns associated with conventional stem cell based approaches [166].

Autologous no myeloid ablative hematopoietic stem cell transplantation (HSCT) give better results in newly diagnosed T1D [167,168]. Although insulin independence was derived but was lost after 4-5years in most recipients and side effects discourage the use of this approach for universal therapy [167].

Stem cells can potentially also be differentiated to β cells in vivo. Use of autologous stem cells avoids alloimmune but not autoimmune responses to transplanted like cells. Lot of effort is invested in the encapsulation techniques to protect the transplanted cells or islets after implantation. For instance Viacyte differentiates ESC's into pancreatic endoderm and has plans to subsequently to get these cells enveloped in a protective permeable device for transplantation (Encapra) [168,169]. Idea is that islet like structure forms and becomes functionally responsive to glucose in vivo. Encapsulation approaches had been shown to protect human fibroblast allograft from rejection in rhesus monkeys [170]. Also implantation of murine β cells encapsulated in a similar device could ameliorate DM in NOD mice [171]. Viacyte formerly known as Novocell published that their differentiated hESC develop glucose responsive insulin producing cells. These can protect against streptozotocin induced DM in mice [172]. But recent data examining the approach in athymic nude rats could not completely continue this. Islet like structures did develop from h ESC differentiation in pancreatic endoderm. Yet the extent of endocrine cell formation and secretory function was considered insufficient for clinical relevance [173]. An alternative, the alginate microcapsule containing porcine islets from Diane cells allows some insulin production upto 9. 5years post transplant [174], and is current being tested in phase I/II(NCT00949173)Living Cell Technologies. Similarly the Cell Pouch is a device which gets implanted s /c. This is prior to delivery of transplanted cells to allow tissue and blood vessel formation (Sernova).

In humans a small scale study showed long term transplants survival and positive effect on metabolic control [174] but extensive clinical trials have not yet begun. Though pro mising 2 problems could arisei) clogging may limit influx of nutrients and glucose and efflux of insulin and ii) Solute mediators that elicit β cell death can still react with the transplants.

Addio, et al. aimed to determine the effects of autologous nonmyeloablative HSC transplantation in 65paients with new onset T1D,enrolled in 2 Chinese centres and one Polish centre pooled and followed up for 48mths. Atotal of 59%of individuals with T1D achieved insulin independence within the first six months after receiving conditioning immunosuppression therapy (with antithymocyte globulin and cyclophosphamide) and a single infusion of autologous HSCs And 32%remained insulin independent at the last time point of their follow up. All treated subjects showed a decrease in Hb A1c levels and an increase in C peptide levels compared with pretreatment. In spite of a complete immune system recovery (i. e leukocyte count) after treatment, 52%of treated individuals experienced side effects. Thus they concluded the following i)that remission of T1D is possible by combining HSC transplantation and immunosupression ;ii)that autologous non myeloablative HSC transplantation represents an effective treatment for selected individuals with T1D;and iii)that it is in 2 mouse models of diabetes after HSC based therapeutics options are required [175].

Clinical Therapies

Insulin Substitution

Many forms of insulin have raised the quality of life in T1DM patients. Insulin although does not provide a cure, it will remain the major therapy in short term and probably will be used as supplementary to other treatments over a long term. Continuous blood glucose monitoring using glucose monitors or artificial pancreas which is closed loop insulin delivery systems make DM management comfortable but still problems remain [176]. With preclinical data a favorable approach use is through use of smart insulin which consists of layered, biocompatible and biodegradable polymer therapeutic which is based 0n and engineered glucose binding molecule. Insulin gets released only when it is unbound by the presence of a particular glucose concentration.

Combination-Human Modulators and Islet Antigen Vaccines

Suboptimal doses of FcR nonbinding anti CD3F (ab')2 along with intranasal administration of pro insulin peptide can reverse DM in 2 models of mouse DM [177]. Tregs get induced which secrete immunomodulatory cytokines like TGFβ-and IL-10 and confer dominant tolerance upon transfer to recent onset diabetic recipients. On follow up experiments greater application of this approach was shown; antiCD3 along with a GAD 65 plasmid vaccination would synergize in a RIP-LMCV model of T1D [177]. But success depended on the genetic

background, maybe due to the way antigen is presented to the Treg [178].

Combination Therapy-Immune Modulation with Compounds Increasing Beta Cell Mass or Function

Exanatide along with anti CD3 combines induction of immune tolerance by FcR nonbinding anti CD3 mAb with stimulation of insulin secretion of the remaining beta cells. Since this approach tackled 2 problems in T1D, there are higher chances of success with this approach as compared to the beta cell regenerative agents like gastric and exenatide trials, which lack the immunomodulatory arm needed to stop autoimmune attack of the pancreatic β cells. Despite that favorable results in diabetic NOD mice have led to a phase II traial of gastrin & extendin [179,180] while an anti CD3+EXENATIDE trial is anticipated.

Cytokine Based Therapeutics

This is a complex matter as varied range of efficacious and side effects are obtained Interfering with majority of cytokines manipulations showed a double effect depending on the timing, dose and route of administration. Eg both IL18 and TNFα Accelerate or inhibit T1D in NOD when administered early or late respectively [46,181,182]. The complex nature of this obstructs the clinical translation of cytokine targeted strategies. One strategy in combined administration of rapamycin with agonist IL-2 Fc and antagonist IL-15c fusion proteins, which causes longterm engrafment /tolerance in allotransplant models [183]. Basic idea is to limit effector T cells activation and expansion by blocking IL15 signals while promoting Treg (IL2 and rapamycin). There is essential role of IL2/IL21R axis in autoimmune DM in NOD [184] along with its role in genetic susceptibility [104].

Conclusion

With the rapidly increasing incidence of this disease globally a major problem lies in early detection of preclinical disease for which staging of Ingler, et al. [7] is very useful. i) Knowing cause is more important than presentation at onset therapy ii) At onsetor near onset therapy one need an immune silencing or modulating component.

1)Therapy can be based on C peptide levels; if >0. 5(Nm) a two component may be sufficient as natural β cell regeneration can still occur.

2) If C petide 0. 2-0. 5it needs additional beta cell regeneration components like exenatide and gastrin

3) If C peptide <0. 2 it suggests need for islet cell transplantation.

It is needed to conduct trials with treatments like anti CD3 which are effective at onset (alone or in combination). Early detection is to preserve beta cell mass and minimize complications of chronic inadequate glycaemic control.

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