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Studies on Autoimmune Diabetes in Latvians and

Other Populations

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To my husband Claes and

our little miracle Ulrika

Contents:

SUMMARY

IDDM is an autoimmune disease, characterized by autoimmune mediated loss of insulin secreting ß- cells. GAD65 and IA-2 are major ß-cell specific autoantibodies and are not found in healthy population. IDDM is associated with certain HLA class II alleles. IDDM is positively associated with HLA DR3 and DR4 in Caucasians. From HLA-DQ molecules, DQ8 (DQA1*0301-DQB1*0302) is the most prevalent IDDM haplotype in Caucasians, followed by DQ2 (DQA1*0201-DQB1*0501). Taken together, DQ8, DQ2 or both account for as many as 89% of Caucasian IDDM patients. DQ6 (DQA1*0102-DQB1*0602) is associated with the protection against IDDM in most populations.

A certain group of NIDDM patients doesn't respond to oral hypoglycemic treatment and insulin therapy is required. Majority becomes insulin - deficient because of autoimmune ß-cell destruction. It is described as latent autoimmune diabetes in adults (LADA) or slow - onset Type 1 (autoimmune) diabetes.

MRDM as a separate form if diabetes mellitus, described in tropical regions and associated with undernutrition. The question remains if autoimmunity and genetic markers are associated with the development of MRDM.

The aim of this thesis was to investigate prevalence of different HLA class II molecules in different types of diabetes and different populations to answer the question if autoimmune diabetes is similar in most populations and if different genetic background in general population is responsible for different incidence of IDDM.

Another question was if GAD65 and IA-2 are main autoantigens in IDDM and autoantibodies against them are associated with IDDM, irrespective of ethnic background. And final question was if slow onset autoimmunity is present among patients diagnosed as NIDDM in different populations.

The study on HLA markers showed that DR3-DQ2 and DR4-DQ8 were positively associated with the disease in Latvian diabetics. The negatively associated DQ taken together were present in more than 75% of healthy controls. The excess frequency of the negative associated DQ molecules in the general population could explain the lower incidence of IDDM in Latvia.

Indian diabetics showed positive association of IDDM with DR3 and DQ2 but not DR4 and DQ8. DQ6 (A*0102-B*0601) showed to be negatively associated with IDDM in South Indians. In the MRDM group - PDDM patients showed positive association with DR3 and DQ2, while FCPD patients had association with DR7 and DQ9.

GAD65ab were present in 57% of South Indian IDDM patients while 97% of Latvian IDDM patients carried either GAD65 or IA-2 autoantibodies. 55% of NIDDM patients carried one of the autoantibodies suggesting high prevalence of slow onset autoimmunity. Antibodies against minor autoantigens (TTG and 21-OH) add 1% of positivity in IDDM group. Still 10% of IDDM patients are negative for any of analyzed autoantibodies, suggesting involvement of other possible autoantigens. Antibodies against new beta cell antigen ICA12 (SOX13) alone, are present in only 3% of IDDM patients and 1% of NIDDM patients and adding ICA12ab to GAD65 and IA-2ab, gives only 1% increase in antibody positivity in both IDDM and NIDDM groups both in Latvian and Swedish diabetics.

LIST OF ORIGINAL PAPERS:

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I   **A. Shtauvere**, I. Rumba, I. Dzivite, C.B. Sanjeevi
        HLA – DR and – DQ gene polymorphism in Latvian patients with insulin-dependent diabetes mellitus.
        Tissue Antigens 1998; 52: 385 – 388

II   C.B. Sanjeevi, A. Kanungo, **A. Shtauvere**, K.C. Samal, B.B. Tripathi
        Association of HLA class II alleles with different subgroups of Diabetes mellitus in Eastern India identify associations with IDDM and Malnutrition – Related Diabetes.
        Tissue Antigens 1999, 54: 83 – 87

III   C.B. Sanjeevi, **A. Shtauvere**, A. Ramachandran, C. Snehalatha, A. Falorni
        Prevalence of GAD65 autoantibodies in South Indian patients with insulin-dependent diabetes mellitus and in their parents.
        Diabetes, Nutr. And Metab. 1997, Vol.10, No.2, 60 - 64

IV   **A. Shtauvere-Brameus**, D.B. Schranz, L. Bekris, W. Hagopian, I. Rumba, A. Falorni, Å. Lernmark, C.B. Sanjeevi
        Clinically classified non-insulin dependent diabetes mellitus (NIDDM) patients show high prevalence of autoimmunity in Latvia.
        In manuscript

V   C. Törn, **A. Shtauvere – Brameus**, C.B. Sanjeevi, W. Hagopian, M. Landin – Olsson
        Autoantibodies to SOX13 are increased in Swedish type 1 diabetic patients.
        Submitted

Abbreviations used in the text:

IDDM — Insulin-dependent diabetes mellitus

NIDDM — Non-insulin dependent diabetes mellitus

MRDM — Malnutrition-related diabetes mellitus

PDDM — Protein-deficient diabetes mellitus

FCPD — Fibro-calculous diabetes mellitus

GDM — Gestational diabetes mellitus

LADA — Latent autoimmune diabetes in adults

IGT — Impaired glucose tolerance

WHO — World Health Organization

ADA — American Diabetes Association

APC — Antigen presenting cells

MHC — Major Histocompatibility Complex

HLA — Human Leukocyte Antigen

GAD — Glutamic acid decarboxylase

ICA512/IA-2 — Protein tyrosine phosphatase

TTG — Tissue transglutaminase

21-OH — 21 - hydroxylase

GABA — Gamma aminobutyric acid

HMG — High mobility group

SOX13 — A transcription factor that is a member of SOX family, which regulate proliferation and differentiation of tissues

APS — Autoimmune polyendocrine syndrome

BMI — Body – mass index

PCR — Polymerase chain reaction

RIA — Radioimmune assay

Ab — Antibody

C-peptide — Connecting peptide

Th1 — Helper T cells 1

Th2 — Helper T cells 2

IFN — Interferon

IL — Interleukin

SMS — Stiff-Man Syndrome

TRIGR — Trial to Reduce Diabetes in Genetically at Risk

DIPP Diabetes Intervention and Prevention Study

ENDIT European Nicotinamide Diabetes Intervention Trial

DENIS German Nicotinamide Diabetes Intervention Trial

DPT Diabetes Prevention Trial

Introduction:

Diabetes Mellitus is a metabolic and vascular disease and the metabolic disorders involve carbohydrates, protein and fat metabolism. In a fully developed clinical expression, it is characterized by fasting hyperglycemia and, in most patients with long standing disease, by microangiopathic and atherosclerotic macrovascular disease and neuropathy (Fajans 1996)

The classification of diabetes has been a topic for discussion for several years. The classification based on clinical criteria (WHO-1985) has been changed to one, based on ethiopathogenesis of the disease (ADA, classification 1997, Alberti 1998) (Table 1).

Table 1: Classification of diabetes mellitus.

WHO-1985	ADA-1997	WHO-1998
IDDM	Type 1 diabetes	Type 1 diabetes
NIDDM	Type 2 diabetes	Type 2 diabetes
MRDM: PDDM	Not specified	Not specified
FCPD	Secondary type of diabetes	Secondary type of diabetes

An International workshop on types of diabetes peculiar to the tropics reviewed the evidence and characteristics of diabetes mellitus seen in undernourished populations (Tripathy 1997). Whilst it appears that malnutrition may influence the expression of several types of diabetes, the evidence that diabetes can be caused by malnutrition or protein deficiency is not convincing. Therefore, the class "Malnutrition-related diabetes (MRDM)" is renamed as "Malnutrition modulated diabetes mellitus (MMDM)" and is considered for inclusion in the classification as Type 1b diabetes. The former subtype:

protein – deficient pancreatic diabetes (PDDM) may be considered as malnutrition modulated or modified form of diabetes, for which more studies are needed. The other subtype – fibrocalculous pancreatic diabetes (FCPD) is now classified as a disease of exocrine pancreas, fibrocalculous pancreatopahy, which can cause diabetes mellitus.

Epidemiology of diabetes mellitus

In the subsequent pages, IDDM and NIDDM are referred as in WHO - 1985.

IDDM is the second most common chronic disease in childhood and the most common form of diabetes in children (1.7 of 1000 children) (Atkinson 1994). The incidence rate of IDDM is increasing constantly in many countries and is expected to be about 40% higher in 2010 than in 1997. The earlier hypothesis of the north – south gradient in the disease incidence is not as strong as previously thought (LaPorte 1985). The highest incidence of this disease is still reported in Northern Europe, particularly in Finland (37.4/100000 per year) and Sweden (34.6/100000 per year) (Onkamo 1999, Padaiga 1997). The lowest incidences are reported in Japan and Korea (<1/100000 per year) (Karvonen 1993). In rest of Europe incidences are between 7.0 and 19.0 cases /100000 per year, except in Sardinia, where the incidence is the highest in the world after Finland (36.0/100000 per year) (Muntoni 1995). In the countries around Baltic Sea, incidence of IDDM is different. The high incidence in Finland and Sweden is followed by surprisingly low incidence in Baltic States - Estonia (10.3/100000 per year), Lithuania (7.1/100000 per year) and Latvia (6.5/100000 per year) (Padaiga 1997). The reason for such a difference in relatively small area is not clear. We know that historically Sweden and Latvia, as well as Finland and Estonia has common background, which has allowed admixture of genes. The possible explanation can be the difference in relative frequency of susceptible and resistance genes in these countries. As well as environmental factors which can possibly initiate or trigger the

process leading to the ß-cell destruction. In Latvia male excess in incidence is recorded (1.01) and age specific risk is observed in 11 - 13 year age range (Padaiga 1997).

According to the published data, based on WHO's (1985) clinical classification of Diabetes, IDDM has a low prevalence in India (Menon 1990, Mohan 1985). In the study of IDDM in children under 15 years of age from South India, the prevalence was estimated 0.26/1000 (Ramachandran 1992). The incidence of IDDM reported from Madras was 10.5 /100000 per year (Ramachandran 1996).

Islets of Langerhans in health and disease

In all mammalians, pancreatic ß-cells are found in the islets of Langerhans, where they form clusters of endocrine cells scattered through the pancreas but mostly in the tail of pancreas. The number of islets is between 100000 to 2.5 million per pancreas, and the size can vary from 50 to 300µm in diameter and they contain from hundreds to several thousand hormone-secreting endocrine cells. Islets are separated by delicate connective tissue. They receive sympathetic and parasympathetic nerves as well as arterial blood supply through intra-islet capillary bed. The islets are densely packed collection of peptide-secreting endocrine cells, of which all of them are involved in metabolic regulation. The insulin-secreting ß-cells are the most abundant and form 70-90% of all endocrine cells of islets. The rest of the cells are alpha cells, which are glucagon producing cells. There are also ∑-cells, which secrete somatostatin and F-cells, which secrete pancreatic polypeptide (PP). All islet cell types tend to share the classical features of other peptide-secreting cells.

Insulin in the pancreatic ß-cells is produced as proinsulin, which is a precursor molecule of insulin (Steiner 1969). It is turned into insulin and pro-insulin connecting peptide (C-peptide), stored in granules and secreted into the portal vein. Insulin is extracted in the liver by 50%, while c-peptide reaches the peripheral circulation and can be used as

indirect measure of insulin secretion. (Faber & Binder 1986). Insulin release itself is associated with the activation of ATP– sensitive potassium (K_{ATP}) channels (Ashcroft 1999.)

In the resting ß-cells, when extracellular glucose levels are low (under 3 mmol/l), K_{ATP} channels are open and the ß-cell is hyperpolarized. Glucose metabolism begins K_{ATP} channel closure, which leads to membrane depolarization, opening of Ca^{2+} channels, triggering the exocytosis of insulin (Figure 1).
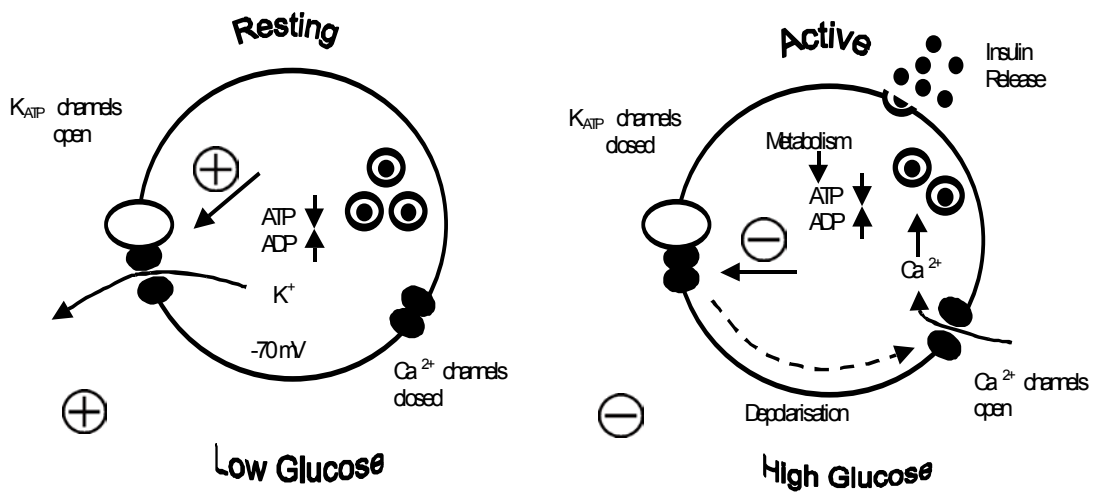

Figure 1: Insulin secretion pattern (adapted from Aschroft FM, 1999)

In IDDM, changes in pancreas are found in correlation with the length of the disease. In early onset IDDM, the size of pancreas is not changed, while in late onset, there is often significant reduction of the pancreatic weight, and also interstitial fibrosis of the exocrine tissue. Already in the early onset IDDM, there is a qualitative reduction in the number of islets per area. There is also a reduction of ß-cell granulation, as well as number of ß-cells and some part of the islets having none. These islets consist of mostly of alpha, $\sum$ and PP cells. The longer the history of disease, the more increase in PP cell frequency. In long term diabetes the islets are small and reduced in volume, due to

complete loss of islet ß-cells. Infiltration of the islets with mononuclear as well as lymphocytic cells ("insulitis") is a common finding. In late onset diabetes insulitis is mostly associated with islet amyloid. The infiltrate mostly consists of T lymphocytes, B lymphocytes and macrophages. In recent onset IDDM three types of islets are described – apparently normal islets, inflamed islets and "pseudoatrophic islets", where there are no ß-cells but all other types of islet cells.

In the natural history of IDDM, 4 stages of the disease are observed in majority of patients:

- Preclinical ß-cell autoimmunity with progressive defect of insulin secretion

- Onset of clinical diabetes

- Transient remission

- Established diabetes associated with acute and chronic complications (Westmark 1996).

IDDM as autoimmune disease

The word "auto" is the Greek word for self. If a person has an autoimmune disease, the immune system mistakenly attacks self, targeting the cells, tissues, and organs of a person's own body (Roitt 1993). Classification of autoimmune diseases specifies organ or tissue specific and non-organ or systemic autoimmune diseases. It depends on whether the self-antigen is localized or found in the entire body. Organ specific autoimmune diseases are more often characterized by the destruction of specific cell types as a consequence of tissue infiltration by lymphocytes and other mononuclear cells.

As an autoimmune disease, IDDM has features characteristic for most of the autoimmune diseases (Rose 1993):

1)   IDDM is associated with specific haplotypes of the MHC complex (HLA class II)

2)   there are found circulating autoantibodies against the autoantigens of ß-cells (GAD65, ICA512/IA-2)

3)   There is mononuclear infiltration of the pancreatic islets, which can be detected histologicaly

The classical symptoms of IDDM are polyuria, polydipsia, polyphagia and weight loss. Symptoms are the results from the organ-specific autoimmune mediated loss of insulin secreting ß-cells, which is chronic process involving both cellular and humoral components, detectable in the peripheral blood years before the clinical onset of the disease (Kukreja 1999). At the time of clinical onset most of the ß-cells are destroyed and islets are infiltrated with inflammatory mononuclear cells.

The first step in the initiation of the disease is presentation of ß-cell specific autoantigens by antigen presenting cells (APC) to CD4+ helper T cells by help of MHC class II molecules. Macrophages secrete interleukin (IL) −12, which stimulates CD4+ T cells to secrete interferon (IFN)-γ and IL-2. IFN-γ stimulates other resting macrophages to release other cytokines (IL-1, TNF-α) and free radicals, toxic to ß-cells. Cytokines also induce the migration of ß-cell autoantigen specific CD8+ cytotoxic T cells, which by help of MHC class I molecules cause ß-cell damage and apoptosis of the cells (Figure 2).
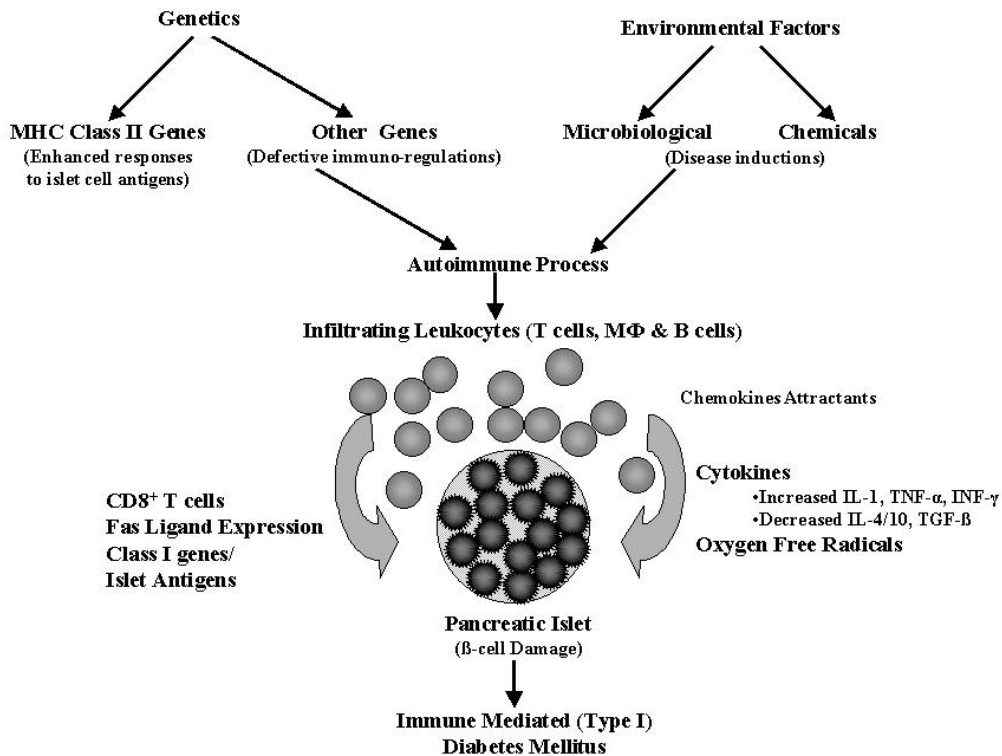
Figure 2: Pathogenesis of autoimmune diabetes mellitus (adapted from Kukreja A & Maclaren NK, 1999)

Cytokine imbalance plays an important role in the development of diabetes. CD4+ T cells form two categories: T helper 1 (Th1) and T helper 2 (Th2) cells.

Th1 cells primarily secrete IFN-γ and IL-2 and promote cell-mediated immunity, while Th2 cells produce IL-4, Il-10 and downregulate Th1 cell activity and mainly are associated with humoral immunity. The immune response itself is regulated by the balance between Th1 and Th2 cell cytokines. Reported data on Th1 and Th2 has proposed that Th1 cells promote the development of diabetes while Th2 could be protective. It has been shown that Th2 T cells lack the ability to destroy ß-cells and trigger disease (Katz 1995).

Malnutrition – related diabetes mellitus

Identification of MRDM as a separate form if diabetes mellitus, with subtypes fibrocalculous pancreatic diabetes (FCPD) and protein-deficient diabetes mellitus (PDDM) is controversial, in terms of the role of malnutrition in pathogenesis of both types. It is known that diabetes in tropical regions and in some developing countries presents clinically differently from IDDM and NIDDM in the western world. The clinical symptoms of both subtypes include onset earlier than 30 yrs of age, body – mass index below 18, moderate or severe hyperglycemia, lack of ketosis on withdrawal of insulin, insulin requirement more than 60 units/day or more than 1.5 units per kg body weight and frequently a history of malnutrition in infancy and early childhood. They may or may not have pancreatic calculi (Sanjeevi 1988). The resemblance with typical insulin dependent diabetes is in acuteness and age of onset, severity of symptoms, no response to oral hypoglycemic agents and requirement of insulin to control metabolic status (Tripathy 1965, Ahuja 1980, Tripathy 1993).

PDDM has also been suggested to be due to selective aminoacid deficiency in dietary intake and destruction of ß-cells from cyanogenic compounds in food, like linamarin and lotaustralin in cassava (McMillan 1979).

FCDP usually occurs due to chronic calculous pancreatopahy, patients might have history of recurrent abdominal pain and staetorrhoea, pancreatic calculi are usually large multiple and intraductal.

One of the main questions is, if malnutrition is really responsible for the disease. Several reports support the contributory role of malnutrition in the ethiopathogenesis of diabetes in malnourished people (Rao 1988, Rao 1984).

Malnutrition results in glucose intolerance, insulinopenia and ß-cell dysfunction. Experimental models have shown that insulinopenia is a result of impaired ß-cell function rather than reduced number of ß-cells (Dixit 1985, Bajaj 1987).

Autoantigens in autoimmune diabetes

The markers of autoimmunity in IDDM are autoantibodies directed to several autoantigens (Lernmark 1996, Palmer 1983, Leslie 1996). There are main groups of autoantigens (Table 2):

Table 2: Islet autoantigens in IDDM

Major autoantigens

Glutamic acid decarboxylase (GAD65)

Protein tyrosine phosphatase (ICA512/ IA-2)

Insulin

Minor autoantigens

ICA12 (SOX13)

ICA69

Carboxypeptidase H

Gangliosides (GM-1)

Sulfatides

Shared antigens

Tissue transglutaminase (TTG)

21- hydroxylase (21-OH)

other antigens

Glutamic acid decarboxylase (GAD)
In 1950's the first descriptions of glutamic acid decarboxylase were available. GAD - what stands for glutamic acid decarboxylase - is important in GABA (gamma aminobutyric acid) formation in different cells. It is enzyme which catalyses the formation of gamma aminobutyric acid, which is the major neurotransmitter in the central nervous system (Martin 1993).

GAD is found in nerves and islet cells as a doublet of proteins mostly referred as GAD65 and GAD67 – isoenzymes with molecular weight of 65,000 and 67,000. They both are encoded by at least 2 homologous genes located on chromosomes 2 and 10 (Karlsen 1991, Michelsen 1991). In GAD65 and GAD67 cDNA amino acid sequences are 65% identical, but in the first 100 aminoacids, the difference is almost 75% and that difference is responsible for different sub-cellular localization of these two isoenzymes. Signals located there are responsible for hydrophobicity of GAD65.
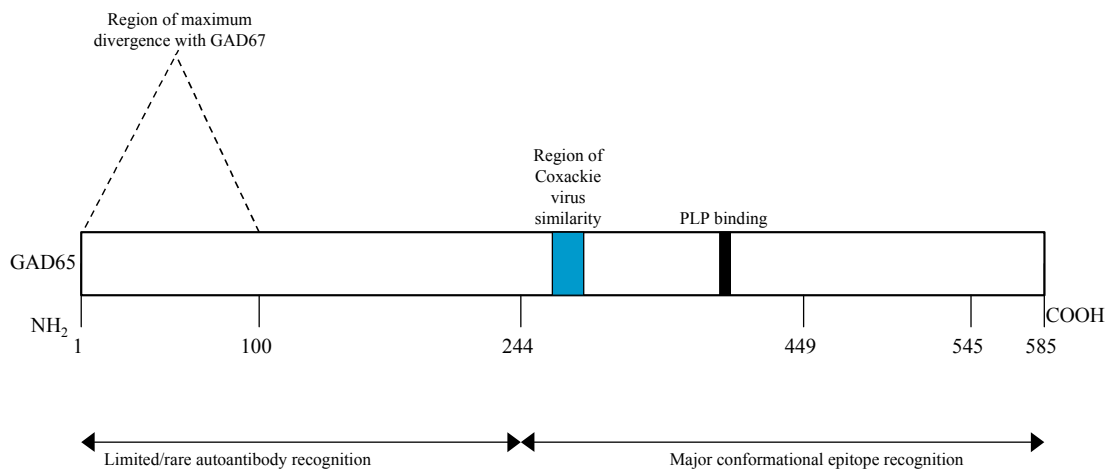

Figure 3: Model of GAD65 protein (adapted from Leslie RDG, 1999)

Both GAD isoforms are synthesized as hydrophilic, soluble molecules. GAD67 later remains soluble, but GAD65 is involved in multistep process, the result of which is the molecule within the membranes of synaptic vesicles in gamma aminobutyric acid containing neurons or small synaptic-like microvesicles of islet ß-cells (Blu 1992).

The presence of GAD and GABA in pancreatic islets are known since 70's, but the function of GABA there is still not clear, but it has been suggested that GABA works as a functional regulator of pancreatic hormone release and is involved in paracrine signaling for communication between islet endocrine cells as well as islet cell development and function (Satin 1998, Leslie 1999).

The reports of GAD distribution show that the cellular distribution of GAD is very restricted. GAD is only observed in pancreatic islet cells, epithelial cells of the fallopian

tube and testis. Both GAD65 and GAD67 are expressed in the central nervous system in humans and also different animals, while GAD65 is present only in human islets in high levels (Petersen 1993). Besides GAD65 is a shared autoantigen in different autoimmune disorders, like Stiff – man syndrome (SMS) and autoimmune polyendocrine syndrome (APS) (Baekkeskov 1990).

The availability of GAD65 and GAD67 cDNA has made it possible to mutate GAD65 to study the autoantibody epitopes (Richter 1993). Several GAD65 epitopes have been identified: N terminal region (from position 39), C-terminal region (aa 450 – 585) and middle region (aa 245 – 499) of GAD65 (Leslie 1999, Baekkeskov 1999 Falorni 2000) (Figure 3). The analysis of various regions of GAD65 by the use of monoclonal anti-islet-cell antibodies or GAD65/GAD67 chimeric proteins reveals that the production of diabetes associated GAD65ab is the result of an autoantigen driven process and autoantibodies are directed to middle (M) and carboxyterminal (C) regions (Richter 1993, Falorni 2000). But the reports show that in different diseases the mechanism of GAD65ab production may be different and directed to broader or different spectrum of epitopes. In Stiff - man syndrome patients high titre autoantibodies to GAD65 are detected and are mainly directed against linear epitopes located in the N-terminal region, M and C-terminal regions of GAD65 (Kim 1994, Butler 1993).

The cloning of human islet GAD65 made the development of sensitive and specific radioimmunoassay to detect GAD65 antibodies in IDDM.

Several reports have shown that GAD65 may be found in 70-85% IDDM patients and 1-2% of healthy controls (Landin-Olsson 1992, Falorni 1995, Hagopian 1995, Sanjeevi 1996)(Table 3). GAD65 antibodies are associated with slower rate of disease progression and these antibodies tend to be present many years after the clinical onset of the disease (Christie 1994, Schmidli 1994). The reason for the persistence of these antibodies in patients' sera after the clinical onset is not clear, but one option is that

exogenous proteins possessing structural or functional homology with GAD65 account for the persistence. In addition, there could be a continuous, but small scale regeneration of ß-cells in the pancreas, which is sufficient to maintain an immune response (Swenne 1992).

Tyrosine phosphatase (ICA512/IA-2)

Protein tyrosine phosphatase - 2 gene is located on chromosome 2q35 and its cDNA encodes 979 aminoacid long transmembrane protein. It has extracellular, transmembrane and intracellular domains. ICA512/IA2 is a member of protein tyrosine phosphatase (PTP) –like proteins, except that this recombinant protein lacks the enzymatic activity, and observations show that by replacing aminoacids in positions 911 and 877 it gains enzymatic activity (Magistrelli 1996).

This islet cell antigen 512 (ICA512/IA-2) was identified from islet DNA expression library by screening the sera from Type 1 diabetes patients. It has been found in normal human brain, pituitary, pancreas and different cell lines (Solimena 1996). The function of IA-2 in islet ß-cells is unknown, but PTP-like proteins has been suggested to play a role in the secretion of granule contents from neuroendocrine organs and as well as in cell growth and proliferation. The gene is expressed in human, mouse and rat insulinoma cells as well as normal islets (Stone 1994, Roberts 2001).

The use of a radioimmune assay has showed that 55-60% of IDDM patients carry antibodies against ICA512/IA-2. Antibodies to IA-2 are associated with rapid progression of IDDM and usually are associated with younger age at onset (Christie 1994). IA-2ab are not so stable and they usually tend to disappear with the longer duration of the disease.

Other antigens (TTG, ICA12, 21OH)

Several other antigens have been named as possible candidates for autoantigens in IDDM, most of them reported in single publications.

It is well known that a common genetic background is responsible for the association of several endocrine autoimmune diseases in so called autoimmune polyendocrine syndromes (APS). The polymorphism in HLA class II DQ genes is associated with genetic risk for IDDM, Addison's disease, as well as coeliac disease (Badenhoop 1995, Winquist 1992, Lampasona 1999, Galli -Tsinopoulou 1999). It is reported that coeliac disease occurs in around 1.1 - 10% of patients with IDDM (Cronon 1997, Saukkonen 2001). That is quite high disease prevalence and is due to shared genetic susceptibility provided by HLA DR3/DQB1*02 allele, but could indicate some gut-associated pathogenesis in IDDM patients. On the other hand IDDM occurs also in coeliac disease patients and in this case antibodies against GAD65 and IA-2 carry approximately 23% of coeliac disease patients (Rapoport 1996).

The main antigen in coeliac disease is tissue transglutaminase (TTG), the presence of antibodies to TTG in IDDM patients can determine silent coeliac disease to be confirmed by biopsy, or can be the marker for autoimmunity outside the typical coeliac disease. That can be possible due to some kind of intestinal immunization or cross-reactivity between transglutaminase and islet antibodies (Ellis 1996). Other alternative could be a direct immunization during ß-cell destruction. It is possible that during active inflammation and ß-cell destruction, transglutaminase, which is expressed in islets, can bind other autoantigens, like GAD65 and IA-2 and subsequently be recognized through a mechanism of determinant spreading.

Addison's disease is also known to be associated with IDDM, but more often as a part of autoimmune polyendocrine syndromes (APS). IDDM and Addison's disease are both present in 15-20% of the cases (Tuomi 1996). Several studies have found strong

association of Addison's disease with HLA-DR3 and DR4 (Partanen 1994) which are also susceptibility DR haplotypes for IDDM. Majority of the Addison's disease patients is positive for 21-hydroxilase (21-OH) antibodies (Winquist 1992). Several studies have shown that IDDM as well as LADA or slow onset Type 1 diabetes patients carry antibodies against 21-OH (Gambelunghe 2000).

Autoimmune thyroid diseases are common disorder associated with IDDM (Landin-Olsson 1989, Lorini 1996). The frequency of thyroid antibodies in IDDM patients are present in about 20% (Lorini 1996). Both GAD65 and TPOab are associated with the presence of HLA-DR3 and DQ2.

Recently a new ß-cell candidate antigen ICA12 (1-622 amino acids long) has been cloned after it was identified by immunoscreening of an islet cDNA library (Rabin 1992). The target protein of ICA12 autoantibodies is described as high mobility group (HMG)- box transcription factor SOX13. SOX (SRY-type High Mobility Group BOX) transcription factors share homology with testis determining factor SRY (sex determining region Y) through their HMG domain, which binds and bends DNA (Harley 1992). SOX genes encode transcription factors with important role in embryonic development (Pevny 1997). SRY initiates testis differentiation in the indifferent gonad and mutations in SRY are present in patients with XY gonadal dysgenesis (Argentaro 2000). SOX13 is detected in human tissues like pancreas, placenta and kidneys (Kasimiotis 2000). Up to now there are only couple of reports on SOX13 autoantibodies in patients with Type 1 diabetes (Steinbrenner 2000, Kasimiotis 2001).

Table 3: Frequency of autoantibodies GAD65 and IA-2 in different populations.

Country	Type	Age	Antibody	Frequency (%)	Study
Sweden	Type 1	0-14	GAD65	70	Landin-Olsson 1995
Finland	Type 1	0-15	GAD65	73	Sabbah 1999
			IA-2	86	
Finland	Type 2	28-83	GAD65	9.3	Tuomi 1999
Sweden	Type 1	0-14	GAD65	70	Hagopian 1995
UK	Type 1	0-21	GAD65	74	Bingley 1997
			IA-2	75	
UK	Type 2	25-34	GAD65	34	Turner 1997
		35-44	GAD65	14	
		45-54	GAD65	9	
		55-65	GAD65	7	
Holland	Type 1	0-40	GAD65	66	Seissler 1998
			IA-2	56	
	Type 2	40-96	GAD65	2.8	
			IA-2	0.1	
Belgium	Type 1	0-9	GAD65	64	Vandewalle 1995
		10-19	GAD65	80	
		20-39	GAD65	78	
Australia	Type 2	>30	GAD65	25	Zimmet 1993
Japan	Type 2	>30	GAD65	11	Fukui 1997
India	Type 2	>30	GAD65 or IA-2	42	Sanjeevi 1998
Japan	Type 1	7-20	GAD65	71	Sera 1999
			IA-2	62	
India	Type 1	0-30	GAD65	40	Singh 2000
			IA-2	22	

HLA in autoimmune diabetes mellitus

As we know today, IDDM is a polygenic disease, associated with several genes on different chromosomes, each one of them contributing to the development of disease. Today up to 18 genes have been identified by gene mapping or linkage analysis (Pozzilli 1996, Davies 1994, Pugliese 2000) (Table 4).

Table 4: List of IDDM genes identified by gene mapping.

Designation	Location on chromosome
IDDM1 (HLA)	6p21.3
IDDM2 (INS)	11p15.5
IDDM3	15q26
IDDM4	11q13.3
IDDM5	6q25
IDDM6	18q21
IDDM7	2q31
IDDM8	6q27
IDDM9	3q21
IDDM10	10p11-q11
IDDM11	14q24.3-q31
IDDM12 (CTLA-4)	2q33
IDDM13	2q34
IDDM14 (Not identified)	-
IDDM15	6q21
IDDM16 (Not identified)	-
IDDM17	10q25
IDDM18	5q31.1-33.1

The most important gene is Human Leukocyte Antigen (HLA) also known as Major Histocompatibility Complex (MHC), which is located on chromosome 6p21.3. MHC is a region of 4 million base pairs, which is 0.1% of the human genome and it contains over 100 genes, which are characterized by high degree of allele polymorphism

(Trowsdale 1988). The human MHC is divided into class I, II and III regions (Figure 4).

The class I genes are located telomeric in the complex. Earlier studies demonstrated association between class I haplotypes and IDDM. But today this is explained by associations due to linkage disequilibrium of class I genes with HLA class II molecules. Class I molecules have single polypeptide chain containing 3 domains associated with ß2 microglobulin. The class I genes classically are HLA-A, -B and -C and their function is to present antigens to CD8+ cytotoxic T cells (Sanjeevi 1996).

HLA class II genes are located at the centromeric end of MHC and occupy a region of 1 million base pairs (Hardly 1986). Class II molecules are heterodimeric proteins, with heavy alpha chain and lighter ß chain. Molecules are expressed on cells of immune system - monocytes, macrophages, B lymphocytes, dendritic cells and activated T lymphocytes.

The major function of MHC molecule is to act as recognition element for T lymphocytes which have an obligatory requirement to co-recognize foreign antigen with self MHC products. As a consequence of this, MHC alleles inherited by an individual are one of the major determinants of immune response phenotype.

For the class II region, DRA is non - polymorphic, but DRB, DQA1 and DQB1 show a high degree of allelic variability. Many of these alleles differ from each other by one or several aminoacids.

For humans in class II HLA-D region 273 alleles of DRB1, 45 alleles of DQB1, 21 alleles of DQA1 as well as DPA1, BPB1, TAP1 and TAP2 are identified (Marsh, 2001). IDDM is strongly associated with HLA-DQ (Wassmuth 1990). IDDM is positively associated with HLA DR3 and DR4 in Caucasians, the frequency of which is usually increased, while frequency of DR2 is decreased (Ronningen 1991, Sanjeevi 1994). From HLA-DQ molecules, DQ8 (DQA1*0301-DQB1*0302) is the most prevalent

IDDM haplotype in Caucasians, detected in 74%, followed by DQ2 (DQA1*0201-DQB1*0501), detected in 52% of Caucasians (Sanjeevi 1995). Several DQ haplotypes are negatively associated with IDDM of which the most negative is DQ6 (DQA1*0102-DQB1*0602) (Kockum 1993). A single copy of this haplotype or DQB1*0602 is adequate to confer significant negative association (Kockum 1993).

Taken together, DQ8, DQ2 or both account for as many as 89% of Caucasian IDDM patients.

In Asian patients, IDDM tends to be associated with different HLA haplotypes, as compared to Caucasian patients. In Japanese IDDM patients, HLA DR4/DQ4 and DR9/DQ9 showed positive association with IDDM (Awata 1990) and HLA DR9/DQ9 and DR3/DQ2 in Chinese diabetics (Hawkins 1987). The study on South Indian IDDM patients showed predominant association of DR3 compared to DR4 with the disease (Serjeantson 1987, Easteal 1990). In the Indian population HLA DQ6 (DQB1*0602) is infrequent, while DQ6 (DQB1*0601) is the most frequent (Sanjeevi 1992, Rani 1999).

The differences in HLA associations between ethnic groups can be due to different prevalence of HLA haplotypes in the general population.

Few genetic studies have been done on MRDM patients (Kambo 1989). Earlier studies in South Indian patients have shown the association of MRDM with HLA – DR7 and DQ9 (Sanjeevi 1992), while Ethiopian patients showed association with DR3 (Abdulkadir 1989). Our study on Eastern Indian diabetic patients showed association of PDDM with DR3 and DQ2, which supports the earlier observations, and FCDP showed association with DR7 and DQ9, the finding which has not been reported earlier (Sanjeevi 1999).

Figure 4: Simplified map of the human MHC on the short arm of chromosome 6.
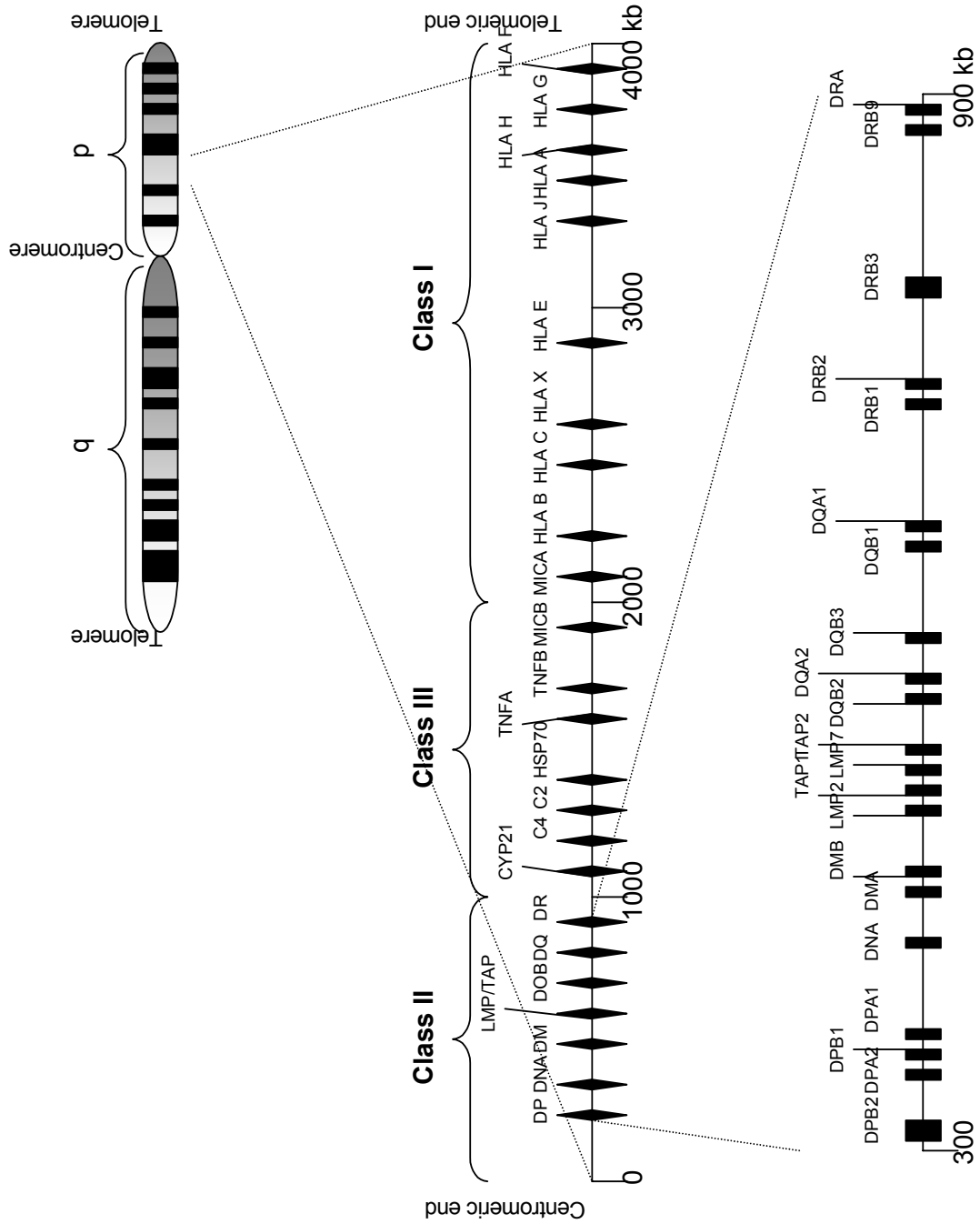
Environmental factors

Since IDDM is a multifactorial disease, besides the genetic predisposition, environmental factors might also play significant role in the development of disease as triggers of ß-cell destruction in genetically predisposed individuals. Several studies around the world have tried to prove the role of environmental factors in the pathogenesis of IDDM.

Environmental risk determinants can be classified into three groups: viral infections (coxackie virus, cytomegalovirus), early infant diet (breast feeding vs. early introduction of cow's milk) and toxins (Ellis 1996, Knit 1999). Other factors which can possibly be triggers for the disease are psychosocial factors as well as climate and ante- and prenatal risk factors (feto-maternal blood group incompatibility) (Åkerblom 1998, Knip 1999, Dahlquist 1999).

The possible way of virus action can be:

- direct cytotoxic effect

- formation of a novel antigen

- induction of MHC by releasing cytokines

- molecular mimicry, by expressing the antigen that shares sequence homologies with pre-existing ß-cell autoantigen

- shifting of T cell response from one subpopulation to another or destroying suppressor T cells or activating the autoreactive cells (Rossini 1993).

The effect of early introduction of cow's milk in infant's diet has been studied widely with controversial results. The study in Finland has shown the association of intake of cow's milk and increased appearance of diabetes in siblings of children with Type 1 diabetes (Virtanen 1998). Also short duration of breast feeding has shown the relation to increased risk to Type 1 diabetes (Virtanen 1991). On the other hand studies in USA

and Germany so not document any effect of early cow's milk intake on Type 1 diabetes (Norris 1996, Hummel 2000).

The possible effect of cow's milk on the development of type 1 diabetes can be because of:

- higher protein concentration in cow's milk (casein)

- main protein in cow's milk – beta lactoglobulin, which is not a component in breast milk

- primary serum albumin aminoacid sequence differs from human

- bovine insulin in cow's milk differs from human insulin by three aminoacids

Other environmental factors, like toxins, psychosocial factors, ante- and perinatal risk factors, have not been studied so widely and are speculated to be triggers of the development of Type 1 diabetes in susceptible individuals.

Aims of the study:

1) To test the frequencies of the HLA-DR and DQ genes associated with susceptibility and protection in IDDM in Latvian population

2) To test the hypothesis that the differences in the incidence of IDDM in Latvian and Sweden is reflected by the increased frequencies of the protective DR and DQ in Latvia.

3) To test the hypothesis that genetic background for IDDM is different in India, that MRDM is an autoimmune disease and immunogenetically different from IDDM.

4) To show that GAD65 autoantibodies are associated with IDDM, irrespective of ethnic background and GAD65 is the main autoantigen in most populations.

5) That Latvian autoimmune diabetes is similar to Swedish autoimmune diabetes by estimating the prevalence of autoimmune markers.

6) To test the prevalence of slow onset autoimmune diabetes among clinically diagnosed Latvian and Indian NIDDM patients.

Materials and methods:

Patients and samples

For the study on Latvian diabetic patients we selected two study groups. In the first group 101 Latvian IDDM children: 55 boys and 52 girls were selected. The age at the disease onset was from several months to 18 years (mean age 10 years) and disease duration less than 5 years. All patients were diagnosed at State Children's Hospital of Medical Academy of Latvia. 111 sex and age matched healthy controls were selected for this study group (mean age 9.4 years).

In the other study group, 100 patients, initially diagnosed as NIDDM were included from different hospitals in Riga, Latvia: 35 were males, 65 were females (mean age 54 years). In this group, age at onset was ≥ 30 years, disease duration less than 5 years. Out of 100 patients, 85 were on oral hypoglycemic agents and 15 were on insulin. Body-mass index (BMI) under 19 was recorded in 1% (1/100) cases, while overweight (BMI > 25,5 in females and 27 in males) was documented in 45% (45/100) cases. In this study group 100 healthy sex and age matched controls were included (mean age 52.4 years).

We collected 5 - 10 ml of intravenous blood samples in EDTA from all the patients and healthy controls. Serum was separated from all blood samples and both serum and blood samples were kept frozen at -20ºC until transported to Karolinska Hospital, Stockholm Sweden. All further analyses were performed at Center for Molecular Medicine, Karolinska Hospital, Stockholm, Sweden.

Two groups were formed also for study on Indian diabetics. One group included 115 IDDM patients from South India: 65 males and 60 females, in the age group 1 – 46 years attending the Diabetes research Center and M.V. Hospital for Diabetes in Madras. All patients were insulin treated since the time of diagnosis. BMI was <19 in 59% and >25 only in 7%. Patients had disease duration 0 – 20 years. 105 unrelated, age and sex

matched healthy subjects were selected for the study as well as 58 fathers and 60 mothers of IDDM patients. In 44 cases both parents were available for the study.

In the other group patients attending the diabetes clinic of the SCB Medical College in Cuttack, Orissa, Eastern India were studied. IDDM (n = 74), NIDDM (n = 215), PDDM (n = 71) and FCPD patients (n = 46) were compared with population based unrelated healthy controls (n = 120).

In the study on Swedish diabetics, 102 newly diagnosed IDDM patients from southern part of Sweden were studied. Mean age was 35 years (range 9 – 89). 99 healthy controls, with the mean age of 25 (range 15 – 34) were also studied.

We collected 5 - 10 ml of intravenous blood samples in EDTA from all the patients and healthy controls for the HLA studies. Serum was separated from all blood samples for autoantibody studies and both serum and blood samples were kept frozen at -20ºC until transported to Karolinska Hospital, Stockholm Sweden. All further analyses were performed at Center for Molecular Medicine, Karolinska Hospital, Stockholm, Sweden.

DNA extraction
Genomic DNA was isolated from peripheral blood leukocytes by standard phenol – chloroform extraction. The DNA was dissolved in 200 μl of sterile double - distilled water.

HLA-DQA1, DQB1 and DRB1 analysis
HLA - DQA1, DQB1 and DRB1 typing was performed by amplifying the genomic DNA with primers specific for DQA1, DQB1 and DRB1 second exon using polymerase chain reaction (PCR) in the programmable thermal cycler (Perkin Elmer Cetus, CT) (Erlich 1989).

For DQA1: 35 cycles of amplification; 94ºC for 1 min for denaturation, 62ºC for 1 min for annealing and 72ºC for 2 min for extension.

For DQB1: 30 cycles of amplification; 95ºC for 1 min for denaturation, 55ºC for 1 min for annealing and 72ºC for 1 min for extension.

For DRB1: 3o cycles of amplification; 94ºC for 1 min for denaturation, 55ºC for 1 min for annealing and 72ºC for 2 min for extension.

The amplification, manual dot blotting on to nylon membranes, 3' end labeling with synthetic oligonucleotide probes for DQA1, DQB1 and DRB1 genes with 32P, prehybridisation, hybridization autoradiography, followed by allele calling was performed (Ronningen 1989).

GAD65, IA-2, TTG, 21-OH and ICA12 analysis

GAD65 and IA-2 autoantibodies, as well as TTG and 21-OH antibodies were detected by radioligand binding with Protein A Sepharose assay (RIA) using 35S-methionon labeled *in vitro* transcribed - translated recombinant human GAD65, IA-2, TTG and 21OH antigens, as described previously (Falorni 1996). cDNA of human IA-2 and cDNA for TTG was kindly donated by Dr. George S. Eisenbarth (BDC, Denver, CO, USA). cDNA for 21OH was a gift from Dr. Alberto Falorni (Perugia, Italy), and cDNA for ICA12 was provided by Dr. W. Hagopian (Seattle, WA, USA). Immunoprecipitation assays used scintillation counting of Protein-A Sepharose pellets (Hagopian 1995) (Figure 5). All samples including positive and negative controls were tested in triplicates. This analysis was performed in Molecular Immunogenetics laboratory, CMM, Karolinska Institute (Stockholm, Sweden).
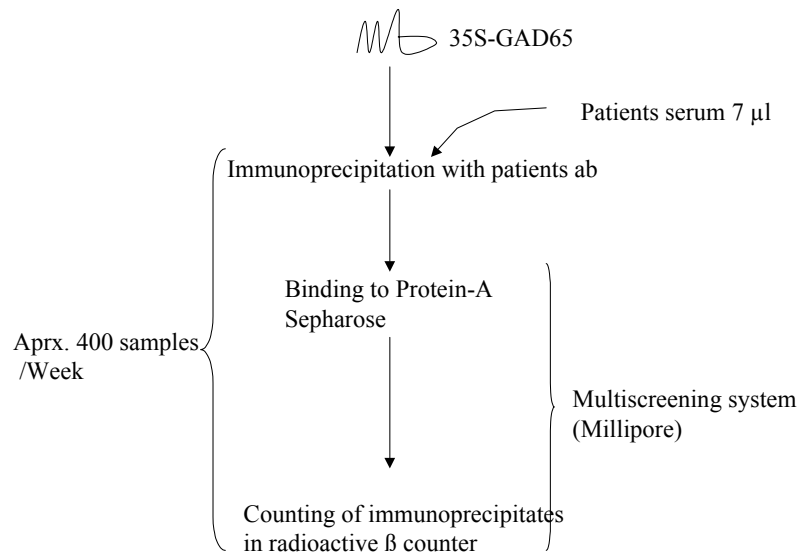
35S-GAD65

Patients serum 7 µl

Immunoprecipitation with patients ab

Aprx. 400 samples /Week

Binding to Protein-A Sepharose

Multiscreening system (Millipore)

Counting of immunoprecipitates in radioactive ß counter

Figure 5: Overview of 35S radioimmunoassay.

Statistical analysis

All the antibody levels were shown as antibody indices. The cut-off values were calculated as the mean + 3D of GAD65, IA-2, TTG, ICA12 and 21-OH index of healthy control sera from each population and for each antibody separately. Comparisons between frequencies were tested using the Fisher's exact test. P values less than 0.05 were considered to indicate statistical significance.

For genetic studies, comparison of haplotype frequencies between the diabetic and control groups was made using Chi-square test. The probability values were corrected (*Pc*) for the number of comparisons made, and considered significant if < 0.05.

Results and discussion:

Paper I:

In 101 Latvian IDDM patients and 111 healthy controls analysis of HLA-DR and DQ polymorphism shows DR3-DQ2 (OR 8.23, *Pc* < 0.05) and DR4-DQ8 (OR 6.12, *Pc* < 0.05) to be positively associated and DR15-DQ6 (OR 0.13, *Pc* < 0.05), DR13-DQ6 (OR 0.06, *Pc* < 0.05) and DR1-DQ5 (OR 0.27, *Pc* < 0.05) to be negatively associated with the disease. In addition, DQ7 (DQA1*0501-DQB1*0301) (OR 0.31, *Pc* < 0.05) was also negatively associated with IDDM. These findings in Latvian IDDM patients are consistent with observations in Scandinavian and other European populations. These finding suggest that IDDM in Latvia is not genetically different from IDDM seen in Scandinavia or Western Europe (Ronningen 1991). Earlier study on distribution of IDDM related HLA alleles in Eastern Baltic region shows that in general Latvian population there is low frequency of highly susceptible HLA alleles and increased frequency of protective HLA alleles (Nejentsev 1998). That was confirmed also in our study. When the negatively associated DQ are taken together in healthy controls, more than 75% of the healthy controls are positive for one of four negatively associated DQ molecules. Even though Latvia and Sweden are geographically close and Swedish genes are present in Latvian population due to historical reasons, the excess frequency of the negatively associated DQ molecules in the general population could explain the lower incidence of IDDM in Latvia. Even though environmental factors play a significant role in the development of the disease, genetic factors play a major role in disease protection.

Paper II:

The study on diabetic patients from Eastern India shows that DRB1*03 is increased in IDDM patients (OR 3.28, *Pc* < 0.05) compared to healthy controls. Interestingly, DRB1*07 was associated with FCPD compared to controls (OR 3.13, *Pc* < 0.05). DQ

analysis showed that IDDM in India is associated with DQ2 (OR 5.27, *Pc* < 0.05), but not DQ8 as in Caucasian populations. PDDM showed association with DQ9 (OR 4.25, *Pc* < 0.05).

In this study we also looked at the relation of autoantibodies to certain genetic markers. Patient groups were divided into GAD65ab positive and GAD65ab negative groups and the frequency of diabetes associated DR-DQ was analyzed. The only significant finding was that DR3-DQ2 was increased in GAD65ab positive NIDDM and PDDM patients compared to GAD65ab negative NIDDM and PDDM patients (OR 8.21, *Pc* = 0.01)

The findings of this study on IDDM patients confirm the findings of the earlier study on South Indian patients (Serjeantson 1987, Easteal 1990, Sanjeevi 1992) on the association of DR3-DQ2 but not DR4-DQ8 with the disease. Earlier studies in South Indian patients have shown the association of MRDM with HLA – DR7 and DQ9 (Sanjeevi 1992), while Ethiopian patients showed association with DR3 (Abdulkadir 1989), what is also shown in our study. These findings suggest the possibility of MRDM coexisting with IDDM and also the possible role of malnutrition in initiation the development of IDDM.

Paper III:

In this study we tested 115 IDDM patients and 105 healthy controls for the presence of GAD65 antibodies. GAD65ab were found in 57% IDDM patients and 4% healthy controls (p<0.001). The frequency of the antibodies in obese IDDM patients was significantly lower, than in normal and low weight patients (12% vs. 60%).

The frequency of GAD65ab in the patients with less that 10 years of duration ranged between 59 and 69% and then dropped to 29% in patients with longer duration. Interesting finding is that all the patients with younger than 5 years of age carried GAD65ab.

When we looked at the parents of the IDDM patients, we found that fathers had higher frequency of GAD65ab than mothers did (29% vs. 20%). Out of GAD65ab positive fathers 29% were diabetic and 29% had IGT. None of the antibody positive mothers were diabetic or had IGT.

This study shows that prevalence of GAD65 antibodies in South Indian IDDM's is lower than in Caucasian populations, where the prevalence is 80-90% in new onset IDDM patients. It could be explained by disease duration more than 5 years in 30 patients, since it is known that GAD65 antibodies tend to disappear several years after the clinical onset of the disease (Christie 1994).

High prevalence of GAD65 antibodies among parents suggests an underlining autoimmune process in healthy first – degree relatives.

***Paper IV*:**

In our population based study on Latvian patients we studied autoantibody markers. The prevalence of autoimmune markers (GAD65, IA-2, TTG, 21-OH, ICA12) was analyzed in both IDDM and NIDDM patient groups. In IDDM patients, we found GAD65ab in 61/87 (70%) when compared to 3/90 matched controls (3%), ($p<0.0001$).

IA-2ab were present in 54/87(62%) when compared to 5/90 (5%) controls, ($p< 0.0001$), while ICA12ab were present in 26/87 (30%) of IDDM patients in compare to 4/90 (4%) healthy controls ($p<0.0001$).

We know that in IDDM autoimmunity plays the main role. Our findings on the presence of autoantibody markers are not different from results obtained in other studies worldwide. Several studies on Caucasians show that GAD65ab may be found in 75 - 80% of new onset IDDM patients. But still 10-20% of clear autoimmune diabetic patients don't carry any of these major autoantibody markers, suggesting that other autoantigens should be involved in the autoimmune process.

While results of autoantibody assays in Latvian IDDM patients essentially confirmed studies in other populations, interesting results emerged in the study involving NIDDM patients. Classical Non-insulin dependent diabetes mellitus (NIDDM) is one of the most common forms of diabetes, usually diagnosed based on clinical criteria. According to WHO data NIDDM is present in almost 2 - 3% of the population in Europe, mainly in the age group of 50 to 60 years (Harris 1987). In classical NIDDM, autoimmunity is not responsible for disease pathogenesis. In NIDDM patients death of ß cells is not so rapid and they may have few or none of the classical symptoms of diabetes and do not always require exogenous insulin for glycemic control (Horton 1995).

However, this description involves only classical NIDDM, the diagnosis of which can be made only by excluding presence of autoimmune markers in the patients sera. In most countries including Latvia diagnosis of NIDDM is based on clinical criteria and patients treated with diet or oral hypoglycemic agents. In a certain group of patients this treatment turns out to be insufficient for diabetes control and insulin therapy is required. Some patients may require insulin because of inadequate treatment, however majority may become insulin - deficient because of autoimmune ß-cell destruction (Groop 1986, Willis 1996, Sanjeevi 1998). Autoimmunity in NIDDM has been described earlier in several publications as latent autoimmune diabetes in adults (LADA) (Zimmet 1994, Tuomi 1997). But lately autoimmune markers in clinical NIDDMs have been described in many studies and it is better identified as slow - onset autoimmune diabetes (Naik 1997, Lohmann 1997).

Our findings show that GAD65ab were present in 30/100 (30%) of NIDDM patients and 2/100 (2%) controls ($p < 0.0001$), while IA-2 antibodies in 40/100 (40%) patients and 4/100 (4%) controls ($p < 0.0001$). Autoantibodies against minor antigens are not significantly increased in NIDDM group when compared to healthy controls.

When autoantibody marker combinations are analyzed in both study groups we can see either GAD65ab or IA-2ab in 76/87 (87%) IDDM patients and 55/100 (55%) NIDDM patients. TTGab are present in 1/87 (1%) IDDM patients and 1/100 (1%) NIDDM patients. This number is very small, and in addition to GAD65ab and IA-2ab contributes to only a further 1% increase of autoimmune markers in both IDDM and NIDDM groups. Neither GAD65ab, IA-2ab nor TTGab and ICA12ab are present in 10% of IDDM patients and 44% of NIDDM patients. Interestingly, while GAD65ab positivity is higher in the IDDM patients, IA-2ab are more prevalent in clinical NIDDM patients.

None of the patients or controls in both study groups carry antibodies against 21-OH.

NIDDM patients also in other populations have also been found to be positive for autoantibody markers, which are indicative of slowly progressing IDDM (Tuomi 1993, Abiru 1996, Kasuga 1996, Niskanen 1995, Sanjeevi 1999). It is not clear whether the reason for the slow onset is due to genetic causes or environmental.

Detection of GAD65 antibody along with IA-2 antibodies and additional minor antibodies as TTG, 21-OH, etc. might be useful for the identification of subjects at risk of developing IDDM and screening individuals without family history of IDDM. So GAD65ab analysis, supplemented with IA-2ab and other minor antibody analysis are likely to increase sensitivity in predicting IDDM (Tremble 1997). Both GAD65ab and IA-2ab are ß-cell specific autoantibodies which characterize autoimmune damage of ß-cells well before clinical onset of IDDM diabetes and as these antibodies are usually not found in healthy population, they may distinguish IDDM from healthy children.

Slow onset autoimmunity in NIDDM can be identified only by antibody positivity to GAD65 or IA-2. But the antibody positivity in NIDDM not always refers to slow onset IDDM. They can be also autoimmune polyendocrine syndrome patients, especially if there are any other autoantibodies present (Gambelunghe 2000). The reason why the

slow onset diabetes is so common in Latvian population is not clear. Majority of these autoantibody marker positive patients were treated with oral hypoglycemic agents. Studies have shown that these patients when treated with small doses of subcutaneous insulin limit pancreatic ß-cell damage and also helps in the recovery of ß-cell function (Kobayashi 1996, Kobayashi 1996, Steffes 1996).

Paper V:

In this study we analyzed 102 newly diagnosed IDDM patients from South of Sweden and 99 healthy controls for the presence antibodies against new antigen ICA12 (SOX13) in relation to ICA, GAD65 and IA-2 antibodies.

The results indicate the presence of ICA12ab in 9.8% IDDM patients compared to 2.0% in healthy controls (p=0.033).

At least on of the four antibodies were present in 67%, but only 2% of IDDM patients carried ICA12ab alone. One of the three major antibodies was present in 65%, so the presence of ICA12ab contributes insignificantly to revealing new cases of autoimmunity.

The frequencies of ICA, GAD65 and IA-2 antibodies were higher in the age group below 35 years, compared to the group above 35 years (71% vs. 40%), while the difference in these age groups was not significant in correlation to ICA12 antibodies.

Our results show that frequency of ICA12ab in type I diabetes patients was 10%. This finding is similar to the results obtained in German population (Steinbrenner 2000). However the German study showed also increased frequency of ICA12ab in healthy population (5.2%), while in our study it was only 2%. We observed also that ICA12ab were more present in patients carrying GAD65ab, while presence of both ICA12 and IA-2ab was observed only in one patient.

Conclusions:

1) HLA-DR and DQ associations with susceptibility to and protection from IDDM in Scandinavian and Western European populations are the same in Latvian patients with IDDM.

2) Decreased incidence of IDDM in the Latvian population could be due to an increased frequency of protective HLA-DR and DQ molecules in healthy population (75% in Latvia vs. 31% in Sweden).

3) GAD65 antibodies are present in 75% of Latvian IDDM patients and 57% of South Indian IDDM patients and support the statement of GAD65 being the main autoantigen in most populations irrespective of ethnic background.

4) Minor and shared antigens alone don't contribute in revealing new cases of autoimmunity. Studies on ICA12 (SOX13) antigen do not support the hypothesis about the importance of this antigen in IDDM.

5) Frequency of autoantibody markers in clinical NIDDM patients both from Latvia and India is increased suggesting the high prevalence of slow onset autoimmune diabetes as well as possibility of overdiagnosis of NIDDM.

6) GAD65 antibodies at diagnosis of diabetes can be predictive for insulin treatment in patients with slow onset autoimmune diabetes.

7) IDDM in Eastern India is associated with HLA-DR3 and DQ2 but not with DQ8 like in Caucasian populations, while DR7 is associated with FCPD and DQ9 with PDDM.

Present and future:

The last 10 years has brought lot of changes in the prediction of diabetes, in terms of determining the nature of autoantigens by detecting autoantibodies. If earlier, the cytoplasmatic anti islet cell (ICA) autoantibody test was considered crucial (Botazzio 1974, Bonifacio 1990), today assays with high specificity and sensitivity are available for three major islet autoantigens: glutamic acid decarboxylase (GAD65), protein tyrosine phosphatase (IA-2) and insulin (IAA). Most of the studies today are aimed at the prediction of Type 1 diabetes in relatives of diabetic individuals, but the problem is that 90% of patients with Type 1 diabetes do not have close relatives with the disease and prediction in the general population by screening should be considered important. Several studies have shown that the presence of three autoantibodies predicts diabetes, since they can appear during a subclinical period that can last for several years before the clinical onset (Tuomilehto 1994). The availability of HLA susceptibility markers, as well as high sensitive autoantibody tests, make possible the identification of individuals at risk for disease.

Other important point is prevention of the disease. Several large studies are going on in different countries aimed on Type 1 diabetes prevention. The biggest studies are:

The Trial to Reduce Diabetes in Genetically at Risk (TRIGR) in Finland, where cow's milk protein is excluded from diet during the first six months in newborns in diabetic families with increased genetic risk.

Another study in Finland is Diabetes Intervention and Prevention Study (DIPP) where children in general population with increased genetic risk in combination with positivity to two autoantibodies are given intranasal insulin.

Earlier it has been shown that nicotinamide – a soluble B group vitamin, has shown to protect ß-cells against toxins, cytokines, which generate nitric oxide, break DNA

strands, activate enzymes and lead to cell death. Nicotinamide improves ß cell regeneration, inhibits enzyme activity, protects cells from lysis after exposure to oxygen radicals (Andersen 1994, Radons 1994). The study - European Nicotinamide Diabetes Intervention Trial (ENDIT) is a multicenter double blinded trial to evaluate the effect of nicotinamide in high risk relatives of individuals with Type 1 diabetes. The aim of this study is to answer the question if nicotinamide can reduce the risk of progression to Type 1 diabetes by 40% in 5 years. Other study – German Nicotinamide Diabetes Intervention Trial (DENIS) – was evaluating the clinical efficacy of high nicotinamide doses in children at high risk for Type I diabetes. In New Zealand, nicotinamide is administered to ICA-positive children in an open study (Knipp 1998).

The study going on in USA, Diabetes Prevention Trial (DPT) is evaluating the protective value of subcutaneous insulin, trying to detect 35% decrease in the disease over 5 year period in ICA positive first and second-degree relatives with a low first phase insulin response. In annual ADA meeting in Philadelphia in June 2001, it was announced that low-dose insulin injections do not delay or prevent type 1 diabetes in people who have a high risk (50 percent or greater) of developing the disease within 5 years (JS Skyler 2001; www.niddk.nih.gov/welcome/releases/).

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