AUTOIMMUNITY IN INIDAN DIABETICS

***C. B. Sanjeevi , **A. Kanungo, ***A. K. Das, ****V. Balaji.**

INTRODUCTION :

Diabetes mellitus was classified by WHO in 1985 into insulin-dependent diabetes mellitus (IDDM), non-insulin-dependent diabetes mellitus (NIDDM) and malnutrition-related diabetes mellitus (MRDM). This classification of diabetes is based on the clinical criteria and not based on the pathophysiologic disease process. IDDM is also referred to as Type 1 diabetes and NIDDM as Type 2 diabetes. These terminologies are used almost in all countries synonymously. However the American Diabetes Association (ADA) in their recent classification, proposed in 1997, have used the terminology Type 1 diabetes and Type 2 diabetes and have dropped MRDM from the classification. After this classification, it has become clear that the terms Type 1 diabetes and Type 2 diabetes are used to describe the disease process, thereby giving the understanding that the IDDM and NIDDM are terms used to describe diabetes as clinical categories. In the current ADA classification, clinically described IDDM patients and the autoantibody positive NIDDM patients are to be referred to as Type 1 diabetes. As there are no markers available for the etiologic diagnosis of Type 2 diabetes, the diagnosis of the Type 2 diabetes is determined by exclusion of Type 1 diabetes.

In this paper we will first describe the markers for autoimmunity in Type 1 diabetes and then discuss these markers in the context of clinical classification followed in India for the diagnosis of diabetes.

MARKERS FOR AUTOIMMUNITY IN TYPE 1 DIABETES MELLITUS :

HLA:

Several genes have been studied in exploring the pathogenesis of Type 1 diabetes, but the most important of them all is the Human Leukocyte Antigen (HLA) complex, also called as Major Histocompatibility complex (MHC). An HLA class II gene, identified as IDDM1 locus, is located in the short arm of chromosome 6. The MHC encompasses a region of four million base pairs. HLA are highly polymorphic membrane bound glycoproteins that play an important role in the immune response of an individual. They bind and present antigenic peptides to CD4 + helper T cells [1]. The allelic variability affects the peptide binding properties of the molecules [2]. The HLA molecule can bind many different antigenic peptides, which are different for different HLA molecules [3]. At the same time, the same peptide can interact with several HLA molecules with different binding affinities [4]. The genes of the MHC are classified into class l, class ll and class lll genes. HLA class I are heterodimeric cell surface molecules with extracellular, transmembranous and intracellular part. They are present in all the nucleated cells in the body. These molecules have a single polypeptide chain containing three domains associated with b_2 microglobulin. The genes encoding these molecules are located in the telomeric region of the MHC complex. The class I genes represent classically HLA-A, -B, -C (Figure 1). Non classical class I genes have been identified, which include HLA-E, -F, -G, [5, 6] and class I pseudogenes HLA-H, -J, -K, and –L [7]. The function of these class I molecules are to present short peptides (8-9 amino acids long) to CD8 + cytotoxic T cells. Association of HLA with Type 1 diabetes was first demonstrated in the world, by the finding of increased frequency of HLA-B8 allele in Indian patients when compared to controls.

The class ll genes are located at the centromeric end of the MHC and they span a region of one million base pairs [8, 9]. The class II molecules are heterodimeric proteins consisting of a a chain and b chain expressed on the cells of the immune system, i.e., monocytes, macrophages, B-lymphocytes, dendritic cells and activated T cells. Products of the HLA class I and Class II loci show high degree of polymorphism [7]. For the class II region, DRA is non-polymorphic, but DRB, DQA1 and DQB1 show high degree of allelic variability. Many of these alleles differ from each other by several amino acids and these polymorphic loci are located in the peptide binding and T cell recognition area of the molecule. As more and more populations are studied, newer polymorphisms and additional combinations of allelic sequences are identified. HLADQ molecules when identified by serological

** Dept of Molecular Medicine University Stockholm, Sweden **Diabetes Care & Research Center, Cuttack-Orissa ***Dept. of Medicine JIPMER, Pondicherry **** Diabetology Dept. Apollo Hospitals, Chennai.*

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techniques were previously called as DQ1, DQ2, DQ3 and DQ4. Subsequently, with improved DNA based typing techniques, DQ1 could be subdivided into DQ5 and DQ6; DQ3 could be divided into DQ7, DQ8 and DQ9. Since DQ molecules is made of DQA chain and DQB chain each one of them being polymorphic, identification of the DQA and DQB subtypes was possible by DNA typing techniques.

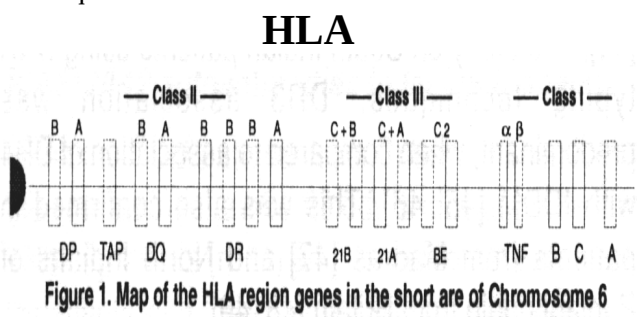

Figure 1. Map of the HLA region genes in the short are of Chromosome 6

Islet Cell antibodies (ICA)

Antibodies reactive against islet cell antigens (islet cell antibodies – ICA) were first described in 1974[10] and provided strong evidence for an autoimmune etiology and pathogenesis for Type 1 diabetes. They were termed as islet cell antibodies, as it was not clear what the antigen was. Even though the ICA was discovered nearly 25 years ago, the antigen or antigens for ICA are still not known. The ICA is islet cell specific and not beta cell specific. ICA belongs to IgG class and staining patterns in immunofluorescence shows two different patterns. When the entire islet fluoresces it is called 'whole-islet ICA' and if the staining is seen specifically in b cell it is called 'restricted ICA'. The major antigen for restricted ICA is Glutamic acid decarboxylase [GAD], as preabsorption with GAD can block ICA reaction [11]. From the current studies on patients with Type 1 diabetes, taken from different population, ICA is present in approximately 80-90% of new-onset patients [12, 13]. In-patients with clinical IDDM [14], clinical NIDDM and gestational diabetes mellitus (GDM), presence of ICA appears to predict later insulin-dependency. Inverse correlation has been reported between the presence of ICA positively and beta-cell function as evidenced by low C-peptide response [13, 15-17]. In Swedish Caucasians, with new onset diabetes, 84% were positive for ICA, suggesting a high prevalence [18].

GAD65 antibodies

Glutamic acid decarboxylase isoform 65 [GAD65] is the major islet autoantigen in Type 1 diabetes. This enzyme catalyses the conversion of inhibitory neurotransmitter g -amino butyric acid (GABA) from glutamate. Studies on prediabetic individuals showed occurrence of GAD65 antibodies (Ab) in a large number of cases [19, 20]. In the first-degree relatives of patients with Type 1 diabetes, the presence of GAD 65 antibodies appeared as a better marker for subsequent onset of clinical disease. The cloning of human islet GAD65 allowed the development of highly sensitive and specific radioimmunoassy to detect GAD autoantibodies in Type 1 diabetes [21-23]. GAD65 antibody positively was observed in 70 to 80% of new onset Type 1diabetes in several population, compared to only 1-2% in healthy controls [13,18, 23-19]. It was found that the prevalence of GAD65Ab was significantly higher among Type 1 diabetes females than among Type 1 diabetes males' [22]. This gender-dependent difference in occurrence of GAD65Ab seems to be more pronounced in children aged less than 12 years, and in this group, GAD65Ab were found in 80% of females and 61% of males [22]. In a study of 312 recent onset, Belgian, Type 1 diabetes patients [28], the prevalence of GAD65Ab amongst children less than nine years of age (64%), was found to be lower than that of ICA (86%) or insulin auto antibodies [IAA] (78%). ICA512 is associated with rapid onset of Type 1 diabetes, but GAD65 antibodies are associated with slower rate of disease progression [30, 31]. However, while the prevalence of ICA and IAA decreased with increasing age at onset of the disease, the prevalence of GAD65Ab remained unchanged (80% and 78% in 10-19 years and 20-39 years age groups, respectively). These data indicate that GAD65Ab may have the highest diagnostic sensitivity for Type 1diabetes amongst patients aged 20-30 years.

ICA512 antibodies

Immunoprecipitates of 64k antigens, when treated with proteases resulted in three distinct fragments called 50k, 37k and 40k [32]. Fragment 50k was found to be derived from GAD65. However, 37k and 40k immunoprecipitated a distinct class of antibodies [33] and these were shown to be derived from islet membrane associated protein. Screening of human islet cDNA expression library, using pooled sera from new onset Type 1 diabetes patients, resulted in precipitation of a number of important proteins like Carboxypeptidase-H, ICA69 and ICA512 [34]. The deduced amino acid sequence of ICA512 encodes a 60kDa protein containing a putative tyrosine phosphatase domain, homologous to the T-cell surface molecule CD45. Some of the properties identified for ICA512 were identified to be similar to 37k/40k antigen. A longer clone was reported independently, called IA-2, and the

nucleotide sequence suggested that ICA512 was a partial sequence of IA-2. The protein it encoded had a predicted molecular weight of 105kDa [35]. Later studies showed IA-2 to be a precursor for the 40k-antigen [36].

However, 37k protein was identified to be a protein related to IA-2 but it is distinct from IA-2 [37]. Studies on the mouse pancreatic endocrine cell line led to the discovery of a protein with high degree of homology to IA-2 and were called 'IA-2b '[38]. A similar protein in the rat model was designated 'phogrin' [39]. It was later shown that IA-2b /phogrin was recognized by antibodies in Type 1diabetes sera and IA-2b /phogrin inhibited the binding of 37k antibodies, suggesting that IA-2b /phogrin was a precursor for 37k antigen. Antibodies to IA-2 are more prevalent than IA-2b /phogrin and Type 1 diabetes sera recognizing IA-2b /phogrin and not IA-2 have not been demonstrated. There is 80% identity in the amino acid sequences of IA-2 and IA-2b PTP like domains and the antibodies in the Type 1 diabetes sera are directed to epitopes in this region [38]. ICA512 is expressed in pancreas and brain. IA-2 and IA-2b antibodies are shown to be associated with rapid progression to Type 1 diabetes [30, 31] and are associated in patients with a younger age at onset.

INSULIN AUTOANTIBODIES :

Insulin is a 51 amino acid molecule, made up of A chain and B chain, connected by a disulphide bond. Antibodies to insulin were initially described in untreated Type 1 and Type 2 diabetes patients in 1963 [40] and subsequently in newly diagnosed Type 1-diabetes patients, before the treatment with insulin [41]. Approximately 40-45% of the new onset Type 1 diabetes patients is positive for insulin autoantibodies (IAA). In prediabetic individuals, IAA when detected with ICA significantly enhances the risk of development of Type 1 diabetes [42]. There are no studies available on the prevalence of IAA in Indian Type 1 diabetes patients.

AUTOIMMUNE MARKERS IN INDIAN DIABETICS

HLA in Indian diabetics.

The first study demonstrating HLA association with Type 1 diabetes was done by an Indian in 1973 [43], who showed HLA-B15 and B-8 to be positively associated and B-7 to be negatively associated with IDDM, thus starting a new area of research, which has still not abated after 25 years. However, in North Indian IDDM patients, B-21 was significantly positively and HLA B-7 was negatively associated [44]. In a study on South Indian patients using DNA typing techniques, DR3 association was predominant, when compared to association of DR4 with IDDM [45, 46]. This was also confirmed in-patient from Madras [47] and North Indians of Punjabi origin from Britain [48,49].

ICA in Indian diabetics

Students on the Indian population however did not show high a prevalence of ICA, as was seen in European Caucasians. In North Indian patients with IDDM, about 40% of the new – onset IDDM were ICA positive [50,51]. It is not clear whether this wide difference in the prevalence of ICA in Caucasian and Indian population is due to genetic differences between the populations or the autoimmunity induced in Indian IDDM is different from that of the Caucasians. It has been shown that ICA are associated with DR4 and DQ8 in Caucasians and the association of Indian IDDM to DR3 rather than to DR4, could be one of the reasons for the low prevalence of ICA in Indian IDDM patients [23].

GAD65 autoantibodies in Indian Diabetics

There are very few studies on the prevalence of GAD65 antibodies in Indian IDDM patients. The prevalence of the GAD65 antibodies in South Indian IDDM patients was 59% in recent onset-IDDM patients (duration less than two years), but the frequency increased to 69% in-patients with duration between six to ten year's [53]. All patients below the age of five years were GAD65 antibody positive. This suggests that the IDDM seen in India is not different from that seen in Caucasians. Obese IDDM patients had significantly less GAD65 antibodies, when compared to undernourished and normal nourished patient's [53]. However, in IDDM patients from Cuttack, GAD65 antibodies were lower in frequency than in South Indian patient's [54]. In this group of patients, the predominant autoantibody was to ICA512 and not GAD65. However, in European Caucasians, the predominant autoantibody is to GAD65 and not ICA512 [55]. In an analysis of NIDDM patients from Cuttack, GAD65 antibodies were present in 7% of patients and their frequency was not significantly different from that of controls. However, the predominant autoantibody in NIDDM patients was ICA512 [56]. In NIDDM patients from Madras, prevalence of GAD65 antibodies decreased with increasing age.

ICA512/IA2 autoantibodies in Indian Diabetics

In Indian IDDM patients from Cuttack, ICA512 was the predominant autoantibody and was present in 43% of patients (n-74) and its incidence was lower in-patients with a shorter duration of disease, (less than four years, 32%) than in-patients of longer duration (more than four years, 60%). This finding is opposite to that found with GAD65 autoantibodies, which has a higher incidence in Indian patients with shorter rather than a longer duration of the disease [54]. In NIDDM patients from Cuttack (n =218), ICA512 autoantibodies are present in 39% of all patients and was seen in 40% of early onset (onset less than thirty-five years) NIDDM patients (n=128) and 38% of late onset (onset more than thirty-five years) NIDDM patients (n=90).

When all the NIDDM patients were divided into different age groups, the maximal ICA512 antibody positivity was present in 20-30 years age group (30/41, 73%) and in 50-60 years age group (14/23, 60%). This finding suggests that the incidence of slow-onset IDDM is higher in Indian patients and that WHO's clinical classification of diabetes does not allow this group of IDDM patients to be properly classified, as it relies on clinical criteria. This finding may suggest that the prevalence of autoimmune diabetes in India is probably as high as that observed in European or American Caucasians and proper follow-up studies are necessary to consolidate this finding. The reason why the slow-onset diabetes is more frequent in Indian patients is not clear. Studies on Swedish patients with IDDM showed that those who were DR3 positive, had a mild disease with less ketonuria at diagnosis, were less often ketoacidotic and more often had partial remission, than patients who were DR4 or heterozygous for DR3/DR4 [57]. The predominant HLA observed in the autoantibody positive Indian NIDDM patients, is DR3 and genetic factors could be one of the reasons for the slow-onset autoimmune form of diabetes in Indian diabetics.

IDDM :

IDDM, one of the most common chronic diseases of the childhood, is caused by autoimmune destruction of pancreatic b cells [58-60]. This destruction occurs over a prolonged period of time and the disease is clinically manifest when almost 90% of the pancreatic islet b cells are destroyed. During this period the patients are clinically healthy, but are positive for autoantibodies and may have a defect in b cell function. Islet infiltration by macrophages and lymphocytes [61-63], as well as islet cell antibodies (ICA) [10,12], glutamic acid decarboxylase (GAD65) antibodies [64-67] and insulin autoantibodies (IAA) [12,41], have been documented at or before the clinical diagnosis and may predict IDDM. The incidence rate of IDDM is reported to be high in several countries, being highest in Finland, Sardinia and Sweden and lowest in Japan [68,69]. Studies on different populations suggest that the country of residence [70] and genetic susceptibility [69,71] may be critical in conferring the risk for IDDM.

The prevalence of IDDM in India has been reported to be low [72,73]. In a large screening programme for diabetes in school children, no case of diabetes was detected [74]. IDDM prevalence was estimated to be 0.26/1000 in children aged less than 15 years, in an urban population from South India [75]. In a retrospective study, the incidence of IDDM from Madras was identified to be 10.5/100000/year, which was higher in boys, when compared to girl's [76]. A nation wide multicentric study conducted in 1975, showed an overall prevalence of 1.8% for NIDDM in population over 15 years and a higher rate was reported in urban versus rural population (21.1% and 1.5%) [77]. However, in a recent study, the incidence of NIDDM was reported to be 5% of the population (age over twenty years) in urban Indians [56]. With the reported high prevalence of slow-onset diabetes amongst NIDDM population in Eastern India [56], how many of these clinical NIDDM are true NIDDM or slow-onset IDDM is not clear.

IDDM is positively associated with HLA-DR4 in Caucasians [79], the frequency of which is increased when compared to normal controls, while the frequency of DR2 is decreased [80,81]. Studies of MHC class II genes have shown that DQ8, when in linkage disequilibrium with DR4 are more strongly associated with IDDM than the DR genes [82] On the other hand, other mechanisms may be important, since DR3 is more strongly associated with IDDM than DQB1 *0201 [81]. IDDM is positively associated with HLA-DQ2, DQ8 in Caucasians. Taken together DQ8 (DQB1 *0302-DQA1 *0301), DQ2 (DQB1 *0501-DQA1 *0201) or both account for as many as 89% of Caucasian IDDM patients with clinical onset before 15 years of the age [83]. These two haplotypes showed the strongest association as confirmed in numerous studies, but it is important to notice that DQ8, DQ2 or both do not account for all (100%) patients developing the disease. Several DQ haplotypes are negatively associated with IDDM, of which the most negative is DQB1 *0602-DQA1* 0102(DQ6). A single copy of this haplotype or of DQB1* 0602 is adequate to confer significant negative association, interpreted to represent protection form IDDM [80, 84-86].

DQ6 molecules (B1* 0603 and B1* 0601) share the same DQa chain (A1* 0103) and both are negatively associated. In the Indian population, DQ6 (B1* 0602) is infrequent but, DQ6 (B1* 0601) is the most frequent allele [87].

NIDDM :

Using clinical criteria for the diagnosis of diabetes, patients who did not present with ketoacidosis and ketonuria were grouped under the category of NIDDM. This resulted in the identification of NIDDM both in the older and younger age groups resulting in the identification of a group called "Early-onset NIDDM", as a possible additional clinical category in the Indian diabetes scenario.

With the advent of assays to detect autoantibodies becoming simpler, more and more patients were identified as autoantibody positive in the NIDDM category. This led to the identification of a group called "Late-onset Autoimmune Diabetes in the Adults or LADA" [88, 89]. This group of LADA, first described by Paul Zimmet [89], subsequently turned out to be present in the younger individuals with diabetes in the NIDDM category. This category has also been described as latent Type 1 diabetes, late-onset Type 1 and ½ diabetes. The Japanese found a high frequency of these patients and referred to them as slow-onset IDDM. This category has come to be addressed more appropriately as slowly progressive IDDM. It is believed that the autoimmune beta cell destructive process proceeds slowly in this form of diabetes. [90].

Several studies have shown that either GAD65 and/or ICA512 antibody positive patients have more rapid decline in C-peptide levels, do not respond adequately to oral drugs and require early insulin treatment. In a study from Japan, in non-obese and insulin-deficient patients with sulphonylurea failures, 11% were positive for GAD65 antibodies, suggesting that autoimmune mechanism may play a role in the secondary failure to Sulphonylurea therapy [91]. When ICA and GAD65 antibodies were compared in relation to beta cell function, the positive predictive value for insulin deficiency in GAD65 antibody positive and ICA positive patients was 39% and 78% respectively. The sensitivity of both antibodies for detecting insulin deficiency was 50%. The specificity for detecting insulin deficiency was 85% for GAD65 antibodies and 97% for ICA. Positively for both GAD65 antibodies and ICA gave a specificity and positive productivity value for insulin deficiency of 99% and a sensitivity of 50% [92] GAD65 antibody index has also been shown to correlate well with glycosylated hemoglobin [HbA1} in IDDM patients. Patients with low GAD65 antibodies have better glycemic control and require less insulin. However the ICA512 index did not correlate with HbA1 [93]. Thus loss of beta cell function in majority (two thirds) of individuals with clinical NIDDM can be predicted by GAD65 antibody and ICA. Early detection of immune markers of beta cell damage creates the potential for immunomodulation to limit such damage [94].

Clinical clues for suspecting Autoimmunity in NIDDM patients.

Antibodies to GAD65 and ICA512 are relatively easy to perform costs less and several hundred samples could be tested in a single day by one individual. These assays provide an easy way to identify the slowly progressive IDDM patients (who fare better with insulin), from the classical NIDDM patients, who can be effectively controlled by oral agents. However, in situations where these assays cannot be performed, there are clues to suspect patients who belong to autoimmune category of clinical NIDDM. These are:

The antibody positive NIDDM patients have significantly lower record of family history of diabetes when compared to antibody negative NIDDM patients, who have a strong family history of diabetes.

Obese NIDDM patients are less likely to be autoantibody positive, when compared to normal weight and under weight patients.

Patients with Sulphonylurea failures are more likely to be autoantibody positive.

Implication in the treatment of Autoimmune vs. Non autoimmune variety of NIDDM

Insulin treatment has been shown to be best for the autoimmune variety of diabetes both in animal models of autoimmune diabetes and in humans. The action of insulin in autoimmune situation has been to reduce hyperglycemia, thereby giving beta cell rest and for its immunologic effect. In the animal model of insulin, parenteral insulin had been shown to protect both NOD mouse model and BB rat model of the disease. In humans with IDDM, DCCT trial provides evidence that parenteral insulin may slow or inhibit IDDM disease process by a metabolic effect [95]. Patients treated by intensive therapy with insulin and by conventional insulin received similar doses and types of insulin. Those treated with intensive insulin resulted in less beta cell stimulation, meaning more beta cell rest, which

resulted in beta cell protection, compared to patients in the conventional therapy group [96]. A pilot study on the antibody positive NIDDM, done in Japanese patients, shows the preventive effect of small doses of insulin given subcutaneously compared to sulphonylurea treated patients. Four of the five patients in the antibody positive NIDDM group became antibody negative after follow-up for 30 months and none out of five in the sulphonylurea group remained antibody positive at the end of 30 months [97]. Currently, NIH sponsored Diabetes Prevention Trial-Type 1 (DPT-1) in USA, addresses the question of parenteral insulin to block or slow the Type 1 diabetes disease process in humans. The disease process in the antibody positive NIDDM patients is recognized as Type 1 disease process, and insulin may be preferred treatment in this group.

Recognizing these findings the ADA has recommended in their recent classification [98], that autoantibody positive patients are to be grouped under the category of Type 1 diabetes and autoantibody negative diabetes patients to be grouped under the category of Type 2 diabetes, which is further subdivided into two categories, one with insulin deficiency and the other with insulin resistance. WHO in their preliminary report [99], of their revised classification, have more or less adopted the ADA classification?

In the Indian diabetes scenario, combining IDDM and antibody positive NIDDM as Type 1diabetes, alters the picture of the incidence and prevalence of Type 1 diabetes and it can no longer be considered as rare or less frequent.

Conclusions :

1. HLA-DR3-DQ2 and DR4-DQ8 are the important susceptibility antigens for IDDM and the degree of DR3-DQ2 and DR4-DQ8 association varied with different populations. The strongest association with IDDM in Indian population is with DR3-Dq2 than DR4-DQ8.

2. GAD65 and ICA512 autoantibodies account for almost all ICA positive, new onsets, IDDM patients and accounts for 89% of new onset IDDM patients. GAD65 autoantibodies are predominant in European Caucasians with IDDM and ICA512 autoantibody is predominant in Indian patients.

3. The increased prevalence of autoantibody markers in Indian NIDDM, suggest that autoimmune diabetes is not as low as shown by epidemiological studies using WHO's 1985 clinical classification of diabetes and this increased prevalence of slow onset IDDM could be due to genetic factors and require special attention in their identification and treatment.

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REFERENCES :

1. Babbit B, Allen P, Matsueda G, Haber E, Unanue E: Binding of immunogenic peptides to IA histocompatibility molecules. Nature 1985, 317:359-61.

2. Buus S, Sette A, Coion S, Miles C, Grey H: The relation between major histocopatibility complex restriction and the capacity of la to bind immunogenic peptides. Science 1987, 235:1353-8.

3. Chicz R, Urban R: Analysis of MHC-presented peptides: Applications in autoimmunity and vaccine development lmmunol Today 1994, 15:155-60.

4. Sette A, Buus S, Apella E, Smith J, Chesnut R, Milles C, et al: Prediction of major histocopatibility binding regions of protein antigens by sequence pattern analysis. Proc Natl Acad Med Sci USA 1989, 86: 3296-300.

5. Koller BH, Geraghty DE, Shimizu Y, DeMars R, Orr HT: HLA-E., A novel HLA class I gene expressed in resting T lymphocytes. J Immunol 1988, 141: 897-904.

6. Geraghty D, Wei X Orr H, Koller B: Human leucocyte antigen (HLA-F) an expressed HLA gene composed of a class I coding sequence linked to a novel transcribed repetitive element. J Exp Med 1990, 171:1.

7. Bodmer JG, Marsh SGE, Albert ED, Bodmer WF, Dupont et al: B Erlich HA, Mach B, Mayr WR, Parham P, Nomenclature for factors of the HLA system, Tissue Antigens 1994; 44:1-18.

8. Hardy DA, Bell Jl, Long EO, Lindsten T, McDevitt HO: Mapping of the class II region of the human major histocompatibility complex by pulsed-field gel electrophoresis. Nature 1986; 323:453-5.

9. Dunham l, Sargent C, Dawkins R, Campbell R: An analysis of variation in the long range genomic organisation of the human major histocompatibility

complex class II region by pulsed field gel electrophoresis. Genomics: 1989: 5; 787.

10. Bottazzo GF, Florin-Christensen A, Doniach D: lslet Cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974; 1279-83.

11. Atkinson M, Kaufmann D, Newman D, et al.: Islet cell cytoplasmic antibodies reactivity to GAD in IDDM. J Clin Invest-1993; 91:350.

12. Landin-Olsson M, Palmer JP, Lernmark A, Blom L, Sundkvist G, Nystrom L, Dalhquist G: Predictive value of islet cell and insulin autoantibodies for Type 1 (insulin dependent) diabetes mellitus in a population-based study of newly diagnosed diabetic and matched control children. Diabetologia 1992; 35:1068-73.

13. Komulainen J, Knip M, Lounamaa R, Vahasalo P, Karjalainen J, Sabbah E, Akerblom H: Poor beta-cell function alter the clinical manifestation of Type 1 diabetes in children initially positive for islet cell specific autoantibodies. Diabetic Medicine 1997; 14:532-7.

14. Landin-Olsson M, Nilsson KO, Lernmark A, Sundkvist G: Islet cell antibodies and fasting C-peptide predict insulin requirement at diagnosis of diabetes mellitus. Diabetologia 1990; 33:561-8.

15. Elamin A, Omer M, Tuvemo T, et al. Islet-cell antibodies and endogenous insulin secretion in Sudanese diabetic children. Diabetes Res Clin Pract 1992; 16:91-96.

16. Manna R, Salvator M, Scueri F, et al. Negative correlation between ICA persistence and beta cell restoration after IDDM diagnosis. Diabetes Research 1988; 9:101-3.

17. Peig M. Gomis R. Ercilla G, Casamitjana R, Bottazzo G, Pujoll-Borrel R. Correlation between residual beta-cell function and islet cell antibodies in newly diagnosed Type 1 diabetes. Diabetes 1989; 38:1396-1401.

18. Hagopian W, Sanjeevi CB, Kockum I, Landin-Olsson M Karlsen A, Sundkvist G, Dahlquist G, Palmer J, Lernmark A: Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. J Clin Invest 1995; 95:1505-11.

19. Baekkeskov S, Landin-Olsson M, Kristensen JK, Srikanta S, Brunining GJ, Mandrup-Poulsen T, de Beaufort C, Soeldner JS, Eisenbarth G, Lindgren F, Sundkvist G, Lernmark A: Antibodies to a Mr 64,000 human islet cell antigen precede the clinical onset of insulin-dependent diabetes. J Clin Invest 1987, 79:926-34.

20. Atkinson MA, Maclaren NK, Scharp DW, Lacy PE, Riley WJ: 64,000 Mr autoantibodies as predictors of insulin-dependent diabetes. Lancet 1990; 335:1337-60.

21. Falorni A, Grubin C, Takei I, Shimada A, Kasuga a, Murayama T, Ozawa Y, Kasatani T, Saruta T, Li L, Lernmark A: Radioimmunoassay detects the frequent occurrence of autoantibodies to the Mr 65,000 isoform Glutamic Acid decarboxylase in Japanese insulin-dependent diabetes. Autoimmunity 1994; 19:113-25.

22. Falorni A, Ortquvist E, Person B, Lernmark A: Radioimmunoassays for glutamic acid decarboxylase and GAD65 antibodies using 35S or 3H recombinant human ligands. I Immune Methods 1995; 186:89-99.

23. Grubin C, Daniels T, BT, et al. Isoform-specific recombinant glutamic acid decarboxylase antibodies in childhood insulin-dependent diabetes mellitus Diabetologia 1994; 37:344-50.

24. Hagopian WA, Karlsen AE, Gottsater A, Landin-Olsson M, Grubin CE, Sundkvist G, Petersen JS, Boel E, Dyrbnerg T, Lernmark A: A quantitative assay using recombinant human islet glutamic acid decarboxylase (GAD-64) showed 64K autoantibody positivity at onset predicts diabetes type. J. Clin, Invest. 1993; 91:368-74.

25. Velloso LA, Kampe O, Hallberg A, Christmanson L, Betsholtz C, Karlesson FA: Demonstration of GAD-65 as the main immunogenic isoform of glutamate decaboxylase in type 1 diabetes and determination of autoantibodies using a radioligand produced by eukaryotic expression. J Clin Invest, 1993.

26. Seissler J, Amann J, Mauch L, Haubruck H, Wolfahrt S, Beig S, Richter W, Holl R, Heinze E, Northenmann W, Scherbaum W: Prevalence of autoantibodies to the 65 and 67 kD isoforms of glutamic acid decarboxylase in IDDM. J Clin Invest 1993, 92: 1394-9.

27. Petersen J, Hejnaes K, Moody A, Karlsen A, Marshall M, Hoier-Madsen M, Boel E, Michaelsen B, Dyrberg T: Detection of GAD65 antibodies in diabetes and other autoimmune diseases using a simple radioligand binding assay. Diabetes 1994; 43:59-65.

28. Sanjeevi CB, Falorni A, Robertson J, Lernmark A: Glutamic acid decarboxylase in IDDM. Diab Nutr Metab 1996, 9:167-82.

29. Sanjeevi CB, Falorni A Robertson, Lernmark A: Glutamic acid decarboxylase in IDDM. Diab Nutr Metab 1996; 9: 167-82.

30. Christie M, Genovese S, Cassidy D, Bosi E, Browm T, Lai M, Bonifacio E, Botazzo G:Antibodies to islet 37k antigen, but not to GAD, discriminated rapid progression to IDDM in endocrine autoimmunity. Diabetes 1994; 43:1254-9.

31. Bingley P, Christie M, Bonifacio E, Shattock M, Fonte M, Botazzo G, Gale E: Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibodies positive relatives. Diabetes 1994, 43:1304-10

32. Christie Mr, Vohra G. Champagne P, Daneman D, Delovitch TL: Distinct antibody specificities to a 64-kD islet cell antigen in type 1 diabetes as revealed by trypsin treatment. J. Exp, Med. 1990; 172:789-94.

33. Cristie M, Hollands J, Brown T, Michelsen B, Delovitch T: Detection of pancreatic islet 64000 Mr autoantigens in insulin-dependent diabetes distinct from glutamate decarboxylase. J. Clin Invest 1993; 92:240-8.

34. Rabin D, Pleasic S, Palmer-Crocker R, Shapiron J. Cloning and expression of IDDM-specific human autoantigens. Diabetes 19925; 41:183-6.

35. Lan M, Lu J, Goto Y, Notkins A: Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, lA-2, from human insulinoma. DNA Cell biology 1994; 13:505-14.

36. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E: Identification of protein tyrosine phosphatase-like lA-2 as the insulin-dependent diabetes related 37/40k autoantigen and a target of islet-cell antibodies. J Immunol 1995; 155:5319-426.

37. Payton M, Hawkes C, Christie M: Relationship of the 37000- and 40000-M (r) tryptic fragments of islet antigens in insulin-dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2. J Clin Invest 1995; 96:1506-11.

38. Lu J, Li Q, Xie H, Chen Z, Borovitskaya a, Maclaren N, Notkins A, Lan M: Identification of a second transmembrane protein tyrosine phosphatase, IA-2, as an autoantigen in IDDM: precursor of the 37kDa tryptic fragment. Proc Natl Acad Sci. Usa 1996; 93:2307-12.

39. Wassmeier C, Hutton J: Molecular cloning of phogrin, a protein tyrosine phosphatase homologue localised to insulin secretory granule membranes. J Biol Chem 1996, 271: 18161-70.

40. Pav J, Prague M, Jexokova Z, Skrha F: Insulin antibodies. Lancet 1963, 2:221.

41. Palmer JP, Asplin CM, Clemons P, Lyen K, K Tatpati O, Raghu PK, Paguette TL: Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 1983, 222: 1337-9.

42. Vardi P, Dib S, Tuttleman M, Connelly J, Grinbergs M, Rabizadeh A, Riley W, Maclaren N, Eisenbarth G, Soeldner J: Competitive insulin Autoantibody RIA. Prospective evaluation of subjects at high risk for development of type 1 diabetes mellitus. Diabetes 1987, 36:1286-91.

43. Singal DP, Blajchman MA: Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in-patients with diabetes mellitus. Diabetes 1973, 22:429-32.

44. Srikanta S. Mehra N, Vaidya M, Malaviya A, Ahuja M: HLA antigens in Type 1 diabetes mellitus in North India. Metabolism 1981; 30:992-3.

45. Serjentson S, Ranford P, Kirk R, Kohonen-corish M, Mohan V, Ramachandran A, Snehalatha C, Viswanthan M: HLA-DR and –DQ DNA genotyping in insulin dependent diabetes patients in South India Disease markers 1987, 5:101-8.

46. Easteal S, Viswanathan M, Serjeantson S: HLA-DP,-DR RFLP types in South Indian insulin-dependent diabetes mellitus patients. Tissue antigens 1990; 35:71-4.

47. Sanjeevi CB, Seshiah V, Moller E, Olerup O: Different genetic background for Type 1 diabetes and mainutrition related diabetes in South Indians. Diabetologia 1992, 35:283-6.

48. Fletcher J, Odugbesan O, Mijovic C, atal: Class II HLA DNA polymorphisms in type 1 (insulin-dependent) diabetic patients in North Indian origin. Diabetologia 1988; 31:343-50.

49. Jenkins D, Mijovic C, Fletcher J, Jacobs KH, Bradwell AR, Barnett AH: Identification of susceptibility loci for type 1 (Insulin-dependent) diabetes by transracial gene mapping. Diabetologia 1990; 33:387-95.

50. Srikanta S, Malaviya A, Mehtra N, Vaidya M, Geevarghese P, Ahuja M Autoimmunity in Type 1 diabetes in North India. J Clin Immunol 1981; 1:169-173.

51. Dabadghao P, Bhatia E, Bhatia V, Jayaraj K, Colman P:Islet-cell antibodies in malnutrition-related diabetes mellitus from North India. Diab Res Clin Prac 1996 1996; 34:73-78.

52. Sanjeevi CB, Hagopian W, Landin-Olsson M, Kockum l, Palmer J, Lernmark A, Dahlquist G: Association between autoantibody markers and subtypes of DR4 and DR4-DQ in Swedish children with IDDM reveals close association of tyrosine pyrophosphatase autoimmunity with DR4 than DQ8. Tissue Antigens (in press), 1998.

53. Sanjeevi CB, Shtauvere A, Ramachandran A, Snehalatha C, Falorno A, Prevalence of GAD65 autoantibodies in South Indian patients with IDDM and their parents. Diab Nutr Metab 1997; 10:60-4.

54. Sanjeevi CB, Shtauvere A, Kanungo A, Samal K, Tripathi B, Falorni A: Antibodies to ICA512 and GAD65 in IDDM patients from Eastern India. Diabetologia 1997, 40(Suppl. 1; Abstr):A72.

55. Shatauvere A, Rumba I Dzivite I Falorni A, Sanjeevi CB. Antibodies to ICA512 and GAD65 in IDDM patients form Latvia. Diabetologia 1997, 40(Suppl. 1;Abstr.):A71.

56. Kanungo A, Shatauvere A, Samal K, Tripathy B, Falorni A, Sanjeevi CB: Antibodies to ICA512 and GAD65 in patients with NIDDM from Eastern India. Diabetologia 1997, 40 (Supply.1):A164.

57. Ludvigsson J, Samuelsson U, Beauforts C, Deschamps I, Dorchy H, Drash A Francois R, Herz G, New M, Schober E: HLA-DR3 is associated with a more slowly progressive from of type 1 diabetes. Diabetologia 1986; 29(4):207-10.

58. Falorni A, Kockum I. Sanjeevi CB, Lernmark A: pathogenesis of insulin dependent diabetes mellitus. Clinical Endocrinology and Metabolism 1995, 9(1): 25-46

59. Greenbaum C, Brooks-Worrel B, Palmer J, Larnmark A: Autoimmunity and prediction of insulin-dependent diabetes mellitus. Diabetes Annual 1994, 8:21-52.

60. Ellis T, Atkinson M: The clinical significance of an autoimmune response against GAD. Nature Med 1993. 2:148-53.

61. Gepts W: Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 1965, 14:619-33.

62. Foulis AK, McGill M, Farquharson A:Insulitis in Type 1 (Insulin-dependent) diabetes mellitus in man-macrophages, lymphocytes and interferon-I containing cells. J Pathology 1991; 165:97-103.

63. Lernmark A, K Oppel G, Stenger D, Vathanaprida C, Falt K, Landin-Olsson M, Baskin D, Palmer J, Grown A, Petersen J, Linsong L, Edenwall H, Mauseth R: Heterogenicity of the human islet pathology in newly diagnosed childhood insulin dependent diabetes mellitus. Macrophage infiltration and expression of HLA-DQ and glutamic acid decarboxylase. Virchwos Arch 1995, 425:631-40.

64. Beakkeshov S, Nielsen JH, Marner B, Bilde T, Ludvigsson J, Lernmark A: Autoantibodies in newly diagnosed diabetes children immunoprecipitate human pancreatic islet cell proteins, Nature 1982; 298:167-9.

65. Baekkeskov S, Ananstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P: Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 1990; 347:151-6.

66. Karlsen AE, Hagopian WA, Grubin CE, Dube S, Disteche CM, Adler DA, Barmeier H, Mathewes S, Grant FJ, Foster D, Lernmark A: Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. Proc Natl Acad Sci Us 1991;88:8337-41.

67. Hagopian WA, Michelsen B, Karlsen AE, Larsen F, Moody A, Grubin CE, Rowe R, Petersen J McEvoy R, Lernmark A: Autoantibodies in IDDM primarily recognize the 65,00M, rather than the 67,000-M, isoform of glutamic acid decarboxylase. Diabetes 1993; 42:632-6.

68. LaPorte RE, Tajima N, Akerblom HK, Berlin N, Brosseau J, Christy M, Drash AL, Fishbein H, Green A, Hamman R, Harris M, King H, Laron Z, Neil A: Geographic differences in the risk of insulin-dependent diabetes mellitus: the importance of registries. Diabetes Care 1985; 8:101-7.

69. Karvonen M, Tuomilehto J, Libman l, LaPorte R, Group F: A review of the recent epidemiological data on incidence of type 1 diabetes mellitus worldwide. Diabetologia 1993; 36: 883-92.

70. Bodansky H, Staines A, Stephenson C, Haigh D, Cartwright R: Evidence for an environment effect in the etiology of insulin-dependent diabetes mellitus in a transmigratory population. Br Med J 1992; 304: 1020-2.

71. Dorman JS, LaPorte RE, Stone RA, Trucco M: Worldwide difference in the incidence of type 1 diabetes are associated with amino acid variation at position 57 of the DQ beta chain. Proc. Natl. Acad. Sci. USA 1990;87:7370-4.

72. Menon P, Viramani A, Shah P, Raju R, Sethi A: Childhood onset diabetes in India: an overview. J Diab Dev Countries 1990; 10:11-6.

73. Mohan V, Ramachandran A, Viswanathan M: Tropical diabetes. In Alberti K, Krall L (eds): Diabetes Annual, vol 1. Amsterdam, Elsevier, 1985; p 82-92.

74. Asha Bai P, Krishnaswami C, Chellamana M, Vijay Kumar G, Subramaninm J: Glycosuria and diabetes mellitus in children and adolescents in South India. Diab Res Clin Prac 1991; 13:31:131-6.

75. Ramachandran A, Snehalatha C, Abdul Khader O, Annie Joseph T, Viswanathan M: Prevalence of childhood diabetes in an urban population in South India. Diab Res Clin Prac 1992; 17:227-31.

76. Ramachandran A, Snehalatha C, Krishnaswamy C, registry Ml: Incidence of IDDM in children in urban population in Southern India. Diab Res Clin Prac 1996; 34:79-82.

77. Ahuja M: Epidemiological studies on Diabetes mellitus in India, In Ahuja M (ed): Epidemiology of diabetes mellitus in developing countries. New Delhi, Interprint, 1979; P 29-38.

78. Ramachandran A, Jali M, Mohan V, Snehalatha C, Viswanathan M: High Prevalence of diabetes in an urban population in South India. Br med J 1988; 297:587-90.

79. Ronningen KS, Sprukland A, lwe T, Vartdal F, Thorsby E: Distribution HLA-DRB,-DQA1-DQB1 genotypes among Norwegian patients with insulin-dependent diabetes mellitus. Tissue Antigens 1991; 37:105-111.

80. Sanjeevi CB, Lybrand TP, Landin-Olsson M, Kockum I, Dahlquist G, Hagopian WA, Palmer JP, Lernmark A: Analysis of antibody markers, DRB1 DRC5,DQA1 and DQB1 genes and modeling of DR2 molecules in DR2 positive patients with insulin dependent diabetes mellitus. Tissue Antigens 1994; 44:110-119.

81. Kockum I, Wassmuth R, Holmberg E, Michelsen B, Lernmark A: HLA-DQ primarily confers protection and HLA-DR susceptibility in Type 1 (insulin-dependent) diabetes studied in population-based affected families and controls. American Journal of Human Genetic 1993; 53:150-67.

82. Nepom GT: Immunogenetics and IDDM. Diabetes review 1993; 1(1):93-103.

83. Sanjeevi CB, Landin-Olsson M, Kockum I, Dahlqvist G, Lernmark A: Effects of the second haplotype on the association with childhood insulin-dependent diabetes mellitus. Tissue antigens 1995; 45:148-52.

84. Caillat-zuemons, Grachon HJ, Timsit J, Assan R, Boitard C, D, Kilali-Saiah I, Bongneres P, Bach JF: Age dependent HLA genetic heterogenecity of Type 1 insulin dependent diabetics mellitun Jour Clin Inves: 1992; 90(6): 2242-50.

85. Sanjeevi CB, DeWeese C, Landin-Olsson M, Kockum I, Dahlquist G, Lernmark A, Lybrand T: Analysis of critical residues of HLA-DQ6 molecules in insulin dependent diabetes mellitus. Tissue Antigens 1997; 50:61-65.

86. Sanjeevi CB, Lybrand T, DeWeese C, Landin-Olsson M, Kockum I, Dahlquvist G, Sundkvist G, Stenger D, Lernmark A: Polymorphic amino acid variations in HLA-DQ are associated with systematic physical property changes and occurrence of insulin dependent diabetes mellitus. Diabetes 1995; 44:125-31.

87. Sanjeevi CB, Olerup O: Restriction fragment length polymorphism analysis of HLA-DRB DQA and DQB genes in South Indians and Swedish Caucasians. Transplantation Proc. 1992; 24: 1687-8.

88. Tuomi T, Groop L, Zimmet PZ, Rowley MJ, Knowledge W, Mackay IR: Antibodies to GAD reveal latent autoimmune diabetes in adults with a non-insulin dependent onset of disease. Diabetes 1993; 42:359-62.

89. Zimmet PZ, Tuomi T, Mackay IR, Rowley MJ Knowles W, Cohen M, Lang DA: Latent Autoimmune diabetes mellitus in adults (LADA); the role of antibodies to GAD in diagnosis and prediction of insulin-dependency. Diabetic Medicine 1994; 11:299-303.

90. Kuzuya T, Matsuda A: Classification of diabetes on the basis of etiologies versus degree of insulin deficiency. Diabetes Care 1997; 20: 219-20.

91. Fukui M, Nakano K, Shigeta H, Yoshimori K, Fujii M, Ktagawa Y, Mori H, Kajiyama S, Nakamura N, Abe Net. Antibodies to GAD in newly diagnosed adult onset diabetes mellitus. Diabetic Medicine 1997; 14: 148-52.

92. Wills JA, Scott RS, Brown LJ, Forbes LV, Schmilli RS, Zimmet PZ, Macky IR, Rowley MJ: Islet cell antibodies and antibodies against GAD65 in newly diagnosed adult onset diabetes mellitus. Diabetes Res Clin Prac, 1996, 33:89-97.

93. Hoeldtke RE, Bryner KD, Horvath GG, Byerly MR, Hobbs GR, Marcovina SM, Lernmark A: Antibodies to GAD and glycemic control in recent onset IDDM. Diabetes Care 1997; 20: 1900-3.

94. Naik RG, Palmer JP: Late-onset type1 diabetes. Current Opinion in Endocrinology and Diabetes. 1997; 4: 308-15.

95. Ahren JA, Kruger DF, Gatcomb PM, Petit WA Jr, Trambolane WV: The diabetes control and complication trial (DCCT): the trial coordinator prespective. Report by the DCCT Research Group. Diabetes Educ, 1996; 15:236-41.

96. Steffes M, Trambolane WV, Becker D, Palmer JP Cleary P for DCCT Research Group: The effect of intensive diabetes treatment on residual beta cell function in the DDCT. (Abstract) Diabetes 1996; 45: (Suppl2): 18.

97. Kobayashi T, Nakanishi K, Murase T, Kosaka K: Small doses of subcutaneous insulin as a strategy for preventing slowly progressive beta cell failure in islet-cell antibody positive patients with clinical features of NIDDM. Diabetes 1996; 45:622-6.

98. The Expert Committee on the Diagnosis and Classification of diabetes mellitus Report of the Expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 1997; 20:1183-97.

99. Albert KGMM, Zimmet PZ for the WHO Consultation: Definition, Diagnosis and Classification of Diabetes Mellitus and its complications. Part 1: Diagnosis and classification of Diabetes mellitus-Provisional report of a WHO consultation Diabetic Medicine 1998; 15:539-53.