UPDATE ARTICLE

IMMUNOLOGY OF DIABETES

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The field of immunology is closely linked to diabetes mellitus in several ways. Basically the subject of immunology and diabetes may be considered under four headings:

I Immunology and the etiology of diabetes
II Animal models in studies on immunology of diabetes
III Immunology and the complications of diabetes
IV Immunology and the treatment of diabetes

IMMUNOLOGY AND THE ETIOLOGY OF DIABETES MELLITUS

Research on the immunological aspects of diabetes mellitus during the past two decades has resulted in the recognition of the two primary types of diabetes namely Insulin Dependent Diabetes Mellitus (IDDM) which was previously known as Type 1 diabetes and Non-insulin Dependent Diabetes Mellitus (NIDDM) which was earlier referred to as Type 2 diabetes. Autoimmunity appears to play a significant role in the development of IDDM. In this section therefore the link between immunology and the occurrence of IDDM will be considered.

There are several evidences for autoimmunity in the etiology of IDDM.

1) Association between IDDM and other autoimmune diseases

Clinical studies have shown that IDDM shows a significant overlap with other autoimmune diseases like Addison's disease (primary adrenocortical failure) and thyroid diseases such as thyrotoxicosis, Hashimoto's thyroiditis and primary hypothyroidism.[1] The prevalence of IDDM in patients with pernicious anemia and myasthenia gravis is also higher than in the genral population. This suggests that similar autoimmune mechanisms may operate in IDDM subjects.

2) Presence of pancreatic islet cell antibodies

a) Islet cell antibodies (ICA)

Evidence of islet cell antibodies (ICA) in the sera of diabetic patients was first described by the Middlesex hospital[2] and Edinburg groups[3] in late 1974 using the immunofluorescence technique. It was shown by these workers that ICA could be detected in 60-80% of IDDM patients at the time of diagnosis. In contrast, only 0.5% of the control population showed ICA positivity. This finding was an important breakthrough because ICA could be used as a fairly reliable marker for IDDM. There are two types of ICA namely the cytoplasmic ICA and the surface ICA. The prevalence rates of these two antibodies vary in different patients. Until recently ICA could not be quantified. Since 1985 three international workshops have been held in order to improve precision within and between laboratories. By distributing sera containing standarized amounts of ICA to all participating laboratories, unknown sera can be diluted to an endpoint which is compared to standard sera. In this way ICA levels can be reported in standarized units (Juvenile Diabetes Foundation or JDF units) allowing comparison between laboratories. It has been shown that when the ICA titre is high (40 JDG units) the chances of developing IDDM are high.

Recent work[4] has shown that apart from the ICA other antibodies may also play a role in the etiology of IDDM. Table 1 lists some of the autoantibodies that have been described in the preclinical stages of IDDM.

Table 1: **Autoantibodies found in preclinical Stage of IDDM**

Antibody	Abbreviation	Year
Cytoplasmic islet cell antibody	ICA	1974
Islet cell surface antibody	ICSA	1978
Antibody to M - 64,000 islet cell protein	64KA	1982
Insulin autoantibody	IAA	1983
Proinsulin autoantibody	PAA	1988
Complement-fixing anti-adrenal medullary antibody	CF-ADM	1988
Islet cell stimulating antibody	ICSTA	1988

From: McCulloch DK, Palmer JP. *Diab Nutr Metab* 1989; 2:245-255.

b) Complement fixing ICA (CF-ICA)

If a non-diabetic person possesses CF-ICA, the chances of his developing IDDM are higher than if he had ICA along. In fact, CF-ICA can act as a marker for future development of IDDM. The CF-ICA is directly responsible for damage to the beta cells of the islets of Langerhans and is therefore a more reliable marker for beta cell damage.

c) Insulin autoantibody (IAA)

Insulin autoantibody (or IAA) was discovered by Gerald Palmer at the University of Washington in Seattle. As the name implies these antibodies bind to insulin either in the islets or in the blood. Like ICA, IAA has been detected in newly diagnosed IDDM as well as in first degree relatives of IDDM. IAA levels help predict how fast the islet cell distribution is progressing and how

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soon a person will develop it. In IDDM children below 5 years of age, almost 100% have IAA.

d) **64K Protein**

Another recently discovered antibody is the 64K antibody which was discovered in 1982 at the Hagedorn Research Laboratory in Denmark. Almost all children and young adults who develop IDDM have 64K antibodies which can be detected up to 7 years prior to onset of diabetes. Very recently it has been shown that the 64K protein is in fact glutamic acid decarboxylase (GAD), a protein found throughout the nervous system. This finding opens up new possibilities for early detection and prevention of IDDM.

3) **Role of T cells in pathogenesis**

An observation that islet cells in diabetics express class II major histocompatibility complex (MHC) antigens provided initial support for the role of altered antigen expression in triggering autoimmunity. In transgenic species expressing class I or class II MHC molecules, 100% of the progeny developed diabetes. However, the characteristic lymphocytic infiltration of islets (insulitis) was not observed. It has been shown in animal models that insulitis is associated with T antigen-specific antibodies. In these models activation of autoimmunity was related to delayed onset of T antigen expression. Triggering of autoimmunity in IDDM may, as in the T antigen model, result from delayed expression of islet antigens, with subsequent failure to establish self-tolerance followed by activation of islet-specific autoimmunity.

4) **Role of HLA in causation of IDDM**

IDDM is strongly associated with certain antigens of the human MHC located on the short arm of chromosome 6. The human MHC has genes that encode three classes of antigens called class I, class II and class III. the class II molecules code for HLA-A, B and C which are expressed on virtually all nucleated cells of the body. Class II genes code for products of the HLA-D region, namely DP, DQ and DR molecules that are consecutively expressed on several haemopoietic cell lineages, some macrophages and dendritic cells. Their expression can be induced on several other cell types, for example endothelial and epithelial cells. Class III genes code for the complement protein factors B, C2 and C4.

IDDM is associated with HLA-DR3 and DR4, the frequency of which are increased (95%) when compared to normal controls (40%), while the frequency of DR2 is decreased. DR1 also shows a positive association with IDDM. It is not known whether the relevant susceptibility or resistance related factors are DR determinants themselves or other class II determinants in linkage disequilibrium with DR or non-class II genes in this genetic region; this is being intensively studied. HLA-Dw specificities, as defined by homozygous typing cells, appear to be genetically subtypic to DR with each Dw specificity being associated with only one DR specificity. The Dw4 subtype of DR4 is increased in DR4 + IDDM compared to controls. In contrast, Dw2 specificity is decreased in DR2 + IDDM when compared to DR2 + controls, making HLA-DR subtyping all the more important.

Serological and Restriction Fragment Length Polymorphism (RFLP) studies of MHC class II genes have shown that HLA-DQ genes, which are in linkage disequilibrium with HLA-DR are more strongly associated with IDDM than the DR genes. In particular a 3.7-kb Bam HI fragment hybridizing with a DQ B probe is more common in DR4 diabetics than in controls. According to Todd and others,[5] it is the amino acid at position 57 in the DQ B molecule which determines susceptibility or resistance to IDDM. The presence of aspartic acid (Asp) at position 57 confers resistance in a dominant manner. Residues at position 57 in both the DQ B alleles are necessary for disease development in 94% Caucasian type 1 diabetics. The requirement of homozygosity of the Asp 57 negative DQ B allele may explain the apparent recessive inheritance of the MHC linked susceptibility to IDDM in man.

HLA studies in India:

The HLA profile in IDDM in South India is quite different from that reported from North India. In the south, HLA-B8 is associated with IDDM,[6] which is similar to the findings in the Caucasian IDDM. However, unlike the latter, there was no association with B15. Among Caucasians, HLA-DR3 and DR4 as well as the combination of DR3 and DR4 are increased in IDDM patients. In North Indian IDDM DR3 is increased whereas DR4 appears to be absent.[7,8] In South Indians DR3, and to a lesser extent DR4, are increased in IDDM patients but not the combination of DR3 and DR4 (Table 2). These studies appear to provide evidence for genetic differences in susceptibility to IDDM between Indians and Caucasians and even between the North and South Indian Populations. They also corroborate the earlier studies of Hammond and Asmal.[9]

Table 2: **HLA Associations in Different Populations**

	Caucasian IDDM	N. Indian IDDM	S. Indian IDDM
HLA System			
B	B8, B15	BW21	B8
DR	DR3, DR4 & DR3/DR4	DR3	DR3/DR4
DQ	DQ-Beta	?	DQ-Beta

We have done extensive genetic studies in South Indian IDDM using the technique of Restriction Fragment Length Polymorphism (RFLP) in collaboration with Hitman of the London Hospital, UK. These studies[10] showed that our IDDM patients show a strong associa-

tion with the HLA-DQ beta gene. Here again, minor differences were noted between Indian and Caucasian subjects.

II. ANIMAL MODELS IN STUDYING IMMUNOLOGY OF DIABETES

Two animal models have been widely used to study the immunology of IDDM. They are the BB rat and the NOD mouse.

BB rat:

Approximately 60% of BB rats develop Type 1 diabetes. Characteristically, diabetes occurs between 60 and 120 days of age and is associated with massive lymphocytic islet infiltrates. Equal numbers of male and female animals develop diabetes and the majority of animals which do not develop diabetes have evidence of islet lymphocytic infiltrates. The BB rat is unique in that in additon to Type 1 diabetes, these animals have a severe T cell lymphopenia best characterized by a total absence of circulating RT6 positive lymphocytes. The inheritance of diabetes is believed to be a recombination between a 'diabetogenic' gene and a 'lymphopenia gene'.

NOD Mouse:

As for the BB rat, for the NOD mouse also, more than one gene is involved in the pathogenesis of Type 1 diabetes. NOD mice develop diabetes after 13 weeks of age and there is a marked excess of diabetes among females (70-80%).

III. IMMUNOLOGY AND COMPLICATIONS OF DIABETES:

The role of immunology in the pathogenesis of late diabetic complications is a matter of controversy. Insulin antibodies following heterologous insulin administration and insulin anti-insulin complexes were thought to play a role in producing vascular damage. Vascular diabetic-like lesions in non-diabetic animals immunized with exogenous insulin have been reported. The fact that diabetic microangiopathy occurs in patients who have never received insulin and that it occurs with the same characteristics in insulin-treated and non-insulin treated diabetics argues against a primary involvement of insulin anti-insulin complexes in the pathogenesis of diabetic microangiopathy. In a preliminary report Irvine *et al* [11] noted in insulin treated diabetics that high titres of insulin antibodies correlated with evidence for immune complexes but that there was no correlation between the two in the presence of moderate or low titres of insulin antibodies. Thus soluble immune complexes, not necessarily comprised of insulin, may play a role in diabetic microangiopathy.

The prevalence of AgAb does not seem to be related to the type of treatment or to insulin antibody titres. The correlation noted in previous reports between high insulin antibody titres and AgAb are not borne out by recent studies. Although a pathogenic role for insulin anti-insulin complexes cannot be excluded, their involvement appears to be a relatively minor one.

IV. IMMUNOLOGY AND THE TREATMENT OF DIABETES

a) Role of insulin antibodies in the management of diabetes:

The conventional insulins available in our country are highly antigenic because they contain about 60% of pure insulin. The rest of the "insulin" is made up of various large molecular weight proteins derived from the exocrine and endocrine part of the pancreas as well as various insulin dimers and metabolites of insulin. Moreover these insulins are a mixture of beef and pork insulins. Use of impure insulins particularly in an interrupted manner may provoke severe antibody formation. We have reported on the frequency of insulin antibody formation by different insulin preparations in India.[12] We have also shown that the insulin antibody titres can be considerably brought down by the use of highly purified monocomponent insulins.[13] With the advent of MC Insulins and Human Insulins, the frequency of antibody mediated insulin related complications eg. insulin resistance, insulin allergy and insulin lipodystrophy have been considerably reduced.

b) Immunotherapy of IDDM:

Approximately 80% of beta cells of IDDM are damaged at the time of clinical diagnosis by the autoimmune process; however, there is still some beta cell mass left and regeneration may be possible. Thus immunotherapeutic efforts have been started which aim to block or reduce the cytotoxic process in order to favour beta cell regeneration. In the immunotherapy of IDDM Pozzilli *et al* [14] distinguish the "pre-cyclosporin period" and the time when cyclosporin came into use. Attempts to treat newly diagnosed IDDM patients with immunotherapy were first made nearly 10 years ago. Initially, clinical trials were conducted in small groups of patients without control groups and the results were unsuccessful. Cyclosporin (CyA) was first introduced in the late 70's for immunosuppression in transplant patients.

CyA is a fungal metabolite with potent immunosuppressive effects without significant myelotoxicity. It blocks lymphocyte function by inhibiting interleukin 2 dependent activity, the T cell proliferation induced by antigenic stimulation and furthermore the release of immunologically active cytokines such as interleukin 1, macrophage procoagulant activity, macrophage activating factor, migration inhibitory factor and gamma interferon. The therapeutic use of CyA in this disease characterized by the presence of activated lymphocytes would therefore seem appropriate. In experimental

models such as the BB Wistar rat and the NOD mouse, CyA prevents the onset of the disease; however, when CyA is discontinued, diabetes may reappear even though in a reduced percentage than expected.

Three large controlled studies with CyA have been carried out in diabetic patients: the European-Canadian study[15] and the other two in France.[16,17] Overall, in the CyA groups total remission (suspension of insulin therapy) at 1 year may be expected between 20 and 30% compared to 10-15% in control groups. These results showed that CyA therapy may change the natural history of the disease in its first year. Despite this achievement, results have given rise to several questions, the most relevant being 1) the nephrotoxicity associated with CyA therapy and 2) the length of treatment with CyA. CyA possesses acute and chronic nephrotoxicity. The former may develop rapidly at CyA blood levels of 1000 ng/ml, is usually detected in transplanted patients and evidence of toxic tubulopathy and peritubular congestion may be found. Chronic toxicity is characterized by intestinal fibrosis and tubular atrophy and occurs only in patients treated with higher doses than those used for diabetes (approximately 300 ng/ml whole blood). Acute nephrotoxicity developed only in a very small number of CyA treated diabetic patients, but the main concern is still the chronic nephrotoxicity.

Although CyA may be successful in inducing remission in IDDM if performed soon after diagnosis, its nephrotoxicity limits its use for long periods. Thus at present the use of CyA is restricted to specialized centres and mainly in the experimental setting and not for routine clinical use.

Table 3 outlines the various immunotherapy methods which have been tried in animal models. Most are too toxic for human use. Azathioprine at 2 mg/kg/day significantly increases insulin-free remission. Azathioprine-prednisone combination has also been used. Although immunosuppressive agents may halt the autoimmune destructive process, only patients with adequate B cell mass will enter insulin-free remission. Again the serious side-effects and toxicity are major limitations. Development of safer immunosuppressive drugs may herald the era of prevention of Type 1 diabetes.

Table 3: **Experimental Immunotherapy in animal models**

Preventive therapy	NOD mouse	BB rat
Anti-T cell antibodies	Yes	Yes
Anti-T helper antibodies	Yes	Yes
Anti-NK cell antibodies	No	Yes
Silica	Yes	Yes
Bone marrow transplantation	Yes	Yes
Thymectomy	Yes	Yes
Antibodies against islet-specific T cells	NT*	NT
Streptococcal OK-432	Yes	Yes
Nicotinamide	Yes	Yes
Azathioprine	NT	NT
Cyclosporin	Yes	Yes
Insulin therapy	Yes	Yes

*NT = not tested.

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