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Review Article

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A REVIEW ON ROLE OF AUTOANTIBODIES (GAD-65 IA-2) IN TYPE 1 DIABETES

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ABSTRACT

Insulin-dependent type 1 diabetes is an autoimmune disease mediated by T lymphocytes recognizing pancreatic islet cell antigens. Glutamic acid decarboxylase 65 (*GAD*-65) appears to be an important auto antigen in this type of disease. However, T cells from both patients with type 1 diabetes and healthy subjects vigorously proliferate in response to *GAD-65* stimulation *ex-vivo*, leading us to postulate that the critical event in the onset of human diabetes is the activation of auto reactive T cells. Thus, it was investigated, whether *GAD-65* reactive T cells in patients with diabetes functioned as previously activated memory T cells, no longer requiring a second, co-stimulatory signal for clone expansion. We found that in patients with new-onset type 1diabetes, *GAD-65*-reactive T cells were strikingly less dependent on *CD-28* and *B7-1* co-stimulation to enter into cell cycle and proliferate than were equivalent cells derived from healthy controls. The hypothesis reveals that these auto reactive T cells have been activated *in-vivo* and have differentiated into memory cells, suggesting a pathogenic role in type 1 diabetes. In addition, it was observed that different effects with selective blockade of either *B7-1 or B7-2* molecules; *B7-1* appears to deliver a negative signal by engaging *CTLA-4*, while *B7-2* engagement of *CD-28* up regulates T cell proliferation and cytokine secretion.

Keywords: Insulin-dependent type 1 diabetes, autoimmune disease, autoantibodies, T lymphocytes and *GAD*-65.

INTRODUCTION

DM is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] Insulin-dependent diabetes (IDDM; type I diabetes) is one of the most serious and common of metabolic disorders, affecting approximately 1 person in 300 in the U.S., while epidemiological studies in Europe suggest that its incidence is increasing.[2,3] The disease is thought to result from the autoimmune destruction of the insulin-producing.

Classification of Diabetes Mellitus

Type 1 diabetes

Immune-mediated diabetes*:* This form of diabetes, previously encompassed by the terms insulin-dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas.[2] Markers of the immune destruction of the β-cell include islet cell auto- antibodies (*ICAs*), auto antibodies to insulin *(IAAs*), auto antibodies to glutamic acid decarboxylase (*GAD-65*) and autoantibody to the tyrosine phosphatase *IA*-2[3,4] One and usually more of these auto antibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected, and it is influenced by the *DRB* genes.[5,6]

This form of diabetes, the rate of β-cell destruction is quite variable, being rapid in infants and children and slow Adults.[7] Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia or ketoacidosis in the presence of infection or other stress. Still others, particularly adults may retain residual β-cell functions sufficient to prevent ketoacidosis for many years. Many such individuals with this form of type 1 diabetes eventually become dependent on insulin for survival and are at risk for ketoacidosis.

Idiopathic diabetes: Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type 1 diabetes fall into this category, of those who do, most are of African or Asian origin. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes.

This form of diabetes is strongly inherited, lacks of immunological evidence for β-cell autoimmunity, and is not *HLA* associated. An absolute requirement for insulin replacement therapy in affected patients may come and go.[8]

Type 2 diabetes

This form of diabetes, previously referred to as non-insulin-dependent diabetes, type 2 diabetes, or adult-onset diabetes, is a term used for individuals who have insulin resistance and usually have relative insulin deficiency.[9-12] At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of

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diabetes, and it is likely that the proportion of patients in this category will decrease in the future as identification of specific pathogenic processes and genetic defects permits better differentiation among them. Although the specific etiology of this form of diabetes is unknown, autoimmune destruction of β-cells does not occur, and patients do not have any of the other causes of diabetes.

Other specific types of diabetes genetic defects of the β-cell

Several forms of diabetes are associated with monogenetic defects in β-cell function, this forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity-onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action.[13-15] Abnormalities at three genetic loci on different chromosomes have been identified. The most common form is associated with mutations on chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor (HNF).[16,17]

Diseases of the exocrine pancreas

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma.[18-20]

Endocrinopathies

Several hormones (e.g., growth hormone, cortisol, glucagon, and epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma) can cause diabetes.[21,22]

Drug or chemical-induced diabetes

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin résistance. Such drug reactions fortunately are rare. There are also many drugs and hormones that can impair insulin action, *e.g.* Include nicotinic acid and glucocorticoids.[23,24] Patients receiving α-interferon have been found to develop diabetes associated with islet cell antibodies and, in certain instances, severe insulin deficiency.[25,26]

Infections

Certain viruses have been associated with β-cell destruction. Diabetes occurs in patients with congenital rubella.[27]

Gestational diabetes mellitus (GDM)

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The definition applies regardless of whether insulin or only diet modification is used for treatment or whether the condition persists after pregnancy. It does not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy.[28]

DIAGNOSTIC CRITERIA FOR DM

The diagnostic criteria for DM have been modified from those previously recommended by the NDDG[29] (National diabetes data group) or WHO[30]. This recommendation is made in the interest of standardization and also to facilitate field work, particularly where the cost and demands on participant time may be excessive. This approach will lead to slightly lower estimates of prevalence than would be obtained from the combined use of the FPG and OGTT. Since insulin antibodies (*InsAb*) were first described, there have been numerous investigations of the possible influence of prolonged heterologous insulin treatment on the course of diabetic microangiopathy. This question is still unanswered. So far there have been a few conflicting studies that have tried to correlate the level of insulin antibodies with the severity of microangiopathy.[31,32] There have also been several contributions in animals showing a direct pathogenic role of exogenous insulin in microangiopathic lesions[32]. On the other, it is well known that the lesions of small blood vessels are present in patients treated with both insulin and oral agents, although differences have been described in the severity and frequency of the lesions. If insulin treatment has a role in aggravating blood vessel damage through immune mechanisms, the formation and deposition of insulin-anti-insulin complexes must be considered.

The study of insulin-anti-insulin complexes is complicated by a few factors.[33] Insulin is a weak antigen and is antigenically bivalent. Insulin complexes are usually small and soluble both in antigen and in antibody excess. Only a small part of these complexes, near the equivalence point, are large enough to be capable of triggering a sequence of events that can result in tissue damage. So far there are no routine methods available to measure these different kinds of complexes. When a few years ago circulating immune complexes (IC) were described in some diabetic conditions and when more recently these complexes were reported to be significantly increased in diabetics with severe microangiopathy compared with those without complications it was natural to suppose that part of the complexes found in patients with microangiopathy would be comprised of insulin.

AUTO-ANTIBODIES

The autoantibody production are varied and not well understood. It is thought that some autoantibody production is due to a genetic predisposition combined with an environmental trigger, such as a viral illness or a prolonged exposure to certain toxic chemicals. There is generally not a direct genetic link however. While families may be susceptible to autoimmune conditions, individual family members may have different autoimmune disorders, or may never develop an autoimmune condition. Researchers believe that there may also be a hormonal component as many of the autoimmune conditions are much more prevalent in women of child bearing age.[34]

The autoimmune response that causes type 1 diabetes begins years before clinical onset of disease, continues for years after diagnosis, and may recur years later. Studies of islets from patients with new-onset type 1 diabetes suggested loss of approximately 90% of insulin-positive cells. Early studies by Faber and colleagues showed that soon after diagnosis, the production of C-peptide in patients with diabetes consuming a standard diet was about one third of that seen in normal individuals. These studies measured 24-hour C-peptide levels, which provide only an estimate of insulin production. The interpretation that insulin secretion was impaired markedly at the time of diagnosis with little evidence of recovery of β-cell mass was prevalent in the late 1980 and early 1990.

A more recent, cross-sectional analysis of data from the Diabetes Care and Complication Trial (DCCT) showed more substantial insulin production in patients. DCCT, a stimulated C-peptide level of greater than 0.2 mol/mL was

associated with improved metabolic control compared with β-cell secretory levels below that level. Moreover, the DCCT suggested that retention of insulin production was a valuable endpoint for treatment of type 1 diabetes because improved metabolic control reduced the development of end-organ complications. Nonetheless, over time, C-peptide responses are impaired severely in most patients. Auto antibodies are found in most patients with new-onset type 1 diabetes, and identify prediabetic individuals at risk for the disease.[35,36]

The role of B-cell Antibodies

Antibodies that target the body's own tissues and cells are known as auto- antibodies. The presence of β-cell or pancreatic islet cell autoantibody confirms that type 1 diabetes is autoimmune in origin. The disease process in IDDM is primarily caused by the destruction of pancreatic β-cells. This cell destruction is thought to result mainly from the action of T-lymphocytes, the key players in autoimmune disease development. The β-cell auto-antibodies that characterize type 1 diabetes may not be responsible for cell destruction. Instead, these antibodies are thought to signal a T-cell mediated immune response that sets the stage for β-cell destruction.[37]

Other antibodies in diabetes

ICA was the first auto-antibodies discovered in patients with diabetes. However, antibodies specific to the β- cell antigens that make up islet cells are more specific. Antibodies to insulin and proinsulin also occur in diabetes. Antibodies to the enzyme glutamic acid decarboxylase (*GAD*-65), which is found in nervous system and pancreatic cells, are also seen in diabetes. *GAD -65* antibodies were first demonstrated in patients with Stiff-Man syndrome[38]. A disorder sometimes seen in patients with diabetes. Antibodies to the islet cell protein tyrosine phosphatase (*IA*-2) and phogrin are also seen in diabetes.[37]

Role of auto-antibodies in type 1 diabetes

The immune mediated form of diabetes is a relatively common disorder that develops in genetically susceptible individuals. The disease is associated with a series of anti-islet auto antibodies and the auto antibodies can be present for years prior to the onset of hyperglycemia. In man evidence is lacking that transplacental passage of anti-islet antibodies increases disease risk. Well characterized, autoantibody assays tested in a series of international workshops are now available, and are the mainstays of prediction of type 1 diabetes, diagnosis of the immune mediated form of diabetes, and are important for the design of trials for the prevention of type 1 diabetes. In addition to anti-islet auto antibodies, patients with type 1 diabetes develop a series of associated autoimmune disorders that are usually detected with screening for additional auto antibodies (e.g. anti-thyroid, anti-transglutaminase, anti-21 hydroxylase, anti-parietal cell). At present it is possible to predict the development of type 1 diabetes and prevent the disorder in animal, but we lack proven therapies for disease prevention in man. The ability to detect specific anti-islet auto antibodies provides the foundation for developing and testing these preventive therapies.[39]

HISTORY OF *IA-2*

IA-2, a member of the protein tyrosine phosphates family, is a major auto antigen in type 1 diabetes, of all newly diagnosed type 1 diabetic patients, 70% have auto antibodies to *IA*-2, and these auto antibodies appear years before the development of clinical disease. The presence of auto antibodies to both *IA*-2 and *GAD* are highly predictive markers for identifying individuals at risk of developing type 1 diabetes[40] and it is estimated that if both autoantibody are present, the likelihood of developing type 1 diabetes . The *IA*-2 molecule is 979 amino acids in length and consists of an intracellular, transmembrane, and extracellular domain. Autoantibody is directed exclusively to the intracellular domain[41]. Genomic structure and chromosome analysis revealed that *IA*-2 is located on chromosome and consists of 23 axon[42]. Although the amino acid sequence of *IA*-2 from non diabetic subjects has been determined. There have been no studies comparing the *IA*-2 amino acid sequence of diabetic subjects who are *IA*-2 autoantibody positive with no diabetic subjects who are *IA*-2 autoantibody negative.[43]

In *IA*-2 that might account for the development of *IA*-2 auto antibodies, genomic DNA from six *IA*-2 autoantibody positive type 1 diabetic patient. Seven pairs of primers covering the 23 axons of the coding region of *IA*-2 were prepared and used to amplify *IA*-2 genomic DNA by polymerase chain reaction (PCR). The PCR products were then cloned, and compared with the sequence of *IA*-2 from nondiabetic siblings and normal control data in the Gene Bank. A total of nine nucleotide substitutions were found, resulting in five amino acid changes. Seven of the nine nucleotide substitutions were found in different axons. The whole axon was amplified from the genomic DNA of 190 type 1 diabetic patients and 190 normal control subjects of Caucasian origin, single-strand conformation polymorphism analysis[43], did not identify any subjects with the same mutations, suggesting that these mutations have very low frequencies in the Caucasian population. Mutations in the promoter region of specific genes or the transcription factors that interact with these genes can influence the time and magnitude of protein expression[44].

GAD AUTOANTIBODY

GAD (glutamic acid decarboxylase) is present in all human islets cells and there are two forms of *GAD* termed *GAD-65*, *GAD*-67 that are 76% homologous in amino acid sequence. *GAD* is also synthesizing in testis ovary and neurons in addition to the islets, though only pancreatic β- cells are destroyed in type diabetes. In mouse islets *GAD* is usually not detectable but in rat it is only expressed in β-cells.[45] It is possible that the autoimmune response to *GAD* is secondary rather than the primary pathogenic importance, though auto antibodies to *GAD* for man is important for the diagnosis and diabetes prediction.[46]

ASSAY OF GAD -65

Sera (25 µl) were incubated in *GAD-65* coated ELISA plate wells followed by washing and incubation with *GAD-65* biotin. After a further wash step, *GAD-65* biotin bound was quantitated by addition of streptavidin peroxides followed by tetramethylbenzidine. Assay calibration was with WHO reference preparation. Using a cut-off for positively of 5 units/ml, sera from 0.7% healthy blood donors (HBDs), 100% selected type 1 diabetes mellitus (DM) patients, 1.6% type 2 DM patients and 3% autoimmune disease controls were *GAD-65* Ab positive in the ELISA. Levels of positivity in an immunoprecipitation assay (*IPA)* based on *GAD-65*, respectively.[47]

IA- 2 AND *GAD* AS AUTO-ANTIGEN

Insulin auto-antibodies: Insulin and its precursor, proinsulin are the major β- cell specific auto antigen in

human islets *GAD-65 IA-2* auto antigen are also present in glucagon and somatostatin producing islets cell in man. Berson and Yalow developed the first radioimmunoassay utilizing sera with anti-insulin antibodies from patient treated with bovine insulin. The presence of anti insulin antibodies; either induced by exogenous insulin injection or by naturally occurring doesn't usually interfere with insulin therapy. Patients with a very rare syndrome termed insulin autoimmune syndrome, also termed as Hirata syndrome, express extremely high levels of insulin autoantibody often with episodes of severe hypoglycemia.[48]

CONCLUSION

Auto-antibodies against the intracellular part of a membrane bound, protein tyrosine Phosphates-like molecule, *IA*-2, are rarely detected in non-diabetic children and Adolescent. A combined analysis of auto-antibodies seems to be a useful way of assessing the future risk of developing the disease. Humoral immune responses are stronger in patients diagnosed under the age of 20 years than in older ones, as reflected by higher autoantibody frequencies in younger patients. Antibodies against *GAD*-65 are present in a majority of patients with type 1 diabetes mellitus and those auto antibodies against other islet cell antigens also exist. Well characterized, high throughout autoantibody assays are now available, and are the mainstays of prediction of type 1 diabetes, diagnosis of the immune mediated form of diabetes, and are important for the design of trials for the prevention of type 1 diabetes.

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