

# IMMUNOLOGICAL RESPONSE IN DIABETES MELLITUS

Pages with reference to book, From 297 To 300

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Diabetes mellitus is a complex syndrome characterized by hyperglycaemia, thickening of basement membrane of the capillaries and a variety of late complications including accelerated atherosclerosis, retinopathy, nephropathy and neuropathy. Primary diabetes mellitus is broadly divided into type I or the insulin-dependent diabetes mellitus (IDDM) and type 2 or the non-insulin-dependent diabetes mellitus (NIDDM). There also exists a rare form of diabetes mellitus which is dominantly inherited, referred to as "maturity-onset" diabetes of the young and is distinct from the more common types of primary diabetes. IDDM has an earlier age of onset (usually less than 30 years), often rapid onset, is ketosis prone, associated with human leukocyte antigens (HLA) and highly associated with anti-islet cell immunity. NIDDM has a later age of onset (usually more than 40 years), often insidious onset, rarely leads to ketosis, is not associated with HLA and rarely, if ever, associated with anti-islet cell immunity<sup>1</sup>. Since IDDM is highly associated with anti-islet cell immunity, some investigators have proposed two subtypes of the type 1 diabetes: type 1a, the more common juvenile form<sup>2,3</sup> thought to be initiated by viruses<sup>4,5</sup> and type 1b<sup>6,7</sup> found in polyendocrine patients in whom viral etiology is less conspicuous and an autoimmune anomaly is likely.

## HLA AND DIABETES<sup>8</sup>

IDDM is one of the many chronic diseases which exhibit marked geographic variation in incidence and the worldwide patterns show that IDDM incidence rates are very high in the Scandinavian countries, like Finland (29.5/100,000 per year), but are extraordinarily low in the oriental populations like China and Japan (0.7/100,000 per year). Worldwide differences in the incidence of IDDM are thought to be associated with amino acid variation at position 57 of the HLA-DQ beta chain. The presence of an amino acid other than aspartic acid at position 57 of the HLA-DQ beta chain is highly associated with susceptibility to IDDM, whereas an aspartic acid at this position appears to confer resistance to the disease<sup>8</sup>. Although genetic differences across populations may be responsible for the geographic pattern of incidence, immunological differences clearly play a role in the pathogenesis of IDDM.

### Autoimmunity in the pathogenesis of IDDM

At least five types of evidences suggest that autoimmunity may play an important role in IDDM. These include: 1) clinical association of IDDM with other autoimmune endocrinopathies, 2) prevalence of autoantibodies to non-pancreatic antigens, 3) pathologic changes in the pancreas of early or untreated diabetics 4) circulating antibodies to endocrine pancreas and 5) evidence of cell-mediated immunity to pancreas.

#### 1. Association of IDDM with other autoimmune endocrinopathies

The earliest and most compelling indirect evidence of auto-immune nature of IDDM came from its association with co-existent autoimmune disease<sup>9</sup> and other endocrinopathies<sup>10</sup> thought to have autoimmune basis<sup>1,11</sup>, including Graves' disease, primary hypothyroidism, pernicious anaemia<sup>7</sup> and idiopathic adrenal insufficiency'. These findings in addition to the high prevalence of auto-antibodies to non-pancreatic antigens form the first clue to the auto-immune process in IDDM.

#### 2. Prevalence of auto-antibodies to nonpancreatic antigens

A high prevalence of auto-antibodies to non-pancreatic antigens has been observed in association with IDDM. Christy et al<sup>1</sup> reported presence of organ-specific auto-antibodies in sera of diabetic

populations. Among the diabetics, they<sup>1</sup> reported 16% individuals as positive for anti-thyroid microsome antibodies, 10% positive for anti-thyroglobulin antibodies, 17% positive for anti-parietal cell antibodies, 2% positive for anti-intrinsic factor antibodies and 1% positive for anti-adrenal cortex antibodies. Among the diabetic patients positive for anti-islet cell antibody, Bottazzo et al<sup>7</sup> reported 93% individuals with thyrotoxicosis as being positive for anti-thyroid microsomal antibodies as against 71% of anti-islet cell antibody positive patients with myxedema. The presence of lymphocyte surface antibodies primarily of IgM class have been reported at onset of IDDM. Furthermore, these antibodies persisted during the first year of insulin treatment and were also detected in first degree relatives of lymphocyte surface antibody-positive patients. It is unlikely that IgM- lymphocyte surface antibodies mark the destructive process in pancreatic beta cell population. They may, instead, express a state of immune reactivity which precedes the formation of IgG autoantibodies and therefore be associated with an event in the development of IDDM<sup>12</sup>. Other non-organ specific auto-antibodies were reported in 21 to 33%<sup>13,14</sup> of IDDM patients at diagnosis. In another study it was found that an anti-immunoglobulin antibody is present in the sera of recent-onset diabetic patients and represents an additional immunological phenomenon with possible physiopathological and clinical significance<sup>15</sup>. Circulating immune complexes have also been detected in 50% of IDDM patients aged 1 to 21 years<sup>14,16</sup> which increase in concentration over the first year of the disease<sup>17,18</sup>. The content of circulating immune complexes may serve as an additional immunological indicator of compensation and prognosis of the disease<sup>17</sup>. It has been found that the steady rise of circulating immune complexes in patients with IDDM is prognostically an unfavourable sign of the development of microangiopathies<sup>16</sup>. Auto-antibodies and circulating immune complex positive sera at diagnosis support the concept that a previous autoimmune disorder exists before the clinical manifestation of diabetes<sup>14</sup>.

3. Pathologic changes in the pancreas of diabetics Pathologic changes, similar to those accompanying other auto-immune endocrine disorders, termed as "insulinitis" have been demonstrated in diabetes mellitus<sup>19,20</sup>. "Insulinitis" includes lymphocytic infiltration of the islets and a halo of lymphocytes around the capsule of the islet. The fact that insulinitis can be induced in experimental animals by injection of homologous or heterologous endocrine pancreas<sup>21</sup> suggests an autoimmune process involved in insulinitis. Recently, examination of frozen blocks of fresh pancreas obtained at postmortem from a child who died shortly after diagnosis of IDDM revealed new information<sup>22</sup>. Using various monoclonal reagents with flouochrome technique, it was found<sup>22</sup> that majority of the mononuclear infiltrate were T cells, mostly T cytotoxic/suppressor cells. Moreover, B cells, IgG and complement deposition were also observed.

#### **4. Serum antibodies to endocrine pancreas**

The existence of auto-antibodies reactive with the endocrine pancreas was first reported in 1974<sup>10</sup>. Several other reports ensued later<sup>2,6,9,10,23-25</sup> indicating a significant incidence of islet cell antibodies (ICAs) in IDDM. ICAs are seldom found in normal persons (Less than 1%) or in patients with NIDDM (about 15%). The percentage of patients with ICAs in their circulation, decreases with time and less than 25% have detectable levels of this antibody two years after the diagnosis<sup>2,6,24,26</sup>. Islet cell surface antibody (ICSA) is distinct from ICAs in that ICAs are directed against cytoplasmic antigens and are non-cytotoxic to viable islet cells while ICSAs are directed against cell surface antigens and in presence of complement are cytotoxic to viable islet cells<sup>27-29</sup>. However, the simple presence of islet cell surface antibody (ICSA) is not sufficient to produce diabetes since first degree relatives of diabetics have been found to be ICSA-positive and not all IDDM patients have cytotoxic ICSAs<sup>27</sup> It is possible that the ICSAs are the host's immune response to foreign antigens that cross-react with surface antigens on beta cells, due to molecular mimicry<sup>2,23,30</sup>. Bottazzo, Dean et al<sup>31</sup> described their findings

about the variable complement-fixing (CF) capacity of ICAs. They provided evidence that the appearance of CF-ICAs in the circulation may be more closely related to the clinical onset of diabetes than that of conventional ICAs. Rotter and Rimoin<sup>32</sup> confirmed a strong association of ICAs with IDDM (38%), compared with 5.3% in NIDDM and 1.7% in non-diabetics in Caucasian patients. In long-standing diabetes<sup>14,33</sup> only 15% were positive for ICA-IgG and all negative for CF-ICA. Few studies have been conducted in non-Caucasian populations which reveals a paucity of ICAs in association with diabetes. The prevalence of ICAs was low in non-Caucasian diabetics from Nigeria<sup>36</sup> and in Pima Indians from Arizona, U.S.A.<sup>35</sup>. In the Saudi Arabian population<sup>25</sup>, the incidence of ICAs was 13% in diabetics diagnosed within six months of onset of symptoms. The frequency of CF-ICAs was one-third of the total ICA-positive cases. More recently it has been confirmed that the development of IDDM in subjects younger than 20 years is associated with the generation of both IgM and IgG cell surface antibodies. The IgM surface antibodies may result from stimulated production of polyreactive natural auto-antibodies and could precede the switch to the formation of monoreactive IgG auto-antibodies<sup>36</sup>. ICAs have been characterized to be of the IgG class and most commonly of the IgG1 subclass<sup>37</sup>.

### **5. Cell mediated immunity in IDDM**

Nerup et al<sup>30</sup> in 1971 described the presence of an organ-specific, species non-specific anti-pancreatic hypersensitivity of cellular type in diabetes mellitus. Moreover, the lack of association of diabetes with genetic polymorphism of the immunoglobulin genes favours the possibility that the islet beta cell destruction in IDDM is mediated by forbidden clones of cytotoxic T cells, rather than of B lymphocytes<sup>38</sup>. Total lymphocytes were reduced in 77% of insulin-dependent diabetic children<sup>33</sup>. However, no alterations in T cell subsets, T4 and T8 were noted at onset in 32 IDDM patients aged 1 to 21 years<sup>14</sup>. A significant elevation of helper to suppressor ratio was observed after a six to twelve months follow-up in the same group of patients<sup>14</sup>. Higher helper to suppressor ratio represents a specific alteration due to metabolic imbalance or to an earlier immunological disorder<sup>14</sup>. Recent data suggests that although the pancreas is accessible to circulating auto-reactive T cells, pancreatic cells are ineffective antigen presenting cells<sup>39</sup> which explains the paucity of cell mediated immune response in IDDM.

### **Autoimmune pathogenesis of IDDM**

It is now well accepted that IDDM is an autoimmune disease but the actual steps in its pathogenesis remain to be confirmed. It has been postulated that some kind of environmental insult to the pancreatic beta cells, most probably, by viruses<sup>40</sup>, results in the production of either an auto-antigen on the pancreatic beta cells and/or the aberrant or inappropriate expression of HLA molecules on pancreatic beta cells. Glucose uptake into pancreatic beta cells by means of the glucose transporter is essential for the normal insulin secretory response to hyperglycaemia<sup>41</sup>. In both autoimmune and non-autoimmune diabetes, this glucose transport is reduced as a consequence of down-regulation of the normal beta cell transporter<sup>41</sup>. Johnson et al<sup>42</sup> tested the possibility that in autoimmune diabetes, circulating immunoglobulins can further impair glucose transport across the beta cell membrane. They<sup>43</sup> measured the uptake of glucose by dispersed islet cells after a 15 minute incubation with purified IgG from 27 patients with newly diagnosed IDDM, 28 normal subjects and 5 patients with NIDDM. The IgG fractions of 96% patients with IDDM, but from none with NIDDM reduced the initial rates of glucose uptake. The inhibitory activity of IgG from the patients with IDDM was abolished by pre-incubation with islet cells and membranes from hepatocytes, which contain the same glucose transporter as beta cells, but not with erythrocytes which do not contain this transporter. Thus concluding that IgG from patients with IDDM of recent onset, but not from those with NIDDM, inhibits glucose uptake by islet cells. The results are consistent with the presence of an antibody against

a protein involved in glucose transport by beta cells that would impair glucose-stimulated insulin secretion<sup>42</sup>. These observations suggest the possibility of the glucose transporter acting as an auto-antigen in IDDM. A 64 kd protein, glutamate decarboxylase has also been evaluated as a possible auto-antigen having pathogenetic role in IDDM<sup>43,44</sup>. It has been shown that auto-antibodies to glutamate decarboxylase are the earliest and most predictive auto-antibody marker of IDDM<sup>45,46</sup>. Earlier, a 29 kd auto-antigen was described in IDDM but it was found that it is not a major marker for IDDM<sup>47</sup>, but may help identify a sub-group of IDDM in which non-islet auto-antibodies occur early in life bearing in mind the fact that 29 kd antigens are present in the exocrine pancreas<sup>47</sup>. Moreover, a strong link of IDDM pathogenesis has been postulated with the major histo-compatibility complex (MHC) expression on pancreatic islet cells. Although physiologically MHC class II expression occurs mainly on the antigen presenting cells, inappropriate or aberrant expression of class II HLA molecules has been described in the target cells of certain autoimmune diseases including pancreatic beta cells in IDDM<sup>22,49</sup>. Thus pancreatic cells become able to present an antigen (auto-antigen) to helper and cytotoxic T lymphocytes<sup>50</sup>. Alpha and delta islet cells do not express class II HLA which is consistent with the sparing of these cells in the cell killing process in IDDM. The exact role of the inappropriate expression of class II HLA molecules in the pathogenesis of IDDM is not yet fully understood but it provides a clue regarding the association of IDDM with certain HLA specificities<sup>51</sup>. Furthermore, faulty or low MHC class I expression has also been hypothesized to be involved in the pathogenesis of autoimmune diabetes<sup>52</sup>. Faustman et al<sup>52</sup> observed defective expression of class I MHC molecules in the pre-diabetic and hyperglycaemic phases of IDDM. The defective expression of MHC class I correlated better with the risk of progression to hyperglycaemia than did the presence of active islet cell autoimmunity or genetic susceptibility per se. Thus presence of low MHC class I expression is correlated to and probably predictive of the risk of progression to hyperglycaemia. Because low MHC class I expression precedes hyperglycaemia and is present in the pre-diabetic phase, the abnormality in MHC class I expression is probably not secondary to the hyperglycaemia or to some other metabolic concomitant of insulin deficiency. It was demonstrated<sup>52</sup> that deficient class I MHC expression and function can by itself cause autoimmune diabetes. The presence of auto-antigens on the pancreatic beta cells and/or the aberrant or inappropriate expression of MHC on beta cell surface may as well be responsible for the subsequent events in the production of the immunological response by the T series and B series of lymphocytes, eventually leading to the destruction of pancreatic beta cells. Pancreatic beta cells, however, have a high functional reserve and only 75 percent of individuals with complement-fixing islet cell surface antibodies or those with high titres (more than 20 juvenile diabetes foundation units) of islet cell antibodies are reported to progress to IDDM in a period of upto eight years<sup>53</sup>.

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