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Immunogenetics of Type 1 Diabetes Mellitus

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Abstract

Type 1 diabetes mellitus (T1DM) is an autoimmune disease arising through a complex interaction of both genetic and immunologic factors. Similar to the majority of autoimmune diseases, T1DM usually has a relapsing remitting disease course with autoantibody and T cellular responses to islet autoantigens, which precede the clinical onset of the disease process. The immunological diagnosis of autoimmune diseases relies primarily on the detection of autoantibodies in the serum of T1DM patients. Although their pathogenic significance remains uncertain, they have the practical advantage of serving as surrogate biomarkers for predicting the clinical onset of T1DM. Type 1 diabetes is a polygenic disease with a small number of genes having large effects, (i.e. HLA) and a large number of genes having small effects. Risk of T1DM progression is conferred by specific HLA DR/DQ alleles [e.g., DRB1*03-DQB1*0201 (DR3) or DRB1*04-DQB1*0302 (DR4)]. In addition, HLA alleles such as DQB1*0602 are associated with dominant protection from T1DM in multiple populations.

A discordance rate of greater than 50% between monozygotic twins indicates a potential involvement of environmental factors on disease development. Viral infections may play a role in the chain of events leading to disease, albeit conclusive evidence linking infections with T1DM remains to be firmly established. Two syndromes have been described in which an immune-mediated form of diabetes occurs as the result of a single gene defect. These syndromes are termed autoimmune polyglandular syndrome type I (APS-I) or autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), and X-linked polyendocrinopathy, immune dysfunction and diarrhea (XPID). These two syndromes are unique models to understand the mechanisms involved in the loss of tolerance to self-antigens in autoimmune diabetes and its associated organ-specific autoimmune disorders. A growing number of animal models of these diseases have greatly helped elucidate the immunologic mechanisms leading to autoimmune diabetes.

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Keywords

Immunology; Genetics; Pathogenesis; Type 1 diabetes

1. Introduction

Type 1 diabetes mellitus is a chronic autoimmune disease in which endogenous insulin production is severely compromised as a result of an immune-mediated injury of pancreatic β -cells (Eisenbarth, 1986). Genetic analyses of T1DM have linked the HLA complex, mainly class II alleles, to susceptibility to T1DM (Morel et al., 1988; Todd et al., 1987). Viral antigens may also play a role in the generation of beta cell autoimmunity (Lonnrot et al., 2000). The latter observations are supported by the increasing seasonal incidence of T1DM in many Western countries (Orchard et al. 1986) and that enteroviruses may be involved in the autoimmune pathogenesis of T1DM (Hyöty, 2002; Lonnrot et al., 2000; Zipris et al., 2007)

Type 1 diabetes was not always considered as the classical organ-specific disease it is now known to be. Insulin-dependent diabetes was known to occasionally occur in the Autoimmune Polyendocrine Syndrome I (APS I), a classic autoimmune syndrome with T-cell and B-cell antibody abnormalities directed at adrenal, parathyroid, gonadal, thyroid and other tissues. However, diabetes mellitus is not a constant, necessary or sufficient feature of APS I (Eisenbarth et al., 2004). This condition is now known to be caused by mutations in the autoimmune regulator gene (AIRE) (Husebye et al., 2010). Bottazzo et al. (1974) reported that sections of human pancreas treated with sera of diabetic patients who also had Addison's disease and myxedema, showed cytoplasmic fluorescence in the islets of Langerhans. This response was termed cytoplasmic islet cell antibodies (ICA) (Bottazzo et al., 1974). Furthermore, the existence of insulin autoantibodies and other autoantibodies against various islet proteins was not uncovered until years later. It was in 1983 that insulin autoantibodies were reported in sera of newly diagnosed patients with T1DM, before any treatment with exogenous insulin (Palmer et al., 1983). In this finding, improvements of the sensitivity of the insulin antibody assay were instrumental for the determination that about one-half of newly diagnosed patients had autoantibodies that bound ^{125}I -labeled insulin.

Type 1 diabetes is primarily a T-cell mediated disease. Following the early discoveries on humoral autoimmunity in T1DM, there has been a remarkable expansion in the detection of T1DM-associated autoantibodies as well as in the characterization of the molecular basis of the antigenicity of their target proteins (Atkinson et al., 2001; Pietropaolo et al., 2001). This expansion has led to the uncovering of specific antigenic determinants, the development of biochemically-defined immunoassays and also to coordinated efforts to standardize assays across laboratories (Bonifacio et al., 2010).

2. Association with other autoimmune diseases

For reasons not fully understood, patients with an organ-specific autoimmune disease have increased risks of developing autoimmune responses against other organs/tissues (Jaberi-Douraki et al., 2014; Pietropaolo et al., 2012). Patients with T1DM are at increased risk for

developing other autoimmune diseases, most commonly autoimmune thyroiditis and celiac disease. Thyroid autoimmunity is particularly common among patients with type 1A diabetes, affecting more than one-fourth of individuals, and 2 to 5 percent of patients with type 1 diabetes develop autoimmune hypothyroidism. Transglutaminase autoantibodies are present in approximately 10 percent of patients, and half of these patients have high levels of these autoantibodies and celiac disease on biopsy (Hoffenberg et al., 2004; Jaeger et al., 2001). In addition, certain alleles (e.g., HLA haplotype DR3-DQ2 or DR4-DQ8 PTPN2, CTLA4, RGS1) confer a genetic susceptibility to both T1DM and celiac disease, suggesting a common biological pathway (Liu et al., 2014; Smyth et al., 2008a). Fewer than 1 percent of children with T1DM have autoimmune adrenalitis. In one study, it was reported that 11 of 629 patients (1.7 percent) with type 1 diabetes but none of the 239 normal subjects had antibodies directed against 21-hydroxylase, a common autoantigen in primary adrenal insufficiency (Brewer et al., 1997). A total of 3 out of 8 patients with anti-21-hydroxylase antibodies had adrenal insufficiency.

Type 1 diabetes can be seen with polyglandular autoimmune disease, especially type II, in which adrenal insufficiency, autoimmune thyroid disease, and gonadal insufficiency are the other major components. Rare syndromes associated with T1DM have shed important light on pathogenesis. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is associated with neonates developing T1DM. These infants usually die of overwhelming autoimmunity, in particular, severe enteritis. This syndrome is an X-linked recessive immunologic disorder characterized by early onset of Type 1A diabetes mellitus, autoimmune thyroiditis, autoimmune enteropathy, hemolytic anemia, atopic dermatitis including food allergies, and immunodeficiency leading to fatal infections. This syndrome is also known by X-linked autoimmunity-immunodeficiency syndrome (XLAAD). It is important to note that this condition, though extremely rare, can be reversed by bone marrow transplantation. X-linked autoimmunity-immunodeficiency syndrome is caused by a mutation of a gene termed *foxp3*, a “master-switch” for the development of regulatory T cells. Studies of the syndrome and the related animal model provide dramatic evidence that regulatory T cells (formerly termed suppressor T cells) have a major physiologic role in suppressing autoreactive T cells. The APS-I syndrome (Autoimmune Polyendocrine Syndrome type 1) is caused by a mutation of the AIRE gene (autoimmune regulator). This gene controls expression of a series of “peripheral” antigens in the thymus, including insulin. It is thought that the gene provides protection from autoimmune disorders, including T1DM, through its influence on the mechanisms involved in central T cell tolerance (Eisenbarth et al., 2004; Husebye et al., 2010).

3. Genetic Susceptibility

In T1DM familial aggregation is indicated by the notion that the overall risk for developing T1DM in North American Caucasian siblings, parents and offspring of individuals with T1DM ranges from 1% to 15% (Allen et al., 1986; Dorman et al., 1995; Wagener et al., 1982; Warram et al., 1984; Warram et al., 1994) as compared to less than 1% for individuals without T1DM relatives and 1.2/1,000 of the general population (LaPorte et al., 1995) (Table 1).

However, over 80% of cases of T1DM occur in individuals with no apparent family history of the disease. In the remaining 20%, this disease aggregates in families. The lifelong risk of T1DM is markedly increased in first degree relatives of patients with T1DM, averaging about 6 percent in offspring, 5 percent in siblings, and 50 percent in identical twins (versus 0.4 percent in subjects with no family history) (Kaprio et al., 1992; Redondo et al., 2002). A monozygotic twin of a patient with T1DM has a higher risk of diabetes than a dizygotic twin, and the risk in dizygotic twin siblings is similar to that in non-twin siblings (Table 1). Alleles at each locus on a single chromosome are usually inherited in combination as a unit. This combination is termed haplotype. Since each individual inherits one set of chromosomes from each parent, each individual has two haplotypes. HLA genes are codominant and follow a simple Mendelian form of transmission in families. Therefore, both alleles are expressed at a given HLA locus. There is a 25% chance that two siblings share the same haplotype and be fully compatible, a 50% chance that they will share one haplotype, and a 25% chance that they share no haplotype and thus be HLA incompatible. Siblings with the highest risk HLA DR and DQ alleles (e.g., DR3/DR4 heterozygotes), who inherit both HLA regions identical by descent to their diabetic sibling, may have a risk of developing islet autoimmunity as high as 70 percent and a similar long-term risk of diabetes (Aly et al., 2006) (Table 1).

3.1. The MHC complex

The short arm of human chromosome 6 (6p21) accommodates a ~3.5 megabase genetic segment containing a group of immune response genes termed the major histocompatibility complex (MHC) (Figure 1) (Pietropaolo et al., 2000; Wucherpfennig et al., 2001). The main genes localized within the MHC encode human leukocyte antigens, or HLA, two molecular classes of cell surface glycoproteins differing in structure, function, and tissue distribution. While MHC class I molecules are expressed in virtually all nucleated cells, class II molecule expression is restricted to B lymphocytes, dendritic cells, macrophages and activated T lymphocytes. Both MHC class I and class II molecules are involved in the presentation of antigens to T cells. Cytotoxic T cells (CD8⁺) mainly recognize antigen in the context of class I, whereas helper/inducer cells (CD4⁺) usually recognize antigen in the context of the class II molecules.

Many immunologically mediated diseases, including certain endocrine syndromes are genetically associated with specific HLA molecules and several hypotheses have been suggested to explain HLA-disease associations (Schwartz, 1996). Some HLA-associated diseases have been linked with polymorphisms of the genes encoding the class II molecule. One typical example is T1DM. Other mechanisms beside those discussed above have been implicated as a result of HLA molecule-peptide interactions, but it should be emphasized that more than one mechanism may be operating concurrently to determine susceptibility to a disease process.

The ability of these class II molecules to present antigens is dependent in part upon the amino-acid composition of their alpha and beta chains. Substitutions at one or two critical positions can markedly increase or decrease binding of relevant autoantigens and therefore the susceptibility to type 1 diabetes (Khalil et al., 1990; Rowe et al., 1994). In particular,

more than 90 percent of patients with type 1 diabetes carry either HLA-DR3, DQB1*0201 (also referred to as DR3-DQ2) or -DR4, DQB1*0302 (also referred to as DR4-DQ8), versus 40 percent of controls with either haplotype; furthermore, about 30 percent of patients have both haplotypes (DR3/4 heterozygotes), which confers the greatest susceptibility (Tisch et al., 1996). The higher risk of this heterozygote compared with the two (DR3/DR3 and DR4/DR4) homozygotes has been attributed to the two transcomplementing DQ heterodimers, including the a (DQA1*0501) and b (DQB1*0302) heterodimer, present only in this heterozygote (Erlich et al., 2008). Prospective studies of HLA-typed general population samples and first-degree relatives of T1DM patients have shown that the risk for DR3/DR4 (or DQ2/DQ8) in these relatives is greater than the risk for the same genotype in the general population (Aly et al., 2006), suggesting that additional loci either within or outside the HLA region also contribute to T1DM risk. Among the relatives of T1DM patients, DR3/DR4 siblings have a greater risk than offspring, and DR3/DR4 siblings who share two HLA haplotypes with the proband have an extremely high risk (Aly et al., 2006). Using the DR3/4 genotype (rather than DR3 or DR4) as a predictive marker will identify approximately 20–40% of future T1DM cases.

The prevalence of HLA-DR2 is decreased in patients with T1DM, and the DQA1*0102/DQB1*0502/DRB1*1601 haplotype accounts for the most part of disease susceptibility in DR2-associated cases of T1DM (Redondo et al., 2002). Therefore, the originally described effect of the DR2 allele in conferring resistance to diabetes is considered to be neutral rather than protective, whereas the real protective effect is provided by the DQ alleles, generally found in linkage disequilibrium with DR2. The effect of HLA alleles on T1DM susceptibility are summarized in Table 2.

A study of T1DM in Filipinos (Mbunwe et al., 2013) revealed that the common HLA*24:02 allele conferred high diabetes risk, unlike the A*24:07 allele. The authors concluded that A*24 is an independent predictor of progression to T1DM in antibody-positive relatives of T1DM patients. Clearly, other markers could be implemented into these predictive algorithms but the sensitivity and specificity of A*24 as genetic marker remains to be established.

With regard to the effect of HLA genotypes in other populations, a meta-analysis of HLA-DQ, DR allele polymorphisms in T1DM revealed that DQA1*0301, DQA1*0501, DQB1*0201, DQB1*0302 are high-risk alleles (all $P < 0.05$) in the Chinese population, whereas the DQA1*0103, DQA1*0201, DQA1*0401, DQB1*0301, DQB1*0402, DQB1*0501, DQB1*0503, DQB1*0601 and DQB1*0602 alleles are considered protective in this population. DRB1*04, DRB1*0301, DRB1*0901 appear to be susceptible alleles, while DRB1*07, DRB1*08, DRB1*12, DRB1*13, DRB1*14, DRB1*16, DRB1*0406 are considered protective alleles in subjects of Chinese descent. Moreover, the DRB1*0405-DQB1*0401 (DR4) and DRB1*1302-DQB1*0604 (DR13) haplotypes are associated with T1DM in Japanese patients (Katahira et al., 2010), whereas the DRB1*0406 allele confers protection in Japanese (Huang et al., 1995), Sardinian (Cucca et al., 2001) and Spanish (Morales et al., 1991) populations. The DRB1*0405 allele seems to confer strong T1DM susceptibility in the majority of ethnic groups, whereas DRB1*0403 DRB1*0406 appears to confer T1DM protection (She, 2001).

3.2. Mechanisms of susceptibility to or protection from T1DM

The importance of HLA class II molecules in playing a role in the pathogenesis of T1DM is also indicated by studies in a transgenic non-obese diabetic (NOD) mouse model, in which the expression of an I-A β chain (the equivalent to the human class II DQB1 locus) transgene carrying Asp 57 instead of Ser 57 protects these mice from developing diabetes (Miyazaki et al., 1990; Slattery et al., 1990). Moreover, expression of Pro 56 instead of the normal His 56 in the I-A β chain has the same effect (Lund et al., 1990). Finally, expression of certain I-E (the equivalent of the human HLA-DR locus) transgenes appears to confer resistance to the disease (Lund et al., 1990; Nishimoto et al., 1987). Of note, the treatment of NOD mice with a monoclonal antibody reacting with the murine class II molecule, also prevents the progression to overt diabetes (Boitard et al., 1988). These findings obtained in an animal model of T1DM, certainly support the role of both HLA-DQ and HLA-DR in human T1DM. The interaction between the components of the trimolecular complex (CD4⁺ T cell receptors, self-peptide, and MHC class II molecules) plays a pivotal role in autoimmune disease pathogenesis. The development of therapies targeting various components of the trimolecular complex for the prevention of type 1 diabetes is actively being pursued (Michels, 2013; Zhang et al., 2014).

The mechanisms by which the class II genes can influence susceptibility to, or protection from, T1DM are still a subject of discussion. Brown et al. (Brown et al., 1993) have characterized the structure of the crystallized HLA class II molecule. One hypothesis is that effective antigen-binding depends on the conformation of the antigen-binding site on the DQ dimer. The two critical residues, DQ α 52 and DQ β 57 are located at opposite ends of the α -helices that form the antigen-binding site of the DQ molecule. Another hypothesis is that a substitution of an amino acid residue at these positions of the DQ molecule leads to conformational changes of the antigen-binding site and, consequently, to a modification of the affinity of the class II molecule for the “diabetogenic” peptide(s). In support of this hypothesis, it is known that in the DR molecule Asp-57 is involved in hydrogen and salt bonding with the antigenic peptide and the Arg-76 position of the α chain, respectively (Lund et al., 1990; Nishimoto et al., 1987; Pietropaolo et al., 2000). Theoretically, modifications in the DR α Arg-76 residue would also alter the antigen-binding site. This is physiologically difficult to observe since the DR α chain is not polymorphic.

The TCR on a given peripheral T-cell is composed of separately encoded α - and β -chains that are disulfide-linked. These dimers must form a molecular complex with the multi-chain CD3 complex to become functionally active at the cell surface (Michels et al., 2011). Throughout the entire life of an individual, T-cells undergo a constant maturation process occurring primarily in the thymus. During this process, precursor stem cells, initially from the fetal liver and then from bone marrow, enter the thymic *anlage*, where they are induced to rearrange their germline TCR α and β genes (Haars et al., 1986). TCR gene rearrangements are essentially random, and most are nonproductive as a result of out-of-frame joints; however, these unsuccessful rearrangements are requisite for the expression of generally a single functional α/β TCR at the cell surface. Furthermore, the essentially random nature of these rearrangements among a large number of variable segments ensures an extremely large (10^{10} – 10^{15}) repertoire of distinct antigen specificities present at the

surface of the unselected thymocyte pool. Once a T-cell expresses a functional TCR at the cell surface, it is subject to either positive or negative selection events in the thymus. Both positive selection and negative selection depend on interactions between the TCR, MHC molecule, and antigenic self-peptide (Figure 2). *Positive selection* occurs as thymic stromal cells bearing MHC molecules (containing self-peptide fragments) engage TCR molecules on the developing thymocytes and direct their continued maturation into functionally mature T-cells. T-cells with “useless” receptors (i.e., those that cannot bind with sufficient affinity to the MHC molecule) are not driven to mature and expand, and eventually die. *Negative selection* refers to the poorly understood set of events that specifically eliminates or alternatively “anergizes” potentially autoreactive cells, thereby inducing “tolerance” to self (i.e., self-tolerance). During negative selection, factors such as affinity for self-antigen and antigen load likely influence the final outcome of cell death or clonal anergy. Thus, the peripheral T-cell repertoire of each person (including each individual of two monozygotic twins) is unique (Davey et al.,1994) and is a consequence of both the random generation of TCRs in the initial unselected thymocyte pool as well as of thymic positive and negative selection events.

Autoimmunity is thought to result from an altered balance between two functionally opposite processes, *tolerance induction* and *immune responsiveness*. They are dependent on the presence of class I and class II molecules that are able to present tissue specific antigenic peptides. In genetically-susceptible individuals, certain class II molecules may ineffectively present self-peptides, thereby leading to inadequate negative selection of T-cell populations that could later become activated to manifest an autoimmune response. Nepom and Kwok (1998) explained the molecular basis of HLA-DQ associations with T1DM exactly on this basis (Nepom et al.,1998). Paradoxically, some self-peptides that normally negatively select T cells, are likely to lead to positive selection when the MHC molecule is, for example, the HLA-DQ3.2.

The HLA-DQ3.2 molecule is encoded by DQA1*0301 and DQB1*0302 genes, that are generally present on the most strongly T1DM-associated haplotype also encompassing HLA-DR4. Due to a characteristic structural motif for peptide binding, the HLA-DQ3.2 can be considered an intrinsically “unstable” MHC class II molecule. If in a DQ3.2-positive individual, the T-cells that are negatively selected in the thymus are only those that recognize DQ3.2-peptide complexes in a “stable” high-affinity configuration, and this in turn leads to the release from the thymus of mature T-cells able to establish a potentially autoimmune repertoire in the periphery.

Small structural changes, then, may result in large functional changes in the antigen-presenting capabilities of the class II molecules. One might conceive that the cells from a person who is heterozygous for both DQ α and DQ β would contain all four chain combinations on their surface. Competition for binding the processed antigen could take place, with effective antigen binding dictated by the conformation of the antigen-binding site on each DQ dimer. Changes at either amino acid DQ α -52 or DQ β -57, located at opposite ends of the alpha helices that form the antigen-binding groove could alter the configuration of the groove. Changes at both positions, would likely inflict a great conformational effect on the molecule’s antigen-presentation capability. Such conformational differences may be

partially responsible for the observed hierarchy in the degree of susceptibility within the group of non-Asp-57 alleles, and for the differences in the degree of protection afforded by each allele within the group of Asp-57 alleles. For example, the protective effect of the Asp-57 DQB1*0502 allele prevails over that of certain susceptible alleles, such as non-Asp-57 DQB1*0501. Conversely, the susceptible allele non-Asp-57 DQB1*0302 dominates over the protective effect of Asp-57 DQB1*0301.

Positive and negative selection events can also explain genetic resistance to T1DM. In many populations, the frequency of the DQB1*0602 allele is rarely found among patients with T1DM (Carcassi et al.,1991; Pugliese et al.,1995). This suggests that this allele may play a protective role in the disease process. During thymic development, an unidentified diabetogenic peptide can preferentially bind to the DQB1*0602 molecule, and because the relatively higher affinity and/or avidity it has with this than with other DQ molecules, it will form HLA-DQ molecule/antigenic peptide/TCR complexes more efficiently than other molecules. This could lead to negative selection and depletion of potentially self-peptide-reactive T cells. Individuals with a typical DQB*0602 allele can then delete these potentially dangerous T cells during thymic maturation and therefore are protected from developing diabetes. At present, carrying a “protective” DQB*0602 allele is considered as a criterion of exclusion for enrolling first-degree relatives of diabetic patients in clinical trials, such as the Diabetes Prevention Trial 1 (DPT-1), which is being carried out in the U.S. This trial has been designed to prevent the progression to the clinical onset of T1DM in individuals considered at high risk to develop the disease (DPT-1 Study Group,1995; Mahon et al., 2009). However, this does not mean that carrying the DQB1*0602 allele confers protection from developing the disease (Greenbaum et al., 2000b; Pugliese et al.,1999). Our results suggests that prediabetics carrying the HLA haplotype DQA1*0102, DQB1*0602 have the tendency to be antibody negative for all islet autoantigens (Pietropaolo et al., 2002). Seven percent of prediabetics in this study carried the HLA haplotype DQB1*0602, which confirms previous observations that the protective effect associated with DQB1*0602 is not absolute (Greenbaum et al., 2000a; Pugliese et al.,1999).

Tolerance to self-molecules is established and maintained through complex mechanisms taking place in both thymus (central tolerance) and peripheral lymphoid organs (peripheral tolerance). Since proteins with tissue-restricted or peripheral expression are traditionally thought to be unavailable for presentation in the thymus, it has been proposed that tolerance to such proteins can only be achieved through mechanisms of peripheral tolerance. Indeed, one attractive hypothesis is that T1DM is essentially due to failure of negative selection of autoreactive T cells, either in the thymus or in the periphery, or because of a breakdown in tolerance to β -cell-specific antigens. This hypothesis has received support following thymic transplantation of islet antigens or expression of putative islet cell autoantigens resulting in prevention of diabetes in both NOD mouse and BB rat models (Gerling et al.,1992; Naji et al.,1981).

There is evidence to suggest that molecules with tissue-restricted expression may also be expressed in the thymus (Pugliese et al.,1997). Genes encoding the T1DM-related autoantigens insulin, the neuroendocrine antigen IA-2, glutamic acid decarboxylase (GAD), and the neuroendocrine antigen ICA69 are transcribed in human thymus throughout fetal life

and childhood (Pugliese et al., 1997). Insulin gene transcription in human thymus was also reported by others and similarly insulin, glucagon, and GAD, ICA69 transcripts were detected in mouse thymus (Mathews et al., 2003). Transcripts of several self-molecules have been detected in the thymus, such as pancreatic and thyroid hormones, neuroendocrine molecules, and other peripheral proteins, raising the concept that self-antigen expression in the thymus may be crucial for the development of self-tolerance.

To investigate ICA69 autoimmunity (Bonner et al., 2012; Pietropaolo et al., 1993), in an elegant recent study two genetically modified mouse lines were generated to modulate thymic ICA69 expression: the heterozygous ICA69del/wt line and the thymic medullary epithelial cell-specific deletion Aire- ICA69 line (Fan et al., 2014). Suboptimal central negative selection of ICA69-reactive T-cells was observed in both lines. Aire- ICA69 mice spontaneously developed coincident autoimmune responses to the pancreas (Figure 3), the salivary glands, the thyroid, and the stomach. These findings established a mechanistic link between compromised thymic ICA69 expression and autoimmunity against multiple ICA69-expressing organs, and identified a potential novel mechanism for the development of multi-organ autoimmune diseases (Figure 5).

3.3. Non-MHC genes

The genetic susceptibility associated with T1DM has been investigated extensively. Polymorphisms of non-MHC genes are reported to influence the risk of type 1A diabetes including, the insulin, the PTPN22 gene, the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), interferon-induced helicase, IL2 receptor (CD25), a lectin-like gene (KIA00350), ERBB3e, and undefined gene at 12q) (Concannon et al., 2009; Cooper et al., 2012; Todd et al., 2007). Genome-wide association studies confirmed the above associations and identified four additional risk loci (BACH2, PRKCQ, CTSH, C1QTNF6) associated with an increased risk of T1DM (Cooper et al., 2008). Furthermore some loci conferring shared risk for celiac disease (RGS1, IL18RAP, CCR5, TAGAP, SH2B3, PTPN22) have been identified (Smyth et al., 2008b). Most loci have small effects, and the variants studied are common.

The Type 1 Diabetes Genetics Consortium (T1DGC), led by Dr. Stephen Rich has captured the majority of the genetic risk for T1DM (Barrett et al., 2009; Howson et al., 2012). Genome-wide association studies of T1DM have identified 55 non-MHC susceptibility loci, which required more detailed mapping in order to identify their candidate genes and causal variants. Using the ImmunoChip, a custom Illumina Infinium genotyping array based upon the robust GWAS results in 12 autoimmune diseases, we performed dense genotyping of regions previously associated with risk of at least one immune-mediated disease in 16,086 T1DM case and control samples and 2,670 families. We identified three novel T1DM loci: 1q32.1, 4q32.3 and 10p11.22/(*ITGB1* and *NRPI*). Using 1000 Genomes Project and ENCODE data, functional impact of the most T1DM-associated variants was predicted. Further, GSEA analysis suggested that T1DM is most similar genetically to multiple sclerosis (MS) and most dissimilar to psoriasis, a result that has biological and possibly clinical implications. In contrast, the genes associated with T2DM appear to be focused on β -cell function, yet only one T2DM gene (*GLIS3*) is associated with T1DM (and with neonatal diabetes).

The insulin gene (*INS*) region on chromosome 11p15 became a premier candidate for genetic association with type I diabetes in the early 1980s (Bell et al., 1981; Owerbach et al., 1982; Rotwein et al., 1981). Insulin's central role in metabolism and blood glucose homeostasis and its unique distinction as the only known β cell-specific antigen made it a likely frontrunner to account for an inherited susceptibility to diabetes.. Julier et al. (1991) provided evidence for genetic linkage for the insulin gene (*IDDM2*) with T1DM in a collection of multiplex families from France, USA and North Africa (Julier et al., 1991). Subsequently, the investigations of Bain et al. (2001) have confirmed the evidence of linkage between *IDDM2* and T1DM (Bain et al., 1992). Importantly, Bain et al. (1992) demonstrated linkage for *IDDM2* independent from the influence of HLA alleles (i.e., *IDDM1*) and the parental source of the *IDDM2* susceptibility allele.

Detailed sequence analysis of the insulin gene region identified a polymorphic locus which consists of a variable number of tandem repeats, or VNTR, present within the 5' regulatory region (promoter) adjacent to the coding sequence of the insulin gene. Each repeat element consists of a 14- to 15-bp DNA segment having the consensus nucleotide sequence A(C/T)AGGGGT(G/C)C(T/C/G)(G/A/T) (G/T/A)G(G/C/T). The number of repeats within sequenced alleles ranges from 26 to >200, with three classes of alleles identified on the basis of overall size: class I, class II and class III. Class I *INS* VNTR alleles consist of 26–63 repeats, averaging 570 bp in length, and are associated with T1DM susceptibility. Class III alleles consist of 140–200 or more repeats and are considered to be protective from diabetes. In size, class III alleles are the largest variants, averaging over 2.2 kb in length. Finally, class II alleles (1.2 kb average length) are too rare in the populations studied to draw any conclusion about their association with T1DM susceptibility (Bennett et al., 1995).

A number of studies have suggested that the *INS* VNTR may have a biological role in the genetic regulation of insulin expression (Kennedy et al., 1995; Lucassen et al., 1994). The proximity of this polymorphism to the *INS* transcriptional start site (<400 bp upstream) makes this an attractive hypothesis.

It has previously been reported that *INS* mRNA levels in the thymus were correlated with VNTR alleles in opposite fashion to that observed in the pancreas (Bennett et al., 1995). *INS* transcripts in *cis* with class III VNTR alleles are transcribed at much higher levels (on average 2–3 fold) than those in *cis* with class I VNTR alleles (Pugliese et al., 1997). The higher transcription levels detected in thymus could underlie the protective effect associated with class III VNTR alleles, as higher insulin levels in the thymus may more efficiently induce negative selection of insulin-specific T-lymphocytes (or improved selection of regulatory T cells). In contrast, homozygosity for diabetes-associated class I VNTR alleles determines lower insulin levels that may be associated with a less efficient deletion of insulin-specific autoreactive T-cells (or impaired selection of regulatory T cells). Proinsulin appears to be the main product of the insulin gene in the thymus (Pugliese et al., 1997). Direct support for the hypothesis that levels of *INS* expression in thymus and lymphoid organs could influence T1DM susceptibility was provided by studies in insulin gene knockout mice and transgenic mice (Fan et al., 2009; Nakayama et al., 2005) and more recently in humans (Durinovic-Bello et al., 2014).

Bottini and coworkers evaluated a functional polymorphism in the *lyp* gene in two series of T1DM patients, one from Denver and one from Sardinia (Bottini et al., 2004). The *Lyp* molecule, coded by the *PTPN22* locus, is a lymphoid tyrosine phosphatase located on chromosome 1p13. The relevant diabetes associated polymorphism appears to be a missense mutation that changes an arginine at position 620 to a tryptophan and thereby abrogates the ability of the molecule to bind to the signaling molecule Csk (Bottini et al., 2004; Bottini et al., 2006; Vang et al., 2005). The *lyp*-Csk complex downregulates T cell receptor signaling and thus loss of this interaction was thought to enhance T cell receptor signaling, though a study by Bottini and colleagues indicates a gain of function with the missense mutation and inhibition of T-cell receptor signaling. Of note, the minor tryptophan encoded allele is associated with a series of autoimmune disorders including T1DM, rheumatoid arthritis and lupus erythematosus (Vang et al., 2005). A number of studies have confirmed the association of this missense mutation with T1DM including a large study from Great Britain. A gain of function with a missense polymorphism probably also explains why the R620W change is the most widely studied polymorphism within the *PTPN22* locus clearly associated with T1DM.

A polymorphism in the *CTLA4* gene was shown to be associated with the risk of T1DM in a meta-analysis of 33 studies involving over 5000 patients (Kavvoura et al., 2005). This chromosomal region 2q33 contains the *CTLA-4* and *CD28* genes, which encode for two molecules that are intimately involved in the regulation of T-cell activation and proliferation. Differential regulation of these molecules could easily affect T-cell function and hence the regulation of immune responses. The *CTLA-4* gene is a strong candidate gene for autoimmune diseases since it encodes for a molecule that functions as a key negative regulator of T-cell activation, and the linked markers encompass a region containing an (AT)_n microsatellite located in the 3' UTR of the *CTLA-4* gene. Moreover, the analysis of an A-G transition in the first exon of the *CTLA-4* gene, coding for a Thr/Ala substitution in the leader peptide, also showed preferential transmission to affected siblings. Although linkage was not observed in families from Sardinia, U.K., and U.S.A., preferential transmission was observed considering all of the above families together (n= 818) (Awata et al., 1998). Further confirmation of association with the *IDDM12*-*CTLA-4* locus came through linkage disequilibrium (association) analysis using a multi-ethnic collection of families with one or more affected children, which included families from Spain, France, China, Korea, and Mexican-Americans. In this study, the transmission disequilibrium test (TDT) revealed a highly significant deviation for transmission of alleles at the (AT)_n microsatellite marker in the 3' untranslated region as well as the A/G polymorphism in the first exon of the *CTLA-4* gene (Marron et al., 1997).

4. Environmental Factors

Environmental influences are another important factor in the development of type 1 diabetes. Perhaps the best evidence for this influence is the demonstration in multiple populations of a rapid increase in the incidence of type 1A diabetes (Gale, 2002; Vehik et al., 2007). The etiology of the increase is unknown. One hypothesis, termed the hygiene hypothesis, relates improved "sanitation" to increasing immune mediated disorders (Bach, 2002). Twin studies indicate that not all monozygotic twins of probands with type 1 diabetes

develop diabetes, although the cumulative prevalence increases with long-term follow-up (Kaprio et al., 1992; Redondo et al., 1999; Verge et al., 1995).

Putative environmental factors include viral, microbial, diet-related, anthropometric and psychosocial factors. Ongoing observational cohort studies such as The Environmental Determinants of Diabetes in the Young (TEDDY) study aims to ascertain environmental determinants that may trigger islet autoimmunity and either speed up or slow down the progression to clinical onset in subjects with evidence for persistent islet autoimmunity (Elding Larsson et al., 2014). The TEDDY study should shed light on the role of environmental factors in T1DM development.

Viruses can cause diabetes in animal models either by directly infecting and destroying beta cells or by triggering an autoimmune attack against these cells (Szopa et al. 1993). The clearest association of viral infection with the development of spontaneous autoimmune diabetes comes from the observation that biobreeding diabetes-resistant (BB-DR) rats, a diabetes resistant strain of rats related to BB rats but without the severe lymphopenia of BB rats, develop diabetes when infected with the Kilham rat virus (Zipris et al., 2007; Alkanani et al., 2014).

Studies suggest a role for innate immune system activation in this model. In a similar manner, polyinosinic:polycytidylic acid (poly-IC) injections (a mimic of double stranded RNA viruses that induces interferon alpha secretion) can induce diabetes in this model and in a mouse model, where induction of interferon alpha is essential for diabetes development (Devendra et al., 2005).

Recent observations revealed the presence of immunoreactive enteroviral capsid protein, VP1, within beta cells in T1DM, which could be associated with a cellular phenotype consistent with the activation of antiviral response pathways and enhanced sensitivity to apoptosis (Richardson et al., 2013; Richardson et al., 2014). However, the role of viral infections as a cause of beta cell loss in human diabetes remains to be elucidated.

The environmental factors leading to T1DM development are far from being elucidated. However, a number of candidates have been identified, including dietary factors (breast feeding vs. infant formula, highly hydrolysed infant formula vs. conventional infant formula, early/late exposure to gluten, vitamin D deficiency etc.), exposure to certain viral elements or helminths anthropometric and psychosocial factors. (Elding Larsson et al., 2014; Nielsen et al., 2014).

The involvement of the gastrointestinal system in T1DM etiology is suggested by differences in intestinal microbiota composition observed in individuals diagnosed with T1DM or with evidence for islet autoimmunity (Nielsen et al., 2014; Reyes et al., 2013). In addition, proof of concept studies conducted in NOD mice provided evidence to suggest that changes in the composition of intestinal microbiota prevent or reduce T1DM incidence (Wen et al., 2008; Hansen et al., 2014).

It is time to carefully design adequately powered longitudinal studies to determine to what extent changes in the gut microbiota or in the metabolome, affect islet autoimmunity and

T1DM progression. Can early warning gut microbiota patterns be identified? Do environmental factors trigger islet autoimmunity, i.e. islet autoantibodies in subjects at genetic risk, accelerate the disease progression in islet autoantibody positive subjects, or both? Do epigenetic effects play a role in the etiology of the disease? Is there a trigger or an accelerator of a pre-existing subclinical state of islet autoimmunity that is evident at the time of clinical diagnosis? All of these questions have yet to be answered.

5. Islet Autoantigens and Humoral Autoimmunity

An ongoing search has identified several autoantigens within the pancreatic β -cells that may play important roles in the initiation or progression of autoimmune islet injury. Seminal studies have suggested that using a combination of humoral immunological markers gives a higher predictive value for T1DM progression, and great sensitivity without significant loss of specificity (Verge et al.1996).

As autoimmunity in T1DM progresses from initial activation to a chronic state, there is often a higher number of islet autoantigens reacting with T cells and autoantibodies. This condition is termed “epitope spreading” (Figure 4). Compelling evidence indicates that islet autoantibody responses against multiple islet autoantigens are associated with progression to overt disease (Verge et al.,1996). A number of additional T1DM-related autoantigens have been identified, which include islet cell autoantigen 69 kDa (ICA69), the islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), chromogranin A (ChgA) the insulin receptor, heat shock proteins, the antigens jun-B,16, CD38 (Pietropaolo and Eisenbarth, 2001), peripherin and glial fibrillary acidic protein (GFAP) etc. (Winer et al., 2003) (Table 3).

During immune system development, lymphocytes that react to self-antigens in the thymus and bone marrow are deleted. However, host molecules, in particular proteins and nucleic acids, are constantly being modified in the course of normal physiological events. A key post-translational modification in autoimmunity appears to be the citrullination of arginine amino acid residues, by the enzymatic deimination of arginine to citrulline (Doyle et al., 2012; Eggleton et al., 2008). This reaction is catalyzed by the enzyme peptidyl arginine deiminase (PAD) (Soderlin et al., 2004). In multiple sclerosis and RA, citrullinated isoforms of myelin basic protein (Moscarello et al., 2007) and fibrin (Masson-Bessiere et al., 2001) have been found in the brain and synovia respectively. It must be pointed out that the detection of anti-citrullinated protein antibodies (ACPA) has proven extremely useful in the early diagnosis and assessment of prognosis in rheumatoid arthritis (RA), and has also led to insights into gene environment effects in autoimmune diseases (Kastbom et al., 2004).

With regard to T1DM, processing of molecules such as insulin within the β -cell generates peptides that are then taken up by APCs either as whole dead β -cells or specifically granules of β -cells for eventual further processing/presentation of islet peptides to self-reactive T cells (Crawford et al., 2011). Furthermore, Stadinski et al. have shown that chromogranin A (ChgA) is an autoantigen in T1DM (Table 1), and that the peptide WE14 from ChgA stimulates diabetogenic CD4⁺ T cell clones (Stadinski et al., 2010). The natural form of the antigen in β cell extracts is far more potent than an unmodified synthetic WE14 peptide,

suggesting that this peptide may be post-translationally modified with a carbonyl group in murine pancreatic islets.

5.1. Insulin Autoantibodies

Insulin is a hormone produced by the pancreatic β -cells, which is not only central to regulating carbohydrate and fat metabolism, but also has a pathological role as T1DM autoantigen. Insulin is the predominant secretory product of pancreatic β cells whose autoimmune destruction leads to insulin deficiency and consequent metabolic de-compensation of glucose homeostasis (Nakayama et al., 2005). Investigations on the immunologically relevant regions of the insulin molecule, conducted in NOD mice, revealed that the 9-23 amino acid sequence of the insulin B chain (termed B9-23) and the effect of intracellular processing of molecules, such as insulin, within the β -cell can lead to formation of immunogenic epitopes (Crawford et al., 2011).

A high titer of insulin autoantibodies (IAA) at younger ages is consistent with the concept that these patients develop a more aggressive disease course. In particular, insulin autoantibodies levels greater than 2000 nU/ml are almost exclusively found in patients who progress to T1DM prior to age 5 years, and less than half of individuals developing T1DM after age 15 years carry detectable levels of IAA. Although these observations emphasize the utility of IAA, particularly in younger populations and justify the need to design trials in such a young group, the success of these intervention strategies depends on the safety and effectiveness of therapeutic regimens.

A study from The Finnish Type 1 Diabetes Prediction and Prevention Study, comprising a large population of 2448 genetically at-risk children (Kimpimäki et al., 2001) demonstrated that IAA are usually the first islet autoantibody to appear in the natural history of T1DM and a powerful identifier of disease progression in children followed from birth (Steck et al., 2011).

5.2. Glutamic Acid Decarboxylase (GAD) Autoantibodies

In an earlier seminal report, incubation of rat islets with radioactively labeled [^{35}S]-methionine and subsequent immunoprecipitation of solubilized membranes with serum from newly diagnosed patients with T1DM or controls showed that an antigen with a molecular weight of 64 kDa was precipitated by sera from T1DM patients (Bækkeskov et al., 1989). The antibodies to this 64 kDa antigen were present in about 80% of new onset patients and in pre-diabetics before the appearance of clinical disease. The nature of the 64 kDa antigen remained unknown until the report by Solimena et al showing autoantibodies to GABA-ergic neurons and pancreatic β -cells in an unusual condition termed Stiff Man syndrome (Solimena et al., 1988). Glutamic acid decarboxylase is the enzyme which catalyzes the conversion of glutamic acid to gamma amino butyric acid (GABA), a potent inhibitory neurotransmitter. This led Bækkeskov et al to rapidly identify GAD as the 64 kDa autoantigen in T1DM (Atkinson et al., 1993; Bækkeskov et al., 1990; Karlsen et al., 1991). Other molecular-related forms of GAD, such as the 67 kDa isoform, have subsequently been identified (Hagopian et al., 1993). Autoantibodies against GAD are a predictor of progression to overt diabetes. When coupled with insulin autoantibodies and islet cell

antibodies (ICA), their ability to predict the likelihood of developing T1DM in asymptomatic first-degree relatives of T1DM patients is quite high.

5.3. IA-2 (ICA512) Autoantibodies

The neuroendocrine antigen IA-2 (ICA512) is another major autoantigen in T1DM (Lan et al., 1994). It is an enzymatically inactive member of the tyrosine phosphatase family, involved in regulating insulin secretion. Assessment of the presence of IA-2 autoantibodies contributes to the predictability of the likelihood of developing T1DM. As shown by Verge et al. and others, ICA512 (IA-2) and its homologue IA-2 β (phogrin) are both neuroendocrine molecules (Achenbach et al., 2004; Pietropaolo et al., 2002; Verge et al., 1996). The deduced ICA512(IA-2) cDNA sequence reveals a 979-amino acid protein with a single transmembrane region and with significant homology to the receptor-type PTP(RT-PTPase). A PTP homologue, termed phogrin, was subsequently identified. Subcellular fractionation of insulinoma tissue showed that both IA-2 and phogrin had a very similar cellular distribution to that of insulin and carboxypeptidase H, and these two molecules are predominantly localized in the secretory granules of neuroendocrine cells (Mziaut et al., 2006; Wasmeier et al., 1996).

While the main immune reactive region of the IA-2 molecule was thought to reside within its intracellular domain (amino acids 601–979), the inclusion of this region together with other reactive epitope regions of the molecule, (encompassing aa residues 256–979), has permitted the development of highly accurate constructs to assess the presence of antibodies against IA-2 (Kawasaki et al., 1997). However, other research has also suggested that humoral autoimmunity against the intracellular as well as the extracellular domain of the molecule is related to a high risk of faster T1DM development (Morran et al., 2010).

5.4. Zinc transporter family member 8 (ZnT8) Autoantibodies

Zinc transporter family member 8 (ZnT8) is a member of the cation diffusion facilitator family, with abundant expression in pancreatic β -cells, although it is also expressed in extra-pancreatic tissues (Chimienti et al., 2004; Wijesekara et al., 2009). In the β -cell it plays an important physiological role since Zn, which is highly concentrated in β -cells, is needed for normal insulin storage. β -cell-specific deletion of ZnT8 in mice results in glucose-intolerance, reduced β -cell zinc accumulation and anomalous insulin granules, as well as blunted first-phase glucose-stimulated insulin secretion, reduced insulin processing enzyme transcripts and increased proinsulin levels (Wijesekara et al., 2010). Its relevance as an important T1DM autoantigen was first described by Wenzlau and coworkers (Wenzlau et al., 2007), following an evaluation of 68 candidate islet autoantigens compiled from multidimensional analyses of microarray mRNA expression profiling. The assessment of the zinc transporter ZnT8 (Slc30A8 encodes ZnT8), indicated that it was targeted by autoantibodies in 60–80% of new-onset T1DM compared with <2% of healthy controls, <3% type 2 diabetic patients and in up to 30% of patients with other T1DM-associated autoimmune pathologies. Interestingly, ZnT8 antibodies were found in 26% of T1DM subjects who had not exhibited antibody positivity to other commonly measured autoantigens such as GAD, (IA-2), insulin, or in the assay for cytoplasmic islet cell antibodies (ICA). Further research has revealed polymorphisms in ZnT8 that are relevant to

its role as a major T1DM autoantigen. There are three polymorphic variants located in the intracellular (C-terminus) domain of the transporter protein, namely Arg 325, Trp325 and Gln 325. Of these variants, Trp325 (W) and Arg325 (R) have been shown to be the major autoantigenic polymorphisms in T1DM and use of a construct containing the W and R variants (ZnT8WR) (Wenzlau et al., 2008) has proven its efficacy as a screen for T1DM associated humoral autoimmunity. More recently, a chimeric construct containing amino acid residues 609–979 of the intracellular domain of IA-2, linked to peptides containing both ZnT8 W and R polymorphisms has been successfully developed and tested as a broader and more economical screen to detect patients exhibiting humoral autoimmunity against IA-2 and/or ZnT8 (Yu et al., 2010).

Furthermore, Stadinski et al. have shown that chromogranin A (ChgA) is an autoantigen in T1DM (Table 1), and that the peptide WE14 from ChgA stimulates diabetogenic CD4⁺ T cell clones (Stadinski et al., 2010; Delong et al., 2012). More recently, the same group provided evidence to suggest that WE14 is recognized by T cells from diabetic subjects vs. controls in a dose dependent manner (Gottlieb et al., 2014). The natural form of the antigen in β cell extracts is far more potent than an unmodified synthetic WE14 peptide, suggesting that this peptide may be post-translationally modified with a carbonyl group in pancreatic islets.

5.5. Relevance as predictors of risk for T1DM, role of age and specificity

Combining both immunologic and metabolic strategies (e.g. oral glucose tolerance test or the first phase (1 + 3 min.) insulin response of an intravenous glucose tolerance), the current opinion is that type 1 diabetes progression can be predicted with 80–100% accuracy within 5 and 10-year follow-up respectively (Xu et al., 2010).

A study on the Diabetes Autoimmunity Study in the Young (DAISY) cohort showed that 89% of children who progressed to T1DM had two or more islet-related autoantibodies (Steck et al., 2011). Importantly, age of diagnosis of diabetes was strongly correlated with age of appearance of first autoantibody and IAA levels. By life-table analysis, children exhibiting two or more autoantibodies showed a nearly linear progression to diabetes ($P < 0.0001$). Children with persistently positive IAA levels had a higher progression rate to overt T1DM (100% by 5.6 years) as compared to children with fluctuating IAA levels (63% by the 10-year follow-up) ($P < 0.0001$). Finally, in children enrolled in the DAISY study followed to the development of diabetes onset, only high IAA titer correlated with rapid progression to T1DM onset ($P < 0.0001$). As a matter of fact, this effect was not evident with respect to the presence of high GAD65 or IA-2/ICA512 autoantibody titer. Therefore, insulin autoantibody levels at the time of diagnosis are inversely related to the age of the patient being highest in those less than 5 years of age and hence, IAA appear to be an early marker of β -cell destruction. The titer of insulin autoantibodies along with the insulin secretory response judged by the first phase insulin levels at 1 and 3 minutes after an intravenous glucose challenge, has also been successfully employed to construct mathematical models to predict likelihood of clinical diabetes in asymptomatic first-degree relatives of patients (Eisenbarth, 1986). Investigators from the DAISY study reported that 5 children were found to have persistent IAA before 1 year of age, and 4 of them went on to

develop the clinical onset of T1DM (all before 3.5 years of age). In contrast, children not exhibiting persistent IAA before the age of 1 year, rarely rapidly developed insulin requirement. When analyzing only children followed from birth, who progressed to diabetes, the two major predictors of age of diabetes diagnosis were the age at which autoantibodies first appeared and the mean level of insulin autoantibodies. These observations emphasize the utility of IAA, particularly in younger populations and justify the need to design trials focused on such a young group. However, the success of these strategies depends on the safety and effectiveness of therapeutic regimens. Indeed, this was the strategy in the trial to prevent development of T1DM (DPT-1), which successfully predicted the development of diabetes in first degree relatives of T1DM patients (Skyler et al., 2001).

6. Role of cellular immunity

A strong indication that T1DM is an autoimmune disease derived from a comprehensive histological examination of pancreata from T1DM patients who had died shortly after diagnosis (Bottazzo et al., 1985; Conrad et al., 1994; Foulis et al., 1986; Gianani et al., 2010). The majority of the subjects had significant lymphocytic infiltration of their islets concordant with loss of β -cell mass. With the advent of monoclonal antibodies capable of identifying distinct lymphocyte sub-populations more detailed immunohistochemical examinations of islet infiltrates became possible. One of the earliest of such studies showed a predominance of CD8⁺ T-cells in the islets of a deceased 12-year old girl with newly diagnosed T1DM, which, together with the observed up-regulation of MHC class I molecules by islet cells, implicated cytotoxic T-cells (CTLs) in β -cell destruction (Bottazzo et al., 1985). Additional studies of pancreas from patients with type 1 diabetes have confirmed preponderance of CD8⁺ T-cells and the presence of B-lymphocytes related to extent of β -cell destruction (Conrad et al., 1994). The JDRF nPOD (Network for Pancreatic Organ Donors with Diabetes) program now allows viewing of pancreatic histology of cadaveric donors directly online (<http://www.jdrfnpod.org/>) (Pugliese et al., 2014).

In T1DM autoimmune responses are influenced by a balance between pathogenic and regulatory T lymphocytes (Bluestone et al., 2005). While the role of autoimmunity in the pathogenesis of type 1 diabetes and the frequent development of autoantibodies are not in question, there is compelling evidence for a major role of cellular immunity in disease pathogenesis. Antigen peptides are presented by major histocompatibility complexes (MHC) on antigen presenting cells that activate and mobilize effector CD4⁺ and CD8⁺ T cell populations that are required for disease induction (Haskins et al., 1988; Mallone et al., 2007). The formation of the immunological synapse containing T cell receptor, MHC, and cognate auto-antigen can be performed by a number of different antigen presenting cells. These can include dendritic cells, macrophages, and B cells.

Naturally-processed epitopes of islet cell autoantigens represent the targets of effector and regulatory T cells in controlling pancreatic beta cell-specific autoimmune responses (Di Lorenzo et al., 2007). In particular, naturally processed HLA class II allele-specific epitopes recognized by CD4⁺ T cells, corresponding to the intracellular domain of IA-2, were identified after native IA-2 antigen was delivered to EBV-transformed B cells and peptides eluted and analyzed by mass spectrometry (Allen et al., 2009; Peakman et al., 2000).

Furthermore, dendritic cell subsets can process and present soluble IA-2 to CD4⁺ T-cells after short-term culture, but only plasmacytoid dendritic cells enhance (by as much as 100 percent) autoantigen presentation in the presence of IA-2 autoantibody patient serum (Allen et al., 2009). The plasmacytoid subset of dendritic cells is overrepresented in the blood close to T1DM onset and shows a distinctive ability to capture islet autoantigenic immune complexes and enhance autoantigen-driven CD4⁺ T-cell activation. This suggests a synergistic proinflammatory role for plasmacytoid dendritic cells and IA-2 autoantibodies in T1DM. Taken together, these observations may lead to identification of novel naturally-processed epitopes recognized by CD4⁺ T cells, which may represent potential therapeutic agents, either in native form or as antagonistic altered peptide ligands, for the treatment of T1DM.

Traditionally, one of the main sources of tolerance induction is the CD4⁺CD25⁺FoxP3⁺ regulatory T cell population, which secretes IL-10 as its hallmark cytokine and induces anergy and apoptosis in activated effector CD4⁺ and CD8⁺ T cells. Alterations in number and function of this regulatory cell population may contribute to the generation of an autoimmune state in Type 1 diabetes (Bluestone et al., 2006). Results from a clinical trial of a humanized anti-CD3 monoclonal antibody in new onset T1DM suggest that this treatment improves metabolic control beyond one year after treatment (Herold et al., 2002; Tang et al., 2013). This monoclonal antibody, termed hOKT3 γ l(Ala-Ala) contains the binding region of OKT3 in which the C_H2 region has been modified by site-directed mutagenesis to alter FcR-binding activity. The outcome was the elimination of T cell activation properties and to induce T cells with regulatory capabilities. The rationale for the clinical trial using the “humanized” hOKT3 γ l(Ala-Ala) monoclonal antibody is based on the observation that in NOD mice anti-CD3 mAb treatment reverses hyperglycemia in newly diagnosed NOD mice as a result of the induction of regulatory T cells (Chatenoud et al., 1994).

The CD40-CD40 ligand (CD40L) interaction is one of the most important receptor-ligand interactions that occurs during a T dependent immune response. CD40L is expressed on a range of cell types including activated T and B cells, dendritic cells, granulocytes, macrophages and platelets. One of the most widely studied reagents used to block CD28/CD80/86 interactions is the Ig fusion protein construct CTLA4-Ig (abatacept). CTLA4 (CD152) is expressed on T cells and also binds to CD80 and CD86 with high affinity. A very promising clinical trial indicated that costimulation modulation with abatacept slowed decline in beta cell function and improved HbA1c in newly diagnosed T1DM patients. This beneficial effect was sustained for at least one year after cessation of abatacept infusions or three years from T1DM diagnosis (Orban et al., 2014). Thus, abatacept appears to hold great promise as an effective therapy to prevent T1DM in subjects at risk of progressing to the clinical onset of the disease.

NKT cells may also play a role in the pathogenesis of T1DM in both humans (Wilson et al., 2000) and NOD mice (Sharif et al., 2002). NKT cells could have a general function of suppressing autoimmunity. For instance, reduced frequencies of these cells were reported in patients with T1DM (Wilson et al., 1998), although others (Lee et al., 2002) did not confirm these results. NKT cell importance in NOD mice has been demonstrated by genetic disruption of CD1, which results in the absence of CD1-restricted invariant NKT cells (Shi

et al., 2001). In these mice, diabetes develops at an earlier age as well as an increased number of memory autoreactive and activated T cells. In addition, administration of the NKT agonist α galactosyl ceramide (α GalCer) dramatically reduces the frequency of diabetes in NOD mice α GalCer also prevents autoimmune recurrence in spontaneously diabetic mice transplanted with syngeneic islet grafts. The proposed mechanism of action consists of a) NKT cell induction of tolerogenic dendritic cells in draining pancreatic lymph nodes; b) induction of regulatory cytokines; (Sharif et al., 2002) and b) NKT cell induction of tolerogenic dendritic cells in draining pancreatic lymph nodes (Naumov et al., 2001).

The importance of B cells in the pathogenicity of T1DM was initially studied in the non-obese diabetic (NOD) mouse model. Initial generation of a transgenic NOD mouse containing an IgM null gene leading to a lack of B cells clearly showed that these mice were highly resistant to T1DM onset as compared to controls (Serreze et al., 1996). However, upon successful reconstitution of the B cell compartment, IgM null NOD mice went on to develop robust diabetes (Vong et al., 2011). In another study using anti-IgM depleting antibodies to eliminate the B cell repertoire, NOD mice receiving treatment with anti-IgM were protected from T1DM (Noorchashm et al., 1997). Furthermore, using B cell receptor transgenics, it was shown that mice having even slightly increased (1–3%) B cell receptor specificity for insulin significantly increased insulinitis and T1DM progression, whereas mice with decreased B cell receptor specificity for insulin were protected from T1DM (Hulbert et al., 2001). B cells typically play important roles in disease progression: autoantibody production and antigen presentation. An early study to determine whether B cells played a role as presenters or antibody secretors was used in IgM null NOD mice. B cell deficient NOD mice receiving transfers of isolated immunoglobulins taken from diabetic NOD donors did not lead to disease onset, but reconstitution of the B cell repertoire from NOD donors led to increased T cell activation against beta cell autoantigens (Serreze et al., 1998). The generation of a NOD transgenic mouse that expressed only membrane-bound IgM capable of presenting antigen was able to induce T1DM in an IgM null NOD mouse as compared to NOD control mice (Wong et al., 2004). Further work using transgenic NOD mice with B cell receptor specificity to the irrelevant hen egg lysozyme (HEL) antigen led to decreased disease progression similar to what was seen in the IgM null NOD mice, showing the importance of B cell specific presentation of diabetes autoantigens over other antigen presenting cells in disease pathogenesis (Silveira et al., 2002). Moreover, one such B cell population that is thought to play an important role in diabetes progression are marginal zone (MZ) B cells, which act as potent antigen presenters to naïve CD4⁺ T cells (Falcone et al., 1998).

More recently, it has been shown that antigen-matured Bregs may maintain tolerance to islet autoantigens by selectively suppressing autoreactive T-cell responses (Kleffel et al., 2014). In the context of T1DM pathogenesis, the inflammatory microenvironment may render this B cell subset functionally impaired or apoptotic.

Other observations suggest that a unique immune phenotype exists in the B cell compartment of healthy individuals who carry the PTPN22 1858T variant (Habib et al., 2012). It has been hypothesized that Lyp620W-mediated effects may contribute to breakdown of peripheral tolerance and the entry of autoreactive B cells into the naive B cell

compartment. Thus, altered B cell homeostasis in subjects who express this variant appears to be characterized by an increase in transitional and D19⁺CD27⁻ IgD⁺IgM⁻ B_{ND} B cells, as well as blunted BCR signaling in naive mature B cells. The identification of the pathways that are impacted by Lyp620W may provide clues for understanding the predisposition to autoimmune diseases associated with this variant, as well as the response to B cell-directed therapies.

7. Concluding Remarks

Type 1 diabetes results from autoimmune destruction/dysfunction of pancreatic β cells. In physiologic conditions there is balance between pathogenic T cells that mediate disease such as T cells with marked conservation of their TCRs (e.g. insulin), and regulatory cells that control autoimmunity. In T1DM and other autoimmune disorders, there is an altered balance between pathogenic and regulatory T cells. Development of therapies targeting specific T and B lymphocytes is underway in animal models of autoimmune diabetes and eventually may be examined in humans.

In T1DM, the use of genome-wide scans has identified over 40 putative loci of statistical significance but for now only linkage to HLA loci seems incontestable. Albeit much excitement has recently been generated by the results of genome-wide scans, for many polygenic disorders including T1DM, careful and rigorous replication as well as association studies in many populations must be conducted before any attempts are made to identify potentially elusive sequence variations that are thought to influence genetic susceptibility.

Post-translational modifications (PTM) enhance immunostimulatory properties (generation of neoantigens, neoepitopes) in autoimmune diseases such as RA and SLE. It is quite possible that in T1DM altered islet neoantigens may play a critical role in enhancing T cell immunogenicity as the attendant hyperglycemic and pro-oxidative metabolic milieu includes abnormal glycosylations and oxidative damage to proteins within pancreatic beta cells (Pietropaolo et al., 2012; Roep et al., 2014; van Lummel et al., 2014).

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Figure 1. Schematic representation of the human leukocyte antigen (HLA) complex on chromosome 6. The genes that encode a protein product are indicated in grey color; the genes encoding nonfunctional products, or products that have not been characterized, are indicated in white color.

Figure 2.

Both positive and negative thymic selections contribute to form the repertoire of mature T cells in the periphery from immature T cells originated in the bone marrow. Subjects carrying HLA-DQ alleles associated with resistance to disease, such as HLA-DQ*0602, will be able to negatively select in the thymus T cells with high affinity to self-peptides (●), so that no autoreactive T cells would be present in peripheral blood and the likelihood of developing diabetes would be reduced. In contrast, subjects who carry susceptibility alleles with low affinity for self-peptides (○), such as HLA-DQ*0302, will negatively select less efficiently autoreactive T cells, which will egress from the thymus and be present (even in small numbers) among peripheral T cells (Modified from Nepon GT and Kwok WT. Diabetes 1998. 47:177).

Figure 3.

Aire-ICA69 mice spontaneously develop anti-islet autoimmune responses. A. Intraperitoneal glucose tolerance test. 12-week old Aire-ICA69 mice ($n = 7$) were challenged with a bolus of 2 g/kg D-glucose, in comparison to ICA69flox/flox mice ($n = 6$). Data are presented as mean $\pm$ SEM. Unpaired Student t test, $*p < 0.05$; $**p < 0.01$. B. Serum insulin levels in the Aire-ICA69 ($n = 7$) and the ICA69flox/flox ($n = 6$) mice. Sera were harvested after overnight fasting and challenged after i.p. injection of 2 g/kg of D-glucose. Data are presented as mean $\pm$ SEM. Unpaired Student t test, $***p < 0.005$; $****p < 0.001$. C. Immunohistochemistry showing lymphocyte infiltration of pancreatic islets in 16-week old Aire-ICA69 mice. Cryosections of pancreata harvested from either the Aire-ICA69 (upper panel), or the ICA1flox/flox (lower panel) mice, were stained with anti-CD4 (red), anti-CD8 and anti-B220 antibodies and counter-stained with anti-insulin (green) antibodies (With permission from the Journal of Autoimmunity).

Figure 4. Autoantigen Epitope Spreading in T1DM

As the severity of symptoms associated with T1DM increases over time, so does the number of autoantigens recognized by the immune system. Epitope spreading begins once the immune system is triggered within the pancreas, leading to the processing and presentation of self-antigens. As β -cell destruction takes place, multiple self-antigens become targets of the immune system. During this process, insulin is thought to be the first antigenic target, followed by other β -cell associated components, such as glutamic-acid decarboxylase 65 (GAD65) and islet-cell antigen-2 (IA-2) and ZnT8. Over time autoantigens are processed differently, creating various recognition epitopes for a given antigen. The tree symbolizes an immune system at birth which lacks autoimmunity. As the tree grows towards autoimmune T1DM, its limbs represent targeted self-antigens which develop. As T1DM progresses, multiple limbs grow off the tree, each from a different antigen. These growing limbs next branch off, representing the unique epitopes recognized from differential processing of similar self-peptides. As T1DM develops, the tree grows towards autoimmunity by increasing both the number of limbs and the number of branches on a given limb, representing the process of epitope spreading observed in disease progression.

Figure 5.

Schematic representation of initiation of the immunologic response to an autoantigen. The antigen binds to a groove in MHC class II molecules on antigen-presenting cells (APCs). This binding allows the antigen to be presented to antigen receptors on autoreactive CD4 inducer or helper T cells which, in T1DM, initiate immune-mediated injury to the pancreatic beta cells. Furthermore, the respective binding of B7 proteins and lymphocyte functional antigen-3 (LFA-3) on APCs to CD28 and CD2 on T cells are important costimulatory pathways that further enhance T cell activation. Other molecules can also participate in the immune response, such as the binding of interleukin-2 to its receptor (IL-2R).

Table 1

Empiric risk of Type 1 diabetes

Population	Type 1 Diabetes Risk %
Low Risk	
No FDR affected, positive for HLA protective genes	0.01
No FDR affected	0.4
FDR Affected, positive for HLA protective genes	0.3
Intermediate Risk	
No affected FDR, positive for HLA risk genes	4
One Affected FDR	5
Father with T1DM	3
Mother with T1DM	5
Sibling with T1DM	8
High Risk	
One affected FDR, positive for HLA high risk genes	10–20
Multiple FDRs affected	20–25
Very High Risk	
Multiple FDRs affected, positive for HLA risk genes	50
Sibling affected, positive for HLA risk genes	30–70
Identical twin affected	30–70

Table 2

HLA Class II DR-DQ genotypes and T1DM Susceptibility in Caucasians

<i>HLA-DR</i>	<i>DQA1</i>	<i>DQB1</i>	<i>DRB1</i>	Susceptibility
<i>DR2</i>	<i>0102</i>	<i>0602</i>	<i>1501</i>	Protective
<i>DR2</i>	<i>0102</i>	<i>0502</i>	<i>1601</i>	Predisposing
<i>DR2</i>	<i>0103</i>	<i>0601</i>	<i>1502</i>	Neutral
<i>DR3</i>	<i>0501</i>	<i>0201</i>	<i>0301</i>	High Risk
<i>DR4</i>	<i>0301</i>	<i>0302</i>	<i>0401</i>	High Risk
<i>DR4</i>	<i>0301</i>	<i>0302</i>	<i>0402</i>	Predisposing
<i>DR4</i>	<i>0301</i>	<i>0302</i>	<i>0403</i>	Lower Risk
<i>DR4</i>	<i>0301</i>	<i>0302</i>	<i>0404</i>	Predisposing
<i>DR4</i>	<i>0301</i>	<i>0302</i>	<i>0405</i>	High Risk
<i>DR4</i>	<i>0301</i>	<i>0301</i>	<i>0401</i>	Neutral
<i>DR4</i>	<i>0301</i>	<i>0303</i>	<i>0401</i>	Neutral
<i>DR7</i>	<i>0201</i>	<i>0303</i>	<i>0701</i>	Protective
<i>DR6</i>	<i>0101</i>	<i>0503</i>	<i>1401</i>	Protective

Table 3

Most characterized islet autoantigens associated with Type 1 diabetes.

	Localization	Humoral Response	Cellular Response
Insulin *	Secretory granules pancreatic β cells. Human thymus and PAE cells (Peripheral Antigen Expressing Cells)	Insulin autoantibodies are found in virtually 100% of young children (<5 years of age) before the onset of Type 1 diabetes. Correlation with younger age and fast rate of progression to insulin requirement in first-degree relatives of IDDM patients. Prophylactic subcutaneous injection of insulin, oral and intranasal administration prevents Type 1 diabetes in NOD mice.	PBLs from humans and NOD mice react with insulin β -chain.
GAD65* and GAD67	Synaptic-like microvesicles of neuroendocrine cells. Present in testis and ovary. Human thymus and PAE cells	A subset of 64-kDa autoantibodies recognize GAD. Autoantibodies to GAD65 are present in 70–80% of prediabetic subjects or newly diagnosed diabetic patients. GAD antibodies are also detected in patients with stiff man syndrome, and with autoimmune thyroid disease. Radioimmunoassay of in vitro transcribed/translated GAD65 useful for large-scale screening.	PBL responses to GAD65 in newly diagnosed diabetic patients and in NOD mice
ICA512(IA-2)* and phogrin (IA-2β)	Neurosecretory granules (pancreatic β cells, CNS, pituitary, adrenal). Human thymus and PAE cells	Autoantibodies to ICA512(IA-2) are present in ~60% of prediabetics or newly diagnosed IDDM patients. Relationship between 37,000 and 40,000 Da tryptic fragments and ICA512(IA-2). Radioimmunoassay of in vitro transcribed/translated ICA512(IA-2) useful for large-scale screening.	PBL responses in newly diagnosed diabetic patients.
ZnT8 (Slc30A8)*	Zn transporter, a member of the cation diffusion facilitator family exhibiting abundant expression in β cells. Expressed also extra-pancreatically	Targeted by autoantibodies in 60–80% of newly diagnosed T1DM patients and in ca. 26% of patients negative for other islet autoantibodies. Relevant polymorphic variants are Trp325 and Arg325	Autoreactive T cells to ZnT8 found in human T1DM
Islet Cell Autoantigen 69 kDa (ICA69)	Predominantly Neuroendocrine tissues. Human and mouse thymus	Autoantibodies to ICA69 can be detected in 43% of prediabetic subjects by Western blotting.	Association between HLA-DR3 and PBL responses in newly diagnosed Type 1 diabetics.
Chromogranin A	Neurosecretory granules. Neuroendocrine tissues	Circulating ChgA found in NOD mice.	Autoreactive T cells to ChgA found in NOD mice
Carboxypeptidase H	Neurosecretory granules	Autoantibodies to carboxypeptidase H found in ~20% of prediabetics.	Present
Ganglioside GM2-1	Pancreatic islet cells	Autoantibodies to GM2-1 detected in ~80% of prediabetic subjects and NOD mice.	?
Imogen 38 (38 kDa)	Mitochondria; widely distributed with variable levels of expression	Presence of circulating antibodies to 38-kDa proteins. Possible presence of antibodies to imogen 38.	PBLs from newly diagnosed diabetics proliferate to imogen 38
Glima 38	Amphiphilic N-Asp glycosylated β cell membrane protein that is expressed in islets and neuronal cell lines.	Autoantibodies to Glima 38 can be detected in 14–22.7% of newly diagnosed diabetics and prediabetics. The majority of these patients are negative for GAD65 and/or ICA512(IA-2) autoantibodies.	?
Peripherin	Neuronal cells	Autoantibody response against peripherin in NOD mice and in patients Type 1 diabetes and other autoimmune disorders.	T cell responses against peripherin in NOD mice.
Heat-shock protein (Hsp60)	Ubiquitously inducible	Antibodies to Hsp60 in prediabetic NOD mice.	Hsp60-reactive T-cells can accelerate disease in prediabetic NOD mice.

* Biochemical autoantibody assays readily available for large screening programs.