Lessons on autoimmune diabetes from animal models

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ABSTRACT

T1DM (Type I diabetes mellitus) results from selective destruction of the insulin-producing β-cells of the pancreas by the immune system, and is characterized by hyperglycaemia and vascular complications arising from suboptimal control of blood glucose levels. The discovery of animal models of T1DM in the late 1970s and early 1980s, particularly the NOD (non-obese diabetic) mouse and the BB (BioBreeding) diabetes-prone rat, had a fundamental impact on our ability to understand the genetics, aetiology and pathogenesis of this disease. NOD and BB diabetes-prone rats spontaneously develop a form of diabetes that closely resembles the human counterpart. Early studies of these animals quickly led to the realization that T1DM is caused by autoreactive T-lymphocytes and revealed that the development of T1DM is controlled by numerous polymorphic genetic elements that are scattered throughout the genome. The development of transgenic and gene-targeting technologies during the 1980s allowed the generation of models of T1DM of reduced genetic and pathogenic complexity, and a more detailed understanding of the immunogenetics of T1DM. In this review, we summarize the contribution of studies in animal models of T1DM to our current understanding of four fundamental aspects of T1DM: (i) the nature of genetic elements affording T1DM susceptibility or resistance; (ii) the mechanisms underlying the development and recruitment of pathogenic autoreactive T-cells; (iii) the identity of islet antigens that contribute to the initiation and/or progression of islet inflammation and β-cell destruction; and (iv) the design of avenues for therapeutic intervention that are rooted in the knowledge gained from studies of animal models. Development of new animal models will ensure continued progress in these four areas.

INTRODUCTION

T1DM (Type I diabetes mellitus) results from a chronic autoimmune process directed against the pancreatic β-cells. The onset of hyperglycaemia is preceded by a protracted preclinical period of islet inflammation that is mediated by macrophages, DCs (dendritic cells), B-lymphocytes and autoreactive T-cells. The disease process develops in individuals with defective mechanisms of self-tolerance and immunoregulation, and is probably

Key words: autoimmunity, genetics, immunology, lymphocytes, non-obese diabetic mice, pathogenesis, Type I diabetes.

Abbreviations: APC, antigen-presenting cell; BB, BioBreeding; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; DCR3, decoy receptor 3; DC, dendritic cell; GAD65, 65 kDa glutamic acid decarboxylase; HA, haemagglutinin; HLA, human leucocyte antigen; ICA69, 69 kDa islet cell antigen; IGRP, islet-specific glucose 6-phosphatase catalytic subunit-related protein; IL, interleukin; LCMV, lymphocytic choriomeningitis virus; liCTLA-4, ligand-independent CTLA-4; mAb, monoclonal antibody; NOD, non-obese diabetic; PLN, pancreatic lymph node; SNP, single nucleotide polymorphism; SOCS-1, suppressor of cytokine signalling-1; T1DM, Type I diabetes mellitus; TCR, T-cell receptor; TEM cell, effector memory T-cell; vIL-10, viral-encoded IL-10.

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triggered by ill-defined environmental factors that set in motion a series of multicellular interactions orchestrated by pro-inflammatory cytokines and chemokines [1,2]. Although it is clear that the genetic elements affording T1DM susceptibility and/or resistance are scattered throughout the genome, the molecular identity of most of these genes remains elusive and is a matter of intense investigation [3,4]. Fine mapping of these genetic elements and precise dissection of the immunological mechanisms underlying certain gene–disease associations have benefited tremendously from the availability of different animal models of T1DM [5,6]. Our current understanding of the events leading to the priming, activation, recruitment and expansion of islet-specific T-cells would not have been possible without these models. In this review, the contribution of studies on animal models to our understanding of the molecular and cellular events underlying the breakdown of self-tolerance and the initiation and progression of autoimmune diabetes are discussed (Figure 1).

ANIMAL MODELS

The two most commonly used models of spontaneous T1DM are NOD (non-obese diabetic) mice and BB (BioBreeding) diabetes-prone rats. Development of T1DM in these animals is associated with features that are shared with the human form of T1DM. Disease onset, for example, is preceded by infiltration of pancreatic islets by mononuclear cells, and is controlled by many QTL (quantitative trait loci), particularly MHC class II genes [6]. Despite the similarities, it is important to recognize that none of these models recapitulates all aspects of diabetogenesis in the outbred human population [7].

It is generally believed that initiation of T1DM in both rodents and humans is preceded by dysregulated activation and expansion of autoreactive T-cells in response to β-cell antigens. To understand the contribution of selective expression of target autoantigens in β-cells to the initiation of T1DM, several groups of investigators have expressed rat insulin-promoter-driven transgenic neo-antigens in the pancreatic islets of mouse strains that were genetically resistant to spontaneous diabetes. Expression of such neo-antigens, including LCMV (lymphocytic choriomeningitis virus) structural proteins [8,9], influenza HA (haemagglutinin) [10], HEL (hen egg lysozyme) [11], OVA (ovalbumin) [12] and MHC class I Kβ [13], was insufficient to trigger disease. These mice, however, developed T1DM upon transfer of transgenic neo-antigen-specific T-cells, or upon systemic activation of endogenous antigen-reactive T-cells via viral infection or antigen immunization [13–17]. Other investigators expressed TCR (T-cell receptor) transgenes cloned from autoreactive T-cell clones to generate animals carrying increased numbers of circulating autoreactive T-cells (against transgenic neo-antigens or endogenous self-antigens) (reviewed in [18]). These animal models allowed investigators to: (i) compare the developmental fate of autoreactive T-cells in diabetes-prone and diabetes-resistant genetic backgrounds; and (ii) investigate whether autoreactive T-cells are programmed to attack or ignore their target tissue(s) depending on the genetic background in which they develop and mature. In this regard, studies in TCR transgenic animals have generated valuable information on the roles of specific genes (deleted or modified by gene targeting) and gene polymorphisms (introgressed from other strains, by breeding) in the development or function of autoreactive T-cells. In the following sections, we discuss the contribution of some of these models to our current understanding of diabetogenesis.

GENETICS

Early studies on the genetics of T1DM in mice aimed at investigating the chromosomal locations of polymorphic loci associated (negatively or positively) with diabetes development [19]. Recent technological advances in genomics, including the establishment of genome-wide microsatellite and SNP (single nucleotide polymorphism) maps and completion of the mouse genome sequencing project, have allowed the identification of chromosomal regions containing Idd (insulin-dependent diabetes; in mice) or Iddm (insulin-dependent diabetes mellitus; in rats) susceptibility loci [19]. However, since these chromosomal intervals tend to contain clusters of genes involved in similar immunological processes, and as polymorphisms associated with T1DM risk segregate as quantitative traits, rather than as loss-of-function mutations, it has been difficult to close in on the aetiological polymorphisms. For example, the Idd5 and Idd9 regions on chromosomes 1 and 4 respectively, carry a number of genes involved in T-cell co-stimulation and survival, such as Casp8, Cd28, Cila4, Icos and Bcl2 [20], or cd30, Tnfα2 and Cdl37 [21] respectively. Another example illustrating the complexity of the immunogenetics of T1DM is Idd1. This region was long thought to afford disease susceptibility and resistance exclusively via polymorphic genes of the MHC region (I-A, or I-A and I-E respectively). Recent studies, however, have confirmed the additional contribution of genes lying outside of the MHC (referred to as Idd16), although their molecular identity remains unknown [22–26]. In rats, susceptibility to T1DM is also linked to the class II MHC region [27]. Although certain MHC-linked genes afford disease susceptibility or resistance to T1DM across species (i.e. Idd1), others are species-specific, suggesting that different genes and mechanisms determine the loss of β-cell tolerance in different models.

The ability of murine MHC molecules to afford T1DM susceptibility or resistance has been explored in detail in MHC-transgenic or -congenic mice. Studies
of congenic NOD mice expressing non-NOD MHC haplotypes and of NOD mice expressing non-diabetes-associated MHC class II transgenes demonstrated a role for class II molecules in providing susceptibility or resistance to T1DM. The pro- or antidiabetogenic properties of human MHC [HLA (human leucocyte antigen)] class II genes have also been examined in HLA-transgenic mice [reviewed in [28]]. Expression of HLA-DR3 and -DQ8 alleles in T1DM-resistant mouse strains was sufficient to trigger disease, provided that the mice also expressed the T-cell co-stimulatory molecule B7.1 in β-cells. Co-expression of HLA-DR4 or -DQ6 molecules in these animals modulated the diabetes susceptibility afforded by the HLA-DQ8 transgene, as predicted by epidemiological studies in humans [29,30]. Taken together, these studies highlighted a direct contribution of human and murine MHC class II alleles to the HLA/H-2-linked T1DM susceptibility/resistance [31].
Only a few of the many non-MHC genes that are associated with T1DM susceptibility or resistance have been mapped. Idd13 on mouse chromosome 2 is associated with a dimorphism of the β2-microglobulin (β2m) gene (β2m<sup>a</sup>, affording T1DM susceptibility; and β2m<sup>b</sup>, affording T1DM resistance). In fact, expression of β2m<sup>a</sup> or β2m<sup>b</sup> transgenes in β2m-deficient NOD mice either inhibited or promoted T1DM development respectively [32]. In another example, the NOD Idd5.1 locus encodes a truncated CTLA-4 (cytotoxic T-lymphocyte-associated antigen-4) molecule that lacks its B7-binding domain [IiCTLA-4 (ligand-independent CTLA-4)] [4]. Current evidence suggests that liCTLA-4 is preferentially expressed in memory and/or regulatory T-cells, although the mechanisms through which it affords T1DM predisposition remain unclear [33]. In humans, T1DM is also associated with ctl-a polymorphism. Pro- and anti-diabetogenic alleles at the human ctl-a locus differ by SNPs that control the ratio between a soluble form of CTLA-4 and its normal full-length counterpart, also through unclear mechanisms [4].

The transcription factor Yy1 has been proposed as a candidate susceptibility locus for Iddm4 in rats. Susceptibility and resistance alleles of Yy1 differ by SNPs in intron 4 [34]. Ia<sup>a</sup> is a strong candidate gene for Iddm2/Lyp. The BB diabetes-prone rat strain carries an allele that encodes a truncated form of IAN-4 (immune-associated nucleotide-4) [35–37]. This polymorphism may be a peculiarity of the rat, since SNPs in the mouse or human orthologues of Ia<sup>a</sup> are not associated with T1DM susceptibility [38]. This demonstrates that T1DM is a genetically heterogeneous disease entity, at least across species.

**DEVELOPMENT OF AUTOACTIVE T-CELLS**

Self-MHC restriction and tolerance are imprinted in the thymus, where maturing T-cells 'learn' how to discriminate between self and non-self. In addition to playing a role in shaping the mature TCR repertoire, MHC molecules present self and foreign antigenic peptides to mature T-cells. Hence it is not surprising that MHC polymorphisms are so strongly associated with genetic susceptibility/resistance to most human and murine autoimmune diseases.

Expression of murine pro-diabetogenic MHC class II molecules in diabetes-resistant genetic backgrounds has been reported to foster the development of autoreactive T-cells [39]. The same appears to be true for expression of T1DM-associated HLA transgenes in NOD mice [40,41], suggesting that the MHC-linked susceptibility to diabetes hinges, in part, on the ability of class II molecules to support positive selection of self-reactive T-cells in the thymic cortex, without being able to induce their subsequent deletion in the thymic medulla. This hypothesis predicts that protective MHC class II molecules should be able to purge the mature T-cell repertoire of self-reactive T-cell specificities. Evidence in support of this idea comes from studies of transgenic mice expressing the islet-specific 4.1-TCR. 4.1-TCR-transgenic T-cells are highly diabetogenic when they arise in the NOD (I-A<sup>d</sup> homozygous) background, but are deleted in NOD mice co-expressing antidiabetogenic MHC class II molecules [by a bone marrow-derived APC (antigen-presenting cell) type, possibly a DC] [42–44]. It is clear, however, that the tolerogenic properties of antidiabetogenic class II molecules do not affect all autoreactive T-cell specificities. For example, protective (and 4.1-tolerogenic) class II molecules did not trigger deletion of thymocytes expressing another islet antigen-specific I-A<sup>d</sup>-restricted TCR (BDC2.5) [39,44]. Because the BDC-2.5 TCR transgene is pathogenic when expressed in immunocompromised, but not wild-type, NOD mice [45], we suspect that protective class II molecules mediate their antidiabetogenic function by selectively targeting highly pathogenic T-cell specificities. Obviously, these observations do not exclude the possibility that antidiabetogenic class II molecules act by promoting the selection of regulatory T-cells. NOD mice expressing an I-A<sup<k</sup> transgene, for example, developed a reduced incidence of diabetes, without abrogating the ability of their splenic T-cells to transfer diabetes into NOD.scid recipients [46]. Whatever the precise mechanisms, MHC class II molecules clearly contribute to diabetes susceptibility and resistance by promoting the development of different TCR repertoires (pro- or anti-diabetogenic respectively).

Studies of CD8<sup>+</sup> T-cell-deficient NOD mice have shown that MHC class I-restricted CD8<sup>+</sup> T-cells play a key role in the initiation of diabetogenesis (reviewed in [47]). In fact, there is evidence that MHC class I genes also affect T1DM risk or protection. For example, systemic expression of an H-2L<sup>d</sup> transgene in NOD mice reduced the incidence and delayed the kinetics of T1DM [48]. A recent study in HLA-transgenic NOD mice has suggested that this is not an idiosyncrasy of murine class I molecules. When the HLA-A2 allele, which is associated with T1DM in humans, was expressed as a transgene in NOD mice it accelerated the onset of diabetes [49].

One of us (P.S.) has proposed previously that pathogenic autoreactive T-cells may be inherently promiscuous for MHC molecules. We reported that the MHC class II-restricted highly pathogenic 4.1-TCR can recognize several different MHC class II molecules, with developmental and functional consequences that correlated precisely with their pro- or anti-diabetogenic effects in non-TCR-transgenic NOD mice [42]. This observation has now been extended to at least some pathogenic MHC class I-restricted TCRs. NOD mice expressing the A14-TCR develop accelerated T1DM [50]. This TCR recognizes its target antigenic peptide in the context of H-2K<sup>k</sup>, but it can also engage antidiabetogenic MHC class II
molecules, such as those encoded in the H-2^{nb1} haplotype. In H-2^{Q/nb1} heterozygous TCR-transgenic animals, this interaction causes deletion of AI4 thymocytes [51]. A role of MHC class II molecules in negative selection of ‘autoreactive’ CD8+ T-cells has also been observed in mice co-expressing a TCR (referred to as clone 4 or CL4) and its target antigen (influenza HA) in pancreatic β-cells [52]. This TCR is highly pathogenic in double-transgenic animals, but undergoes central tolerance in mice expressing I-Ea4/-I-Eβb heterodimers [52]. Development and function of islet-reactive T-cells, such as those expressing pathogenic MHC class II- (4.1 and BDC-2.5) and class I-restricted TCRs (8.3 and AI4), is also modulated by non-MHC-linked genetic elements [53–55]. In summary, we can conclude from the above studies that pro-diabetogenic MHC alleles foster the development of autoreactive T-cells; these alleles foster positive selection of autoreactive T-cells in the thymic cortex yet fail to delete these cells in the thymic medulla. These studies have also shown that expression of pro-diabetogenic MHC class II genes contributes to the development of both CD4+ and CD8+ autoreactive T-cells, owing to the MHC promiscuity of certain highly pathogenic autoreactive T-cells.

**ACTIVATION AND RECRUITMENT OF DIABETOGENIC T-CELLS**

T1DM is a slow-progressing autoimmune disease that, in most cases, evolves over a period of months (in rodents) or years (in humans). This may be so because the peripheral precursor frequencies of pathogenic autoreactive T-cells in predisposed individuals probably fall below a certain critical (pathogenic) threshold. Significant expansion of these T-cell populations in the periphery may allow them to overcome immune regulation. This scenario has been visualized by tracking the development, circulation, activation and recruitment of a dominant population of autoreactive CD8+ T-cells in NOD mice (also referred to as ‘8.3-like’) that recognizes residues 206–214 of IGRP (islet-specific glucose 6-phosphatase catalytic subunit-related protein) [56–60]. Studies using peptide/MHC class I tetramers have revealed that the size of the circulating 8.3-like T-cell pool expands prior to the onset of clinical disease. Notably, mice in which this 8.3-like population does not expand do not develop diabetes.

Where do islet-reactive T-cells become activated? Since pancreatic β-cells do not express MHC class II or co-stimulatory molecules, they are probably unable to prime naive autoreactive T-cells. Rather, these T-cells probably need to engage their corresponding target antigens on professional APCs (most likely DCs) to undergo productive activation. It has been recently shown that removal of the PLNs (pancreatic lymph nodes) in young NOD mice prevents the onset of diabetes [61]. The role of PLN-associated APCs in the activation of diabetogenic T-cell specificities has also been examined in detail using CD4+ (BDC2.5 and 4.1) and CD8+ (8.3) T-cells derived from TCR-transgenic NOD mice. These studies have conclusively shown that islet-specific T-cells engage cognate peptide/MHC complexes in the PLNs, suggesting that β-cell autoantigens are shed from β-cells by a T-cell-independent insult, uptaken by DCs, shuttled to the PLNs and eventually cross-presented to local T-cells [62,63]. An important observation of these studies is that β-cell-reactive T-cells do not proliferate in the PLNs of ≤3-week-old hosts, and that the magnitude of this antigen cross-presentation event correlates with the extent of β-cell apoptosis in islets [63]. Because the age-at-onset of this cross-presentation event is preceded by a temporal increase in the rate of β-cell apoptosis (or removal of apoptotic β-cells by macrophages) [64,65], it has been suggested that shedding of T1DM-initiating β-cell antigens into the milieu occurs during the neonatal period, when this islet remodelling process takes place.

Activation of islet-specific T-cells is followed by clonal expansion, recruitment into pancreatic islets and, eventually, β-cell destruction. However, not all islet-reactive T-cells are diabetogenic. NOD mice expressing TCRαβ transgenes (G206 and G286) directed against epitopes of GAD65 (65 kDa glutamic acid decarboxylase), for example, did not develop T1DM. In fact, the transgenic T-cells of these mice exhibited antidiabetic properties in adoptive transfer models [66,67]. Since thymocytes recognizing-peptide/MHC complexes with high-affinity/avidity generally undergo negative selection and since the pathogenicity of islet-reactive TCRs is proportional to their ligand-binding affinities/avidities [56,59,60], it is likely that most peripheral autoreactive T-cell clonotypes recognize ligands with low-affinity/avidity. There is evidence suggesting that low-avidity autoreactive T-cells are not only innocuous, but also able to effectively compete with their high-avidity counterparts for antigen and APCs, and thus able to protect against disease [59]. It has also been shown that high-affinity/avidity autoreactive T-cells undergo partial tolerance even in diabetes-prone genetic backgrounds, explaining why these T-cell populations need to expand to be able to effect clinically significant β-cell damage [54,60]. We have proposed that the onset of hyperglycaemia is preceded by progressive expansion of small peripheral pools of high-avidity clonotypes and that this expansion displaces the initially larger pools of protective low-avidity clones, leading to progressively accelerated loss of β-cells and overt diabetes [56,59,60].

Central tolerance of β-cell-reactive T-cells has also been observed in mice expressing transgenic neo-antigens in β-cells, owing to expression of the transgenic neo-antigen in the thymus [8,9,68]. Co-expression of these transgenic neo-antigens with TCR transgenes derived from neo-antigen-specific T-cell clones fostered T1DM development in some, albeit not all, models. For example,
transgenic LCMV-reactive CD8+ T-cells ignored the neo-antigen-expressing β-cells, unless the mice were infected with LCMV [8]. In contrast, double-transgenic mice expressing HA in β-cells and a high-affinity HA-specific TCR in T-cells (CL4) developed T1DM shortly after birth [16,69,70].

There is extensive evidence from different animal models that the pathogenic activity of autoreactive T-cells is controlled by T-cell intrinsic and extrinsic genes. The pathogenic activity of AI4 CD8+ T-cells in TCR-transgenic NOD mice is inferior to that of the same T-cell population developing in TCR-transgenic H-2β7-congenic C57BL/6 mice [54]. A more exaggerated example of this phenomenon has been observed for the class II-restricted BDC2.5 TCR. BDC2.5-TCR-transgenic, H-2β7-congenic C57BL/6 mice develop diabetes, whereas BDC2.5 NOD mice do not, or do so with very low incidence [71]. The mechanisms underlying these differences remain elusive. It has been suggested that a subpopulation of BDC2.5-TCR-transgenic NOD T-cells co-expressing transgenic and endogenous TCRs somehow inhibits diabetogenesis in these mice [72]. Because low-avidity autoreactive T-cells can inhibit the pathogenic activity of their high-avidity counterparts [56,59,60], it would be reasonable to suspect that the ‘inhibitory’ BDC2.5 cells proposed by Kanagawa et al. [72] recognize self-antigen with low-avidity, owing to dilution of the transgenic TCR by endogenous TCR molecules on the cell surface. A double transgenic mouse co-expressing RIP (rat insulin promoter)–HA and HA-specific TCR transgenes [14] is another example of genetically controlled T-cell pathogenicity. T1DM developed only when the transgenes were expressed in the B10.D2, but not BALB/c mouse backgrounds.

The expansion of pathogenic autoreactive T-cell populations need not be driven by antigen. Under normal circumstances, the size of the peripheral T-cell pool is stable. Loss of peripheral T-cells in the course of antigenic responses or during T-cell turnover is compensated by thymic T-cell output or by homoeostatic proliferation of peripheral T-cells [73]. The BB diabetes-prone rat suffers lymphopenia owing to a loss-of-function mutation in the Ian5 gene (also referred to as Lyp, for lymphopenia, or Iddm2), which causes premature death of recent thymic emigrants (RTE) [35–37]. This results in an unusual high rate of T-cell division in the periphery that plays an essential role in the diabetes susceptibility of BB diabetes-prone rats [74]. Very early evidence suggested that lymphopenia promoted diabetogenesis by impairing the development of the CD4+ RT6+ (ART2+) T-cell population [75–77]. However, whether or not these T-cells have bona fide regulatory activity or, rather, inhibit diabetogenesis by ‘repleting’ the peripheral T-cell pool and thus by inhibiting homoeostatic proliferation of pathogenic T-cell specificities is unclear. In fact, most mature T-cells in normal rats are RT6+ [77], and RT6+ cells exhibit pathogenic activity when transferred into BB rats [78,79]. Furthermore, transfer of large numbers of naïve T-cells into BB diabetes-prone rats inhibits T1DM [80], and induction of lymphopenia in diabetes-resistant rats promotes T1DM [81].

A potential role for abnormal T-cell homoeostasis in the pathogenesis of T1DM in NOD mice has been examined recently. Although NOD mice do not carry a mutant Ian5 gene, it has been proposed that diabetogenesis is driven by homoeostatic proliferation of T-cells, caused by partial lymphopenia and fuelled by IL (interleukin)-21 [82]. T-cell transfers or complete Freund’s adjuvant-induced increases in the size of the peripheral T-cell pool inhibited this process and blunted T1DM progression. Although reduced T-cell counts have also been reported in human diabetics [83,84], the contribution of homoeostatic proliferation of T-cells to the pathogenesis of T1DM remains highly hypothetical.

### AUTOANTIGENS

Numerous islet antigens have been associated with human T1DM. The sera of diabetic patients and their first-degree relatives contain autoantibodies against several β-cell antigens, including insulin, GAD65, ICA69 (69 kDa islet cell antigen) and the protein tyrosine phosphatase-like antigen IA-2 [85]. The presence of high titres of autoantibodies against multiple islet antigens is a good predictor of progression to overt disease, presumably because it reflects the magnitude of ongoing β-cell destruction [86,87].

The role of these β-cell autoantigens in the pathogenesis of T1DM has been examined in animal models. Initial evidence supported a pathogenic role for GAD65-specific T-cells in T1DM. Spontaneous CD4+ and CD8+ T-cell responses to GAD65 have been described in NOD mice [88–90], and therapeutic manipulation of GAD65-reactive T-cells alters the natural course of T1DM. For example, whereas intra-thymic injection of certain GAD65 peptides accelerated the disease process [91], intranasal administration of these and other GAD65 peptides afforded protection [92]. Likewise, vaccination of NOD mice with GAD65 peptides, proteins or GAD65-encoding DNA reduced the incidence of disease [93,94]. The observation that NOD mice expressing an antisense GAD65 transgene in β-cells were diabetes-free [95] also supported a role for GAD65 in diabetogenesis. However, there is also strong evidence arguing against an important role for GAD65 in the pathogenesis of T1DM: (i) increased expression of transgenic GAD65 in the islets of NOD mice does not accelerate the disease process [96]; (ii) NOD mice expressing GAD65 systemically develop diabetes with normal incidence and kinetics, despite being tolerant to GAD65 [97]; and (iii) GAD65-deficient NOD mice develop diabetes essentially like their GAD65-competent counterparts [98]. Taken together, these
GAD65-reactive CD4+ T-cells have regulatory, rather than pathogenic, activity [66,67,99–103].

There is compelling data suggesting that insulin is a key target autoantigen in T1DM. Islet infiltrates contain insulin B-chain-reactive CD4+ and CD8+ T-cells, and these T-cells can adoptively transfer disease to syngeneic hosts [104–106]. Thymic expression or intra-thymic injection of the insulin B-chain in young NOD mice reduces the incidence of diabetes [91,107], suggesting that negative selection of insulin-reactive thymocytes inhibits disease initiation. The notion that insulin autoreactivity might contribute to the earliest stages of diabetogenesis is supported further by recent studies of pro-insulin-1- and/or pro-insulin-2-deficient NOD mice, and of NOD mice overexpressing pro-insulin-2. The mouse genome encodes two forms of pro-insulin, pro-insulin-1 and pro-insulin-2. Although pro-insulin-2 is expressed in both pancreatic islets and thymus, pro-insulin-1 is primarily expressed in pancreatic islets. Pro-insulin-2-deficient NOD mice develop accelerated T1DM, suggesting that pro-insulin-2 autoreactivity affords diabetes resistance, perhaps via regulatory T-cells [108]. In contrast, pro-insulin-1-deficient NOD mice develop a lower incidence of diabetes, suggesting a pathogenic role for pro-insulin-1 autoreactivity [109]. In agreement with some of these observations, NOD mice overexpressing pro-insulin-2, which delete pro-insulin-2 (and some pro-insulin-1)-reactive T-cells, develop a significantly reduced incidence of diabetes [97]. Notably, introduction of both pro-insulin deficiencies into a mouse expressing an insulin transgene that is immunologically ‘invisible’ abrogated the development of insulitis and T1DM [110]. This is unlike what has been described for GAD65 and ICA69, which are dispensable for the development of T1DM [98,111]. Based on these observations, it seems likely that T-cell autoreactivity to insulin is a key event in the initiation of the diabetogenic response. In fact, there is also evidence for an important role of T-cell autoreactivity against insulin in human T1DM. For example, a polymorphic VNTR (variable nucleotide tandem repeat) located upstream of the insulin gene (also known as IDDM2) affords T1DM susceptibility or resistance by influencing the levels of expression of pro-insulin in the thymus [112]. Furthermore, pancreas-associated lymph nodes in long-standing diabetic patients contain a relatively high frequency of insulin A-chain-reactive CD4+ T-cells [113].

The development of NOD mice expressing TCR transgenes cloned from islet-infiltrating T-cells has afforded the opportunity to search for other antigenic targets possibly involved in the initiation and/or progression of T1DM. There is compelling evidence that initiation and progression of T1DM is CD8+ T-cell-dependent [114,115]. A substantial fraction of all islet-associated CD8+ cells in NOD mice use highly homologous TCRα chains (Vα17–Ja42) and recognize the mimotope NRP-A7 in the context of the MHC molecule Kd [115,116]. These T-cells are already a significant component of the earliest NOD islet CD8+ infiltrates [56,117], are diabeticogenic [42], target a peptide from IGRP (IGRP206–214, similar to NRP-A7) [58], and are very common in the periphery [57]. Importantly, progression of insulitis to diabetes in NOD mice is invariably accompanied by cyclic expansion of the circulating IGRP206–214-reactive CD8+ T-cell subset [57], and by avidity maturation of its islet-associated component [56]. It is also noteworthy that islet-associated CD8+ T-cells in pre-diabetic and diabetic NOD mice target multiple epitopes of IGRP [59]. We estimate that approx. 40% of all islet-associated CD8+ T-cells in acutely diabetic NOD mice recognize IGRP epitopes [59]. Recently, it has been shown that autoreactive CD4+ T-cells also target IGRP and that treatment of NOD mice with I-Ak-binding IGRP peptides affords protection from T1DM [118]. When considered together, these data strongly support the idea that IGRP is a dominant β-cell autoantigen in murine T1DM and suggest that IGRP-reactive T-cells play a major role in the progression of islet inflammation to overt clinical disease.

Insulin, GAD65 and IGRP are not the only antigenic targets of islet-associated CD4+ and CD8+ T-cells in T1DM [51,119–122]. A recent study has shown that pathogenic autoreactive CD4+ T-cells (BDC6.9) can also recognize strain-specific autoantigenic epitopes [123]. Additional autoreactive T-cell populations of unknown antigenic specificity have been identified. Finally, it is important to note that anti-islet T-cell autoimmunity is not exclusively directed against pancreatic β-cells. Cell-mediated and humoral immune responses targeting peri-islet neural elements have been identified in both T1DM patients and NOD mice [124–126]. It has also been shown that damage and loss of sympathetic nerves in diabetic BB rats leads to islet neuropathy [127,128].

**INDUCTION OF IMMUNOLOGICAL TOLERANCE IN ISLET TRANSPLANTATION**

Animal models of T1DM have also been instrumental in enabling investigations on the efficacy of experimental therapeutic approaches. There is a long list of interventional strategies that are capable of blunting or delaying disease progression. Many of these strategies, however, need to be used very early on in the disease process, long before the onset of hyperglycaemia, to be effective [7]. For example, blockade of the B7/CD28 or CD40/CD154 T-cell co-stimulatory pathways in NOD mice before 4 weeks of age afforded near complete protection from T1DM [129,130], but did not protect mice older than 8 weeks [129,131]. Although some approaches can inhibit the progression of β-cell destruction when applied late in

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the disease process, only a few (i.e. islet transplantation) have the ability to restore normoglycaemia in overtly
diseased individuals [132–137]. There are numerous
immunosuppressive strategies capable of supporting the
long-term survival of transplanted islets, but many of
these have the potential for long-term complications.
Induction of islet-specific immunological tolerance is
therefore a preferred, yet much more challenging goal
than generalized immunosuppression [138,139].

When grafted into non-immunosuppressed diabetic
NOD mice, syngeneic islets are rejected within days
owing to a rapid anamnestic response of the host against
the transplanted β-cell autoantigens [140,141]. It has been
shown that memory T-cells can survive in the periphery
for long periods of time without the need to engage
certain antigen/MHC ligands [142] and without dividing[143]. Since memory T-cells are recruited rapidly
to sites of inflammation upon antigen re-encounter and
since they respond more vigorously to antigen than their
naive T-cell precursors (particularly when the amounts
of antigen and/or stimulatory resources are limiting)
[144,145], it is not surprising that transplanted islets
cannot withstand the wrath of recurrent autoimmunity.

Blockade of T-cell co-stimulation using CTLA4–Ig or
an anti-CD154 mAb (monoclonal antibody) can inhibit allo-
- and xenogeneic responses, but is ineffective at
inhibiting recurrent autoimmunity in diabetic NOD mice
[131,139,146–149]. This probably reflects, in part, the
presence of relatively large numbers of islet-specific T_{EM}
cells (effector memory T-cells) in diabetic NOD mice.
T_{EM} cells undergo rapid activation upon antigen re-exposure,
even in the absence of co-stimulation [150].

Although cytokine receptors sharing the common γ-chain,
such as IL-7R, IL-15R and IL-21R, play key roles in the development and survival of memory T-cells
[150], treatment with an anti-(γ-chain) antibody failed
to prolong the survival of islet grafts in diabetic NOD mice,
unless the CD28/B7 and CD40/CD154 pathways
were also blocked [131]. Anti-CD45RB mAb treatment
is another example of a tolerogenic strategy that is not
effective at inhibiting recurrent autoimmunity in islet-
grafted NOD mice. Anti-CD45RB mAb induces T-cell
apoptosis and islet allograft tolerance in conventional
mouse strains [151,152], but does not protect islet grafts
in diabetic NOD mice, unless it is given along with anti-
CD154 antibodies [137]. Similar observations have been
made with depleting anti-CD4 mAbs [134]. Presence of
memory T-cells is therefore not the only reason diabetic
NOD mice are resistant to induction of tolerance. In fact,
there is evidence suggesting that genetic susceptibility
to T1DM contributes to both the disease and resistance
to tolerance. For example, co-stimulatory blockade and
induction of donor-specific hyporesponsiveness
cannot effectively prevent disease recurrence in islet-
grafted NOD mice, and this resistance maps to the Idd3
region, which contains the genes for il2 and il21 [139,147].

The last few years have witnessed the development
of islet cell engineering strategies aimed at fending anti-
islet immune responses. Expression of a CTLA4–Ig trans-
gene in allogeneic pancreas grafts increased graft survival
in diabetic BB rats [138]. Expression of DCR3 (decoy
receptor 3; a member of the tumour necrosis factor re-
sceptor superfamily that neutralizes apoptotic signals) in
islet β-cells of NOD mice prevented the development
of insulinitis and T1DM, and rendered the mice resistant
to disease transfer by splenocytes from diabetic donors.
Unfortunately, islets from DCR3 transgenic mice failed
to survive for extended periods of time in diabetic NOD
mice, indicating that the expression levels of DCR3 might
not be sufficient to protect islet grafts. Alternatively,
graft destruction involves killing mechanisms that are not
targeted by DCR3 [140]. SOCS-1 (suppressor of cytokine
signalling-1), which blocks JAK/STAT (signal transducer
and activator of transcription) signalling, has also been
expressed as a transgene in NOD β-cells. As was the
case for islet grafts expressing DCR3, SOCS-1 transgenic
islets did not withstand recurrent autoimmunity in islet-
grafted diabetic NOD mice [153]. Numerous other
immune modulators have been delivered into islet cells
ex vivo prior to transplantation. They include IDO
(indoleamine 2,3-dioxygenase), which is an essential
component of a negative feedback regulatory mechanism
designed to control inflammatory responses [154], and
vIL-10 (viral-encoded IL-10) [155], among others.
Although these strategies are handicapped by difficulties
associated with gene delivery and expression, they share
the advantage that their immune-suppressing effects are
local rather than systemic. Although systemic treatment
of islet-grafted diabetic NOD mice with vIL-10 and
TGF-β1 (transforming growth factor β1) clearly delays
the progression of recurrent autoimmunity [155,156],
these strategies may cause general immune suppression,
rather than islet graft-specific tolerance.

Inefficacy of the above therapeutic strategies contrasts
sharply with the indefinite survival of islet grafts from
β2 microglobulin- (and hence MHC class I) deficient
NOD donors in diabetic recipients [141,157]. This is
consistent with the idea that destruction of islets in
spontaneous disease, and possibly syngeneic islet grafts
as well, is largely an MHC class I-dependent process
[47,158]. MHC class I blockade thus seems to be an
attractive avenue for therapeutic intervention in islet
transplantation.

**CONCLUSIONS**

The pathogenesis of autoimmune (T1DM) diabetes is
enormously complex. The disease process involves
poorly understood interactions among many different
cells types of the immune system that are controlled by
numerous genetic polymorphisms scattered throughout
the genome. For the most part, these polymorphisms are
thought to be normal genetic variants that, in isolation, do not afford significant risk to the development of autoimmunity, but that, in combination, considerably heighten one's predisposition to disease. It is likely that different combinations of T1DM-associated genetic polymorphisms (within the population) lead to pathogenically different forms of T1DM. Thus any one inbred animal model of diabetes cannot possibly teach us everything that there is to know about all forms of diabetes that might exist in the human population. These animal models, however, help us identify many of the generic, signalling and immune pathways capable of creating diabetes risk when dysregulated. Genetic manipulation of diabetes-prone mice and rats allows precise dissection of these pathways, hence an ever-clearer understanding of the complex puzzle of events driving the diabetogenic immune response. In this review, we have summarized much of what we have learned over the last two decades about the immunopathogenesis of T1DM based on the study of genetically manipulated NOD mice and/or BB rats.

ACKNOWLEDGMENTS

We apologize to authors whose work could not be cited because of space constraints. The authors are supported by grants from the Canadian Institutes of Health Research, the Natural Sciences and Engineering Research Council of Canada, the US National Institutes of Health, the Juvenile Diabetes Research Foundation, and the Canadian Diabetes Association. P.S. is a Scientist of the Alberta Heritage Foundation for Medical Research. We thank all the members of our laboratories for excellent technical assistance and stimulating discussions. The Julia McFarlane Diabetes Research Centre is supported by the Diabetes Association (Foothills).

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