Animal models for type 1 diabetes

H.Wang
Department of Animal Science, University of Wyoming, Laramie, WY 82071

ABSTRACT
Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), is pancreatic autoimmune disease mainly due to the massive destruction of insulin producing beta cells. Different from type 2 diabetes, the cytokine induced beta cell death is the major feature in type 1 diabetes. The pathogenesis factors include genetic defects, beta cell autoantigens, and dysregulation of immune homeostasis. Clinical manifestations of T1D are fasting hyperglycemia, glycosuria and polyuria, which indicate 90% destruction of the beta cells in the pancreas. Many significant pathophysiology discoveries for the diabetes derives from the surgical animals and finally to the murine models of spontaneous autoimmune diabetes.

Key Words: autoimmunity, animal models, type 1 diabetes, lymphocytes, tolerance

INTRODUCTION
Type 1 diabetes (T1D) is an immune-mediated disease, which involves the recognition and destruction of insulin producing beta cells in pancreas by aberrant developed T lymphocytes, leading to the systemic decrease of insulin level. (Human juvenile type diabetes) onset is typically in the early lifetime, and the incidence is increasing. Research works using diabetic animals lead to the discovery of the pathogenesis of diabetes such as the beta cells, insulin, and the immune system in the disease. In general, the autoimmune character for type 1 diabetes susceptibility in animal models and humans is highly associated with major histocompatibility complex (MHC) region, which is T cell dependent.

Considering the difficulty in studying T1D in human, investors advocate the study of animal models that can easily be controlled and tested in consistent environment.

Although animal models of type 1 diabetes are imperfect reflections of human disease, there are however sufficient similarities between the pathogenesis of type 1 diabetes in human, mice and rats contributing to the efforts to animal studies. Here we will discuss in detail about the two most intensively studied rodent animal models of T1DM, the Nonobese Diabetic (NOD) mice and Biobreeding (BB) rats. In these models, the T lymphocytes are the effectors for the beta cell attacking and destruction. For a better understanding of the etiology of T1DM or therapeutic interventions to cure or prevent the development of T1DM, it is really important to explore in the rodent models for ultimate goal to apply to human juvenile type diabetes.
NOD mice:

General characteristics:

NOD mouse is an established model of spontaneous insulin-dependent diabetes mellitus (IDDM). In 1980 Shionogi Aburai Laboratories established the NOD inbred strain, by six generations of the selective breeding for sublines of the outbred Jcl:ICR line of mice for the selection of cataract mouse (1). Some mice develop spontaneous diabetic. There was a marked sex difference in the incidence of the development of diabetes, with the accumulative incidence of the onset at 30 weeks old female 80% and male less than 20%. Before onset, T1DM showed progressively infiltration of leukocytes to the islet of Langerhans, termed insulitis. The insulitis started from peri-insulitis, and progressed to intra-islet infiltration and destruction of beta cells, with beta-cell antigen specific CD4+ T and CD8+ T cells. After onset of diabetes, the pathologic changes in NOD mice were similar with those in human T1D.

The development of type 1 diabetes in the NOD mice is a complex process involving the genetic and environmental factors. The chromosome region encoding genes related to susceptibility named insulin dependent diabetes susceptibility (Idd). Up till now, numerous reports indicated that more than 30 loci on 17 chromosomes encoding several genes contribute to T cell mediated autoimmune type 1 diabetes in both humans and NOD mice. Individual Idd locus function is well studied. Some are related to the regulatory elements, such as the Idd5 affects the gene spicing and dysfunction of CTLA-4 and Idd3 for IL-2 and IL-21 genes (2). However those genes were not dysfunctional in NOD mice, the susceptibility loci in NOD mice contributes to the complexity of autoimmune diseases and the loss of immune tolerance.

In addition, the susceptibility of autoimmune diabetes was liked to MHC haplotype in NOD mice. A lot of antigen processing and presenting genes are encoded by MHC complex, and it is well studied that H2$^g7$ promotes the predominant genetic susceptibility (3). In NOD mice the H2$^g7$ class I gene products fails to mediate the negative selection of CD8+ T cells in thymus. Studies show that homozygosity of the H2$^g7$ haplotype of the MHC may be the leading cause of diabetes development in NOD mice (4, 5). Also Idd7 interacts with the H2$^g7$ class I gene products to determine whether the CD8+ T cells in thymus undergoes negative selection. Thus, the unique H2$^g7$ MHC contributes to the susceptibility of diabetes in NOD mice, involves the thymic negative selection, regulatory elements (2), Th1/Th2 ratio (6-8) or the polymorphism of immune mediators, such as TNF-a.

Another important factor for the pathogenesis of T1D is the autoantibodies, recognizing the autoantigens includes insulin, GAD, insulinoma-associated protein 2 (IA-2), and heat shock protein 60 (Hsp60) (9, 10). Recent reports indicate that insulin and GAD65 are also observed in the thymus, where the T cells mature. The appearance of these autoantigens in peripheral lymphoid tissues or endocrine tissues may lead to the generation of autoantigen specific T cells.
T cells:
T cells play a key role in the development and progression of type 1 diabetes, with CD4+ T and CD8+ T cells. CD4+ T cells function throughout the whole process of disease, and mediate the direct destruction of beta cells. In this aspect, many studies show that CD4+ T cells transfer disease and antibody against CD4 can be used to prevent the onset of diabetes in NOD mice. Meanwhile, CD8+ T also plays an important role in the induction of the autoimmune response. NOD CD8+ T cells increased IFN-gamma and impaired the IL4 production in both adult and young mice (11). CD8+ T cells may function as the final effector cells, and the disease requires both CD4+ and CD8+ T cells. The landmark study of low activating regulatory CD8+ T with nanoparticles coated with K\textsuperscript{d} containing islet peptide (IGRP\textsubscript{206-214}) inhibited the development of type 1 diabetes and reverse the established disease(12). In all, therapeutic strategy for the interference the development of autoimmune pathology involving the stimulation of low-avidity autoantigen-specific regulatory CD8+ T cells(12).

B cells:
Another type of immune cell considered to contribute to T1D is the B lymphocyte. B cell depletion in new onset T1D patients indicated that this slowed the destruction of insulin-producing pancreatic beta cells, because B cells can influence the T cell response to islet antigens. Also like T cells B cells could produce variety of cytokines, such as the pro-inflammatory cytokines and regulatory cytokines. They can function differently in the NOD mice. However, new evidence suggests that B1 cells may contribute to diabetes pathogenesis(13). Thus, the role of B cells in autoimmune diabetes is complex. Effector B cells play an important role in the pathogenesis of the autoimmune disease; however the regulatory B cells could interference the progression. It has been shown that blockage of B cells had positive effects in mice, with anti-CD20, toxin-conjugated anti-CD22, and BCMA-Fc(14). The mechanisms involved may relate to the killing of B cells subsets and decreasing the autoreactive cells, also the inducing of regulatory cells.

Dendritic cells (DCs):
The safeguards of the system tolerance involve the central and peripheral tolerance, in which DCs play a critical role in processing the self-antigen to activate the autoreactive lymphocytes. The development of type 1 diabetes in NOD mice coupled with the elevated number of DCs and macrophages in the inflamed islets. Many studies supported the central role of DCs for the initiation and maintenance of self-destruction of beta cells(15). It was found that DCs from NOD mice had higher costimulatory feature contribute to the Th1 responses, which lead to the activation of autoreactive T cells(16). DC dependent intervention in NOD mice associated with the increased levels of IL4, IL10 and IFN-gamma in the in vitro islet cell culture system(17). DC stimulation generated CD4\textsuperscript{+}CD25\textsuperscript{+} tregs could inhibit diabetes in NOD mice(18).
**Trafficking of lymphocytes:**

An increasing number of studies indicate that cell trafficking might involve in the process of development of autoimmune diabetes. An important family of mediators, chemokine, is the major proteins function to attract the immune cells to the local site. B cells of NOD mice are impaired by a severe inflammation response, which is caused by early infiltrate of macrophages and dendritic cells, then followed by T cells, NK cells and B cells into the islets. Chemokines play a key role in the migration of leukocytes to sites of inflammation. The accumulation of monocytes in the islet leads to the destruction of insulin-secreting Beta cells\(^1\). It is reported that Th1, but not Th2 cells are associated with pathogenesis of islet beta cells and disease origination. Polarization of chemokine expression of CD4 cells in the microenvironment of the pancreas leads to different inflammatory infiltrates that determine the fate of insulin-producing \(\beta\)-cells\(^2\). It is also shown that Th1 cells express MCP-1, MIP-1\(\alpha\) and CXCL10\(^3\). Both Th1 and Th2 cells express RANTES, MCP-3 and MCP-5 in islets of NOD mice\(^4\). Researchers also observed an elevated ratio of macrophage inflammatory protein-1alpha (MIP-1alpha): MIP-1beta in the pancreas correlated with severity of diabetes in NOD mice\(^5\). Treatment of IL-4, as a prevention of diabetes in NOD mice causes the polarization of Th2 responses in the pancreas, as determined by decreased CCR5 expression in islets and a high ratio of MIP-1beta and MCP-1: MIP-1alpha in the pancreas\(^6\). It is reported that CXCL10 impairs \(\beta\) cell function and viability in diabetes. Treatment of human islets with CXCL10 decreased \(\beta\) cell viability, impaired insulin secretion, and decreased insulin mRNA. This effect is not mediated by the common CXCL10 receptor CXCR3, but via the TLR4\(^7\). Receptors of chemokine also influence the development of autoimmune diabetes in the NOD mouse. Using the knockout mice of CCR2 and CCR5, researchers demonstrated that the infiltration of monocytes might be mediated by CCR2 in NOD mice and CCR2 deficient mice showed a delayed onset of diabetes. Therefore, CCR2 could be the potential target of therapies against Type 1 diabetes. In contrast, CCR5 deficient mice aggressive destructive insulitis and altered leukocyte migration is also appeared in the islets\(^8\).

**Tolerance disturbance and regulatory cells**

Autoimmune diseases are believed to have dysregulated immune tolerance pathways. The immune tolerant involves the central tolerance and peripheral tolerance mechanisms, and autoimmune diseases are derived from defects in both central and peripheral tolerant complex. As the T cells are the key factor in the pathogenesis of diabetes in NOD mice, the thymic selection of the T cells may lead to the abnormal activating of autoreactive T cells. It has been demonstrated that decreased expression of thymic insulin related to higher risk for T1D in human\(^9\). Thus, the thymic insulin expression has a critical role in the development of autoimmune diabetes, but the mechanisms are not clear. During the thymic selection, autoantibody GAD65 also exists in thymus. It has been observed in NOD mice that T cells could recognize the peptides of this autoantibody, and injection of GAD65 in thymus showed a protective function for the diabetes onset. While further studies have not got the similar results by overexpression of GAD65 in APCs or using...
GAD65 knockout mice, while there was no change of the diabetes susceptibility(25). NOD mice have several defects in the thymic negative selection, I-Ag7 molecule was found to be intrinsically unstable and poor peptide binding property in NOD mice. This might relate to the poor negative selection of the self-reactive immune cells. Another phenomenon found in the NOD thymocytes is that anti-CD3 mediated negative selection is less sensitive in NOD thymus(26).

T cells reactive to self-antigens exist in all normal individuals, but only those susceptible to autoimmune disease had abnormal autoreactive immune cells. Peripheral tolerance plays an important role in the preventing of autoimmune disease. For example, defects in several important proteins in the peripheral tolerant, such as IL2 and CTLA-4, may lead to the development of autoimmune diseases in mice. The critical role of regulatory T cells has been demonstrated in many organ specific autoimmune diseases. Evidences show that depletion of regulatory T cell subsets (CD45RB\textsuperscript{low}, CD25\textsuperscript{+}, and CD62L\textsuperscript{+}) promotes the clinical manifestations including colitis and autoimmune diabetes (27). However, there is a time-limited change of CD4\textsuperscript{+}CD25\textsuperscript{+} tregs to suppress the development of autoimmune diabetes in NOD mice. In this regard, treg cells in NOD mice dispense with IL10 and IL4 to mediate the suppressive function. Recent studies show that TGF-beta impulse in the islet would induce the Tregs and suppress diabetes (28). The generation of Tregs may rely on the thymus where the self-antigens (like insulin, GAD and I-A2) were presented to the thymocytes. After Tregs exported to peripheral, they would contract the auto-antigens and proliferate to maintain the immune homeostasis.

**Treatment:**

The NOD mice used extensively as a preclinical model for the therapeutic regents of type 1 diabetes. Despite the auto antigen used to reduce clonal expansion showed effect during the pre-diabetic phase and drugs like cyclosporine reserve diabetes without a durable effect, therapies that induce long-term tolerance of the immune system are really needed. Up till now, more than 175 agents showed protective effects in type 1 diabetes of NOD mice(29). For the human patients, type 1 diabetes must be treated with systemic administration of insulin. However, insulin treatment could not delay the onset of diabetes, similar as BCG and nicotinamide (30-32). A potent immunosuppressive agent anti-CD3 antibody reverses the diabetes in NOD mice, which indicates that either by stimulating or inducing de nove of the Tregs may be the promising therapeutic strategy. Treg cells induction included anti-CD3 reagents oral administration of islet-antigens, and vitamin D and D3 in NOD mice(33). Together, to induce the antigen specific tolerance, Tregs are a key target for investigation. And NOD mice are indispensible for the investigations. NOD mice helped to gain insight of the dysregulation of immune responses and redirection of the tolerance in autoimmune diabetes. As shown in the following graph, summarized the regulation of effector and regulatory cells(33).
Rat (mainly diabetes prone BB rats):

**General characteristics:**

Rat model had been continued because the genetic analysis of the rat and the interactions between genetics and environment(34). The discovery of low dose parenteral and oral insulin administration was found in the rat model. Besides NOD mice, rat was the model contributes to the extensive genetic data of human autoimmune diabetes, involving at least 27 genetic loci and variety of genetic defects. The principal rat models for type 1 diabetes includes: (1) the diabetes prone BB rat, (2) the LETL rat, (3) the KDP rat, and the (4) congenic LEW rat(34). Diabetes-prone BB (BBDP) rats were most extensively studied and derived from outbred Wistar rats in a Canada, in which the rats developed spontaneous hyperglycemia and ketoacidosis(35, 36). BB rats of both sexes spontaneously develop pancreatic insulitis at about 55–120 days of age.

The pathogenesis of BB rat is due to MHC class II alleles encoded by RT1u haplotype(37). The MHC susceptibility in BB rat was called *iddm*, and heterozygosity required for the diabetic pathogenesis. The spontaneous diabetes in BB rat may due to the autoantigens presented by RT1u. Another important genetic defect in diabetes prone is the recessive mutation on chromosome 4, involving *Ian4L4* or *Iddm2* causing lymphopenia (Lyp)(38). There are several other *iddms* on different chromosome, playing a critical role in the pathogenesis of autoimmune diabetes, such as *iddm4, iddm6, iddm5 and iddm3*. *Ian4* was discovered as diabetic gene, that mutation of *Ian4* cause the death of
CD4+CD25+ regulatory T cells(39). Different from NOD mice that virus infection against type 1 diabetes, the BB rat is virus induced type 1 diabetes.

T cells and B cells function:
It was reported that all BB rats of different sex or presence of insulin dependent diabetes had the severe T lymphopenia (40). However, the number of B cells was normal as well as the level of serum immunoglobulin. The number of both helper T cells and cytotoxic T cells were decreased in all BB rat compared to the wild type rat. Researcher also observed the inversion of ratio in helper T cells and cytotoxic T cells(41). Consequently, the dysregulation of immune responses caused by the abnormality of T cells were noted. As a result of T cells impairments, BB rat cannot reject to the allografts via the major and minor histocompatibility (36, 42). The lymphocytes from spleen and Peripheral blood could not proliferate to mitogens (43). Researchers also observed that the reduced T cells are due to large number of CD4+T cells and CD8+T cells almost disappeared in BB rat, partially as a result of the short life span of this cell subpopulation (44). The diabetes susceptibility gene mutation decreased the number of thymic T cells, which migrate to the periphery with a decreased the life span than the peripheral T cells.

Tolerance disturbance, regulatory cells and treatment
Regulatory T cells played an important role in diabetes of BB rat. It is reported that adoptive transfer of CD4+CD25+T cells prevented diabetes in 80% of rats, as the natural Tregs. However, another subpopulation of cells that was CD4+CD45RC–CD25– also joined in the regulation of autoimmune diabetes (45). The Gimap5 mutation leads to impaired function of regulatory T cells post thymically and the proliferation of diabetogenic T cells(39), but it is not found in human type 1 diabetes(46). There are several prevention strategies found in BB rat model: transfusion of Tregs before insulitis, retrovirus to stimulate high IL4 level, costimulation blockage such as anti-CD28 antibodies, or intrathymic transplantation of islets. But whether they could be applied to human is still not yet clear.
Reference:


