An update on the use of NOD mice to study autoimmune (Type 1) diabetes

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Abstract

The widely used nonobese diabetic (NOD) mouse model of autoimmune (Type 1) diabetes mellitus shares multiple characteristics with the human disease, and studies employing this model continue to yield clinically relevant and important information. Here, we review some of the recent key findings obtained from NOD mouse investigations that have both advanced our understanding of disease pathogenesis and suggested new therapeutic targets and approaches. Areas discussed include antigen discovery, identification of genes and pathways contributing to disease susceptibility, development of strategies to image islet inflammation and the testing of therapeutics. We also review recent technical advances that, combined with an improved understanding of the NOD mouse model's limitations, should work to ensure its popularity, utility and relevance in the years ahead.

Keywords

antigens; autoimmune diabetes; humanized mice; NOD mice; therapies; Type 1 diabetes

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The nonobese diabetic (NOD) mouse model of (autoimmune) Type 1 diabetes first appeared in the scientific literature in 1980 [1]. As it became available to researchers worldwide, this inbred model of spontaneous disease was rapidly embraced and remains a mainstay of current Type 1 diabetes research (FIGURE 1). The model is not without its critics, whose feelings stem primarily from the disappointing realization that while multiple manipulations have been reported to prevent disease in NOD mice [2], this goal has not yet been achieved in people. However, the disease in the mouse model has numerous striking similarities to that in humans, and these serve to explain the continued utility of NOD mice as a research tool. These similarities include common antigenic targets (TABLE 1), the expression of class II MHC molecules (i.e., I-A^{g7} and HLA-DQ8) displaying related peptides [3] and genetic polymorphisms affecting shared pathways [4]. Indeed, there are numerous instances

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where discoveries made in NOD mice were found to parallel the human disease. As just one example, the β-cell protein islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) was first identified as a target of islet-reactive CD8^{+} T cells in NOD mice [5]. Subsequent work revealed that IGRP peptides are also targeted by CD8^{+} T cells in patients with Type 1 diabetes [6–8]. In addition to antigen discovery, other areas of utility for the NOD mouse include identification of disease susceptibility genes, testing of therapeutics and identification of new targets, exploration of environmental influences on disease and the development of imaging strategies (FIGURE 2). The purpose of this article is to provide an update on progress made in these areas, with the help of NOD mice, from 2005 to the present day. Special mention will be made of those developments having established or potential applicability to Type 1 diabetes in humans. Recent technical advances, such as the availability of NOD-derived embryonic stem (ES) cells [9,10] and the development of increasingly 'humanized' NOD strains [11,12], will also be discussed, as they are likely to further increase both the popularity and the utility of the NOD mouse model in the study of Type 1 diabetes.

Antigen discovery

Our understanding of disease pathogenesis has been greatly enhanced through the study of Type 1 diabetes antigens in NOD mice (TABLE 1). A large body of evidence now supports a key role for insulin autoreactivity in diabetogenesis [13]. Pathogenic responses to insulin in NOD mice are directed at $Ins1/2B_{9-23}$ and $Ins1/2B_{15-23}$ in CD4^{+} [14] and CD8^{+} T cells [15], respectively. NOD mice have been generated that are null for Ins1 and Ins2 but express a transgenic *Ins2* gene in which $InsB_{16}$ has been altered from Y to A [16], preventing both binding of $InsB_{15-23}$ to H-2K[d] and activation of $InsB_{9-23}$-specific CD4^{+} T cells. The transgenic mice do not develop Type 1 diabetes or any signs of β-cell-specific autoimmunity, suggesting the importance of $InsB_{9-23}$ and/or $InsB_{15-23}$ in the development of Type 1 diabetes in NOD mice. Indeed, in NOD mice, establishment of tolerance to insulin can lead to prevention of diabetes [17] as well as remission of established disease [18].

Studies in NOD mice have also suggested structural and antigen-processing characteristics of autoantigens, which may contribute to pathogenicity. $InsB_{9-23}$ binds weakly to the NOD MHC class II molecule I-A[g7] in two adjacent peptide-binding registers, which are recognized by different subsets of CD4^{+} T cells [19]. Similarly, a subset of pathogenic $InsB_{9-23}$-reactive T cells does not respond to exogenous insulin processed by antigen-presenting cells, but does respond if the antigen-presenting cells are in contact with β-cells or have been fed secretory granules purified from β-cells [20]. The use of a monoclonal antibody specific for $InsB_{9-23}$ but not proinsulin or insulin recently revealed $InsB_{9-23}$-positive granules in a subset of β-cells [20]. Taken together, these results suggest an antigenic property of $InsB_{9-23}$, which is specifically conferred by β-cells, possibly reflecting a conformational or posttranslational modification of the peptide. Along these lines, a natural cleavage product of the neuroendocrine protein chromogranin A, WE14, was recently identified as the target of the highly pathogenic NOD-derived CD4^{+} T-cell clone BDC2.5 [21]. Binding of WE14 to I-A[g7] is atypical in that it is mediated by the C-terminal portion of the peptide-binding groove and seemingly by regions outside of the binding groove of I-A[g7]. Whether and how this unusual binding may permit escape of BDC2.5-like T cells during thymic negative selection awaits additional studies. However, some possibilities in this regard include low-affinity binding to I-A[g7] (poor antigen presentation) or inefficient interactions of the WE14/I-A[g7] complex with the T-cell receptor. These studies of insulin and chromogranin A have therefore contributed to a model of diabetes pathogenesis in which autoreactive T cells escape thymic negative selection because recognition of their cognate antigens is compromised due to weakly or atypically bound

peptides, and/or due to β-cell-dependent constraints on antigen processing or presentation [22].

Several islet autoantigens targeted in Type 1 diabetes have yet to be identified. For example, CD4^{+} and CD8^{+} T-cell clones of unknown specificity that are pathogenic in transfer systems, transgenic mice and retrogenic mice, and are known to recognize islet antigens, have been isolated from NOD mice [23,24]. Moreover, the islet-infiltrating T-cell repertoire is thought to be complex, especially at later stages of inflammation [25], but also antigen specific [26]. In addition, known CD8^{+} T-cell specificities account for a minority of infiltrating CD8^{+} T cells [27]. Previous methods for Type 1 diabetes antigen identification have been laborious and slow, relying on biochemical or genetic screens, or on the testing of candidate antigens whose identity was suggested by extensive prior investigations (TABLE 1). A recent bioinformatic search for genes highly and preferentially expressed in islet β-cells identified the Type 1 diabetes antigen ZNT8 [28], suggesting that the computational prediction of Type 1 diabetes antigens is feasible. Indeed, pancreatic duodenal homeobox 1 (PDX1) was another high-ranking potential antigen predicted in this study, which was recently identified independently as a Type 1 diabetes antigen [29]. Analysis of islet transcripts represented in the Unigene database suggests the existence of a large set of islet-specific genes that is enriched for known Type 1 diabetes antigens (FIGURE 3). To our knowledge, every β-cell-specific protein examined to date is a target of either antibody or T-cell responses in either NOD mice or humans, and most are shared (TABLE 1). Some islet-specific, non-β-cell antigens have also been described [30]. If the relationship between islet specificity and antigenicity proves to be robust, hundreds of unidentified islet-specific antigens may await discovery (FIGURE 3). We therefore advocate a systematic examination of islet-specific genes as potential Type 1 diabetes antigens. Of course, the number of potential T-cell and antibody epitopes among these antigens will be considerably larger.

Nonobese diabetic mice provide the only model that can address this scale of antigen discovery efficiently. Importantly, NOD mice are amenable to high-throughput technologies that can interrogate the relevant pathogenic cell types directly. The recent finding that entry and accumulation of T cells in the islets of NOD mice requires the T cells to be specific for islet antigens [26] supports the use of islet-infiltrating cells in antigen discovery efforts. NOD mice can also be used to test the pathogenic hierarchy of candidate antigens by tolerogenic [17], gene ablative and/or mutational approaches [16], while HLA and human antigen transgenic and knock-in mice should help in the translation of these findings to human disease. Finally, retrogenic mice should enable rapid assessment of novel T-cell receptor specificities with respect to pathogenicity as they are discovered [23]. A detailed knowledge of the pathogenic response in NOD mice should inform the development of therapies, clinical trials and prognostic markers for Type 1 diabetes.

Identification of disease susceptibility genes

Nonobese diabetic mice and derivative congenic and recombinant congenic strains have been used extensively for the mapping of autoimmune diabetes susceptibility loci (designated *Idd* for insulin-dependent diabetes) and nearly 50 such loci have been reported to date [31]. In some cases, a candidate gene that is probably responsible (alone or in part) for the disease-promoting effect of an *Idd* locus has also been identified [31]. For *Idd5.1*, one such gene is *Ctla4* [32,33]. In NOD mice, a polymorphism that affects RNA splicing results in diminished production of a CTLA-4 isoform that lacks the ligand-binding domain [33] and signals negatively in T cells [34]. Importantly, *Ctla4* is also associated with susceptibility to Type 1 diabetes in humans, although the molecular explanation is thought to be different and to involve decreased production of a soluble form of CTLA-4 [33] that is capable of inhibiting T-cell proliferation by binding to CD80 and CD86 [35].

Nonobese diabetic mouse studies greatly facilitated the progress that was recently made in the elucidation of another shared pathway for Type 1 diabetes development in NOD mice and humans, namely, that involving IL-2 and its receptor [4]. While *Il2* has long been suspected to be a causal gene for the effect of *Idd3* on diabetes development in NOD mice, compelling evidence for this was only recently provided [36]. Sequencing of the *Il2* gene from a panel of diabetes-resistant and susceptible mouse strains revealed numerous disease-associated single-nucleotide polymorphisms, although none were nonsynonymous or obviously capable of altering RNA splicing. However, thymocytes, splenocytes and anti-CD3-stimulated splenocytes from NOD mice all contained lower amounts of *Il2* mRNA when compared with their counterparts isolated from NOD.B6 *Idd3* mice. Reduced IL-2 production correlated with a smaller proportion of Foxp3$^+$CD4$^+$ T cells in the pancreatic lymph nodes and reduced functional competence of CD4$^+$CD25$^+$ regulatory T cells, suggesting a mechanism for the association of *Idd3* with diabetes susceptibility. Importantly, susceptibility to Type 1 diabetes in humans has been mapped to the gene encoding IL-2Rα (CD25) [37,38], and defects in regulatory T cells have been reported in Type 1 diabetes patients [39,40]. These results illustrate the utility of NOD mice in the identification of disease susceptibility genes (or affected pathways) with human relevance, and they also highlight the power of the mouse model in clarifying the mechanisms responsible for the observed disease associations.

Exploration of environmental influences

The cumulative diabetes incidence observed for female NOD mice housed at different institutes varies wildly (i.e., from 15 to 100%), suggesting that the environment can influence the development of autoimmune diabetes [41]. While such environmental factors remain largely ill defined, the finding that reduction in microbial load increases diabetes incidence in NOD mice [42] fostered interest in the potential protective nature of microbes (or their components) that continues today. Indeed, deliberate infection of NOD mice with a variety of specific organisms, including *Schistosoma mansoni* [43], *Mycobacterium avium* [44], *Trichinella spiralis* [45], *Heligmosomoides polygyrus* [45] and *Salmonella typhimurium* [46], inhibits diabetes development. As reviewed [47], the potential mechanisms responsible for these protective effects include Th2 skewing, increased regulatory T-cell and natural killer T-cell activity, and modulation of antigen-presenting cells towards a diabetes-protective phenotype.

Recently, the seminal observation was made that interaction between the intestinal flora and the innate immune system in NOD mice can influence the development of Type 1 diabetes [48]. In this study, NOD mice genetically deficient in MyD88, an adaptor protein for multiple Toll-like receptors, were found to be resistant to diabetes development and to exhibit altered gut flora. Strikingly, transfer of this altered flora rendered germ-free NOD mice resistant to Type 1 diabetes. Interestingly, a probiotic compound (VSL#3; a mixture of *Bifidobacterium* and *Lactobacillus* species and *Streptococcus salivarius* subsp. *thermophilus*) [49] and the fungal cell wall constituent zymosan [50] were among the agents tested in NOD mice from 2005 to the present that have been shown to delay or prevent disease (TABLE 2). As knowledge in this area continues to expand, informed application to Type 1 diabetes in humans may be suggested.

Testing of therapeutics & identification of new potential targets

In 2005, a comprehensive review of all preventive or therapeutic interventions that had been tested in female NOD mice became available to the scientific community [2]. This heroic effort included all interventions (subject to certain inclusion criteria, such as the availability of diabetes incidence data), regardless of whether they had a favorable, neutral or

unfavorable influence on disease course. Importantly, the authors were among the first to highlight the fact that protocol differences (e.g., in terms of relative dose and disease stage) between NOD mouse studies and human trials probably contributed to the disappointing results reported in patients [2], a notion subsequently affirmed by others [51]. As a result of collective extensive experience with the NOD mouse model, the field is far more aware now than it was one or two decades ago of its limitations and of the factors that need to be considered when translating therapies to the human disease. It is now recognized that a regimen that prevents disease in NOD mice when begun at 4 weeks of age will not necessarily be successful in reversing ongoing autoimmunity in a patient. Differences between mouse and human immunology are also better appreciated [52]. Despite its limitations, the NOD mouse model has aided in the development of promising interventions for Type 1 diabetes patients, for example, anti-CD3 treatment. The ability of anti-CD3 to reverse Type 1 diabetes was discovered in NOD mice [53], and subsequent human trials have demonstrated the capacity of anti-CD3 to slow the rate of loss of insulin production and reduce exogenous insulin requirements in patients [54,55].

Given the belief that NOD mouse studies will continue to suggest therapeutic strategies to be considered for translation to patients, we have compiled representative interventions that have recently (reported in 2005 or later) been shown to prevent (TABLE 2) or reverse (TABLE 3) spontaneous Type 1 diabetes in female NOD mice. While not exhaustive, these compilations are intended to illustrate the variety of approaches being pursued. Their examination suggests some general trends. The first is a growing interest in B cells, evidenced by the use of multiple reagents to deplete or manipulate these cells in NOD mice in order to prevent (TABLE 2) or reverse disease (TABLE 3). B-cell-depleting reagents successfully employed include anti-CD20 [56,57], anti-BLyS [58], BAFF/APRIL blockade [59] and cytotoxin-coupled anti-CD22 [60]. Although it has been known for nearly 15 years that B cell-deficient NOD mice do not develop diabetes [61], B cells have been relatively neglected by investigators, as Type 1 diabetes has long been viewed as a strictly T-cell-mediated disease. However, we now know that B cells act as efficient antigen-presenting cells that permit the development of T-cell responses to β-cell antigens such as GAD65 [62]. Thus, their manipulation would seem to be a rational strategy. Indeed, the use of anti-CD20 (rituximab) in new-onset Type 1 diabetes in patients was recently explored [63]. Although as disease was not reversed, further protocol optimization is warranted, some clinical benefit was achieved.

Examination of TABLE 2 and TABLE 3 also reveals that, in contrast to most recent prevention studies in NOD mice, several reversal studies have employed combination therapies (TABLE 3), including anti-CD3 plus either an insulin peptide [64] or exendin-4 [65]; lisofylline plus exendin-4 [66]; EGF plus gastrin [67]; glucagon-like peptide-1 plus gastrin [68]; and a dipeptidyl peptidase (DPP)-4 inhibitor plus a proton pump inhibitor [69]. Multiple ongoing or planned clinical trials in patients with Type 1 diabetes have also adopted this combination approach [70], for example, GAD65 in combination with the proton pump inhibitor lansoprazole and the DPP-4 inhibitor sitagliptin [201] and autologous cord blood cells in combination with vitamin D and omega-3 fatty acids [202]. When considering the utility of the NOD mouse in suggesting clinical interventions, it is important to note that several of the agents listed in TABLE 2 and TABLE 3 are the subject of ongoing or planned studies in Type 1 diabetes patients or at-risk individuals. These include proton pump and DPP-4 inhibitors [201], mesenchymal stem cells [203], anti-CD3 [204,205], granulocyte colony-stimulating factor (G-CSF) [206], dendritic cells [207], lisofylline [208] and intranasal insulin [209,210].

In addition to the execution of preclinical studies in NOD mice, more 'basic' research conducted in this model can help in the identification of molecules, cell types or pathways

previously understudied from a Type 1 diabetes perspective that could potentially be targeted for clinical benefit. Examples from the period covered by this review include IL-21 [71–73], Th17 cells [74], and the glucocorticoid-induced TNF receptor [75] and inducible costimulator pathways [76].

Development of imaging strategies

In recent years, the NOD mouse model of Type 1 diabetes has become a valuable tool for the development of imaging protocols that permit the visualization of disease-relevant phenomena such as islet inflammation [77–79] and β-cell death [80]. Non-invasive methods that are being developed to detect islet inflammation include both direct visualization of islet-infiltrating T cells [77,78] and indirect examination of the consequences of inflammation, such as microvascular alterations [79]. As an example of the former, anionic magnetic nanoparticles, which are efficiently internalized by cells, were used to label T cells isolated from the spleens of diabetic NOD mice [77]. Upon transfer of the labeled cells to nondiabetic NOD recipients, their homing to the pancreatic lymph nodes and pancreas was successfully visualized by *in vivo* MRI for at least 20 days posttransfer. No alterations in T-cell survival or function were noted as a result of the labeling procedure. While suggesting a number of research applications, the need to label diabetogenic T cells *ex vivo* would appear to limit the eventual translation of this technique to the imaging of spontaneous islet inflammation in patients. By contrast, administration of antigen-coated super-paramagnetic iron oxide nanoparticles to NOD mice allowed visualization of the islet accumulation of naturally arising T cells by *in vivo* MRI [78]. This advance was made possible by the identification of $IGRP_{206-214}/H\text{-}2K^d$ as the target of a prevalent population of diabetogenic T cells in NOD mice [5], as the antigen used to coat the nanoparticles was a mimotope of this peptide/MHC complex. Continuing efforts to identify antigens recognized by islet-reactive T cells in Type 1 diabetes patients has led to the identification of peptides that could potentially be used to move this technique from mouse to man. These include $IGRP_{265-273}$ and $InsB_{10-18}$, as both have been identified in multiple studies as T-cell targets in HLA-A*0201-positive individuals [6,7,81–85]. They also have identical sequences in mice and humans and are recognized by islet-infiltrating T cells isolated from HLA-A*0201-transgenic NOD mouse models [11,12,86], supporting the idea that they are disease-relevant. These characteristics should facilitate reagent testing in NOD-based models before translation to patients.

Nonobese diabetic mice have also been used to demonstrate the indirect visualization of islet inflammation as a consequence of inflammation-induced microvascular alterations [79]. Such alterations permit administered magnetic nanoparticles to leak from the vasculature and be taken up by macrophages in the insulitic lesions. The resulting accumulation of nanoparticles allows islet inflammation to be detected by MRI. Importantly, when diabetic NOD mice were treated with a regimen of anti-CD3 that leads to remission in approximately half of all recipients after 2–4 weeks, MRI visualization of islet inflammation permitted early identification (after only 8 days) of animals that would ultimately have a favorable clinical response [79].

Methods to image β-cell death are also being developed, with the NOD mouse model also contributing to this area. When Cy5.5-labeled annexin V was administered to NOD mice between the ages of 3 and 12 weeks, pancreatic accumulation of the probe was observed upon *ex vivo* near-infrared fluorescence imaging of pancreata obtained from 8- and 12-week-old mice [80]. Although *in vivo* imaging was not utilized in this study, the authors discussed their expectation that methodological advances will eventually render this technique suitable for noninvasive imaging of β-cell death. The ability to visualize both islet inflammation and β-cell death would present new opportunities to better understand the

disease process, permit monitoring of these events in at-risk individuals and those undergoing preventive or reversal therapies, and facilitate the development and testing of strategies to interfere with disease development.

Recent technical advances

The inability to generate NOD ES cells capable of efficient germline transmission has severely hampered the use of gene knock-in technology in NOD mice and has prohibited the direct testing of the diabetes-promoting activity of candidate genes present in *Idd* loci. In addition, knock-out strains generated using non-NOD ES cells followed by repeated backcrossing to NOD mice may give misleading results due to the contribution of non-NOD DNA flanking the knock-out allele [87,88]. These technical hurdles now appear to have been overcome with the recent publication of two independent reports of the derivation of NOD ES cells exhibiting germline transmission [9,10]. One approach employed a serum-free and feeder cell-free system in which small-molecule inhibitors of differentiation (the glycogen synthase kinase inhibitor Chir99021 and the mitogen-activated protein kinase inhibitor PD0325901) were combined with leukemia inhibitory factor, a regulator of ES cell self-renewal and pluripotency [10]. Multiple ES cell lines were derived from NOD embryos using this strategy and maintained to passage 14 with a normal karyotype and continued expression of the pluripotency factors Nanog and Oct4. Chimeric mice were readily obtained when the NOD ES cells were injected in C57BL/6 blastocysts. Upon mating of the chimeric mice with NOD partners, efficient germline transmission was observed, as determined by coat color and verified by flow cytometric detection of NOD class I and class II MHC molecules and molecular analysis of microsatellite markers. In addition, NOD ES cell-derived female mice developed spontaneous diabetes at a rate and incidence equivalent to that of female NOD mice housed in the same facility. Finally, germline transmission of NOD ES cells that had been genetically modified was also confirmed. Another approach to the generation of NOD ES cell lines also employed small-molecule inhibitors but in a system containing mouse embryonic fibroblasts as feeder cells [9]. One of the lines so derived recently became available from The Jackson Laboratory (Bar Harbor, ME, USA). In addition, bacterial artificial chromosome libraries have recently been prepared for the two commonly used NOD substrains (NOD/ShiLtJ and NOD/MrkTac) [89]. These libraries will greatly facilitate the construction of efficient targeting vectors to be used in conjunction with the newly developed NOD ES cells. It should be noted that the production of gene-targeted mice using NOD-derived ES cells has not yet been reported.

Another recent advance that has increased the utility and the clinical relevance of the NOD mouse model is the development of strains that have been 'humanized' to varying degrees. One aspect of this humanization has been the development of NOD mice expressing the human class I MHC molecule HLA-A*0201 [12], which is a Type 1 diabetes-associated allele in patients [90–92], in the absence of murine class I MHC molecules. These NOD.β2m$^{-/-}$.HHD mice have been used to map epitopes of IGRP and preproinsulin that are recognized by islet-infiltrating HLA-A*0201-restricted CD8$^+$ T cells [12,86]. At least some of these peptides are now known to also be targeted by CD8$^+$ T cells in Type 1 diabetes patients [7,93], thus demonstrating the utility of this HLA-transgenic NOD mouse strain. A further humanized version of this strain, designated NOD.β2m$^{-/-}$.HHD.Ins2$^{-/-}$, has recently been described [11]. In these mice, HLA-A*0201 is expressed in the absence of murine class I MHC molecules. In addition, Ins2 deficiency leads to reduced thymic expression of insulin and serves to mimic the situation in humans bearing predisposing alleles of the insulin gene [94,95].

Ideally, a humanized mouse model of Type 1 diabetes would incorporate human immune cells. To this end, Roep and coworkers reported the transfer of human β-cell-specific CD4$^+$

T-cell clones to NOD-*scid* mice [96]. Human antigen-presenting cells were also transferred, as the mice did not express the human MHC restriction element for the clones in this case. In these experiments, the T cells accumulated in the pancreas, pancreatic lymph nodes and spleen, but not in other sites. While encouraging, only early peri-insulitis could be observed in the recipient mice. Recently, NOD.*scid.IL2rγ^{null}* mice have emerged as a much improved recipient for the engraftment of human peripheral blood mononuclear cells [97] or CD34^{+} cells [98]. Multiple laboratories are currently working to incorporate human antigens, human β-cell-specific T cells and HLA class I molecules into this model system.

Expert commentary & five-year view

As we mark the 30th anniversary of the NOD mouse model of Type 1 diabetes, it should be with gratitude for all it has taught us and excitement in anticipation of the secrets it has yet to reveal. NOD mice have contributed to our knowledge in a variety of areas, including antigen discovery and the identification and validation of therapeutic targets. The model is not perfect, and some of its imperfections, such as differences in lifespan compared with humans, have no ready remedies. However, the field is now more aware of the limitations of the model and how best to design investigations that will minimize the impact of these limitations. For example, many protocols are capable of preventing disease in NOD mice if treatment is initiated prior to the onset of insulitis. Yet, over the last few years, many researchers have wisely turned instead to the more clinically relevant situation in which attempts are made to reverse insulitis or even diabetes itself. This logical trend should continue in the future. Disease reversal is likely to require not only attention to the destructive autoimmune process, but also some manipulation of the remaining β-cell mass (e.g., with agents such as glucagon-like peptide-1 and gastrin) to facilitate its recovery and augmentation, and studies in the NOD mouse will continue to be a critical component of these efforts.

Recent advances that should increase the utility of the NOD mouse model include the development of NOD-derived ES cells reported to be capable of efficient germline transmission. It should now be possible to knock out genes directly in the NOD background, eliminating the need for time-consuming and costly backcrossing and preventing artifact results due to the carry-over of genetic material from the original knock-out strain. NOD ES cells will also facilitate the efficient generation of knock-in mice that will aid in the elucidation of the contribution of specific genes to diabetes susceptibility and resistance. One could also envision knock-in of human genes, for example, those encoding autoantigens. Such work could become part of a more general push to increase the clinical relevance of the NOD mouse model by humanizing it in various ways, including the introduction of human MHC molecules and immune cells. These technical advances should significantly enhance the utility and importance of the NOD mouse model and its ability to yield clinically relevant results and information. Cooperation among Type 1 diabetes researchers engaged in mouse, rat and human investigations will also go far to facilitate progress towards an improved understanding of the disease and tangible benefits for patients and at-risk individuals.

Key issues

- The nonobese diabetic (NOD) mouse model of Type 1 diabetes, although imperfect, exhibits multiple striking similarities to the disease in humans.

- Investigations utilizing NOD mice continue to advance our understanding of disease pathogenesis and to yield information that is applicable to Type 1 diabetes in humans.

- Areas of utility for the NOD mouse model include antigen discovery, identification of disease susceptibility genes, testing of therapeutics, exploration of environmental disease influences and the development of imaging strategies.

- The availability of NOD-derived embryonic stem cells will now permit knock-in and knock-out strains to be developed directly on the NOD background, thus greatly enhancing the utility of the model.

- Increasingly humanized NOD-derived strains, incorporating human MHC molecules, antigens, predisposing factors and even human immune cells, are being developed and probably represent the future direction of research.

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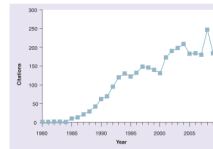
Figure 1. The nonobese diabetic mouse model continues to be a mainstay of Type 1 diabetes research
The PubMed database was searched using the search string 'NOD mice, diabetes.' The plot depicts retrieved citations for each year, excluding review articles.
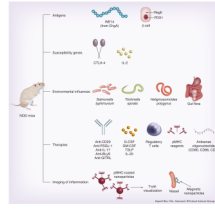
Figure 2. Recent contributions of the nonobese diabetic mouse model to our understanding of Type 1 diabetes have occurred in the indicated areas
Examples of discoveries covered within this review are shown. See text for details and references. G-CSF: Granulocyte colony-stimulating factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor; NOD: Nonobese diabetic; PDX1: Pancreatic duodenal homeobox 1.
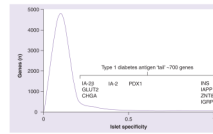
Figure 3. Islet-specific genes remain largely unexplored as Type 1 diabetes antigens
Islet specificity was calculated as the frequency of a given transcript within the Unigene mouse islets expressed sequence tag library 16013 [178] divided by the cumulative frequency of that transcript in all tissues represented by the Unigene 'profiles' dataset excluding whole pancreas [211,212]. The islet library contained 24,059 expressed sequence tags corresponding to 6451 unique genes. Gene bins are 0.1 islet specificity units wide. Known islet-specific Type 1 diabetes antigens are positioned along the smoothed distribution according to their islet specificity values. Approximately 700 genes span the region bounded by IA-2β at the left and INS at the right, suggesting a rich source of as-yet unidentified Type 1 diabetes antigens.

Table 1

Original descriptions and discovery strategies for Type 1 diabetes antigens.

Antigen	Discovery strategy	Human responses		NOD responses	
		Antibody	*T cell*	*Antibody*	*T cell*
Insulin	A	[99]	[100]	[101]	[102]
GT3	A	[103]			
HSP-60	A	[104]	[105]	[106]	[106]
GAD65	A	[107]	[108]	[109]	[109]
IAPP	A	[110]	[111]		
CPE (CPH)	E	[112]	[113]	[109]	[109]
IA-2 (ICA512)	E	[114]	[115]	[116]	[116]
GLUT2	A	[117]	[118]		[119]
Jun-B	E	[120]	[120]		
GAD67	A	[121]	[122]		[123]
ICA69	E	[124]	[125]		[126]
Sulfatide	A	[127]			
Peripherin	A			[109]	[109]
GD3	A	[128]			
Imogen38	E		[129]		
DNA TopII	A	[130]			
Glima38	A	[131]			
IA-2β (phogrin)	A	[132]	[133]		[134]
GM2–1	A	[135]			
Osteopontin	R	[136]			
CD38	A	[137]			
SOX13	E	[138]			
HIP/PAP	E				[139]
S100β	A		[30]		[30]
GFAP	A	[30]	[30]	[30]	[30]
HSP-90	A	[140]			

Antigen	Discovery strategy	Human responses		NOD responses	
		Antibody	*T cell*	*Antibody*	*T cell*
HSP-70	A	[141]	[141]		
IGRP	B	[142]			[5]
REG	A	[143]			
DMK	R				[27]
Importin β	R	[144]			
REGII	A				[145]
ZNT8	C	[28]			
CAII	A	[146]			
LAC	A	[146]			
AMY2A	E	[147]			
HSP10	E	[148]			
PDX1	A	[29]		[29]	[29]
CHGA	B				[21]

Antigens are listed in the order of their discovery. The earliest descriptions of NOD or human antibody or T-cell responses are referenced.

The strategy used to initially discover each antigen is denoted as follows: A: Purified candidate antigen challenge; B: Biochemical fractionation; C: Computational prediction; E: Expression cloning; R: Random peptide library.

NOD: Nonobese diabetic.

Table 2

Strategies that delay or prevent diabetes in nonobese diabetic mice (2005–present).

Agent	Details	Diabetes incidence		Age (weeks)	Ref.
		Control (%)	Treated (%)		
G-CSF	200 µg/kg sc. daily for 5 days at 4, 8, 12 and 16 weeks	75	8	30	[149]
Myeloid progenitors expressing proinsulin	Generated in GM-CSF and TGF-β from bone marrow of NOD mice expressing Ins2 under the control of a class II MHC promoter; 2×10^6 cells iv. at 4 weeks	83	33	34	[150]
FTY720	Sphingosine-1-phosphate analog; 1 mg/kg by oral gavage 5-times/week at 23–37 weeks	78	0	37	[151]
Altered peptide ligand of IGRP$_{206-214}$	100 µg NRP-I4 ip. every 2 weeks at 4–8 weeks and every 3 weeks thereafter	60	0	30	[152]
Anti-PSGL-1	Apoptosis-inducing anti-PSGL-1 (TAB4); 50 µg ip. at 8, 9, 10, 18, 19 and 20 weeks	83	29	27	[153]
GAD65$_{260-279}$ (an ignored determinant)	Also known as GADp18; 100 µg in IFA at 12 weeks and also 10 days later	93	27	45	[154]
Probiotic bacteria	VSL#3 (3×10^{11} viable lyophilized bacteria/g); 3 mg by oral gavage 3-times/week at 4–32 weeks	81	21	32	[49]
CXCL10 neutralization	CXCL10 antibodies induced by plasmid DNA vaccination; 100 µg im. by electroporation (50 µg each side) at 4 and 6 weeks	58	26	30	[155]
p38α-specific MAPK inhibitor	Indole-5-carboxamide (SD-169); 600 mg/kg in chow at 8–18 weeks	60	10	18	[156]
Regulatory T cells	CD4$^+$CD25$^+$CD62L$^+$ cells from NOD.BDC2.5 mice expanded *in vitro* with antigen-pulsed DCs and IL-2; 5×10^4 cells at 13 weeks	88	33	44	[157]
CCL4	Intradermal gene transfer using EBNA1/*oriP*-based vector; 1 µg weekly at 9–14 weeks	78	30	35	[158]
(S,R)-3-phenyl-4,5-dihydro-5-isoxazole acetic acid	VGX-1027; 20 mg/kg ip. 6-times/week at 12–25 weeks	81	31	25	[159]
Cytopiloyne	25 µg/kg ip. or im. 3-times/week beginning at 4 weeks	67	0	30	[160]
Apoptotic β-cells	1×10^5 UVB-irradiated NIT-1 cells iv. weekly at 10–12 weeks	100	62	30	[161]
GM-CSF	100 ng ip.; 3-times/week for 3 weeks and 2-times/week thereafter	86	21	52	[162]
Anti-CD20	B cell-depleting anti-CD20 (MB20–11); 250 µg iv. at 5, 7 and 9 weeks	100	36	40	[57]
Microspheres carrying antisense oligonucleotides	Antisense oligonucleotides to CD40, CD80 and CD86; sc. proximal to pancreatic lymph nodes weekly at 5–13 weeks	100	14	40	[163]
Heparanase	35 µg ip. 2-times/week at 6–14 weeks	75	13	22	[164]
TSLP	500 ng sc. daily for 6 days at 3 weeks	100	25	50	[165]
TSLP-treated DCs	5×10^6 iv. at 3 weeks	87	25	45	[165]
Inosine analog	INO-2002; 100 mg/kg by oral gavage daily at 5–30 weeks	80	35	30	[166]
GAD65-IgFc DNA (gene gun)	Gold particles coated with 1 µg DNA intradermally weekly at 10–13 weeks	80	50	36	[167]

Agent	Details	Diabetes incidence			Ref.
		Control (%)	Treated (%)	Age (weeks)	
Imatinib (Gleevec®)	1.5 mg by oral gavage daily at 12–19 weeks	71	25	30	[168]
Anti-BLyS (BAFF)	B-cell-depleting anti-BLyS (10F4); 100 µg ip. at 4 weeks and again 5 days later; 15 µg biweekly at 8–25 weeks	79	26	40	[58]
Zymosan	Fungal cell wall component; 100 µg ip. 3-times/week every other week at 12–22 weeks	90	40	42	[50]
Galectin-1	100 µg ip. 3-times/week at 5–36 weeks	78	0	37	[169]
IL-25 (IL-17E)	1 µg sc. daily for 25 days at 10 weeks	90	30	26	[74]
Anti-IL-17	Neutralizing anti-IL-17 (50104); 100 µg ip. every other day for 12 days at 10 weeks	90	40	24	[74]
Mesenchymal stem cells (allogeneic)	10^5 BALB.B cells ip. or iv. at 4 weeks	100	59	36	[170]
BAFF/APRIL blockade	150 µg BCMA–Fc ip. 2-times/week at 9–15 weeks	55	0	50	[59]
Galectin-9	100 µg pCR3.1-I-Gal-9 plus 100 µg lipofectamine iv. weekly at 5–8 weeks	86	15	40	[171]
GAD65$_{217-236}$/I-A^{g7} dimers	50 µg iv. for 3 days at 12 and 15 weeks	100	20	35	[172]
GAD65$_{290-309}$/I-A^{g7} dimers	50 µg iv. for 3 days at 12 and 15 weeks	100	10	35	[172]
Anti-GITR ligand	Neutralizing anti-GITR ligand (YGL 386.2); 1 mg ip. weekly at 11–14 weeks	100	33	32	[75]
Immature DCs pulsed with InsB$_{15-23}$ and InsB$_{9-23}$	Generated in GM-CSF, IL-10 and normal mouse serum; 10^6 ip. weekly at 5–12 weeks	83	25	40	[173]
Humanin	2 mg/kg ip. daily beginning at 5 weeks	60	30	20	[174]
Newborn blood (allogeneic)	C57BL/6 whole blood (25–50 × 10^6 nucleated cells) iv. at 12 weeks	80	30	28	[175]
ECP-treated splenocytes	2 × 10^5 cells iv. weekly at 8–12 weeks	100	73	30	[176]
Toxin-coupled IGRP$_{206-214}$/H-2K^d tetramers	Saporin-conjugated tetramers; 34 pmoles iv. per injection; three injections, 6 days apart, beginning at 8 weeks	100	50	30	[177]

Studies are listed in chronological order. All employed female NOD mice.

APRIL: A proliferation-inducing ligand; BAFF: B-cell-activating factor belonging to the TNF family; BCMA: B-cell maturation antigen; BLyS: B-lymphocyte stimulator; CCL: CC chemokine ligand; CXCL: CXC chemokine ligand; DC: Dendritic cell; EBNA: Epstein–Barr virus nuclear antigen; ECP: Extracorporeal photopheresis; GAD: Glutamic acid decarboxylase; G-CSF: Granulocyte colony-stimulating factor; GITR: Glucocorticoid-induced TNF receptor; GM-CSF: Granulocyte–macrophage colony-stimulating factor; IFA: Incomplete Freund's adjuvant; IGRP: Islet-specific glucose-6-phosphatase catalytic subunit-related protein; im.: Intramuscularly; ip.: Intraperitoneally; iv.: Intravenously; MAPK: Mitogen-activated protein kinase; NOD: Nonobese diabetic; PSGL: P-selectin glycoprotein ligand; sc.: Subcutaneously; TSLP: Thymic stromal lymphopoietin; UVB: Ultraviolet B.

Table 3

Strategies that reverse new-onset diabetes in nonobese diabetic mice (2005–present).

Agent	Details	Disease reversal			Ref.
		Number	%	Follow-up (weeks)	
FTY720	Sphingosine-1-phosphate analog; 3 mg/kg by oral gavage daily	5/10	50	24	[151]
EGF plus gastrin	1 µg/kg/day EGF plus 3 µg/kg/day gastrin ip.; 2 injections/day for 2 weeks	5/6	83	12	[67]
Anti-CD3 plus hInsB$_{24}$-C$_4$	40 µg anti-CD3 F(ab')$_2$ (145–2C11) iv. on days 0–4 plus 40 µg hInsB$_{24}$-C$_4$ in. on days 0, 2, 7 and 12	17/31	55	9	[64]
Lisofylline plus exendin-4	27 mg/kg/day lisofylline plus 18 nM/day exendin-4 sc. by osmotic mini-pump for 28 days	5/9	56	24	[66]
Insulin-coupled fixed splenocytes	5×10^7 cells iv.	10/19	53	25	[18]
Regulatory T cells	CD4$^+$CD25$^+$CD62L$^+$ cells from NOD.BDC2.5 mice expanded in vitro with antigen-pulsed DCs and IL-2; 0.8–1.5 $\times$ 10^6 cells on days 0 and 14	4/11	36	24	[157]
Anti-CD3 plus exendin-4	10 µg anti-CD3 (145–2C11) iv. for 5 days plus 0.075 µg exendin-4 ip. for 10 days	20/45	44	3	[65]
Anti-human CD20	NOD/hCD20 mice; B cell-depleting anti-hCD20 (2H7); 500 µg followed by 3 injections of 250 µg at 3-day intervals	4/14	29	19	[56]
Microspheres carrying antisense oligonucleotides	Antisense oligonucleotides to CD40, CD80 and CD86; sc. proximal to pancreatic lymph nodes 2-times/week for 25 days	7/15	47	8	[163]
α1-antitrypsin (Aralast™)	2 mg ip. every 3 days; 5 injections	21/24	88	39	[179]
Cytotoxin-coupled anti-CD22	B cell-depleting N-acetyl-calicheamicin dimethyl acid-conjugated anti-CD22 (Cy34.1); 160 µg/kg ip.; 2 injections, 5 days apart	6/10	60	14	[60]
Imatinib (Gleevec®)	1.5 mg by oral gavage daily for 10 weeks	8/17	47	35	[168]
Sunitinib (Sutent®)	2 mg by oral gavage daily for 9 weeks	5/6	83	9	[168]
GLP-1 plus gastrin	100 µg/kg GLP-1 plus 1.5 µg/kg gastrin ip. twice daily for 3 weeks	7/7	100	8	[68]
DPP-4 inhibitor plus proton pump inhibitor	10 mg/kg DPP-4 inhibitor by oral gavage once daily plus 30 mg/kg proton pump inhibitor sc. twice daily for 12 weeks	6/8	75	12	[69]
Nanoparticles carrying peptide/MHC complexes	NRP-V7/H-2K^d or MimA2/H-2D^b; NRP-V7 and MimA2 are mimotopes of IGRP$_{206–214}$ and DMK$_{138–146}$, respectively; iv. 2-times/week until euglycemic for 4 weeks	8/11	73	13	[180]

Studies are listed in chronological order. All employed female NOD mice except where noted. Follow-up for disease reversal is from time of disease onset. In some studies, mice were treated with insulin that was later withdrawn; see references for details.

DMK: Dystrophia myotonica kinase; DPP: Dipeptidyl peptidase; GLP: Glucagon-like peptide; hIns: Human insulin; IGRP: Islet-specific glucose-6-phosphatase catalytic subunit-related protein; in.: Intranasally; ip.: Intraperitoneally; iv.: Intravenously; NOD: Nonobese diabetic; PDGFR: PDGF receptor; sc.: Subcutaneously.