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REVIEW

Chapter I.3

Comparative Genetics: Synergizing Human and NOD Mouse Studies for Identifying Genetic Causation of Type 1 Diabetes

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■ Abstract

Although once widely anticipated to unlock how human type 1 diabetes (T1D) develops, extensive study of the nonobese diabetic (NOD) mouse has failed to yield effective treatments for patients with the disease. This has led many to question the usefulness of this animal model. While criticism about the differences between NOD and human T1D is legitimate, in many cases disease in both species results from perturbations modulated by the same genes or different genes that function within the same biological pathways. Like in humans, unusual polymorphisms within an MHC class II molecule contributes the most T1D risk in NOD mice. This insight supports the validity of this model and suggests the NOD has been improperly utilized to study how to cure or prevent disease in patients. Indeed, clinical trials are far from administering T1D therapeutics to humans at the same concentration ranges and pathological states that inhibit disease in NOD mice. Until these obstacles are overcome it is premature to label the NOD mouse a poor surrogate to test agents that cure or prevent T1D. An additional criticism of the NOD mouse is the past difficulty in identifying genes underlying T1D using conventional mapping studies. However, most of the few diabetogenic alleles identified to date appear relevant to the human disorder. This suggests that rather than abandoning genetic studies in NOD mice, future efforts should focus on improving the efficiency with which diabetes susceptibility genes are detected. The current review highlights why the NOD mouse remains a relevant and valuable tool to understand the genes and their interactions that promote autoimmune diabetes and therapeutics that inhibit this disease. It also describes a new range of technologies that will likely transform how the NOD mouse is used to uncover the genetic causes of T1D for years to come.

Keywords: type 1 diabetes · genetics · NOD mouse · HLA · MHC · mt-ND2 · epistasis

1. Introduction

The fortuitous development of the NOD mouse [1] resulted in the generation of the most widely used animal model for the study of autoimmune diabetes. There are many similarities between autoimmune diabetes in the NOD mouse and in humans (**Table 1**). Due to the high level of use, this inbred mouse strain has been the subject of a multitude of reviews, books, and commentaries since its introduction over thirty years ago. These works have detailed the strengths of the NOD mouse model and have also pointed to significant weaknesses. Our purpose here is to provide some considerations for those working with the NOD to promote use of this model in a way that will benefit the understanding of how genetics impact human autoimmune disease.

2. Use of NOD mice in preclinical trials

We have recently published an extensive review on the similarities of diabetes development in

humans and NOD mice [2], including comparisons of the immunologic and genetic contributions (**Table 1**). However, emerging evidence from translational efforts forms a unique starting point for a comparison of human and NOD mouse autoimmunity. The NOD mouse was used early and has since been employed very often to test agents to prevent and/or reverse the disease. In general, there are three types of models, namely:

1. Early prevention where a therapy is initiated at 4-8 weeks of age, which would be before establishment of overt islet destruction.
2. Late prevention, 8-12 weeks, with the intent to arrest active pathogenesis.
3. Reversal of disease in mice with clinical symptoms that are treated with the intent of restoring glycemic control.

While many agents can prevent T1D when used early in NOD mice, the number of agents that are effective in late prevention declines, and only a handful have been demonstrated to reverse established disease.

In 1982, the first study demonstrating the therapeutic potential of a compound on T1D was published [3]. This study demonstrated that nicotinamide had the ability to both prevent T1D in prediabetic NOD mice and to reverse it in mice that exhibited hyperglycemia. However, the translation of this preclinical protocol to humans failed to have any preventative effect [4]. A pattern has followed with success in the NOD followed by failure in translating these "cures" to man in trials utilizing a number of agents; a partial list would include the agents daclizumab, anakinra, or teplizumab [5].

Several factors could have contributed to the lack of translation, including clear differences in dosing when moving from mouse to man, a lack of assessment of beta-cell secretory function in mouse, the absence of immunologic or metabolic biomarkers, and an extreme difference in the timeline for treatment initiation [5-7]. The timing of the treatment may be essential for disease remission. Preclinical trials using anti-CD3 monoclonal antibodies in the NOD mouse had a 10% success when the therapy was initiated in mice with a blood sugar greater than 350 mg/dl, compared with reversal in 23 of 35 (66%) mice with circulating glucose levels less than 350 mg/dl [8]. This has prompted investigators to screen NOD mice intensively for onset so that treatments could be initiated as close to that point as possible [8, 9], resulting in inflated efficacy percentages. In contrast, an inclusion criterion for clinical trials is less than 100 days post onset. Therefore, targeting patients immediately after diagnosis of diabetes may result

Abbreviations:
ADA – American Diabetes Association
ALR – alloxan-resistant
ATG – anti-thymocyte globulin
B2m – beta2-microglobulin
BC1 – backcross one
BCR – B cell receptor
BM – bone marrow
CPE - carboxypeptidase E
CTLA-4 – cytotoxic T lymphocyte antigen 4
DSB - double strand break
ESC – embryonic stem cell
FokI – Flavobacterium okeanokoites enzyme
GAD65 – glutamic acid decarboxylase 65
G-CSF – granulocyte colony-stimulating factor
GWAS – genome-wide association studies
HbA1c - glycated hemoglobin
HLA – human leukocyte antigen
HSC – hematopoetic stem cell
HSP60 – heat shock protein 60
IA-2 – islet cell antigen 512
IAPP - islet amyloid polypeptide
ICA69 – islet cell autoantigen 1
IDDM1 – insulin-dependent diabetes mellitus 1
IGRP – islet-specific glucose-6-phosphatase catalytic subunit-related protein
IL – interleukin
MHC – major histocompability complex
mt – mitochondrial
mtDNA – mitochondrial deoxyribonucleic acid
mt-ND2 – mt encoded NADH dehydrogenase 2
NADH – nicotinamide adenine dinucleotide hydrogen
NGS – next generation sequencing
NHEJ – non-homologous end joining
NOD – nonobese diabetic
NOR – nonobese resistant
NSG – NOD scid gamma
nPOD – Network for Pancreatic Organ Donors with Diabetes
Pdx1 - pancreatic and duodenal homeobox 1
PTPRN - protein tyrosine phosphatase, receptor type, N
PTPN22 – protein tyrosine phosphatase, non-receptor type 22
ROS – reactive oxygen species
RNA – ribonucleic acid
RNAi – RNA interference
shRNA – short hairpin RNA
siRNA - small interfering RNA
SNP – single nucleotide polymorphism
SSRP – simple sequence repeat polymorphic
T1D – type 1 diabetes
TALEN – transcription activator-like effector nuclease
TCR – T cell receptor
VNTR – variable number of tandem repeat
WGS – whole genome sequencing
ZFN – zinc finger nuclease
ZnT8 – zinc transporter 8

in increased efficacy [6, 7]. Furthermore, a lack of consistency in methodologies has marked studies employing NOD mice for reversal studies. An exceptional commentary appearing in *Science Translation Medicine* has proposed a detailed plan for improving use of the NOD for preclinical studies [10].

While efforts to move from bench to bedside have met with difficulties, some success has resulted from the bedside-to-bench approach. The only pharmacologic therapy known to reverse T1D in humans and to provide for increased C-peptide production (alongside of gaining exogenous insulin independence) is a method that is commonly termed the "Brazilian protocol" [11-17]. This method utilized a combination therapy of anti-thymocyte globulin (ATG), granulocyte colony-stimulating factor (G-CSF), cytoxan, and autologous stem cell infusion; it has reported remarkably promising results. Yet, the mechanisms underlying the effectiveness of this treatment regimen remain unclear. Recent work has demonstrated ATG as effective in reversing NOD mice with recent onset disease [18]. Furthermore, enhanced efficacy in this reversal process can be obtained by adding G-CSF to this therapy [9]. The combination of ATG and G-CSF was able to reverse the majority of recent onset diabetic NOD mice with blood glucose levels ≤450 mg/dl (~65%; n = 73), yet mice with blood glucose levels >450 mg/dl also exhibited a significant reduction in reversal rate (36%, n = 39). While we conclude, similar to the summary of the anti-CD3 preclinical trials [8], that these blood glucose levels represent the level of beta-cell mass at onset, these data convey our inadequate knowledge of the natural history of beta-cell failure in the NOD mouse. They also highlight, that even under conditions of genetic homogeneity the onset of disease is dissimilar; not unlike that observed in monozygotic twins where the concordance is 65% [19]. This suggests, as discussed elsewhere in this edition, that environmental factors in combination with susceptibility alleles act to control timing and perhaps severity of T1D onset.

Further, efforts such as the Network for Pancreatic Organ Donors with Diabetes (nPOD) [20, 21] and those in Europe [22-27] are highlighting differences between the insulitic lesion comparing individuals with T1D and autoantibody-positive organ donors. This high level of heterogeneity in disease, even amongst twins or the NOD mouse, should refocus the attention on the genetic causes. It is the genetic similarities where the NOD mouse likely gains the greatest amount of traction for assisting in the understanding of human T1D.

3. Similarities in genetic causation

T1D is a polygenic disease, with over 50 genetic linkages identified in both human and mouse that are associated with this autoimmune disease (recently reviewed in [2]). The identification of loci contributing to T1D has been accomplished through arranged marriages of NOD with more than 10 inbred mouse strains (**Table 2**). Similar to the genome wide studies in human populations, some loci are unique to a specific outcross partner, while others, such as genes within the major histocompatibility complex (MHC) locus, appear to contribute in most of these studies. A major reason that the NOD mouse has been thought to serve well as a model for the human form of T1D are the similarities in genes/loci that impact disease, comparing those identified in human genome-wide scans or genome-wide association studies (GWAS) to the regions of the genome associated with causation in NOD. As there is not enough space here for a comprehensive review of all linkages, we will highlight some specific linkages as they provide useful examples. These examples will go from almost identical disease-associated allotypes, to gene systems that are similar in function, and genetic changes that impact similar pathways in human and mouse.

4. HLA and MHC: master genes for susceptibility

A preponderance of evidence implicated acquired immune cells, specifically both $CD4^+$ and $CD8^+$ T lymphocytes as final effectors of beta-cell death. Autoreactive responses of $CD4^+$ and $CD8^+$ T cells to over 15 different antigens have been measured in T1D patients and at risk individuals. In the NOD mouse, immune responses have been measured against 8 antigens [2]. The T cell receptor (TCR) of a T lymphocyte recognizes a particular peptide antigen in the context of a specific MHC molecule, also known as human leukocyte antigen (HLA). This TCR-peptide-MHC interaction is therefore essential for T1D pathogenesis [28]. Likewise, polymorphisms in MHC/HLA are very highly associated with disease susceptibility in both human and mouse.

The linkage to HLA class II, termed *insulin-dependent diabetes mellitus 1 (IDDM1*) [29], and the class II MHC allele in NOD [30] are by far the most significant susceptibility loci [31]. DQB alleles with Ser, Ala, or Val at amino acid residue 57 are associated with T1D susceptibility, while those alleles containing an Asp residue are considered

Table 1. Characteristics of spontaneous type 1 diabetes diagnosed in man and the NOD mouse

Characteristic	Human type 1 diabetes	NOD mouse
Age at onset	Less than 1 year to >50 years of age [124]	12-30 weeks of age
Diagnosis	ADA diagnosis criteria: - HbA1C ≥ 6.5% or - Fasting plasma glucose ≥ 126 mg/dl (7 mmol/l) or - 2-h plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test or - Random blood/plasma glucose ≥ 200 mg/dl (11.1 mmol/l) or - Patient with classic symptoms of hyperglycemia confirmed by the presence of autoantibodies [125]	Can vary widely between laboratories. Recently suggested to be standardized as: 2 measures of blood glucose that are ≥250 mg/dl on subsequent days [10]
Insulitis*	$CD8^+$ cytotoxic T cells, macrophages, B cells, and $CD4^+$ T cells [27]. Very few NK cells. No neutrophils [126].	$CD4^+$ T cells, B cells, $CD8^+$ T cells, macrophages, and dendritic cells [127].
Ketoacidosis	Controlled with insulin	Absent
Autoantigens and candidate autoantigens	INS, HSP60, IAPP, Slc30a8 (ZnT8), CPE, GAD2 (GAD65), G6PC2 (IGRP), PDX-1, PTPRN (IA2), HSP90AB1, PTPRN2 (IA2beta), REG3A, ICA1 (ICA69), IMO38 (MRPS31), PRPH, SOX13, GAD1 (GAD67)	INS, GAD65, IGRP, PDX-1, ICA69, IA-2, DMPK**, chromogranin A**
Autoantibodies	Mark individuals at-risk: risk increases with the production of antibodies to each additional antigen.	Role for autoantibodies is unclear. High-affinity autoantibodies are associated with elevated risk.
MHC-linked	Alleles of class HLA-II DR/DQ and class I HLA-A/B	MHC class II Ab^{g7}/Ea^{null}, class I K^d
Reported non-MHC linkages	>50	>40
Lymphopenia	No	No
T lymphoaccumulation	No	Yes
Increased frequencies of beta-cell autoreactive T cells in peripheral blood	Yes	Yes
Role of T regulatory cells	Functional differences yet to be conclusive. Total numbers are not decreased.	Potential decrease in suppressive function. Total numbers are not decreased.
B cells	Likely an importance cell type for disease pathogenesis in most cases.#	B cell deficiency eliminates type 1 diabetes [128, 129].$
Neutrophils	Numbers are reduced in those at risk [130].	Numbers are reduced, however long-term neutrophil depletion does not impact disease onset [131].
NK cell number	No correlation with susceptibility.	No correlation with susceptibility [132].
NK cell function	No correlation with susceptibility.	Poor lytic function.
NK T cell number	Numerical and functional deficiencies are not consistently linked with susceptibility [133].	Numerical and functional deficiencies linked with heightened susceptibility in congenic mice [134, 135].
Hemolytic complement	Dysregulation linked with complications.	NOD mice are C5-deficient, which does not impact T1D onset [136].

Legend: * Listing order by frequency of cell type. ** Not yet identified as autoantigens in humans. # A single individual with X-linked agammaglobulinemia (severe hereditary B cell deficiency) developed diabetes with T cell reactivity to GAD65 and IA-2 [137]. $ B cell-deficient NOD mice (NOD.129S2-*Ighm*^{tm1Cgn}/Dvs) are strongly resistant to spontaneous autoimmune diabetes. These mice are susceptible to mild insulitis and, on treatment with cyclophosphamide, develop diabetes.

Table 2. Type 1 diabetes susceptibility loci mapped in the NOD mouse

Locus	Chromo-some	Interval (Mb)	Congenic strain effect on T1D	Candidate genes	Potential human ortholog	Reference
Idd1	17	33.133-35.405	B10 resistance	*H2*^{g7} class I and II [#]	IDDM1 HLA 6p21	[138]
Idd2	9	32.308-98.698	B10 resistance	*Cd3, IL18, Cyp19*	IDDM3 15q25.1	[139]
Idd3	3	36.627-37.277	B6 resistance	*IL2, IL21*	4q27	[71, 140]
Idd4.1	11	69.76-71.152	NOR resistance	?		[141]
Idd4.2	11	72.729-73.645	NOR resistance	?		[141]
Idd4.3	11	44.553-55.855	C57L resistance	*IL3, IL4, IL5, Irf1, Csf2*		[142]
Idd5.1	1	60.833-62.840	B10 resistance	*Ctla4, Icos, Als2cr19, Nrp2*	IDDM12 2q33.2	[143]
Idd5.2	1	73.984-75.465	B10 resistance	*Slc11a1, Cxcr2*	IDDM13 2q34	[143]
Idd5.3	1	66.530-70.084	B10 resistance	*Acadl, Ikzf2*		[75]
Idd5.4a	1	77.143-147.307	B10 susceptibility	*Cd55*	1p31.2	[75]
Idd5.4b	1	152.632-157.938	B10 susceptibility	?		[75]
Idd6.1	6	146.378-149.517	C3H resistance	?		[144]
Idd6.2	6	143.560-146.378	C3H resistance	*Iapp*		[144]
Idd6.2	6	137.404-146.386	B6 resistance	*Lrmp*		[145]
Idd6.3	6	146.262-147.388	C3H resistance	*Arntl2*		[144]
Idd7	7	21.0-43.0	B6 susceptibility	?	7p12.1	[146]
Idd7	7	Peak at 19.997	B10 susceptibility	?		[147]
Idd7	7	Peak at 19.997	NON-susceptibility	?		[79]
Idd8	14	Peak at 21.66	B10 susceptibility	?		[147, 148]
Idd9.1	4	128.365-131.179	B10 resistance	*Lck*		[149]
Idd9.2	4	144.968-149.098	B10 resistance	?		[150]
Idd9.3	4	149.300-150.522	B10 resistance	*Tnfrsf9*		[151]
Idd10	3	99.699-100.577	B6 resistance	*Cd101*		[152]
Idd11	4	125.017-132.983	B6 resistance	AK005651		[153]
Idd12	14	Peak at 35.170	B6 resistance	?		[148, 154]
Idd13	2	114.118-158.330	NOR resistance	*Pxmp4*		[134, 155, 156]
Idd13-β2m	2	114.118-130.275	NOR resistance	*B2m*		[157]
Idd13-other gene	2	121.973-134.812	NOR resistance	*Il1a, Il1b, Pcna*		(134)
Idd14	13	25.424-120.284	B6 susceptibility	?	IDDM15 6q21	[158]
Idd15	5	Peak at 8.798	NON-resistance	?		[79]
Idd16	17	26.318-29.405	B6 resistance	*H2*^{g7}-linked		[159]
Idd17	3	79.484-87.106	B6 resistance	?		[160]
Idd18.1	3	108.986-109.590	B6 resistance	*Ntng1, Vav3*		[161][*]
Idd18.2	3	102.747-104.054	B6 susceptibility	*Ptpn22*	1p13.2	[161][*]
Idd18.3	3	108.050-109.054	B6 resistance	*Csf1*		[161][*]
Idd18.4	3	100.911-101.864	B6 resistance	*Cd101*		[161][*]
Idd19	6	117.439-128.468	C3H susceptibility	?		[162, 163]
Idd20	6	83.595-91.990	C3H resistance	?		[162]
Idd21.1	18	69.192-90.722	ABH resistance	?	18q22.2	[164]
Idd21.2	18	64.618-74.588	ABH susceptibility	*Dcc*	IDDM6	[164]
Idd21.3	18	0-21.671	ABH resistance	?		[164]
Idd22	8	Peak at 90.626	ALR resistance	?		[43]
Idd23	17	3.924-26.318	B6 resistance	?	6q25.3, 6q27	[159]
Idd24	17	35.340-44.938	B6 resistance	?		[159]
Idd25	4	Peak at 133.341	NOR resistance	?		[165]
Idd26	1	19.802-40.319	NOR resistance	?	2q11.2	[165]
Idd27	7	86.521-127.029	CBA resistance	?		[94]
mt-Nd2^{m1}	mtDNA	3914-4951 bp	ALR resistance	Identification[#]	*mt-ND2*^{m1}	[42]
N.A.	7	117.936-152.524	C57L resistance	?	16p11.2, 11p15.5	[94]
N.A.	2	Peak at 77	129 susceptibility	?		[95]
N.A.	5	Peak at 31	129 resistance	?		[95]
N.A.	15	Peak at 31	129 resistance	?		[95]
N.A.	15	Peak at 55	129 resistance	?		[95]
N.A.	19	Peak at 50	129 resistance	?		[95]

Legend: [*] Revised interval not published, refer to T1Dbase (www.t1dbase.org). [#] Confirmed.

protective. These risk HLA molecules are included within HLA haplotypes that are significantly enriched in the human form of the disease. Likewise, the only class II MHC allele expressed on the surface of cells from NOD mice, H2-A^{g7}, is a non-asp 57-containing allele [32]. The lack of a full complement of MHC class II molecules is an important difference when compared to the human population, including individuals with T1D, which in general express HLA allotypes from all class II loci.

The association of T1D with the MHC class II region was confirmed through the generation of NOD mice congenic for protective MHC haplotypes. Several congenic NOD strains have been generated by selective breeding using simple sequence repeat polymorphic (SSRP) or microsatellite markers to introgress the MHC region from diabetes resistant mouse strains into the NOD genetic background. In cases where the NOD allele at H2-A was replaced, the resulting mice were resistant to T1D. These congenic mice confirmed the importance of the contributions of this region of chromosome 17 to T1D pathogenesis. However, due to the presence of hundreds of genes in addition to the MHC class II loci, these congenic systems have not yet been able to directly implicate the MHC class II A^{g7} allele. Use of transgenic NOD mice expressing a genetically engineered H2-A^{g7} where serine at position 57 was replaced with an aspartic acid, have helped to address the importance of this single amino acid in NOD mice [33]. These NOD-A^{g7Asp} mice exhibited a significant reduction in T1D incidence, from ~55% to ~15% by 40 weeks of age. While the protection was present, the lack of complete penetrance of the protection provided by Asp-57 suggests that there is more to the contribution of MHC/HLA class II alleles than this amino acid residue. Furthermore, as these studies were performed using a "low-incidence" colony, it is not clear how protective this amino acid substitution would be in a colony with a T1D incidence of up to 90% in the control NOD mice [34]. This underscores the importance of the interplay between genes and environment for T1D onset [35].

The homology of the NOD allele to the predisposing alleles in humans is a major strength of the NOD model. It is believed that the non-asp-containing alleles cause a local rearrangement within the peptide-binding site that alters the peptide-binding specificity [36]. Yet, data demonstrating a clear mechanism for how A^{g7} or the non-asp-containing HLA alleles cause T1D remains elusive. It should be noted that mechanistic studies using human cells are complicated by high genetic diversity at the HLA loci and by differences in allele frequencies [37]. The ability to limit these variables and to perform invasive studies indicates that the NOD is an excellent system to study the impact of class II MHC on T1D pathogenesis. As discussed below and elsewhere in this volume, the emergence of NOD embryonic stem cells and knock-in technologies such as zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN) will provide ample opportunities for investigators to directly probe the MHC contributions to T1D.

4.1 mt-ND2 and mt-Nd2

A hypothesis-driven approach to a biologic problem can create self-fulfilling prophecies as experiments can be designed or results interpreted towards supporting rather than testing a postulate. T1D is an autoimmune disease studied by immunologists. When loci are mapped immunologists likely point to genes influencing immune function as candidates rather than taking a non-biased approach. Certainly, there is excellent support for genes involved in immunological processes in T1D as many of the genetic determinants are expressed in cells from the hematopoietic compartment, including immune cells [38]. On the contrary, genes may also influence the susceptibility of the beta-cell to immune-mediated destruction. For this to occur, it may take a unique outcross partner. To better understand the role of beta-cell-expressed T1D genes, NOD mice were outcrossed to the alloxan-resistant (ALR) mouse, which was derived to have beta-cells that are extremely resistant to destruction [39-49].

By outcrossing ALR to NOD mice, following these progeny for onset of clinical and pathological features of T1D, and then performing a genome-wide screen, three loci in the nuclear genome and an association with the mitochondrial genome (mtDNA) were identified. Sequencing of the entire mtDNA from several strains identified a single nucleotide polymorphism (SNP) as the only difference between NOD and ALR [42]. This C to A SNP results in a leucine to methionine amino acid substitution. The affected gene, *mt-Nd2*, is a subunit of complex I of the mitochondrial electron transport chain. To determine the role of the ALR-derived *mt-Nd2*^{a} allele, NOD mice with the ALR mtDNA were generated. Using adoptive transfer models, it was determined that, while the gene is expressed systemically, the protective allele, *mt-Nd2*^{a}, has an impact on T1D onset only at the beta-cell level.

Study of the mitochondria of these NOD.mt^{ALR} conplastic mice (i.e. mice where the cytoplasmic genomes have been exchanged) demonstrated a significant difference in basal and stimulated mitochondrial reactive oxygen species (ROS) production from complex I of the mitochondrial electron transport chain [50-53]. As mitochondrial ROS production has been tied to the induction of apoptosis, the reduction in mitochondrial ROS production was directly linked to protection against beta-cell apoptosis [39, 50, 51, 54]. After exposure to autoreactive CD8$^+$ T cells, Fas-agonistic antibody, or proinflammatory cytokines, NOD.mt^{ALR} beta-cells failed to increase mitochondrial ROS, exhibited an absence of caspase 8 activation, and remained viable [39, 55]. The importance of ROS in this system was confirmed by adding hydrogen peroxide (H_2O_2) in combination with cytokines or FAS-agonistic antibody. Addition of H_2O_2 with the pro-apoptotic agents results in caspase 8 activation in NOD.mt^{ALR} beta-cells and decreased viability. This effect was caspase-dependent as inhibition of caspase 8 after incubating NOD.mt^{ALR} beta-cells with H_2O_2 and FAS-agonistic antibody blocked cell death.

Similar to these findings in the NOD mouse, a C to A transversion at mtDNA nucleotide position 5178 within the nicotinamide adenine dinucleotide hydrogen (NADH) dehydrogenase subunit 2 gene (*mt-ND2*), resulting in a leucine to methionine amino acid substitution at amino acid residue 237, had been reported as being protective against T1D onset in humans [56]. In a population-based study of 385 Japanese patients (154 male and 231 female) with T1D and 469 age- and sex-matched controls, the methionine-containing allele was associated with protection from T1D ($p = 0.017$) and a significantly reduced frequency ($p = 0.0046$) of the T1D-associated auto-antibodies (GAD, IA-2, and IAA). To study the impact of *mt-ND2*^{a} on human beta-cell destruction, cytoplasmic hybrid (or cybrid) human beta-cells were generated by fusing mitochondrial DNA-depleted human beta-cells with platelets [57, 58], because they are anuclear. Platelet donors were chosen based on their mtDNA haplotype [59-61]. Individuals from haplogroup D served as donors for the mt-ND2^a-containing human beta-cell lines, while platelets from donors with haplogroup M9 were fused to produce the mt-ND2^c-containing human beta-cells. For these cells, M9 was chosen as it is the most genetically related haplogroup to D [62]. These novel human beta-cell lines were then tested for resistance in assays similar to those described above for the mouse beta-cells. When the protective *mt-ND2*^{a} is present, human beta-cells exhibit reduced mitochondrial ROS production and resistance to apoptosis induced by autoimmune mediated stress, including autoreactive CD8$^+$ cytotoxic T lymphocytes [55]. This is in accord with the findings using NOD.mt^{ALR} beta-cells. These studies highlight the importance of crosstalk between research involving samples from the NOD mouse with those from human subjects, and emphasize the important fact that genes influencing T1D may modulate the disease by impacting beta-cell function and/or viability.

4.2 Homology in gene systems and pathways in genetic pathogenesis

MHC class II is perhaps an extreme example where an orthologous amino acid change contributes to T1D susceptibility in both humans and mice. While the majority of loci linked to T1D in NOD mice remain undiscovered, of those identified to date not all have been associated with T1D in humans (**Table 2**). For example, *beta2-microglobulin* (*B2m*) has been confirmed as an *Idd* gene in the mouse [63], but has not been confirmed as a T1D gene in humans. Similarly, genes have been identified in humans as causative, but the syntenic region in mouse has not been associated with disease. An example is the insulin gene, *INS*, in the *IDDM2* interval. It is widely believed that a variable number of tandem repeats (VNTR) in the *INS* promoter plays a significant role in human T1D [64]. A low number of repeats (30-44; class I) is associated with risk of T1D compared to alleles with more than 110 repeats (class III). An intermediate length VNTR (class II) is rare. The VNTR regulates insulin expression in a tissue-specific fashion. A class I VNTR results in high pancreatic islet insulin expression and low expression in the thymus, while class III VNTRs are associated with the opposite (low pancreatic expression and a two-fold increase in thymic expression). The elevated expression in the thymus associated with the class III VNTR has been associated with elevated immune regulation to insulin [65].

In the mouse, there are two genes that encode insulin, these are insulin I (*Ins1*; chromosome 19) and insulin II (*Ins2*; chromosome 7). At the time of writing, neither insulin gene had been linked to T1D, as the biological phenomenon associated with expression of these two isoforms exists in all mouse strains tested. In the mouse, both *Ins1* and *Ins2* are expressed in pancreatic islets. However, only *Ins2* is expressed in the thymus [66]. Genetic ablation of *Ins2* in the NOD mouse results in an

accelerated onset of diabetes with an observed failure of tolerance to insulin [67]. These *Ins2*-deficient NOD mice could be employed for the development of therapies that produce tolerance, which could be translated for induction of tolerance in individuals with a class I VNTR.

In other cases, while the gene linked to T1D may differ between these species, an identical pathway is affected. CD25, a subunit of the high-affinity trimeric IL-2 receptor, has been linked in humans, while the gene encoding IL-2 itself, *Il2*, is likely the gene contributing disease susceptibility within the *Idd3* region. In both human and mouse, investigators portend that the disease-associated alleles create a reduction in IL-2 signaling [68, 69]. Deficiency of IL-2 or IL-2 signaling in human or mouse results in lymphoproliferative disease [68, 70], highlighting the importance of this signaling pathway in immune homeostasis. In the mouse, $Il2^{NOD}$ is associated with a two-fold reduction in IL-2 production, a level that was confirmed as contributory for disease using IL-2 haploinsufficient NOD-mice [71]. The human risk allele of CD25 is associated with reduced expression. In both cases, the lack of IL-2 signaling has been proposed to contribute to reduced Treg activity and a reduction in activation-induced cell death of effector T cells [68]. In either case, the exact genetic mechanism for reduced expression remains to be elucidated.

The identification of loci and genes that impact T1D in the NOD mouse has allowed for disease modulation using congenic and transgenic approaches. Certainly, the NOD MHC haplotype ($H2^{g7}$) is necessary, but not alone sufficient, to bring on T1D when congenically introgressed into a different genetic background. In fact, a combination of genes necessary for disease induction on a different background has not yet been identified. This strongly suggests that there are essential, but unidentified, genetic interactions present that greatly modulate susceptibility and resistance.

5. Gene-gene interactions and epistatic effects

T1D genetic research is largely focused on uncovering individual genes that impact the disease. However, it is the combined effect of multiple T1D susceptibility and resistance alleles, which often interact with each other, that ultimately determine an individual's risk [72]. The difficult task of unraveling this complexity has been carried forward mainly in animal models, including NOD strains congenically expressing different combinations of *Idd* loci from T1D resistant strains (**Table 2** and [73]). These animals have proven a useful tool for understanding how, when expressed on an autoimmune-permissive background, different disease resistance loci modulate T1D or various subphenotypes of the disease. Congenic mice have also uncovered numerous gene-gene interactions and gene-masking effects between *Idd* loci that impact T1D [69]. These effects are normally hidden in a segregating population such as backcross one (BC1) or F2 mouse generations or humans, and are therefore very difficult to detect using conventional genetic association studies [69].

One of the most striking examples of how genetic interactions affect T1D development is the synergism between the partially disease-protective *Idd3* and *Idd5* congenic intervals. Combining these two loci on the NOD background results in near complete elimination of diabetes and insulitis [74]. However, combining *Idd3* with various *Idd5* subregions (*Idd5.1*, *5.2*, *5.3*, and *5.4*) results in a range of disease-protective effects (reviewed in [69]). These include *Idd3/Idd5.1* mice that were not more protected than *Idd3* mice and *Idd3/Idd5.1/Idd5.3* mice that were equally resistant to T1D compared to the *Idd3/Idd5* strain, but develop more insulitis.

Other studies have detected strong gene-gene interactions within the *Idd5* locus. One is between *Idd5.1* that is suggested to encode a protective allele of *Ctla4* and *Idd5.4* that encodes a B10-derived susceptibility allele without known causative gene product. Hunter *et al*. found that the *Idd5.4* allele has a dramatic effect on T1D susceptibility in the presence of *Idd5.2* and *Idd5.3*, but no effect when protective alleles at *Idd5.1* are also present [75]. This suggests that *Idd5.4* can mask the effects of protective alleles at *Idd5.2* and *Idd5.3*, but that the *Idd5.4* susceptibility allele in turn is masked by the protective effect of *Idd5.1/Ctla4*. One interpretation of these findings is that the B10-derived *Idd5.4* susceptibility allele may modulate an immune event that is counter-regulated by *Ctla4*.

Congenic mice are also useful for cell transfer experiments to determine how epistatic interactions affect diabetogenic immune responses within specific cell types. This strategy was used to reveal complex genetic interactions within *Idd9* and *Idd11* on distal chromosome 4 that control how B cells contribute to disease [76, 77]. For these studies, lethally irradiated B cell-deficient and T1D-resistant NOD.IgH^{null} mice were transplanted with syngeneic bone marrow (BM) and B cells from

various chromosome 4 subcongenic donors. T1D incidence was subsequently monitored to establish whether B cells expressing different subcongenic intervals from the nonobese resistant (NOR) strain protected recipient mice from disease compared with standard NOD B cells. These studies identified four adjacent regions (designated R1, R2, R3, and R4) that interactively control the efficiency with which autoreactive B cells become tolerized or induce disease [77]. Several of these interactions appear to alter immunological responses involved in various tolerance induction mechanisms. For instance, NOR-derived alleles at R1 and R4 combine to increase the efficiency of B cell anergy, with counterbalancing negative regulation by R2. The NOR-derived congenic regions also contain genes that increase the diabetogenic capacity of B cells. These are encoded within R2 and R4 that interact to promote B cell hyperresponsiveness to B cell receptor (BCR) stimulation. However, the pathogenic effect of this interaction must normally be masked by the large number of other diabetes resistance alleles within the chromosome 4 interval.

Similar complex interactions appear to contribute to many other T1D subclinical phenotypes. Fox *et al*. described how the T cell-dependent progression of insulitis from a benign to a destructive state requires NOD-derived alleles at both the *Idd5* and *Idd13* loci [78]. Indeed, (NOD x NOR)F2 intercross mice that inherit at least one NOD-derived allele at *Idd5* and *Idd13* display invasive insulitis. In contrast, animals homozygous for NOR-derived C57BLKS/J alleles at either *Idd5* or *Idd13* develop mild peri-insulitis. Syntenic intervals that map to regions of human chromosomes 2q [79-82] and 15q [83, 84] may interact to control the same processes, which could explain why both are considered central regulators of disease pathogenesis.

There is also strong evidence that genetic loci that independently influence the risk for T1D interact in important ways to modulate disease progression in humans. One recent study tested interactions between 38 T1D-associated non-HLA regions with various HLA class II genotypes in a large collection of T1D samples [85]. HLA*non-HLA gene interactions were selected for testing because HLA class II genes have the largest effects on T1D, and therefore have a higher probability of showing interactions with a non-HLA locus. The results identified SNPs within two T1D-associated genes, *PTPN22* and *CTLA4*, that alter the predicted disease risk of various HLA haplotypes. The interaction between *PTPN22* and HLA class II genotypes confirms earlier work showing that the effect of a susceptibility allele at *PTPN22* is greater in low-risk compared to high-risk HLA class II genotypes [86-88].

In another study, Winckler *et al*. determined how different combinations of non-HLA susceptibility genes stratify islet autoimmunity and/or T1D risk [89]. Children of T1D patients were genotyped for 12 T1D-associated genes (*ERBB3*, *PTPN2*, *IFIH1*, *PTPN22*, *KIAA0350*, *CD25*, *CTLA4*, *SH2B3*, *IL2*, *IL18RAP*, *IL10*, and *COBL*) and followed prospectively from birth for the development of islet autoantibodies and disease development. This strategy predicted with surprising accuracy the risk for developing islet autoantibodies and T1D, and the progression from islet autoimmunity to T1D especially in children carrying high-risk HLA genes. The authors anticipated that some gene combinations would be more useful than others. Therefore, the prevalence of individual gene SNPs in different combinations was examined to determine which subpopulations were more predictive of disease. SNPs of four genes (*CTLA4*, *PTPN22*, *IL18RAP*, and *IFIH1*) were present in almost all combinations, while the *IL-2* SNP was infrequently detected. It was also determined that a collection of 8 genes (*IFIH1*, *CTLA4*, *PTPN22*, *IL18RAP*, *SH2B3*, *KIAA0350*, *COBL*, and *ERBB3*) predicted with greatest accuracy the chance of high-risk HLA carriers developing disease. The worse predictive power of all 12 SNPs may reflect a higher frequency of gene-gene interactions that mask the influence of individual T1D susceptibility alleles.

These and other GWAS studies make clear how some genes are strongly modulated by masking effects and gene-gene interactions, while others are not. Interpreting how statistically significant effects and interactions for T1D genes translate into biological actions, presents a challenging problem that experimentation with human samples alone are unlikely to solve. Therefore, future strategies to identify how crosstalk between genes underlies the development of T1D should include the continued use of congenic mice, with a particular focus on genes already known to impact both the mouse and human disease. These efforts should be greatly enhanced by the availability of new genetic tools (discussed below) that will allow the modification of two or more genes within congenic intervals, including the replacement of mouse genes with human variants.

6. Improving the efficiency of candidate gene identification

Enthusiasm for the use of NOD mice to identify genes that contribute to T1D has been tempered by the many difficulties this approach entails. The first limitation is the large number of mice needed to detect *Idd* loci when hyperglycemia or insulitis levels are used as an indicator of disease [73]. This is because very few F2 or first backcross (BC1) progeny generated from outcrossing NOD mice with T1D-resistant strains develop spontaneous autoimmune diabetes even when the diabetogenic $H2^{g7}$ MHC is fixed in all segregants [90]. A second limitation has been that even when *Idd* loci are detected and delineated, the T1D resistance or susceptibility genes they contain are difficult to validate with available technologies. This is particularly true for *Idd* loci containing multiple tightly linked genes not easily separated through genetic recombination. For these reasons relatively few mouse *Idd* genes have been identified to date [73]. As a consequence, there has been a significant decline over the last decade in the number of large-scale mouse studies to identify genes contributing to T1D.

Nonetheless, there remain compelling reasons for more investment in mouse genetics. They include the fact that of the small number of mouse *Idd* genes identified most also contribute to human T1D or function within the same biological pathways (see section entitled "Similarities in genetic causation" above). There is also good evidence that, just like in individual patients, differing genetic subset combinations drawn from an overall larger pool of possible susceptibility variants can contribute to disease pathogenesis in a threshold fashion in mice [72]. These subsets of genes likely control T1D susceptibility by eliciting perturbations at differing nodes within a common set of immunoregulatory pathways. Evidence for this possibility includes the previous observation that nominally resistant mouse strains can carry gene variants, which when expressed in the proper combinatorial context, contribute more strongly to T1D development than the corresponding allele from disease susceptible NOD mice (**Table 2** and reviewed in [73]). Although genetic studies using inbred mice are costly because of the large number of mice required, they remain a powerful method of detecting rare T1D susceptibility alleles that are impractical to identify through GWAS analyses, which require tens or hundreds of thousand of human subjects [31, 72]. Thus, for the reasons outlined above the question is not whether pursuing the identity of T1D susceptibility and resistance alleles is worthwhile, but rather how to make this process more efficient. Considerable encouragement comes from a new generation of genetic tools that may circumvent many of the most intractable obstacles that traditionally limited the identification of *Idd* candidate genes. Some of these are described in the following sections.

6.1 NOD embryonic stem cells

Until recently, no robust embryonic stem cell (ESC) lines capable of germ-line transmission could be generated from NOD mice due to difficulties in controlling the differentiation of these cells in culture [91-94]. Without this tool it is necessary to validate T1D gene candidates using fully germline competent ESC lines derived from other mouse strains. This practice introduces into the genetic background of the NOD mouse variable numbers of linked genes, some of which impact T1D [95]. In 2009, new culture methods were described that enable the derivation of NOD ESC capable of germ-line transmission [96]. Non-genetically manipulated mice generated from these cells were susceptible to T1D onset. The cells were grown based on a previous discovery that inclusion of the small molecule inhibitors PD0325901 and Chir99021 to a defined serum-free culture medium could facilitate ESC isolation from a wide number of strains [97]. This development should allow for much more efficient validation of genes within *Idd* loci because these genes permit the replacement of candidate alleles to be carried out individually and selectively. Indeed, these cells have been used to begin testing the importance of candidate genes within the MHC locus [98]. The same culture techniques were used to generate germ-line-competent ESC from immune-deficient NOD scid gamma (NSG) mice [99]. This resource should enable the future modification of the NSG strain to better accept engraftment of human tissues and cells.

Additionally, an ESC line from T1D susceptible NOD.CBALs-Tyr^{+}/LtJ (Agouti NOD) mice has already been used to generate a genetically modified NOD stock at The Jackson Laboratory (Dr. Lenny Shultz, personal communication). This strain's background carries a wild type Tyr (tyrosinase) allele from CBA mice, which allows for expression of the Agouti allele [94]. This dominant coat color marker provides an alternative for tracking germ-line transmissions by laboratories that routinely use albino blastocysts. After deliv-

ery of the DNA construct into the Agouti NOD-ES cells, these cells can be injected into a blastocyst derived from an albino mouse, and chimeric progeny bred with NOD.

6.2 Zinc finger nucleases (ZFN)

While germ-line-competent NOD ESC lines amenable to genetic modification have become available, recent development of other genetic tools will further facilitate gene-mapping studies in NOD mice. Among them, the ZFN technology has emerged as a powerful means to specifically target genes in a variety of cells and organisms [100]. The benefits of using the ZFN mutagenesis technology over the ESC approach include its applicability to various genetic backgrounds, its high targeting efficiency, and a short period of time required to generate a knockout animal [100].

ZFNs are fusion proteins containing a sequence-specific DNA-binding zinc finger domain and a nuclease domain [100, 101]. Engineered ZFNs specifically recognize and bind a defined target gene sequence within the nucleus of a cell and introduce a double strand break (DSB) [102, 103]. The cellular DNA repair machinery then fixes these breaks, most frequently via the non-homologous end joining (NHEJ) mechanism, resulting in small deletions of the gene sequence (few to hundreds of base pairs) and disruption (knockout) of the target gene [102, 103]. Injected as synthetic mRNAs, ZFNs typically work at the one-cell embryo stage, resulting in single-step, whole animal gene disruption, and infrequent mosaics [104]. More precise genetic engineering can be achieved as well because a DSB also stimulates repair via a homology-directed repair (HDR) mechanism if a homologous DNA template is cointroduced into the cell [105]. It is expected that the ZFN technology will provide an unprecedented approach to identify causal variants within previously known *Idd* regions or to study the function of a particular gene in the NOD model.

6.3 Transcription activator-like effector nucleases (TALEN)

The recently developed transcription activator-like effector nucleases (TALEN) technology allows for the direct targeting of any gene in zygotes of the inbred mouse strain of choice [106-109]. This rapidly evolving genetic modification method is more efficient than conventional gene targeting technologies. It employs DNA-binding TALEN repeat modules designed to have precise specificity for particular nucleotide sequences that are coupled with a modified Flavobacterium okeanokoites enzyme, FokI, nuclease domain. When combined, this creates site-specific double-stranded breaks with remarkable efficiency (~15% in mouse zygotes). TALEN technology has been employed to genetically alter mouse strains previously recalcitrant to the development of stable ESC, including NOD (personal communication with Dr. Dave Serreze). TALENs, like ZFN, represent another new and versatile technology for future manipulation of gene expression in NOD mice. It is reasonable to expect that, because of their efficiency and ease of use, engineered nucleases will significantly replace ESC as a method to edit the NOD genome.

6.4 RNA interference

While NOD ESC, ZFN, and TALEN technology will likely transform future efforts to test T1D candidate genes, RNA interference (RNAi) has already proven useful for manipulating gene expression in NOD mice without introducing genetic contamination from other strains. This approach is based on a well-established transgenesis methodology that entails the direct introduction of short hairpin RNA (shRNA) containing constructs into NOD zygotes by viral transduction [96, 110, 111]. The shRNA-containing constructs are designed to silence genes that impact T1D. shRNA is a sequence of RNA that contains a tight hairpin turn. This structure is cleaved by intracellular machinery into small interfering RNA (siRNA) that knocks down any mRNA bearing a complementary sequence [112]. Several companies are developing viral libraries that produce shRNA that integrate into the host genome and ensure stable gene silencing after integration. The silencing cassette can be incorporated into many different types of vectors, including lentiviral, adenoviral, or retroviral vectors. RNAi has already provided valuable insight into how expression of the T1D candidate genes IL-17 [113], PTPN22 [114], soluble CTLA4 [115], and Slc11a1 (*Nramp1*) [116] contribute to disease.

Although the effectiveness of RNAi is well established, there are some important limitations of this technology. One is the so-called “off targeting” effect that lowers the specificity of RNAi and produces false positive results [112]. This occurs when genes with incomplete complementarity with the transgenically expressed shRNA are inadvertently downregulated, which may result in data misinterpretation and even toxicity [112]. Another drawback is that many of the shRNA molecules

capable of triggering RNAi do not shut down gene activity completely. Because of these problems RNAi experiments require stringent controls to minimize the risk of data misinterpretation, including transducing NOD zygotes with scrambled shRNA designed not to target any transcripts. It is also recommended that results obtained using shRNA-expressing NOD mice be confirmed by additional non-RNAi experiments.

6.5 Retrogenic mice

Retrogenics refers to a method of transgenically expressing genes in hematopoetic stem cell (HSC)-derived cell types by transducing bone marrow using retroviral vectors before adoptive transfer into lethally irradiated recipient mice [117]. This technology has helped to uncover the contribution of diabetogenic TCRs of different specificities to insulitis development in the NOD mouse [118]. This discovery entailed the transduction of bone marrow cells with retroviral vectors, containing TCRα and TCRβ chains of interest, linked with a 2A viral peptide cleavage sequence that allows for the stoichiometric translation of both TCR peptides within a single vector [119]. The advantages of retrogenic technology are manifold, including the low cost and short time interval required to generate retrogenic mice compared with transgenic animals [117]. This approach also avoids the possibility of founder effects that often complicate results obtained using transgenic mice, as each retrogenic animal is a founder [117]. Because of the flexibility of this technique, it is likely that retrogenics will be adapted by T1D researchers for a variety of additional applications to speed the identification of the genetic causes of T1D, including overexpressing in HSC-derived cell types, human TCRs, other *Idd* gene candidates, or the shRNA that silence them.

6.6 Next generation sequencing

Applications for next generation sequencing (NGS) hold enormous potential for accelerating the discovery of T1D causative genes. One of this technology's most important contributions has been for whole genome sequencing (WGS) of the NOD mouse and some of the different strains this stock has been outcrossed to for detection of *Idd* loci. These sequence data have provided a means of accurately identifying whether T1D candidate genes contain SNPs that affect protein expression levels, structure, and/or function. DNA sequences for NOD mice and 16 additional strains are publically available through the Sanger Institute's database. However, some mouse strains, including NOR, B10, and ALR, used to generate many of the stocks congenic for T1D resistance loci on the NOD background have yet to be sequenced. When the sequences of these stains are available, their DNA code should provide much more accurate predictions of which alleles within *Idd* loci contribute to disease than current SNP-based methods.

Although the efficiency of WGS continues to rapidly improve, this technology is likely to remain too expensive and time-consuming to replace conventional linkage studies. At the time of writing, the cost for sequencing the genome of a single mouse (or mouse strain) including the bioinformatics time for assembly is less than $10,000 (by several institutes, including Centrillion Biosciences Inc., Palo Alto, CA, USA). In future, a more cost-effective alternative will be to sequence whole mouse exomes using NGS. Exome sequencing captures allelic variants within the ~1% of the genome where most disease variations are located [120, 121] at a current cost of ~$1300 at 50X coverage for a 50Mb array. While this approach is efficient for identifying protein-coding sequences that underlie Mendelian disorders and *de novo* mutations, it is also useful for detecting polymorphisms that alter risk for, rather than cause, complex genetic disease including T1D [121]. The principal disadvantage of exome sequencing is that much of the 99% of the genome that remains unsequenced contains genetic material that contributes in many critical ways to gene regulation and no doubt T1D development. Whole-exome sequencing also requires additional steps of exome enrichment compared to WGS [120, 121]. This significantly adds to the cost of each sample.

For this reason, it is often favorable to sequence the transcriptome instead. This process, also known as RNA sequencing (RNA-seq) refers to the NGS of cDNA transcribed from all RNAs in a cell or tissue [122]. RNA-seq provides a method of detecting transcribed genes and other non-coding RNA without the need for any additional enrichment steps [122]. Transcripts may also be mapped against reference genomic DNA to obtain additional information, including transcription localization and the relative frequency of different splice variants [122]. A significant advantage of transcriptome sequencing is that this technology directly detects all cDNA sequence, and therefore has very low if any background and no upper limit for quantification. In contrast, because of intrinsic experimental limitations, microarray analyses cannot detect all transcription products and lacks

sensitivity for genes expressed at high and low levels [122]. While RNA-seq represents a sizable advance over microarray analysis for detection of how polymorphisms within *Idd* candidates affect gene expression, widespread use of this technology has been limited by cost, bioinformatics challenges, and a prerequisite for sequencing information to detect and evaluate transcripts. However, it is likely that most of these obstacles will be overcome in time.

7. Conclusions

The genetic pathogenesis of T1D is complex. While sub-phenotypes of T1D (i.e. autoantibodies) may appear in humans and mice not at genetic risk, full pathogenesis requires complex interactions of susceptibility alleles along with a network of synergizing factors, including the environment. Shared between NOD mice and humans is the paramount role of the MHC class II alleles (Tables 1 and 2). Yet, the MHC alone is not sufficient. There are substantial contributions of non-MHC genes in both species. NOD mice also have lost tolerance to an array of self-proteins that is similar to the responses observed in humans, including insulin, glutamic acid decarboxylase 65 (GAD65), IA2 (ICA512 or protein tyrosine phosphatase, receptor type, N), and ICA69 (islet cell autoantigen 1) and IGRP (glucose-6-phosphatase, catalytic subunit, 2 (G6pc2)) protein (**Table 1**). Furthermore, a role for environmental and dietary factors is linked. Early gluten exposure has been associated with T1D onset in humans and is essential for T1D to initiate in the NOD mouse [123].

As has been pointed out, there are key differences between disease development in NOD and that in humans. Most notable, NOD mice exhibit an extremely aggressive form of T1D. Insulitis is present at weaning and the insulitic infiltrates are heavy. In comparison, islet inflammation appears to be a rare event in man [20-27]. However, based on genetic homogeneity throughout the NOD colonies held worldwide, the mouse strain represents a single individual with diabetes or a family with a given genetic susceptibility. Without this aggressive disease, NOD mice would be significantly more difficult to study and the interpretation of results from genetic studies would present unique challenges. Understanding the differences and focusing on similarities, specifically those that modulate genetic pathogenesis, should allow for those in this field to continue to make progress with this model to better understand the human condition.

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