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Thinking bedside at the bench: the NOD mouse model of T1DM

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Abstract

Studies over the past 35 years in the nonobese diabetic (NOD) mouse have shown that a number of agents can prevent or even reverse type 1 diabetes mellitus (T1DM); however, these successes have not been replicated in human clinical trials. Although some of these interventions have delayed disease onset or progression in subsets of participants, none have resulted in a complete cure. Even in the most robust responders, the treatments do not permanently preserve insulin secretion or stimulate the proliferation of β cells, as has been observed in mice. The shortfalls of translating NOD mouse studies into the clinic questions the value of using this model in preclinical studies. In this Perspectives, we suggest how immunological and genetic differences between NOD mice and humans might contribute to the differential outcomes and suggest ways in which the mouse model might be modified or applied as a tool to develop treatments and improve understanding of clinical trial outcomes.

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease that occurs when the immune system destroys β cells of the islets of Langerhans in the pancreas. Pancreatic β cells decline in number and function until they do not produce sufficient levels of insulin to meet metabolic demands. As a result of the inaccessibility of the target tissue and lymphoid organs in humans and the utility of studies of mouse genetics for dissecting mechanisms, studies in rodent models have become guideposts for understanding human T1DM and planning clinical interventions. Before the nonobese diabetic (NOD) mouse model was available, rodent models that shared features with human autoimmune diabetes mellitus included the BioBreeding/Worcester rat, diabetes mellitus induced with lymphocytic choriomeningitis virus and diabetes mellitus induced with multi-dose streptozotocin.^{1–3} However, as diabetes mellitus develops spontaneously in NOD mice and because of the range of tools and genetic information available for mouse studies, the NOD mouse has become the model of choice for preclinical studies. Interestingly, the other rodent models have a number of features, such as the phenotype and islet cellular infiltrates, that more

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Both authors contributed equally to all aspects of the manuscript.

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closely mimic human disease than the NOD mouse. Over the past 10 years, several authors have reviewed the progress and pitfalls of the NOD model. These studies have emphasized the immunopathological findings in islet cells as well as the lessons that were learned from the model regarding genetic susceptibility.^{4–14}

The NOD mouse was discovered in 1974 and was subsequently inbred.¹⁵ This model has been a critical tool that has developed our understanding of disease mechanisms and has been used to focus strategies, including the use of broad spectrum immune suppressive regimens attempted in patients (such as ciclosporin,^{16–18} azathioprine^{19,20} and azathioprine with prednisone²¹) and agents that specifically target cell populations and mediators. Drugs such as metabolic agents, antibodies that target cell surface immune ligands such as CD20, CD3, or co-stimulatory molecules on lymphocytes, as well as cytokines, antigens and others, were first tested in NOD mice;⁴ several were found to partially prevent, delay or reverse T1DM, at least in some patients. In clinical studies, some of these agents (such as anti-CD3 monoclonal antibodies [mAbs],^{22–24} anti-CD20 monoclonal antibody [mAb]²⁵ and alefacept²⁶) have had successes in patients with new (within 3 months of diagnosis) or even recent-onset (>3 months from diagnosis) T1DM in attenuating the decline of provoked C-peptide responses, generally with improved HbA_{1c} levels and reduced requirements for exogenous insulin. Some of these patients have even responded robustly, becoming insulin independent or maintaining their levels of C-peptide responses for >2 years.

However, other clinical trials have either failed or yielded surprising outcomes in light of the NOD studies, including improvement (abatacept [CTLA4-Ig]^{27,28}), described below, or deterioration (combination therapy with rapamycin and IL-2²⁹) of T1DM. Furthermore, even if the treatment is successful, the results are not permanent. For instance, the C-peptide response declines with time, often at a similar rate in the control and treated groups after a period of stabilization in the latter. Nonetheless, these discrepancies between the mouse and human studies have left investigators with unanswered questions about future directions to pursue a cure for T1DM. In this Perspectives, we discuss preclinical studies in NOD mice and the results of clinical trials with a particular focus on the reasons for the differences between the NOD mouse model and humans with T1DM, as well as the design and interpretations of preclinical studies that might account for failures in clinical translation.

Summary of recent studies

Preclinical (NOD) and clinical studies have reported concordant and discordant results (Table 1). Some of the agents that were effective in prevention and reversal of diabetes mellitus in NOD mice, such as porcine oral³⁰ and parenteral insulin,³¹ GAD65(alum),^{32–34} sitagliptin and lansoprazole,³⁵ anti-IL-1,³⁶ IL-1 receptor antagonist agents^{37–39} and antithymocyte globulin (ATG),⁴⁰ did not show efficacy in well-designed clinical trials with standard end points that compared provoked C-peptide responses in patients treated with the drug or control individuals given placebo.^{41–47} Other agents, such as Fc receptor nonbinding anti-CD3 mAbs^{48–52} and anti-CD20 mAb,^{53,54} prevented and/or reversed diabetes mellitus in preclinical studies but were only partially effective in clinical trials, delaying onset or disease progression.^{22–24,55–57}

Alefacept (LFA3-Ig), which targets memory T cells, was originally tested in NOD mice that had received an insulinoma graft that was modified to secrete LFA3-Ig to test whether immunomodulators could improve graft survival. The growth of the tumour cells and survival were improved in NOD mice transplanted with the graft transfected with a transgene encoding LFA3-Ig.⁵⁸ In a clinical trial, alefacept also showed modest effects on C-peptide responses in patients with T1DM.²⁶

Two trials have reported paradoxical findings. Abatacept had shown efficacy in human autoimmune diseases other than T1DM, such as rheumatoid arthritis.^{59,60} However, in NOD mice, mouse CTLA4-Ig (expressed from a transgene)⁶¹ or anti-B7.1 mAb, which blocks a CD28 ligand, accelerated the progression of diabetes mellitus in female mice, and anti-B7.2 mAb or human abatacept showed modest protection when given to 6–8 week old mice but not when given to mice >8 weeks of age.⁶² However, patients with new-onset disease treated with abatacept had a statistically significant improvement in provoked C-peptide response compared with patients given placebo.^{27,28} Rapamycin administered in combination with IL-2 also reduced the incidence of diabetes mellitus in female NOD mice,⁶³ but caused a transient decline in the C-peptide response in nine patients with recent-onset T1DM and residual insulin production who had been treated with this combination regimen.²⁹

Immunological issues

Mice and humans are separated by 65 million years of evolutionary history⁶⁴ and have differences in their adaptive and innate immune systems that are relevant to the use of mouse models. Interferons have been proposed to be important mediators of an initial innate immune response to islet antigens that occurs in young NOD mice around the time of weaning; however, in mice, interferons do not activate STAT4 or induce a type 1 T helper cell (T_H1) response in adaptive immune cells as they do in humans. NOD mice have additional unique features that distinguish their immune responses from human responses such as their failure to produce the mRNA or protein for the complement inhibitor factor-H related protein-C.⁶⁵ IL-10, which is thought to be an important regulatory cytokine, is only produced during a type 2 T helper cell (T_H2) response in mice but is produced during both T_H1 and T_H2 responses in humans.⁶⁴ These distinctions might account for the different outcomes observed in mice and humans treated with immune agents. For example, unlike in mice,⁶⁶ regulatory T cells (as well as effector T cells) were depleted by thymoglobulin administration in human patients and this reduction might have affected the balance of effectors and regulatory cells.^{47,64}

Humans and mice have other relevant genetic discrepancies that affect the function of the immune system. Importantly, NOD mice are inbred and 80% of female mice develop diabetes mellitus but only 20% of male mice develop the disease; this sexual dimorphism and inbreeding is not seen in humans.^{15,67} T-cell antigen recognition using class II major histocompatibility complexes (MHC) is known to confer susceptibility to T1DM in mice and humans. The binding pocket of the NOD class II allele (H2-A^{g7}) is similar to the binding pocket of the analogous human MHC molecule (HLA-DQ) but NOD mice do not express the other class II MHC molecule I-E.^{9,11} Surprisingly, HLA-DQ8 transgenic NOD

mice, which express the human MHC susceptibility gene, develop autoimmune myocarditis.⁶⁸ In addition, the class II human MHC alleles associated with T1DM have a greater degree of polymorphism than the NOD MHC genes. Furthermore, the HLA complex includes several loci that independently confer risk of T1DM. Thus, the total genetic burden of susceptibility alleles (homozygous or heterozygous) differs between individuals.¹⁰

The efficacy of antigen-specific treatments might differ depending on the MHC alleles involved and the way in which peptides bind in the MHC groove.^{69–71} Recognition of antigens by autoreactive T cells is generally of low avidity and, therefore, seemingly minor differences in peptide binding between alleles have profound effects on responses to that antigen. For example, a single amino acid difference in the B chain of human insulin seems to account for the ability of porcine, but not human, insulin to prevent the development of diabetes mellitus in NOD mice.⁷² These genetic differences might account for the heterogeneity in responses to oral insulin among at-risk relatives of patients.⁷³ Furthermore, the extended MHC haplotype might affect autoimmune responses, as illustrated by the dominant protective effect of HLA-DR2 alleles on the risk of developing T1DM in humans. Finally, the genetic heterogeneity, sex and environmental factors in humans can affect responses to drugs, but these variations are not well-modelled in mice.

A number of other immunological differences in the immune systems of mice and humans might be relevant. For instance, CD28 is expressed on all CD4⁺ and CD8⁺ T cells in mice, whereas CD28 is expressed on all CD4⁺ but only 50% of CD8⁺ T cells in humans.⁷⁴ This disparity suggests that the effects of abatacept, which blocks CD28 co-stimulation by B7.1 and B7.2 ligands of activated antigen-presenting cells, might differ in mice and humans, particularly for low avidity interactions that occur with autoantigen recognition. NOD mice have impairment of natural killer (NK) cell function that might account for the failure to identify the toxicity of IL-2 treatment in the preclinical studies of the rapamycin and IL-2 combination therapy.^{10,29,75} In addition, the insulinitis observed in humans with T1DM and in NOD mice are qualitatively and quantitatively different. Even in individuals with new-onset T1DM or those at very high risk of developing T1DM, insulinitis can be found in <10% of the islets (versus >50% in NOD mice⁷⁹) and the loss of β -cell mass in the prediabetes period is less than in NOD mice.⁷⁶ The insulinitis seen in human disease is characterized by fairly small numbers of CD8⁺ T cells and macrophages, whereas high numbers of B cells and CD4⁺ T cells predominate in NOD mice.^{11,77}

Disease kinetics

The kinetics of the disease process are important for the timing of when therapies are delivered but also differs between NOD mice and humans. Humans >21 years old at the time of diagnosis have a slower reduction in levels of C-peptide than individuals who are diagnosed at <21 years old.⁷⁸ By contrast, the rate of loss of β cells in NOD mice is rapid;⁷⁹ however, the effects of age on their loss has not been well-studied. The reason for the differences in the rate of progression in humans before or after age 21 years has not been identified but could involve immunological or β -cell differences, such as the diversity of their immune repertoire (expected to be reduced in older patients), the expression of relevant antigens by younger β cells, or even their susceptibility to cell death. Nonetheless, this

heterogeneity is very important for the design of clinical trials. Studies in young adults require a greater number of participants than studies in older patients to show a statistically significant clinical response. Clearly, if the disease mechanisms are different in younger and older patients, the effects of immune interventions are also likely to differ. Anti-CD3 mAb is most effective in younger (that is, age <15 years) patients.²⁴ In NOD mice, anti-CD3 mAb shows the greatest efficacy when it is given at the time of peak β -cell death, either in prediabetic NOD mice or in recipients of islet allografts.⁷⁹ However, when anti-CD3 mAb was administered to patients with established autoimmune responses (that is, 4–12 months after diagnosis and particularly those aged >15 years) the effects were less robust than in younger patients with new-onset disease, which suggests a window of treatment opportunity is present in humans that is not defined by observations in mice.²⁴

In addition, it is very difficult to extrapolate from preclinical studies to the clinical setting because no biomarkers clearly define the stage of the disease, as well as for practical reasons. As an example, disease prevention with parenteral insulin in NOD mice was effective when it was initiated at a very young age (4 weeks).³¹ However, in humans, the antigen can usually only be given after the appearance of autoantibodies, when insulin secretion is impaired.⁴² NOD mouse follicular B cells downregulate CD20 when they infiltrate the islets of Langerhans.⁸⁰ The inefficiency of anti-CD20 in late-stage diabetes mellitus might be due to a similar phenomenon if the same process occurs in humans. This finding also suggests that the optimal timing for this therapy is before the appearance of autoantibodies, for example, relatives of patients with T1DM could be given this treatment in the first few years of life.

Target cell issues

A number of preclinical studies have suggested that successful therapies in mouse models, such as rapamycin with IL-2,⁶³ α 1-anti-trypsin,⁸¹ anti-lymphocyte serum with exendin-4⁸² and a dipeptidyl peptidase-4 inhibitor with a proton pump inhibitor,⁸³ can cause β -cell regeneration. These findings suggest that T1DM could be cured by both arresting ongoing destruction and permitting spontaneous or enhanced β -cell proliferation. Whether or when β -cell proliferation occurs in humans is still unknown and clinical data offers little evidence to suggest this occurs. For instance, the recovery of β -cell function after disease onset is most consistent with recovery of function of β cells rather than growth of new cells.^{79,84} Rapamycin combined with IL-2 can improve β -cell mass in NOD mice,⁶³ but can also be toxic to β cells,⁸⁵ which suggests that either outcome would be possible if this treatment is used in patients.

Of great clinical importance is the notable β -cell function at the time of onset of T1DM and even for long periods of time after disease onset.⁷⁸ The Joslin Medalist study reported that even 50 years after a diagnosis of T1DM, 67.4% of patients had detectable levels of C-peptide.⁸⁶ As residual insulin production is a key factor in determining the clinical outcomes of the disease,⁸⁷ the absence of chronic residual insulin production in the setting of autoimmunity in NOD mice is a shortfall that limits the usefulness of this model in studying the effects of agents at later stages of the disease.

Improving NOD studies

Studies in NOD mice have provided a basic understanding of disease mechanisms and immunological tolerance and have enabled the identification of autoantigens, as well as giving insights into the identity and function of pathogenic T cells and the contribution of genetic polymorphisms that would never have been possible without this model. Furthermore, disease-initiating events can only be studied in the mouse model and preclinical studies have, in fact, accurately predicted the success of a number of clinical studies and identified the successful mechanisms.^{22,25,27,28,48,49,51,53,55,61,62,74,88} Has the model lost its relevance to clinical T1DM or is the problem in the interpretation of the findings? How could the limitations be addressed?

The design of preclinical studies is as critical as the design of clinical studies and studies testing the same regimens should be consistent with respect to key issues such as timing, dosing of agents and sample size. Improving the design of preclinical studies might help ensure confidence in the outcomes of the mouse studies. Preclinical studies are generally performed at a single site and under very controlled housing conditions with sufficient group sizes to achieve statistical significance, but do not capture acquired differences between facilities or even inherent biological variation. Even studies of mice purchased from the same vendor and same lot, and housed under similar conditions, show unexpected results that might be due to differences in the microflora between housing locations and other uncontrolled factors (Herold, K. C. and collaborators, unpublished work). Studies that are reproduced at different times, between sites, with sample sizes larger than the 6–20 mice used in experimental groups in most reports represent a robust test more akin to clinical conditions than the current format of preclinical studies. This strategy would capture variance between sites as well as minimize the idiosyncratic and environmental differences and biological variation. Parallel mechanistic experiments designed to study the targeted pathway would also provide a strong indication that a false positive conclusion had not been reached.

The dosing of agents and timing of administration are important considerations that are often taken for granted, with clinical trials being designed on the basis of practicality or body weight rather than the data from the preclinical studies. The lower dose (per kg) of parenteral insulin given to relatives of patients with T1DM in the Diabetes Prevention Trial versus NOD mice is an example of this phenomenon.⁴² Mice received 220 µg (211 U/kg per dose) of human recombinant insulin at weeks 4, 12, 20 and 28,³¹ whereas humans received recombinant human ultralente insulin twice a day at 0.125 U/kg and participants were hospitalized every 12 months and received 0.015 U/kg/h of parenteral insulin for 4 days.⁴² Rather than body weight, using body surface area has been suggested to be a more reliable approach for transitioning from animal to human doses.⁸⁹ The appropriate timing for interventions in T1DM is challenging, as no absolute measure of disease progression exists that can be compared between mice and humans. However, the time point during disease progression when the efficacy of agents is maximal, reflected by the frequency of antigen-specific T cells in mice or autoreactive T cells and autoantibodies in humans, might help to guide timing of interventions in humans. A critical need (and a limitation of NOD studies) is a model of the deterioration in β -cell function after remission in the new-onset setting. A

more direct measure of β -cell death, such as by measurement of unmethylated insulin DNA released from dying β cells, might provide clearer information on the relationship between interventions and the pathological consequences.⁹⁰

The genetic and environmental heterogeneity of humans undoubtedly accounts for the mixed responses to drugs in patients, but could also contribute to the different outcomes of studies in NOD mice; however, these factors are difficult to model. A direct approach to this problem might involve developing and studying ‘humanized’ mice, that is, mice with human immune cells or even molecules from humans that are involved in immune responses. One example of this approach has been the introduction of the ϵ chain of the human CD3 molecule into mice to enable the study of the mechanisms of action of anti-CD3 mAb.⁹¹ In this example, signalling through the CD3 ϵ chain by a mAb in clinical testing could be evaluated directly. The use of the CRISPR–Cas9 system for introducing genes into mice might greatly accelerate and expand the development of humanized models by enabling investigators to introduce genetic polymorphisms related to human disease. In addition to introducing single or multiple human genes into mouse immune systems, the responses of the human cells to drugs can be investigated in detail in humanized mice reconstituted with human immune cells.^{92–94} Currently, a humanized mouse model of T1DM does not exist, but investigators have described immune phenotypes and pathological responses in mice that have been reconstituted with stem cells and immune effector cells from patients.⁹⁵ Importantly, these models seem to be useful surrogates for understanding the effects of immune modulators that have been used for the treatment of human T1DM, such as anti-CD3 mAb or even induction of autoimmune disease by human T cells with anti-CTLA-4 mAb.^{93,94}

Conclusions

The rationales for clinical trials require scientific guidance and a small animal model that can be used to address experimental questions and to test the usefulness of therapeutics. Discrepancies between the preclinical studies and the clinical outcomes have highlighted the pitfalls of the NOD model and its use in informing the design of clinical studies. However, the problems are more than the differences between the mouse and human diseases. They include the design and interpretation of the preclinical studies, environmental factors and genetic heterogeneity that affect human disease but cannot be modelled in mice, as well as very practical issues such as drug dosing and the timing of interventions. Human and mouse immune and β cells have important differences, some of which are particular to NOD mice rather than all animal models, which might account for the different outcomes in mice and humans. Nonetheless, the model continues to provide essential guidance to investigators to enable them to develop and test fundamental disease mechanisms such as the role of antigen-specific immune cells, identification of islet antigens and modulation of immune responses by genetic polymorphisms associated with T1DM. In this regard, the NOD mouse model is invaluable— it is the incubator for developing and testing new ideas and for developing new strategies for treatment.

Acknowledgments

The path from small preclinical studies to human trials is perilous for the reasons noted. The biological differences need to be considered in the design of the clinical studies. Improving the robustness of the experimental design of preclinical studies, with replication across laboratories and consideration of timing and dosing of experimental agents, might improve the application of preclinical findings. Ultimately, a small animal model in which human immune cells and other cells can be manipulated would be most valuable. As our strategies for treatment become more refined and precise, so must the models that are used to test them.

References

1. Herold KC, Montag AG, Fitch FW. Treatment with anti-T-lymphocyte antibodies prevents induction of insulinitis in mice given multiple doses of streptozocin. *Diabetes*. 1987; 36:796–801. [PubMed: 3556279]
2. Like AA, Rossini AA. Streptozotocin-induced pancreatic insulinitis: new model of diabetes mellitus. *Science*. 1976; 193:415–417. [PubMed: 180605]
3. Like AA, Rossini AA, Guberski DL, Appel MC, Williams RM. Spontaneous diabetes mellitus: reversal and prevention in the BB/W rat with antiserum to rat lymphocytes. *Science*. 1979; 206:1421–1423. [PubMed: 388619]
4. Shoda LK, et al. A comprehensive review of interventions in the NOD mouse and implications for translation. *Immunity*. 2005; 23:115–126. [PubMed: 16111631]
5. Wicker LS, et al. Type 1 diabetes genes and pathways shared by humans and NOD mice. *J Autoimmun*. 2005; 25 (Suppl):29–33. [PubMed: 16257508]
6. Roep BO. Are insights gained from NOD mice sufficient to guide clinical translation? Another inconvenient truth. *Ann NY Acad Sci*. 2007; 1103:1–10. [PubMed: 17376838]
7. Chaparro RJ, Diloranzo TP. An update on the use of NOD mice to study autoimmune (type 1) diabetes. *Expert Rev Clin Immunol*. 2010; 6:939–955. [PubMed: 20979558]
8. Thayer TC, Wilson SB, Mathews CE. Use of nonobese diabetic mice to understand human type 1 diabetes. *Endocrinol Metab Clin North Am*. 2010; 39:541–561. [PubMed: 20723819]
9. Driver JP, Serreze DV, Chen YG. Mouse models for the study of autoimmune type 1 diabetes: a NOD to similarities and differences to human disease. *Semin Immunopathol*. 2011; 33:67–87. [PubMed: 20424843]
10. van Belle TL, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiol Rev*. 2011; 91:79–118. [PubMed: 21248163]
11. Driver JP, Chen YG, Mathews CE. Comparative genetics: synergizing human and NOD mouse studies for identifying genetic causation of type 1 diabetes. *Rev Diabet Stud*. 2012; 9:169–187. [PubMed: 23804259]
12. King AJ. The use of animal models in diabetes research. *Br J Pharmacol*. 2012; 166:877–894. [PubMed: 22352879]
13. In't Veld P. Insulinitis in human type 1 diabetes: a comparison between patients and animal models. *Semin Immunopathol*. 2014; 36:569–579. [PubMed: 25005747]
14. Jayasimhan A, Mansour KP, Slattery RM. Advances in our understanding of the pathophysiology of type 1 diabetes: lessons from the NOD mouse. *Clin Sci (Lond)*. 2014; 126:1–18. [PubMed: 24020444]
15. Makino S, et al. Breeding of a non-obese, diabetic strain of mice. *Jikken Dobutsu*. 1980; 29:1–13. [PubMed: 6995140]
16. Assan R, et al. Metabolic and immunological effects of cyclosporin in recently diagnosed type 1 diabetes mellitus. *Lancet*. 1985; 1:67–71. [PubMed: 2857024]
17. Bougnères PF, et al. Limited duration of remission of insulin dependency in children with recent overt type I diabetes treated with low-dose cyclosporin. *Diabetes*. 1990; 39:1264–1272. [PubMed: 2210078]
18. Jenner M, Bradish G, Stiller C, Atkison P. Cyclosporin A treatment of young children with newly-diagnosed type 1 (insulin-dependent) diabetes mellitus. London Diabetes Study Group. *Diabetologia*. 1992; 35:884–888. [PubMed: 1397785]

19. Harrison LC, Colman PG, Dean B, Baxter R, Martin FI. Increase in remission rate in newly diagnosed type I diabetic subjects treated with azathioprine. *Diabetes*. 1985; 34:1306–1308. [PubMed: 3905463]
20. Cook JJ, et al. Double-blind controlled trial of azathioprine in children with newly diagnosed type I diabetes. *Diabetes*. 1989; 38:779–783. [PubMed: 2656346]
21. Silverstein J, et al. Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. *N Engl J Med*. 1988; 319:599–604. [PubMed: 3045545]
22. Herold KC, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med*. 2002; 346:1692–1698. [PubMed: 12037148]
23. Herold KC, et al. A single course of anti-CD3 monoclonal antibody hOKT3 γ 1(Ala–Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. *Diabetes*. 2005; 54:1763–1769. [PubMed: 15919798]
24. Herold KC, et al. Teplizumab (anti-CD3 mAb) treatment preserves C-peptide responses in patients with new-onset type 1 diabetes in a randomized controlled trial: metabolic and immunologic features at baseline identify a subgroup of responders. *Diabetes*. 2013; 62:3766–3774. [PubMed: 23835333]
25. Pescovitz MD, et al. Rituximab, B-lymphocyte depletion, and preservation of β -cell function. *N Engl J Med*. 2009; 361:2143–2152. [PubMed: 19940299]
26. Rigby MR, et al. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Diabetes Endocrinol*. 2013; 1:284–294. [PubMed: 24622414]
27. Orban T, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2011; 378:412–419. [PubMed: 21719096]
28. Orban T, et al. Costimulation modulation with abatacept in patients with recent-onset type 1 diabetes: follow-up 1 year after cessation of treatment. *Diabetes Care*. 2014; 37:1069–1075. [PubMed: 24296850]
29. Long SA, et al. Rapamycin/IL-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs β -cell function. *Diabetes*. 2012; 61:2340–2348. [PubMed: 22721971]
30. Zhang ZJ, Davidson L, Eisenbarth GS, Weiner HL. Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc Natl Acad Sci USA*. 1991; 88:10252–10256. [PubMed: 1946445]
31. Muir A, et al. Insulin immunization of nonobese diabetic mice induces a protective insulinitis characterized by diminished intraislet interferon- γ transcription. *J Clin Invest*. 1995; 95:628–634. [PubMed: 7860747]
32. Tian J, et al. Modulating autoimmune responses to GAD inhibits disease progression and prolongs islet graft survival in diabetes-prone mice. *Nat Med*. 1996; 2:1348–1353. [PubMed: 8946834]
33. Tisch R, Liblau RS, Yang XD, Liblau P, McDevitt HO. Induction of GAD65-specific regulatory T-cells inhibits ongoing autoimmune diabetes in nonobese diabetic mice. *Diabetes*. 1998; 47:894–899. [PubMed: 9604865]
34. Boettler T, et al. The clinical and immunological significance of GAD-specific autoantibody and T-cell responses in type 1 diabetes. *J Autoimmun*. 2013; 44:40–48. [PubMed: 23770292]
35. Suarez-Pinzon WL, Cembrowski GS, Rabinovitch A. Combination therapy with a dipeptidyl peptidase-4 inhibitor and a proton pump inhibitor restores normoglycaemia in non-obese diabetic mice. *Diabetologia*. 2009; 52:1680–1682. [PubMed: 19455306]
36. Drage M, et al. Nondepleting anti-CD4 and soluble interleukin-1 receptor prevent autoimmune destruction of syngeneic islet grafts in diabetic NOD mice. *Transplantation*. 2002; 74:611–619. [PubMed: 12352875]
37. Sandberg JO, Eizirik DL, Sandler S, Tracey DE, Andersson A. Treatment with an interleukin-1 receptor antagonist protein prolongs mouse islet allograft survival. *Diabetes*. 1993; 42:1845–1851. [PubMed: 8243831]
38. Sandberg JO, Eizirik DL, Sandler S. IL-1 receptor antagonist inhibits recurrence of disease after syngeneic pancreatic islet transplantation to spontaneously diabetic non-obese diabetic (NOD) mice. *Clin Exp Immunol*. 1997; 108:314–317. [PubMed: 9158104]

39. Gysemans C, et al. Prevention of primary non-function of islet xenografts in autoimmune diabetic NOD mice by anti-inflammatory agents. *Diabetologia*. 2003; 46:1115–1123. [PubMed: 12879250]
40. Simon G, et al. Murine antithymocyte globulin therapy alters disease progression in NOD mice by a time-dependent induction of immunoregulation. *Diabetes*. 2008; 57:405–414. [PubMed: 18039815]
41. Pozzilli P, et al. No effect of oral insulin on residual β -cell function in recent-onset type I diabetes (the IMDIAB VII). IMDIAB Group. *Diabetologia*. 2000; 43:1000–1004. [PubMed: 10990077]
42. Diabetes Prevention Trial—Type 1 Diabetes Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N Engl J Med*. 2002; 346:1685–1691. [PubMed: 12037147]
43. Ludvigsson J, et al. GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus. *N Engl J Med*. 2012; 366:433–442. [PubMed: 22296077]
44. Griffin KJ, Thompson PA, Gottschalk M, Kylo JH, Rabinovitch A. Combination therapy with sitagliptin and lansoprazole in patients with recent-onset type 1 diabetes (REPAIR-T1D): 12-month results of a multicentre, randomised, placebo-controlled, phase 2 trial. *Lancet*. 2014; 2:710–718. [PubMed: 24997559]
45. Sumpter KM, Adhikari S, Grishman EK, White PC. Preliminary studies related to anti-interleukin-1 β therapy in children with newly diagnosed type 1 diabetes. *Pediatr Diabetes*. 2011; 12:656–667. [PubMed: 21518168]
46. Moran A, et al. Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. *Lancet*. 2013; 381:1905–1915. [PubMed: 23562090]
47. Gitelman SE, et al. Antithymocyte globulin treatment for patients with recent-onset type 1 diabetes: 12-month results of a randomised, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol*. 2013; 1:306–316. [PubMed: 24622416]
48. Hayward AR, Shreiber M. Neonatal injection of CD3 antibody into nonobese diabetic mice reduces the incidence of insulinitis and diabetes. *J Immunol*. 1989; 143:1555–1559. [PubMed: 2527266]
49. Herold KC, et al. Prevention of autoimmune diabetes with nonactivating anti-CD3 monoclonal antibody. *Diabetes*. 1992; 41:385–391. [PubMed: 1532369]
50. Hayward AR, Shreiber M. Reduced incidence of insulinitis in NOD mice following anti-CD3 injection: requirement for neonatal injection. *J Autoimmun*. 1992; 5:59–67. [PubMed: 1532716]
51. Chatenoud L, Thervet E, Primo J, Bach JF. Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. *Proc Natl Acad Sci USA*. 1994; 91:123–127. [PubMed: 8278351]
52. Chatenoud L, Primo J, Bach JF. CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. *J Immunol*. 1997; 158:2947–2954. [PubMed: 9058834]
53. Hu CY, et al. Treatment with CD20-specific antibody prevents and reverses autoimmune diabetes in mice. *J Clin Invest*. 2007; 117:3857–3867. [PubMed: 18060033]
54. Xiu Y, et al. B lymphocyte depletion by CD20 monoclonal antibody prevents diabetes in nonobese diabetic mice despite isotype-specific differences in Fc γ R effector functions. *J Immunol*. 2008; 180:2863–2875. [PubMed: 18292508]
55. Keymeulen B, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med*. 2005; 352:2598–2608. [PubMed: 15972866]
56. Aronson R, et al. Low-dose otelexizumab anti-CD3 monoclonal antibody DEFEND-1 study: results of the randomized phase III study in recent-onset human type 1 diabetes. *Diabetes Care*. 2014; 37:2746–2754. [PubMed: 25011949]
57. Ambery P, et al. Efficacy and safety of low-dose otelexizumab anti-CD3 monoclonal antibody in preserving C-peptide secretion in adolescent type 1 diabetes: DEFEND-2, a randomized, placebo-controlled, double-blind, multi-centre study. *Diabet Med*. 2014; 31:399–402. [PubMed: 24236828]
58. Brady JL, Yamashita K, Lew AM. Enhanced survival of grafts genetically endowed with the ability to block CD2 and B7. *Cell Transplant*. 2001; 10:175–181. [PubMed: 11332632]
59. Kremer JM, et al. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. *N Engl J Med*. 2003; 349:1907–1915. [PubMed: 14614165]

60. Kremer JM, et al. Treatment of rheumatoid arthritis with the selective costimulation modulator abatacept: twelve-month results of a phase iib, double-blind, randomized, placebo-controlled trial. *Arthritis Rheum.* 2005; 52:2263–2271. [PubMed: 16052582]
61. Lenschow DJ, et al. CD28/B7 regulation of Th1 and Th2 subsets in the development of autoimmune diabetes. *Immunity.* 1996; 5:285–293. [PubMed: 8808683]
62. Lenschow DJ, et al. Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. *J Exp Med.* 1995; 181:1145–1155. [PubMed: 7532678]
63. Rabinovitch A, Suarez-Pinzon WL, Shapiro AM, Rajotte RV, Power R. Combination therapy with sirolimus and interleukin-2 prevents spontaneous and recurrent autoimmune diabetes in NOD mice. *Diabetes.* 2002; 51:638–645. [PubMed: 11872661]
64. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. *J Immunol.* 2004; 172:2731–2738. [PubMed: 14978070]
65. Mehta G, Ferreira VP, Skerka C, Zipfel PF, Banda NK. New insights into disease-specific absence of complement factor H related protein C in mouse models of spontaneous autoimmune diseases. *Mol Immunol.* 2014; 62:235–248. [PubMed: 25033230]
66. Neff KS, Richards SM, Williams JM, Garman RD, Ruzek MC. Murine antithymocyte globulin T-cell depletion is mediated predominantly by macrophages, but the Fas/FasL pathway selectively targets regulatory T cells. *Transplantation.* 2011; 92:523–528. [PubMed: 21804441]
67. Young EF, Hess PR, Arnold LW, Tisch R, Frelinger JA. Islet lymphocyte subsets in male and female NOD mice are qualitatively similar but quantitatively distinct. *Autoimmunity.* 2009; 42:678–691. [PubMed: 19886740]
68. Taylor JA, et al. A spontaneous model for autoimmune myocarditis using the human MHC molecule HLA-DQ8. *J Immunol.* 2004; 172:2651–2658. [PubMed: 14764740]
69. Mohan JF, et al. Unique autoreactive T cells recognize insulin peptides generated within the islets of Langerhans in autoimmune diabetes. *Nat Immunol.* 2010; 11:350–354. [PubMed: 20190756]
70. Yin L, et al. Recognition of self and altered self by T cells in autoimmunity and allergy. *Protein Cell.* 2013; 4:8–16. [PubMed: 23307779]
71. Collado JA, Guitart C, Ciudad MT, Alvarez I, Jaraquemada D. The repertoires of peptides presented by MHC-II in the thymus and in peripheral tissue: a clue for autoimmunity? *Front Immunol.* 2013; 4:442. [PubMed: 24381570]
72. Homann D, Dyrberg T, Petersen J, Oldstone MB, von Herrath MG. Insulin in oral immune “tolerance”: a one-amino acid change in the B chain makes the difference. *J Immunol.* 1999; 163:1833–1838. [PubMed: 10438916]
73. Skyler JS, et al. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial—Type 1. *Diabetes Care.* 2005; 28:1068–1076. [PubMed: 15855569]
74. Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. *Annu Rev Immunol.* 1996; 14:233–258. [PubMed: 8717514]
75. Brauner H, et al. Distinct phenotype and function of NK cells in the pancreas of nonobese diabetic mice. *J Immunol.* 2010; 184:2272–2280. [PubMed: 20130214]
76. Coppieters KT, et al. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. *J Exp Med.* 2012; 209:51–60. [PubMed: 22213807]
77. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Analysis of islet inflammation in human type 1 diabetes. *Clin Exp Immunol.* 2009; 155:173–181. [PubMed: 19128359]
78. Greenbaum CJ, et al. Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite Type 1 Diabetes TrialNet data. *Diabetes.* 2012; 61:2066–2073. [PubMed: 22688329]
79. Sherry NA, et al. Effects of autoimmunity and immune therapy on β -cell turnover in type 1 diabetes. *Diabetes.* 2006; 55:3238–3245. [PubMed: 17130466]
80. Serreze DV, et al. Loss of intra-islet CD20 expression may complicate efficacy of B-cell-directed type 1 diabetes therapies. *Diabetes.* 2011; 60:2914–2921. [PubMed: 21926271]

81. Koulmanda M, et al. Curative and β cell regenerative effects of $\alpha 1$ -antitrypsin treatment in autoimmune diabetic NOD mice. *Proc Natl Acad Sci USA*. 2008; 105:16242–16247. [PubMed: 18852471]
82. Ogawa N, List JF, Habener JF, Maki T. Cure of overt diabetes in NOD mice by transient treatment with anti-lymphocyte serum and exendin-4. *Diabetes*. 2004; 53:1700–1705. [PubMed: 15220193]
83. Suarez-Pinzon WL, Rabinovitch A. Combination therapy with a dipeptidyl peptidase-4 inhibitor and a proton pump inhibitor induces β -cell neogenesis from adult human pancreatic duct cells implanted in immunodeficient mice. *Cell Transplant*. 2011; 20:1343–1349. [PubMed: 21396168]
84. Akirav E, Kushner JA, Herold KC. β -cell mass and type 1 diabetes: going, going, gone? *Diabetes*. 2008; 57:2883–2888. [PubMed: 18971435]
85. Zhang N, et al. Sirolimus is associated with reduced islet engraftment and impaired β -cell function. *Diabetes*. 2006; 55:2429–2436. [PubMed: 16936190]
86. Keenan HA, et al. Residual insulin production and pancreatic β -cell turnover after 50 years of diabetes: Joslin Medalist Study. *Diabetes*. 2010; 59:2846–2853. [PubMed: 20699420]
87. Palmer JP, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve β -cell function: report of an ADA workshop, 21–22 October 2001. *Diabetes*. 2004; 53:250–264. [PubMed: 14693724]
88. Yu L, et al. Rituximab selectively suppresses specific islet antibodies. *Diabetes*. 2011; 60:2560–2565. [PubMed: 21831969]
89. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J*. 2008; 22:659–661. [PubMed: 17942826]
90. Akirav EM, et al. Detection of β cell death in diabetes using differentially methylated circulating DNA. *Proc Natl Acad Sci USA*. 2011; 108:19018–19023. [PubMed: 22074781]
91. Kuhn C, et al. Human CD3 transgenic mice: preclinical testing of antibodies promoting immune tolerance. *Sci Transl Med*. 2011; 3:68ra10.
92. Brehm MA, et al. Human immune system development and rejection of human islet allografts in spontaneously diabetic NOD-Rag1null IL2r γ null Ins2Akita mice. *Diabetes*. 2010; 59:2265–2270. [PubMed: 20570944]
93. Waldron-Lynch F, et al. Teplizumab induces human gut-tropic regulatory cells in humanized mice and patients. *Sci Transl Med*. 2012; 4:118ra12.
94. Vudattu NK, et al. Humanized mice as a model for aberrant responses in human T cell immunotherapy. *J Immunol*. 2014; 193:587–596. [PubMed: 24943216]
95. Viehmann Milam AA, et al. A humanized mouse model of autoimmune insulinitis. *Diabetes*. 2014; 63:1712–1724. [PubMed: 24478396]
96. Bergerot I, Fabien N, Mayer A, Thivolet C. Active suppression of diabetes after oral administration of insulin is determined by antigen dosage. *Ann NY Acad Sci*. 1996; 13:362–367. [PubMed: 8610991]
97. Chaillous L, et al. Oral insulin administration and residual (β -cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial. *Lancet*. 2000; 356:545–549. [PubMed: 10950231]
98. Hartmann B, Bellmann K, Ghiea I, Kleemann R, Kolb H. Oral insulin for diabetes prevention in NOD mice: potentiation by enhancing Th2 cytokine expression in the gut through bacterial adjuvant. *Diabetologia*. 1997; 40:902–909. [PubMed: 9267984]
99. Grinberg-Bleyer Y, et al. IL-2 reverses established type 1 diabetes in NOD mice by a local effect on pancreatic regulatory T cells. *J Exp Med*. 2010; 207:1871–1878. [PubMed: 20679400]
100. Koulmanda M, et al. Modification of adverse inflammation is required to cure new-onset type 1 diabetic hosts. *Proc Natl Acad Sci USA*. 2007; 104:13074–13079. [PubMed: 17670937]
101. Tang Q, et al. Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. *Immunity*. 2008; 28:687–697. [PubMed: 18468463]
102. Suarez-Pinzon WL, et al. Combination therapy with glucagon-like peptide-1 and gastrin restores normoglycemia in diabetic NOD mice. *Diabetes*. 2008; 57:3281–3288. [PubMed: 18835930]

Table 1

Comparisons of preclinical and clinical studies

Treatment	Preclinical results*	Clinical trial results	Problems in translation
Parental insulin	Prevention of diabetes mellitus in NOD mice ³¹	No prevention of T1DM in high-risk individuals ⁴²	Dosing regimens not comparable
Oral insulin	Prevention of T1DM in NOD mice that received porcine insulin ³⁰ Recombinant human insulin reduces lymphocytic infiltration ⁹⁶ Single amino acid change at position 30 in the B chain of human insulin prevents tolerance to insulin in NOD mice ⁷²	Did not prevent progression to T1DM overall but significant improvement in patients with higher titres of anti-insulin antibodies ⁹⁷	Specificity of oral insulins for conferring tolerance in T1DM was confirmed in a follow-up study with mice that occurred while human trials were already underway ⁷² Only certain forms of insulin induce the production of cytokines ^{72,98}
Teplizumab and oteplixumab (FcR non-binding anti-CD3 mAbs)	Prevented diabetes mellitus induced with low-dose streptozotocin in mice ⁴⁹ Reversed diabetes mellitus ⁵¹ Prevented NOD pups from developing T1DM if injected as neonates ⁴⁸	Treatment maintained increased C-peptide levels and reduced insulin requirements in recent-onset T1DM ^{22,55} Reduced β -cell death ²²	Effects were not permanent as in mice but some individuals showed robust responses Does the permanent reversal in NOD mice model these patients?
Rituximab (anti-hCD20)	Prevented the onset of T1DM and reversed hyperglycaemia in transgenic NOD mice expressing hCD20 ⁵³ Induced regulatory B cells ⁵³	Improved C-peptide response at 1 but not 2 years ²⁵	Transient effect of drug treatment. Disease recurred with recovery of B lymphocytes ²⁵ Effects of drug treatment on antigen-specific responses unclear ²⁵ Selective effects on autoantibody responses ^{25,88}
Abatacept (CTLA4-Ig)	Prevented onset of T1DM in prediabetic NOD mice but not effective when administered after 8 weeks of age ⁶² CTLA4-Ig transgene accelerated T1DM in NOD mice ⁶¹	Successful delay in decline of C-peptide responses ^{27,28}	Kinetics of disease and role of positive and negative T cell co-stimulation for autoimmune responses at different stages? Differences in expression of CD28 on T cells ⁷⁴
Rapamycin and IL-2	Treatment of NOD mice with the combination of rapamycin and IL-2 prevented T1DM incidence ⁶³ In treated mice, there was increased FoxP3 ⁺ T _{REG} cells ^{63,99–101}	Trial discontinued due to poor initial results ²⁹	Rapamycin could be toxic for human β cells, ⁸⁵ which might be augmented by IL-2 ²⁹ The increases in NK cells might account for β -cell losses ²⁹ The effects of IL-2 on NK cells in NOD mice might not be appreciated because of mutations in pancreatic NK cells in NOD mice ⁷⁵
Antithymocyte globulin	Prevented and delayed the progression of T1DM ⁴⁰ With exendin-4 (glucagon-like peptide 1 receptor agonist), reversed diabetes mellitus in NOD mice ⁸²	β -cell function was not preserved ⁴⁷ Relative proportion of T _{REG} cells was not enhanced ⁴⁷	No evidence for establishment of regulatory mechanisms Complement deficiency in NOD mice might explain why induced T _{REG} cells could survive in mice but not in humans ⁶⁶
Alefacept (soluble LFA3-Ig)	Promoted the survival and growth of insulinoma grafts ⁵⁸	No difference in 2 h C-peptide response but a statistically significant increased 4 h C-peptide response ²⁶ Decreased number of effector and memory T cells ²⁶	Clinical end point chosen for analysis (2 h vs 4 h C-peptide response to a mixed meal) accounted for statistical outcome ²⁶ Mice do not have the <i>CD58</i> gene that binds CD2 but have <i>CD48</i> , which binds CD2 with lower affinity ⁶⁴
Sitagliptin and lansoprazole	Reversed diabetes mellitus by restoring normal glucose levels in mice ³⁵	No effect on loss of C-peptide responses in new-onset patients ⁴⁴	Effect on autoimmunity not clear ⁴⁴ Differences in the proliferative capacity of human and murine β cells

Treatment	Preclinical results*	Clinical trial results	Problems in translation
	Induced β -cell growth of human islets transplanted into the exocrine pancreatic ducts of mice ⁸³ Reduced insulin autoantibodies and results in a cytokine shift in infiltrating leukocytes from IFN- γ to TGF- β ¹⁰²		

* Data are from published analyses. Abbreviations; FcR, Fc receptor; mAbs, monoclonal antibodies; NK, natural killer; NOD, nonobese diabetic; T1DM, type 1 diabetes mellitus; TREG, regulatory T cells.