



APPLICATION OF *IN SILICO* MODELING AND BIOSIMULATION TO TYPE 1 DIABETES RESEARCH

Table of Contents

Overview..... 1

In Silico Modeling and Biosimulation..... 2

 Overview..... 2

 Applying Biosimulation to Basic Research..... 2

Type 1 Diabetes PhysioLab Platform..... 3

 Model Overview..... 3

 Virtual NOD Mice..... 4

 Virtual NOD Mouse Responses 5

 Platform Limitations..... 6

Methodologies 7

 Representation of Islet iTreg Lifecycle 7

Case Studies 11

 Identifying Key Mechanisms for Anti-CD3 Efficacy..... 11

 Investigating mechanisms of disease heterogeneity..... 12

 Dosing and Timing Effects of Anti-CD40L Therapy..... 13

Reference List..... 15

Overview

The Diabetes Research Center (DRC) is an initiative of the American Diabetes Association (ADA) and Entelos, Inc. to enhance the understanding of diabetes and its treatment. The DRC seeks to support innovative basic research by providing academic researchers access to the Center’s *in silico* modeling and biosimulation capabilities, which includes the Type 1 Diabetes PhysioLab® platform, a computer-based mathematical model of the non-obese diabetic (NOD) mouse.

The DRC is soliciting letters of intent (LOI) for research projects to be conducted in the second half of 2007. LOIs should include a description of the applicant’s current area of type 1 diabetes (T1D) research (1-2 pages) and specific research questions to be addressed (< 1 page). LOI’s will be reviewed by an ADA-sponsored scientific review committee. Projects of scientific value will be selected, and researchers will be invited to develop a detailed research plan in collaboration with DRC staff. The review committee will then award research grants. The *in silico* research results provided by the DRC are expected to complement laboratory research and enhance the understanding of diabetes pathogenesis and treatment.

This document provides an overview of *in silico* modeling and biosimulation, the Type 1 Diabetes PhysioLab platform, and the DRC’s modeling approach. This unique research capability is then illustrated through the presentation of three case studies.

LOI receipt deadline: 15 February 2007; Award announcement: 1 June 2007.

In Silico Modeling and Biosimulation

Overview

Modeling and simulation is an investigative research approach that can be used to understand complex biological systems. Simulations of biological systems, in particular, are termed “biosimulations.” Entelos developed its proprietary PhysioLab modeling and biosimulation software platform to enable researchers to study the dynamic behavior of complex biological systems.

Each Entelos PhysioLab platform is constructed to describe whole-system behaviors (*e.g.*, how an animal can become diabetic) and the components that give rise to them (*e.g.*, how autoreactive T cells kill insulin-producing β cells). Biosimulations are performed by computing the future state of the dynamic system based on different initial conditions (*e.g.*, the development of diabetes as a consequence of whether or not innate regulatory T cells are ‘knocked out’). This approach provides a powerful means of investigating the impact of hypotheses regarding how specific components drive the behavior of the system as a whole (*e.g.*, the effect of varying regulatory T cell cytokine production on diabetes progression). Moreover, this approach can be used to explore the underlying mechanisms that give rise to observed biological variability (*e.g.*, the heterogeneity seen in diabetes progression rates).

Entelos’ large-scale computer models quantitatively and mechanistically represent the interactions of biological components (*e.g.*, proteins, cytokines, cell populations, etc.) using mathematical equations. By systematically altering the model values that define cell populations and functions and running biosimulations, researchers can investigate the specific contribution of cell types or pathways on disease pathogenesis (*e.g.*, how does inhibiting B cell antigen presentation affect diabetes outcome?). Insights generated by this approach can be used to design laboratory experiments and/or interpret existing research data. In summary, *in silico* research is complementary to current experimental approaches and has the potential to dramatically enhance the way basic research is conducted today.

Applying Biosimulation to Basic Research

Developing a mathematical model of biological processes and disease states requires researchers to systematically confront and resolve gaps in their knowledge regarding relevant physiology. Once these “knowledge gaps” (*i.e.*, biological uncertainties) are identified, their impact on the system and its dynamics can be explored *in silico* by formulating and evaluating hypotheses to explain them. Each of these hypotheses is explicitly represented in the model as a “virtual mouse.” In the Type 1 Diabetes PhysioLab platform, virtual mice represent the cellular functions and biochemical interactions in tissue compartments considered critical for the pathogenesis of T1D in NOD mice. Biosimulations conducted on virtual mice enable the researcher to determine if and how a knowledge gap affects disease outcome. If there is no effect, resolution of that knowledge gap will likely not facilitate understanding of the disease process. However, if the knowledge gap has a profound effect on disease outcome (*e.g.*, diabetes *vs.* protection), then biosimulation results can assist the researcher in designing laboratory experiments to resolve the biological uncertainty. This process is faster and more flexible than running comparable laboratory experiments, allowing researchers to rapidly develop and test “*what if*” scenarios.

Additionally, *in silico* approaches can complement laboratory-based methods, and help address complex scientific questions more effectively and efficiently. For example, results of *in silico* experiments allow researchers to not only understand the effect of their research question on disease outcome, but also the mechanistic underpinnings that give rise to that outcome. This mechanistic understanding can be applied to choose the optimal experimental approach needed to refute or confirm their hypotheses. As a result, data collection efforts are better focused, assays are better designed, and the resulting data are more effectively interpreted. This information can then feedback to further validate model components and predictions. Ultimately, this two-way exchange between *in silico* and laboratory research can lead to better use of resources.

Type 1 Diabetes PhysioLab Platform

The Type 1 Diabetes PhysioLab platform was developed over a two-year partnership between Entelos and the ADA. Scientific guidance and oversight was provided by a Scientific Advisory Board comprised of leading experts in the field of diabetes: Mark Atkinson, Ph.D. (University of Florida); Jeffrey Bluestone, Ph.D. (University California, San Francisco); George Eisenbarth, M.D., Ph.D. (University Colorado at Denver and Health Science Center); Diane Mathis, Ph.D. (The Joslin Diabetes Center); and Aldo Rossini, M.D. (University of Massachusetts). This section provides a brief overview of the platform and its components, an introduction to the concept of virtual mice, and a discussion of platform limitations.

Model Overview

The Type 1 Diabetes PhysioLab platform is a large-scale, mechanistic mathematical model of the female non-obese diabetic (NOD) mouse designed to support research in type 1 diabetes. In this platform, virtual NOD mice are constructed using a series of nonlinear ordinary differential and algebraic equations that mathematically represent cellular functions and biochemical interactions in tissue compartments considered critical for the pathogenesis of type 1 diabetes (Figure 1). The model encompasses two main tissues: pancreatic lymph nodes (PLNs) and pancreatic islets. Antigen and antigen-presenting cells traffic from the islets to the PLN, and conversely, lymphocytes activated in the PLN can enter the blood and be recruited to the islets.

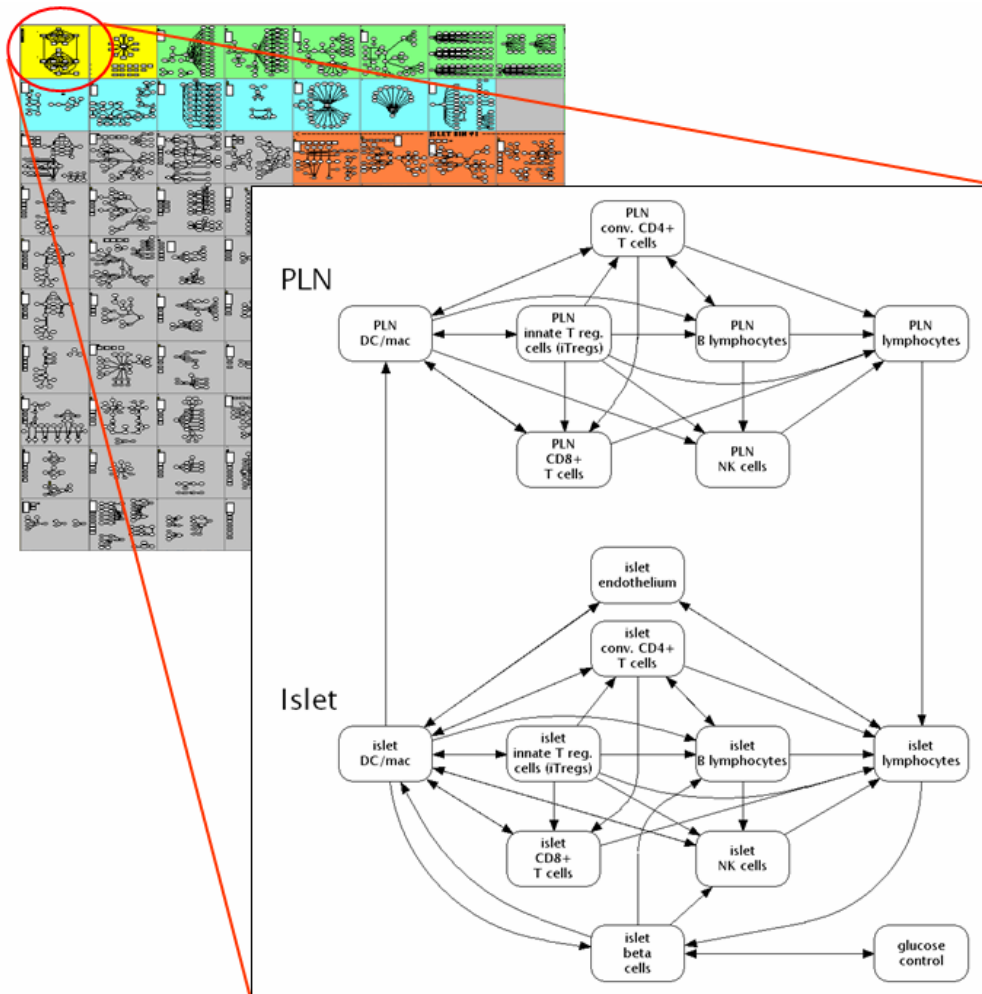


Figure 1: High-level summary diagram from the Type 1 Diabetes PhysioLab platform illustrating the major tissues, cell types, and interactions represented in virtual NOD mice.

The leukocyte cell types represented in both the PLN and islet compartments are listed in Table 1. The islet compartment also includes the pancreatic β cell population. For each cell type represented in the model, turnover (*e.g.*, proliferation and apoptosis), differentiation, activation, and trafficking, as well as functions important for disease pathogenesis (*e.g.*, mediator secretion, antigen presentation, and contact-mediated cytotoxicity) are included as appropriate. Additional elements depicted within the platform include specific drugs, compounds and therapies, and experimental measurements (*i.e.*, model outputs). Gut (intestinal) tissue and a prototypical gut-associated lymphoid tissue (GALT) are also represented to capture orally administered therapies.

Table 1: Components represented in the Type 1 Diabetes PhysioLab platform.

Cell types	Major Functions/Pathways
CD4+ T cells Th1 Th2 aTregs CD4+CD25+ T cells iTregs Natural killer T cells (NKT) CD8+ T cells B lymphocytes Natural killer (NK) cells Dendritic cells (DCs) Macrophages	Depletion of inflammatory cell populations No inflammatory infiltration Depletion of various B cell functions No maternal autoantibodies Depletion of endogenous autoantibodies PLN-ectomy
Outputs	Drugs/Compounds/Therapies
Age at diabetes onset Occurrence of remission Age at remission onset Beta-cell mass Percentage of islets infiltrated Plasma insulin Plasma glucose Autoantibody concentration Cell populations and activation states in islets and PLN Soluble mediators in islets and PLN	Exogenous IL-10 Anti-CD3 Anti-B7.1/B7.2 Anti-CD8 LipCl2MDP TGF- β Anti-CD40L Exendin-4 Rapamycin Anti-IL-2 Oral Insulin

Virtual NOD Mice

The PhysioLab platform currently contains virtual NOD mice phenotypes representing variations in disease progression rate (*i.e.*, early-, average-, and late-onset). To develop virtual mice, quantitative data are obtained from published *in vivo* and *in vitro* studies and used to define parameter values for cell populations and biological functions. In cases where data are not available, values are assigned to ensure that high-level behaviors are consistent with known biology at both the subsystem (*e.g.*, PLN CD4+ T cell expansion dynamics) and the system levels (*e.g.*, dynamics of diabetes onset, appropriate responses to published disease-modulating agents).

Individual virtual mice within the PhysioLab platform are calibrated to reproduce the high-level behaviors of an untreated female NOD mouse (*untreated phenotype*; Table 2) and its response to a set of published interventions (*interval validation*). Each mouse is then tested to see if it responds appropriately to another set

of interventions that were not used in the development of the platform (external validation). Virtual mice are used for *in silico* research only when their behavior is consistent with the external validation criteria.

Table 2: Summary of the tests used to (a) assess the behavior of virtual NOD mice and calibrate the underlying representation of the biology to achieve the expected phenotype (*internal validation*), and (b) validate virtual NOD mice by evaluating their response to protocols not used in their development (*external validation*).

Untreated Phenotype	Internal Validation	External Validation
PLN expansion dynamics Islet infiltration dynamics Developmental increase in β cell mass Multiple modes of β cell death Normoglycemia until diabetes onset Rapid onset of hyperglycemia Diabetes onset between 12-35 wks	Anti-CD8 Anti-CD3 Exogenous IL-10 Anti-B7-1 + anti-B7-2 LipCI2MDP No diab. CD4+ T cells No diab. CD8+ T cells No diab. iTregs No diab. B cells No maternal autoantibodies No endog. autoantibodies	Anti-CD40L TGF- β Exendin-4 Rapamycin Anti-IL-2

Virtual NOD Mouse Responses

In the Type 1 Diabetes PhysioLab platform, virtual NOD mice exhibit different disease phenotypes (*e.g.*, early, average, and late-onset of diabetes), as well as variations in the underlying pathophysiology (*e.g.*, NK cells having net pro-inflammatory *vs.* anti-inflammatory activity). Comparison of the untreated behaviors of virtual mice to published data show that the dynamics of diabetes onset, as well as PLN lymphocyte expansion and islet infiltration are consistent with available data (Figure 2). Together with internal and external validation tests, these comparisons indicate that the virtual NOD mice reasonably represent known phenotypic diversity and underlying biology. Further, the simulation results illustrate the ability of the model to provide longitudinal data on disease processes from the whole-animal level to the cellular/molecular level.

The full value of biosimulation is realized when researchers are able to predict biological responses beyond available data and understand the mechanistic underpinnings of the predicted response, then use this information to plan laboratory experimentation. Ultimately biosimulation and laboratory experimentation are meant to be used in an iterative research process. The speed of *in silico* experiments (minutes to hours) further allows the rapid investigation of multiple hypotheses. Together, the transparency and speed of biosimulation allows researchers to narrow in on the critical experiments that will provide definitive data to address their question of interest.

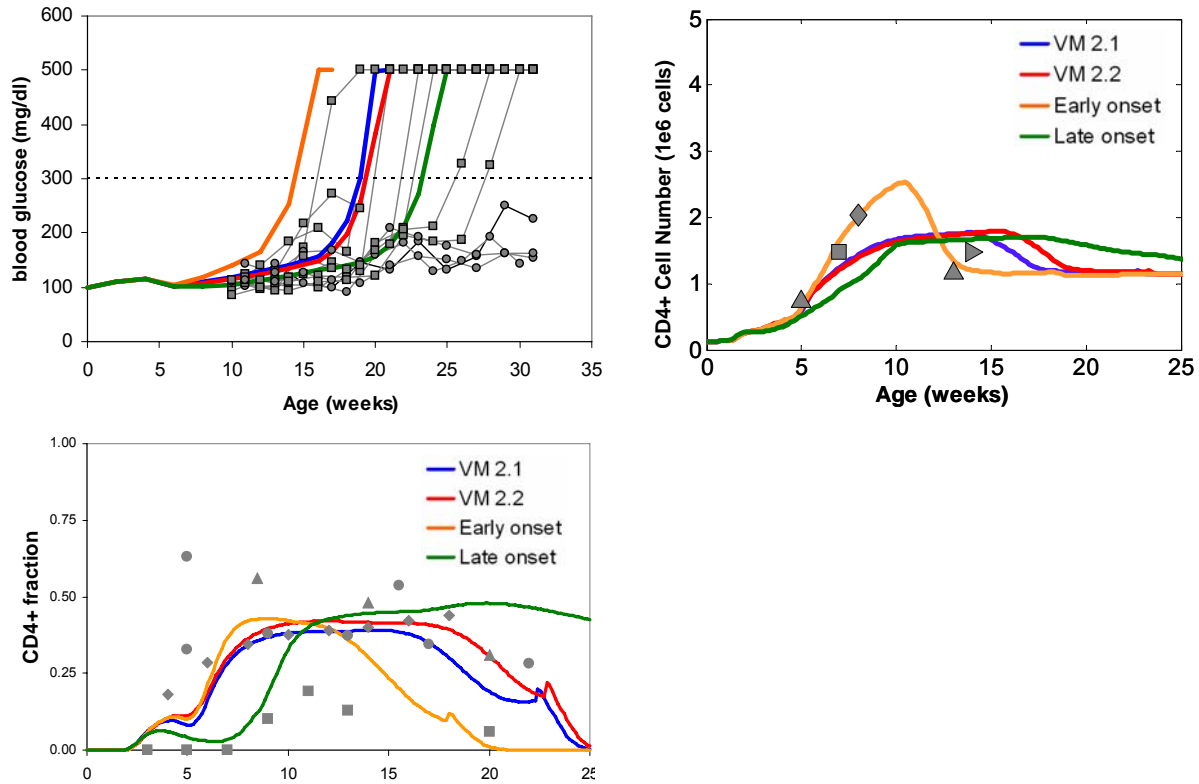


Figure 2: Response of virtual NOD mice during disease progression. Simulated blood glucose profiles (*upper left*), leukocyte expansion in the PLN (*upper right*), and leukocyte infiltration into pancreatic islets (*bottom*) all fall into the expected biological response range. Solid lines represent virtual mouse responses and symbols designate reported data points.

Platform Limitations

The Type 1 Diabetes PhysioLab platform encompasses key cell types and tissues involved in the disease. Biology for which there are insufficient supporting data is either not represented, or represented in a consolidated manner. These include:

- Thymus - modeled as a source of naive T cells only, positive and negative selection not explicitly represented
- Other peripheral lymph nodes and the spleen - not explicitly represented
- Blood compartment - modeled only to the degree necessary to track the flow of cells
- Regulatory CD8+ T cells and gamma/delta cells - not explicitly represented
- Interactions among multiple distinct antigen-specific clones – diabetes-specific lymphocytes are modeled in bulk; the relative contribution of a clone can be investigated
- Memory T or B cells – not explicitly represented, some functionality (*e.g.*, lesser dependence on costimulation, greater cytokine production) is represented in the effector T cell populations

Methodologies

The Type 1 Diabetes PhysioLab platform was developed using a top-down modeling approach. Briefly, the process begins with a detailed literature review to determine what key elements should be represented in the platform and identify the appropriate data that describe these elements (platform scope). Once the platform scope is defined the overall mathematical structure is developed. The model is then calibrated to ensure that quantitative data and overall behaviors are accurately captured. This section provides a detailed description of that approach, using development of the islet innate regulatory T cell (iTreg) life cycle compartment to illustrate the process. It also provides a sense for how experimental data are converted to mathematical equations which are then used to construct the platform.

Representation of Islet iTreg Lifecycle

In the Type 1 Diabetes PhysioLab platform, the iTreg population represents CD4+CD25+ T cells and Natural Killer T (NKT) cells and includes the following functionalities: (1) activation through antigen exposure and cell contact in the PLN and pancreas; (2) population dynamics in the PLN, including influx, proliferation, apoptosis, and exit; (3) population dynamics in the pancreas, including influx and apoptosis; (4) secretion of soluble suppressive mediators in the PLN and pancreas; (5) modulation of antigen-presenting cell function through cell contact in the PLN and pancreas; and (6) suppression of other cell types through cell contact in the PLN and pancreas.

Representation of iTregs in the Type 1 Diabetes PhysioLab platform is calibrated using data from *in vitro* studies of iTreg function and is constrained by the overall behavior of the virtual NOD mouse regarding pathogenesis (*e.g.*, appropriate timing of insulinitis, diabetes onset) and therapeutic responses (*e.g.*, anti-B7.1/B7.2). The parameter values assigned to individual elements within this portion of the platform were determined using the following experimental data:

- The fraction of iTregs in the thymus, PLN, and pancreas ranges from 4-10%^{1,2,3,4,5}
- Activated iTregs are capable of suppressing CD4+ T cells^{6,7,8,9,10}, CD8+ T cells^{11,12}, B lymphocytes^{13,14}, NK cells^{12,15}, and DCs/macrophages^{16,17,18,19} in an antigen non-specific manner, either through cell-cell contact or by secretion of suppressive cytokines such as TGF-beta and IL-10
- Cytokine synthesis rates of iTregs are based on *in vitro* IL-10 and TGF-beta secretion data^{8,20}
- iTregs are less prone to activation-induced cell death (AICD) than conventional CD4+ T cells²¹
- iTreg proliferation is regulated by different factors, including TGF-beta^{22,23,24}
- *In silico* iTreg depletion results in disease exacerbation comparable to what is observed in B7.1/B7.2 or CD28 knockout NOD mice²⁵

The iTreg lifecycle is represented in a manner analogous to that of conventional CD4+ T lymphocyte lifecycle, with initial activation and expansion occurring in the PLN. Although the site of initial iTreg activation and expansion is not definitively known, this representation is consistent with data reported in the literature^{26,27}. Recently activated iTregs are then recruited to pancreatic islets, where they may undergo further activation and expansion. In the islets, iTregs interact with other cell types *via* soluble mediators and/or cell-cell contact. These interactions depend on the number and activation state of the iTregs.

One aspect of T1D disease heterogeneity is the presence of islets that are at different stages of disease progression (*i.e.*, uninfiltated, mildly infiltrated, heavily infiltrated, destroyed). This heterogeneity is reproduced in the Type 1 Diabetes PhysioLab platform by the inclusion of ten different islet bins. Each bin represents a portion of the total islets in the pancreas. Islet bins are progressively infiltrated over the course of disease progression. One islet bin, called the “uninvolved islet bin,” provides the capability of having a proportion of the islets remain infiltrate-free throughout disease. A graphical representation of the processes governing iTreg population dynamics, including recruitment, proliferation, and apoptosis, in a single islet bin (islet bin #1) is shown in Figure 2. Other islet bins (islet bin # 2-9) are represented in an identical manner.

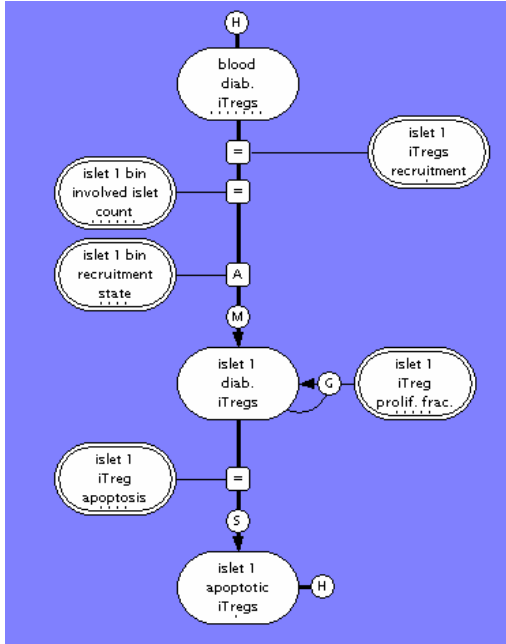


Figure 3: Representation of iTreg lifecycle for one islet bin in the Type 1 Diabetes PhysioLab platform.

Mathematically, the iTreg population dynamics can be represented as:

$$\frac{dX_I}{dt} = R_r + R_p - R_a \quad \text{equation 1}$$

where, X_I is the number of activated iTregs in the islet, dXI/dt represents the rate of change of the number of activated iTregs in the islet, R_r the rate of recruitment from the blood, R_p the rate of proliferation, and R_a the rate of apoptosis.

Derivation of recruitment rate (R_r)

As represented in Figure 2, the rate of recruitment of iTregs into the islet depends on the number of iTregs in the peripheral blood and is governed by three nodes: *islet 1 iTreg recruitment* (r_r), *islet 1 bin recruitment state* (r_s), and *islet 1 bin involved islet count* (i_c). The node *islet bin recruitment state* specifies whether or not the islets are infiltrated by autoimmune lymphocytes. Thus,

$$r_s = 0 \text{ if bin is not infiltrated;} \\ r_s = 1 \text{ if bin is infiltrated}$$

Further, each islet bin can exhibit a range of infiltrations, with some islets being infiltrated while others are infiltration free. The node *islet 1 bin involved islet count* determines the number of infiltrated (involved) islets in bin 1 and is calculated as:

$$i_c = f_{i1} T_I \quad \text{equation 2}$$

where, f_{i1} represents the fraction of all pancreatic islets that are included in bin 1 and T_I represents the total number of islets in the pancreas. The node *islet 1 iTreg recruitment* represents the rate of iTreg influx into islets and is the sum of two components: (1) a constitutive influx of iTregs that is independent of adhesion molecule upregulation on endothelial cells, and (2) an iTreg influx that is dependent on the upregulation of endothelial cell adhesion molecules, represented as:

$$r_r = r_r^{\max} (f_c + U_{EC} (1 - f_c)) \quad \text{equation 3}$$

where, r_r^{\max} represents the maximum recruitment rate, f_c the constitutive fraction, and U_{EC} the effect of endothelial cell adhesion molecules on recruitment.

The iTreg population in the peripheral blood is dependent on the rate of activated iTregs entering the peripheral blood from the PLN (g), the recruitment into the different islet bins (R_{ri}), the half life ($t_b^{1/2}$), and the loss of cells due to apoptosis. The dynamics of the iTreg circulating population is represented as:

$$\frac{dX_b}{dt} = g - \sum_i R_{ri} - \frac{\ln(2)X_b}{t_b^{1/2}} \quad \text{equation 4}$$

The recruitment of the iTregs into the islets is thus represented as:

$$R_r = r_r r_s i_c X_b \quad \text{equation 5}$$

Derivation of proliferation rate (R_p)

The rate of iTreg proliferation in the islets is determined by the fraction of the cells entering mitosis (f_p) and the proliferation rate constant (p_c). The proliferating fraction is driven by the activation of iTregs by antigen-presenting cells and is further regulated by both soluble mediators and cell-cell contact. The proliferation rate is represented as:

$$R_p = f_p p_c X_I \quad \text{equation 6}$$

Derivation of apoptosis rate (R_a)

Similarly, the rate of apoptosis is determined by the fraction of the cells entering the apoptotic cascade (f_a) and the apoptosis rate constant (a_c). The apoptotic fraction accounts for both death by neglect and activation-induced cell death and is regulated by soluble mediators. The apoptosis rate is represented as:

$$R_a = f_a a_c X_I \quad \text{equation 7}$$

The population dynamics of the apoptotic iTregs in the islets is determined by the rate of apoptosis of the activated iTregs and the rate of elimination via phagocytosis and degradation characterized by a half life ($t_a^{1/2}$). The apoptotic cell population is represented as:

$$\frac{dX_a}{dt} = R_a - \frac{\ln(2)X_a}{t_a^{1/2}} \quad \text{equation 8}$$

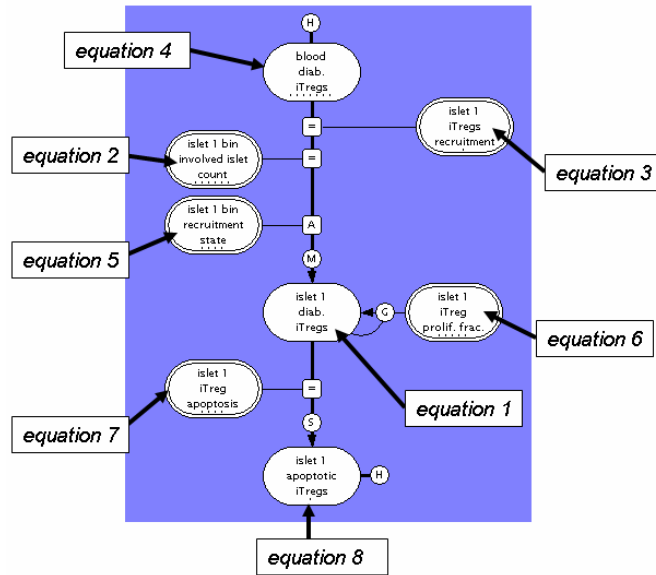


Figure 4: Representation of the iTreg lifecycle in the Type 1 Diabetes PhysioLab platform showing the location of derived mathematical expressions.

Parameter value designations

The parameter values described for the equations above are derived from the following data:

- The total number of pancreatic islets (T_I) is specified as 2000^{28}
- The rate of iTreg apoptosis is assumed to be similar to that of CD4+ T cells²⁹, suggesting a half life ($t_b^{1/2}$) of approximately 46 hours
- The proliferation rate constant of iTregs is assumed to be similar to effector CD4+ T cells and specified as $\ln(2)/8 \text{ h}^{-1}$ ³⁰, corresponding to the reported cycle time of 8 hours³¹
- The apoptotic rate constant is specified as $\ln(2)/5 \text{ h}^{-1}$, corresponding to the observation that 52% of activated murine T cells are apoptotic after 5 hours³²

In summary, the mathematical representation of iTregs in the Type 1 Diabetes PhysioLab platform characterizes the cell type’s lifecycle based on all available data. As many aspects of iTreg population dynamics that are still unknown, the virtual iTreg cell population is internally validated to match publicly available data demonstrating 4-10% iTregs within the total CD4+ T cell population of the PLN at around 6-8 weeks of age. In addition, the overall behavior of the modeled iTreg population is internally validated to match the reported effects of iTreg deficiency and dysregulation. Specifically, an imposed deficiency in the iTreg population via a ‘depletion’ experiment or blockade of iTreg activation via anti-B7.1/B7.2 therapy exacerbates disease, resulting in accelerated diabetes onset in the virtual NOD mouse^{25,33}.

The parameter values assigned in the underlying mathematic expressions reproduce the observed behaviors described for the iTreg lifecycle. Because there is a range in the data reported, more than one set of parameter values can satisfy the constraints of the experimentally observed iTreg behavior. To investigate potential variability in iTreg parameters, these parameters can be modified to represent alternate virtual mice. Further, they can be modified as other experimental data and constraints become available.

Case Studies

Research using the Type 1 Diabetes PhysioLab platform enables scientists to perform experiments *in silico* prior to doing the laboratory experiment. In addition, the PhysioLab platform allows researchers to ask questions that could be difficult to investigate in a laboratory, or would require significant time and/or resources to address. For example, armed with the platform's capabilities, researchers can assess and predict (1) the contribution of key cell types to disease pathogenesis and prevention, (2) the time- and dose-dependent effects of treatments on disease prevention or remission, and (3) different hypotheses to identify and design key laboratory experiments. To illustrate how the Type 1 Diabetes PhysioLab platform can be applied to basic research, a selection of case studies is provided below.

Identifying Key Mechanisms for Anti-CD3 Efficacy

The success of anti-CD3 therapy to induce remission in newly diabetic NOD animals has propelled this treatment into diabetic humans. Currently, a non-mitogenic form of anti-CD3 antibody is in phase II clinical trials to test its efficacy in recent onset diabetic human patients.¹ However, the critical mechanism(s) mediating anti-CD3-induced remission are not completely elucidated. Elucidation of the mechanisms critical for anti-CD3-mediated remission in mice could lead to more targeted treatments in humans. To gain insight into this question, we investigated the actions of anti-CD3 therapy using the Type 1 Diabetes PhysioLab platform.²

The anti-CD3 antibody most commonly used to induce remission in newly diabetic NOD mice is a mitogenic antibody that induces proliferation, cytokine production, and apoptosis of T cells. Interestingly, a key factor that appears critical for remission is the ability of anti-CD3 to induce the accumulation and activity of regulatory T cells^{34,35}. The various pharmacodynamic activities attributed to that antibody were implemented in the Type 1 Diabetes PhysioLab platform. Virtual mice subjected to anti-CD3 therapy reproduced the variable efficacy observed in laboratory experiments (data not shown). The *in silico* research provided a novel insight that preferential induction of regulatory T cells and a more tolerant recurrent infiltrate were necessary, but not sufficient to induce diabetes remission in the virtual NOD mice. Analysis of simulation results demonstrated that restoration of normoglycemia required recovery of β cell mass and β cell function in addition to altered T-cell activity (data not shown).

A second novel insight concerns the interaction between regulatory T cells and antigen-presenting cells (APCs) in determining the character of the pancreatic insulinitis. Consistent with experimental data, PhysioLab simulations predicts that insulinitis declines in recent onset diabetic virtual NOD mice during anti-CD3 treatment, yet returned following cessation of treatment (Figure 5). Further, results indicate that sustained disease remission is attained in virtual mice with either (1) enhanced iTreg functionality due to colocalization of regulatory T cells with activated T cells in the PLN and/or (2) a therapy-induced increase in the ratio of *suppressive* to *inflammatory* APCs that results in preferential priming of regulatory T cells *versus* inflammatory Th1-like T cells.

¹ <http://www.immunetolerance.org/research/autoimmune/trials/herold1.html>

² The results of this study were recently published (Young *et al.* 2006).

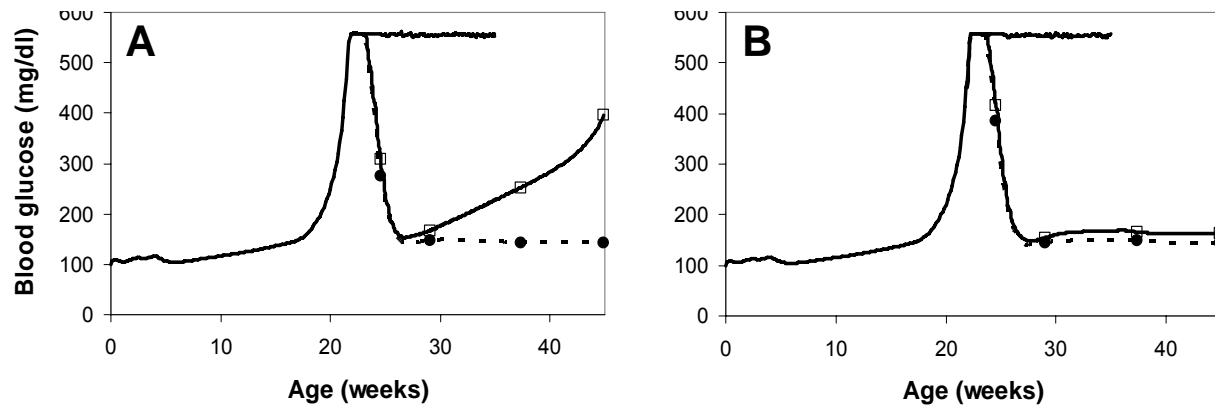


Figure 5: Predicted blood glucose responses to anti-CD3 treatment in a virtual NOD mice with low regulatory T cell activity (*left*) and high regulatory T cell activity (*right*). Solid line: untreated; solid line with open squares: treatment with anti-CD3 antibody; dashed line; treatment + more suppressive APC phenotype. An altered APC phenotype is required for sustained disease remission following anti-CD3 antibody administration in the virtual mouse with low regulatory T cell activity (*left*), while no change in APC phenotype is required for sustained disease remission in the virtual mouse with high regulatory T cell activity (*right*).

Results of our *in silico* research predict that in addition to the known direct effects of anti-CD3 antibody on T cells, sustained remission of diabetes required: (1) rapid β cell recovery and (2) a regulatory pancreatic environment resulting from the interaction between regulatory T cells and antigen-presenting cells. These findings suggest a number of focused laboratory studies to confirm the predicted increase in β cell activity (*e.g.*, proliferation, insulin secretion) and changes measure iTreg activity and antigen-presenting cell phenotype.

Investigating mechanisms of disease heterogeneity

The Type 1 Diabetes PhysioLab platform can also be used to investigate hypotheses regarding the underlying mechanisms leading to disease heterogeneity and the impact of these hypotheses on efficacy of therapies such as exogenous IL-10. While the timing of diabetes onset in NOD mice is known to vary, the underlying biological mechanisms responsible for the variable rates of disease progression are not fully elucidated. Heterogeneity in diabetes-onset times ranging from 12 to 35 weeks of age have been shown in the literature³⁴ and on the websites of NOD mouse suppliers, (<http://www.taconic.com/>; <http://www.jax.org>). The average-onset virtual mouse in the Type 1 Diabetes PhysioLab platform develops frank diabetes at approximately 19 weeks (plasma glucose concentration ≥ 300 mg/dl), which is consistent with the average timing of reported diabetes onset.

To begin exploring the mechanisms responsible for the observed heterogeneity in disease progression, an alternate virtual mouse that develops diabetes at an earlier age was created. Published data suggest that a progressive loss of immunosuppressive regulation is associated with disease progression^{18,34,35}. These data were used as the basis for hypotheses for why some NOD mice develop overt diabetes more rapidly than others. Results show that a disease-dependent loss of immunosuppressive function by iTregs and an increase in TNF- α and IFN- γ production by CD4⁺ and CD8⁺ T cells were sufficient to recapitulate early-onset diabetes. This virtual NOD mouse represents a more aggressive phenotype, developing diabetes at approximately 15 weeks (data not shown).

While the early-onset virtual mouse pathophysiology (*e.g.*, PLN expansion, islet infiltration) remained consistent with the reported values, an IL-10 protocol that was efficacious in the average-onset virtual mouse, failed to protect the early-onset virtual mouse (Figure 6). An implication of this finding is that IL-10 is less effective when significant islet inflammation and β cell destruction has occurred, *i.e.*, efficacy is dependent on the state of disease progression at the start of therapy. Further, simulation results indicate that earlier

administration of the treatment protocol can prevent NOD mice from developing diabetes by initiating treatment during the period when the disease is still sensitive to IL-10's protective function (data not shown).

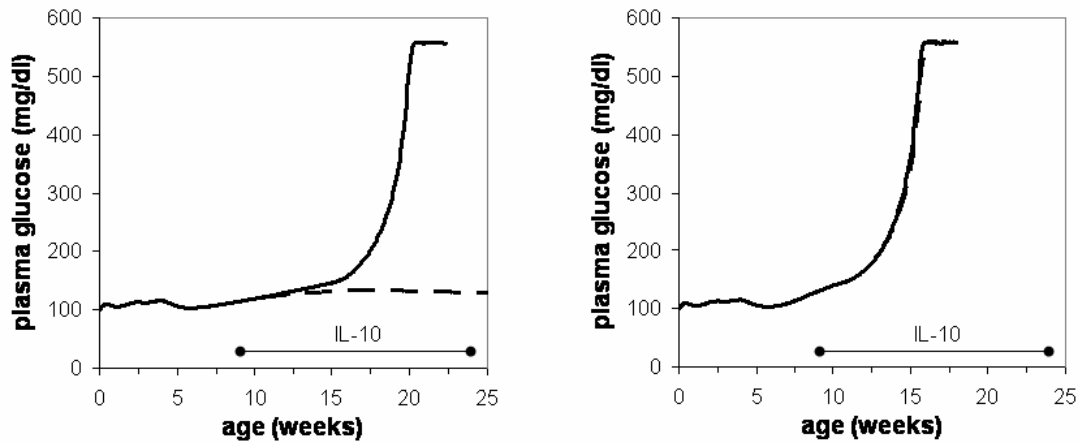


Figure 6: Simulated blood glucose responses of the average- (*left*) and early-onset virtual mouse (*right*) to exogenous IL-10 therapy. Solid lines: untreated; Dashed lines: treated with 1 µg/day IL-10 from 9 to 24 weeks of age. The early-onset virtual NOD mouse treated with IL-10 from 9 to 24 weeks of age is not protected from diabetes.

Dosing and Timing Effects of Anti-CD40L Therapy

The Type 1 Diabetes PhysioLab platform can also be utilized to gain insight into the mechanism of action of interventions and to optimize treatment protocols. Such results can help interpret and resolve conflicting data in the T1D literature.

Several anti-CD40L therapy protocols for the treatment of T1D have been tested in NOD mice³⁶. Data indicate that early treatment is required for protection; whereas late treatment has no effect. However, some of the reported data indicate different responses to similar protocols^{36,37}. In the Type 1 Diabetes PhysioLab platform, representation of the effects of the anti-CD40L on several pathways were implemented and tested. Simulation results indicate that anti-CD40L-mediated inhibition of either (1) conventional CD4+ T cell costimulation, (2) B lymphocyte activation, or (3) DC and macrophage activation alone was sufficient to protect NOD mice from spontaneous diabetes, whereas inhibition of CD8+ T cell activation or endothelial cell surface molecule expression alone were not sufficient for protection (data not shown).

To understand why anti-CD40L treatment is effective early but not late, varying effects on the critical pathways with treatment start time was explored. The pathway considered in this case study is the inhibition of CD4+ T cell costimulation by anti-CD40L antibody that results in a reduction of the CD4+ T cell priming signal. Simulation results show that early treatment (beginning at 2-3 weeks of age) exhibits a 60% down-regulation of the CD4+ priming signal, due primarily to inhibition of costimulation by CD40L (Figure 7). The ability of anti-CD40L to down-regulate the CD4+ priming signal decreases as the treatment start time increases, with no effect observed for treatments starting beyond 6 weeks of age. These findings suggest that the time-dependent effects of anti-CD40L on critical pathways can explain apparent discrepancies reported in the literature.

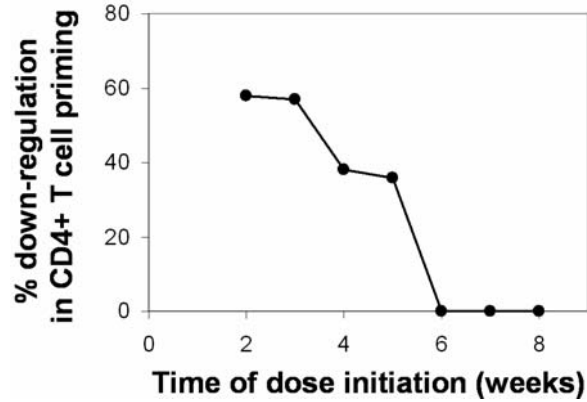


Figure 7: Decrease in percent down-regulation of the conventional CD4+ T cell priming signal by a 250 µg dose of CD40L with increase in time of dose initiation.

Finally, the Type 1 Diabetes PhysioLab platform was used to predict optimum treatment protocols for anti-CD40L therapy in NOD mice. For example, Balasa *et al.* 1997³⁶ demonstrated that six 250µg doses over a period of 9 weeks starting at 3 weeks of age resulted in protection from diabetes. Results from simulated variations of the Balasa *et al.* protocol showed that treatment could be started as late as 4.8 weeks of age and still confer protection from diabetes (data not shown). Furthermore, a reduction in the number of doses did not compromise efficacy, and protection was observed even after a single dose of 250 µg given as late as 4.5 weeks of age. Together, these results demonstrate the ability of biosimulation to make optimal dose and dose timing recommendations that can inform the design of treatment protocols.

Reference List

1. Sakaguchi,S. The origin of FOXP3-expressing CD4+ regulatory T cells: thymus or periphery. *J Clin Invest* **112**, 1310-1312 (2003).
2. Shi,F.D. *et al.* Germ line deletion of the CD1 locus exacerbates diabetes in the NOD mouse. *Proc. Natl. Acad. Sci. U. S A* **98**, 6777-6782 (2001).
3. Berzins,S.P., Venanzi,E.S., Benoist,C. & Mathis,D. T-cell compartments of prediabetic NOD mice. *Diabetes* **52**, 327-334 (2003).
4. Belghith,M. *et al.* TGF-beta-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. *Nat Med* **9**, 1202-1208 (2003).
5. Koarada,S., Wu,Y. & Ridgway,W.M. Increased entry into the IFN-gamma effector pathway by CD4+ T cells selected by I-Ag7 on a nonobese diabetic versus C57BL/6 genetic background. *J Immunol* **167**, 1693-1702 (2001).
6. Thornton,A.M. & Shevach,E.M. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. *J Immunol.* **164**, 183-190 (2000).
7. Takahashi,T. & Sakaguchi,S. Naturally arising CD25+CD4+ regulatory T cells in maintaining immunologic self-tolerance and preventing autoimmune disease. *Curr Mol Med* **3**, 693-706 (2003).
8. Tang,Q. *et al.* Distinct roles of CTLA-4 and TGF-beta in CD4(+)CD25(+) regulatory T cell function. *Eur J Immunol* **34**, 2996-3005 (2004).
9. Bour-Jordan,H. *et al.* Costimulation controls diabetes by altering the balance of pathogenic and regulatory T cells. *J Clin Invest* **114**, 979-987 (2004).
10. Thornton,A.M., Piccirillo,C.A. & Shevach,E.M. Activation requirements for the induction of CD4+CD25+ T cell suppressor function. *Eur. J Immunol.* **34**, 366-376 (2004).
11. Camara,N.O., Seville,F. & Lechler,R.I. Human CD4+CD25+ regulatory cells have marked and sustained effects on CD8+ T cell activation. *Eur. J Immunol.* **33**, 3473-3483 (2003).
12. Trzonkowski,P., Szmit,E., Mysliwska,J., Dobyszek,A. & Mysliwski,A. CD4+CD25+ T regulatory cells inhibit cytotoxic activity of T CD8+ and NK lymphocytes in the direct cell-to-cell interaction. *Clin Immunol.* **112**, 258-267 (2004).
13. Seo,S.J. *et al.* The impact of T helper and T regulatory cells on the regulation of anti-double-stranded DNA B cells. *Immunity.* **16**, 535-546 (2002).
14. Lim,H.W., Hillsamer,P., Banham,A.H. & Kim,C.H. Cutting edge: direct suppression of B cells by CD4+ CD25+ regulatory T cells. *J Immunol.* **175**, 4180-4183 (2005).
15. Ghiringhelli,F. *et al.* CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner. *J Exp. Med.* **202**, 1075-1085 (2005).
16. Cederbom,L., Hall,H. & Ivars,F. CD4+CD25+ regulatory T cells down-regulate co-stimulatory molecules on antigen-presenting cells. *Eur. J Immunol.* **30**, 1538-1543 (2000).
17. Misra,N., Bayry,J., Lacroix-Desmazes,S., Kazatchkine,M.D. & Kaveri,S.V. Cutting edge: human CD4+CD25+ T cells restrain the maturation and antigen-presenting function of dendritic cells. *J Immunol.* **172**, 4676-4680 (2004).
18. Fallarino,F. *et al.* Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol.* **4**, 1206-1212 (2003).
19. Mellor,A.L. *et al.* Specific subsets of murine dendritic cells acquire potent T cell regulatory functions following CTLA4-mediated induction of indoleamine 2,3 dioxygenase. *Int Immunol.* **16**, 1391-1401 (2004).
20. Gregori,S., Giarratana,N., Smioldo,S. & Adorini,L. Dynamics of pathogenic and suppressor T cells in autoimmune diabetes development. *J Immunol* **171**, 4040-4047 (2003).

21. Banz,A., Pontoux,C. & Papiernik,M. Modulation of Fas-dependent apoptosis: a dynamic process controlling both the persistence and death of CD4 regulatory T cells and effector T cells. *J Immunol* **169**, 750-757 (2002).
22. Huber,S. *et al.* Cutting edge: TGF-beta signaling is required for the in vivo expansion and immunosuppressive capacity of regulatory CD4+CD25+ T cells. *J Immunol* **173**, 6526-6531 (2004).
23. Peng,Y., Laouar,Y., Li,M.O., Green,E.A. & Flavell,R.A. TGF- β regulates in vivo expansion of Foxp3-expressing CD4+CD25+ regulatory T cells responsible for protection against diabetes. *Proc Natl Acad Sci U S A* **101**, 4572-4577 (2004).
24. Marie,J.C., Letterio,J.J., Gavin,M. & Rudensky,A.Y. TGF-beta1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. *J Exp. Med.* **201**, 1061-1067 (2005).
25. Salomon,B. *et al.* B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* **12**, 431-440 (2000).
26. Walker,R. *et al.* Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- T cells. *J Clin Invest* **112**, 1437-1443 (2003).
27. Fisson,S. *et al.* Continuous activation of autoreactive CD4+ CD25+ regulatory T cells in the steady state. *J Exp Med* **198**, 737-746 (2003).
28. Bock,T., Pakkenberg,B. & Buschard,K. Genetic background determines the size and structure of the endocrine pancreas. *Diabetes*. **54**, 133-137 (2005).
29. Kelly,M.A., Rayner,M.L., Mijovic,C.H. & Barnett,A.H. Molecular aspects of type 1 diabetes. *Mol Pathol* **56**, 1-10 (2003).
30. Walker,L.S., Chodos,A., Eggena,M., Dooms,H. & Abbas,A.K. Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells in vivo. *J Exp Med* **198**, 249-258 (2003).
31. Swain,S.L. Helper T cell differentiation. *Curr Opin Immunol* **11**, 180-185 (1999).
32. Zhang,X. *et al.* Unequal death in T helper cell (Th)1 and Th2 effectors: Th1, but not Th2, effectors undergo rapid Fas/FasL-mediated apoptosis. *J Exp Med* **185**, 1837-1849 (1997).
33. Lenschow,D.J. *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* **181**, 1145-1155 (1995).
34. Chatenoud,L. CD3 antibody treatment stimulates the functional capability of regulatory T cells. *Novartis. Found. Symp.* **252:279-86; discussion 286-90.**, 279-286 (2003).
35. Kohm,A.P. *et al.* Treatment with nonmitogenic anti-CD3 monoclonal antibody induces CD4+ T cell unresponsiveness and functional reversal of established experimental autoimmune encephalomyelitis. *J Immunol.* **174**, 4525-4534 (2005).
36. Yu,L. *et al.* Early expression of antiinsulin autoantibodies of humans and the NOD mouse: evidence for early determination of subsequent diabetes. *Proc. Natl. Acad. Sci. U. S A* **97**, 1701-1706 (2000).
37. Pop,S.M., Wong,C.P., Culton,D.A., Clarke,S.H. & Tisch,R. Single cell analysis shows decreasing FoxP3 and TGFbeta1 coexpressing CD4+CD25+ regulatory T cells during autoimmune diabetes. *J Exp. Med.* **201**, 1333-1346 (2005).
38. Balasa,B. *et al.* CD40 ligand-CD40 interactions are necessary for the initiation of insulinitis and diabetes in nonobese diabetic mice. *J Immunol* **159**, 4620-4627 (1997).
39. Molano,R.D. *et al.* Prolonged islet graft survival in NOD mice by blockade of the CD40-CD154 pathway of T-cell costimulation. *Diabetes* **50**, 270-276 (2001).