MATHEMATICAL BIOSCIENCES
AND ENGINEERING
Volume **6**, Number **4**, October **2009**

doi:10.3934/mbe.2009.6.753

pp. **753–778**

MODELING DYNAMIC CHANGES IN TYPE 1 DIABETES PROGRESSION: QUANTIFYING β-CELL VARIATION AFTER THE APPEARANCE OF ISLET-SPECIFIC AUTOIMMUNE RESPONSES

Patrick Nelson

University of Michigan, Department of Mathematics
Center for Computational Medicine and Bioinformatics
100 Washtenaw Ave, Ann Arbor, 48109-2218, USA

Noah Smith

University of Michigan, Department of Mathematics
530 Church St., Ann Arbor, 48109, USA

Stanca Ciupe

Duke University, Laboratory of Computational Immunology
Durham, NC 27708, USA

Weiping Zou

University of Michigan, Department of Surgery
C558 MSRB II, Ann Arbor, MI 48109, USA

Gilbert S. Omenn

University of Michigan, Departments of Internal Medicine and Human Genetics,
School of Public Health, The Brehm Center for Type 1 Diabetes Research and Analysis
Center for Computational Medicine and Bioinformatics
100 Washtenaw Ave, Ann Arbor, MI 48109-2219, USA

Massimo Pietropaolo

University of Michigan, Laboratory of Immunogenetics, Department of Internal Medicine
The Brehm Center for Type 1 Diabetes Research and Analysis
1500 East Medical Center Drive, Ann Arbor, MI 48109, USA

(Communicated by Yang Kuang)

2000 *Mathematics Subject Classification.* Primary: 58F15, 58F17; Secondary: 53C35.

Key words and phrases. Type 1 Diabetes, β-cell variation, islet marker antibodies, mathematical modeling.

Abstract. Type 1 diabetes (T1DM) is a chronic autoimmune disease with a long prodrome, which is characterized by dysfunction and ultimately destruction of pancreatic β-cells. Because of the limited access to pancreatic tissue and pancreatic lymph nodes during the normoglycemic phase of the disease, little is known about the dynamics involved in the chain of events leading to the clinical onset of the disease in humans. In particular, during T1DM progression there is limited information about temporal fluctuations of immunologic abnormalities and their effect on pancreatic β-cell function and mass. Therefore, our understanding of the pathoetiology of T1DM relies almost entirely on studies in animal models of this disease. In an effort to elucidate important mechanisms that may play a critical role in the progression to overt disease, we propose a mathematical model that takes into account the dynamics of functional and dysfunctional β-cells, regulatory T cells, and pathogenic T cells. The model assumes that all individuals carrying susceptible HLA haplotypes will develop variable degrees of T1DM-related immunologic abnormalities. The results provide information about the concentrations and ratios of pathogenic T cells and regulatory T cells, the timing in which β-cells become dysfunctional, and how certain kinetic parameters affect the progression to T1DM. Our model is able to describe changes in the ratio of pathogenic T cells and regulatory T cells after the appearance of islet antibodies in the pancreas. Finally, we discuss the robustness of the model and its ability to assist experimentalists in designing studies to test complicated theories about the disease.

1. **Introduction.** Type 1 diabetes is an autoimmune disease, often diagnosed early in life and characterized by destruction of the insulin-secreting cells in the pancreas. As a consequence, patients become insulin-dependent and must follow a rigid insulin regimen to survive. The overall risk for developing Type 1 diabetes in North American Caucasian siblings, parents and offspring of individuals with Type 1 diabetes, ranges from 1% to 15% as compared to 0.12% in the general population [1]. However, over 80% of cases of Type 1 diabetes occur in individuals with no apparent family history of the disease. In the remaining percent, this disease aggregates in families. The prevalence of T1DM in the United States is estimated to be approximately 120,000 individuals age less than 20 years and approximately 300,000-500,000 individuals all ages. The incidence of T1DM is at least 30,000 new cases each year [2]. Since T1DM is an autoimmune disease with a long pre-clinical course [3], the predictive testing of individuals prior to the onset of the disease process has provided a real opportunity for identification of risk markers and the design of therapeutic intervention. The progress made in molecular immunology technology has provided a new stage for the investigation of etiological components, either genetic or immunologic, prior to the clinical diagnosis of T1DM.

T1DM is a chronic autoimmune disease in which β-cells are gradually destroyed by pathogenic (autoreactive) T cells. This process is the end result of complex interactions among genetic, immunologic, and environmental factors [4]. There is compelling evidence in [5] suggesting that T1DM results from an altered balance between pathogenic T cells mediating disease and regulatory T cells (Tregs) controlling autoimmunity [6]. Type 1 diabetes is a polygenic disease for which there are a small number of genes with large effects (i.e., HLA) and a large number of genes with small effects [7, 8]. Risk of T1DM progression is mainly conferred by specific HLA DR/DQ alleles [e.g., DRB1*03-DQB1*0201 (DR3) or DRB1*04-DQB1*0302 (DR4)] [9, 10, 11]. Conversely, the DQB1*0602 allele is associated with dominant $(80\% - 95\%)$protection from T1DM [11].

Although autoreactive $CD4^+$ and $CD8^+$ T cells are required for the initiation and progression of the disease [12, 13], the cellular dynamics leading to disease progression are not well understood. It has been postulated that in T1DM there is an imbalance of pathogenic (effector) T cells and regulatory T cells (Tregs) [6]. Regulatory T cells (formerly suppressor T cells) are a specialized sub-population of T cells that suppress activation of the immune system thereby maintaining the homeostasis and tolerance to self molecules. Tregs represent less than 2% of the T cells in the peripheral blood. Using a number of experimental protocols, Treg cells can be expanded *in vitro* and *in vivo* and eventually could be harnessed therapeutically to treat T1DM or facilitate tolerance of transplanted pancreatic islets [14].

The fundamental pathophysiology shared by all patients with T1DM is the progressive loss in the ability of pancreatic β-cells to secrete insulin in response to glucose [3]. This progressive decline in β-cell function may be secondary to a defect of regulatory T cells. A number of studies have demonstrated that any approach aiming to achieve immune hypo-responsiveness or tolerance in established T1DM will have to address the β-cell mass and function remaining at the time of clinical diagnosis of T1DM to permit a recovery of a metabolically-functional mass over the long-term [15, 16].

Convincing findings from prospective studies in first degree relatives of T1DM probands have shown a long latent period between the first appearance of circulating autoantibodies directed against islet autoantigens and clinical onset [17, 18]. In T1DM a long prodrome offers a wide window of opportunities for identifying individuals at risk and conducting intervention to delay or even prevent the clinical onset of the disease. Algorithms based on immunologic and metabolic measurements have been developed in an effort to improve prediction of type 1 diabetes. However, during the natural history of the disease the mechanisms determining the imbalance between pathogenic T cells and regulatory T cells and are far from been resolved. Thus, in the following sections, we model their fluctuations occurring during the progression to disease onset. We pose questions about dynamic changes in the number and function of pathogenic (effector) and regulatory T cells in relation to pancreatic β-cell mass and function. These conjectures will lay the groundwork to identify gaps in the current knowledge of the pathoetiology of T1DM. Once these knowledge gaps during disease progression are identified, their dynamics can be further explored by formulating and evaluating hypotheses which may lead to the design of new experimental approaches with the potential to dramatically enhance our understanding of the disease process and interventions that prevent progression of T1DM.

2. **Model development.**

Mathematical modeling has played a critical role in our understanding of various pathogenic aspects of human diseases, such as infectious diseases [19, 20, 21, 22], cancer [23, 24], cardiac arrhythmias [25], and diabetes [26, 27]. Modeling in diabetes has looked at the kinetics of glucose-induced insulin secretion and sensitivity [28, 29, 30, 31, 32, 33], bursting properties of pancreatic β-cells [34, 35] and glucose-calcium oscillations in β-cells [36]. Only recently has mechanistic modeling begun to explore specific pathways associated with the effects of T cells in autoimmune diabetes [37] or in the chain of events causing β-cell destruction that leads to T1DM [38]. Work by Wang *et al.* studied the heterogeneity

between young- and adult-onset type 1 diabetes [39], and Entelos Inc. developed a large scale model of a virtual NOD mouse [40].

The NOD mouse represents a relevant animal model of autoimmunity in T1DM. Immunologists analyzed the effect of genes on immunity using this model of spontaneous diabetes and they generated numerous transgenic mice on the NOD background to address specific immunologic questions. Although these models provided important clues in understanding the mechanisms of autoimmunity in T1DM diabetes, they have significant limitations such as numerous differences in the structure of the immune system between mouse and man, which likely result in discrepancies on how the immune system responds to physiologic and pathologic stimuli [41]. A large number of immunomodulatory strategies were and are currently applied to prevent diabetes in animal models of the disease, such as the NOD mouse [42, 43] and the BB rat [44, 45]. Although a large number of these therapeutic strategies may delay or prevent diabetes in NOD mice, the most promising ones are now being tested in humans [46, 47]. Until proven otherwise, both NOD mouse and BB rat are the closest models of human autoimmune diabetes and, most of the time, these are the only tools where we can test our hypotheses experimentally

A recent paper by DeGaetano et al. provided a nice review of the models used by [48, 49]. Another work by Athanasius et al. [50] looked at the early stages of T1DM in mice and assumed that macrophages act more slowly in NOD mice than in normal mice and that this may lead to secondary necrosis of β-cells which can trigger autoimmunity. Our model does not consider macrophages but instead considers other immune cells. Similarly work by [39] considers macrophages based on the Copenhagen model developed by Freiesleben De Blasio [48]. They assume some local insult to the β-cells causes a cascade of events that eventually leads to β-cell destruction. They include β-cell autoantigens but do not include a T cell response. Our model is different in two aspects. First, we do not model the β-cell autoantigens but instead consider the islet marker antibodies. These antibodies, produced by B cells, are in response to these autoantigens but, very importantly, can be measured and used as a predictive tool. Our model implicitly allows for the appearance of these antibodies at specific time points during the disease. We assume in our model that their appearance will correlate with the beginning of the auto immune response. We should note that we cannot provide the specific means for the appearance of these antibodies. Do they come from a viral infection, oxidative stress, normal tissue remodeling, or from an unfolded protein response is not known. We are considering a multiscale model that does look at the possibility that the initial trigger for autoimmunity is coming from the unfolded protein response.

Our intention is to study the relationship between immune cells and regulatory T cells by specifically looking at the ratio of pathogenic T cells and regulatory T cells, to determine the level of β-cell decrease after the appearance of islet antibodies in the pancreas, and to make predictions about the key parameters that are controlling this behavior prior to the clinical onset of T1DM. We do so by developing a model that accounts for glucose, insulin, functioning β-cells, dysfunctional β-cells, normal regulatory T cells, defective regulatory T cells, IL-2, and pathogenic T cells. The key components of this model, which make it unique compared to earlier works, are its ability to track the concentration and functionality of both the β-cells and the regulatory T cells and to quantify the concentration of β-cells with the islet marker antibodies. Both of these aspects are critical if we wish to find a way to better control this disease or even reverse it.

2.1. **Glucose and insulin.** Insulin and glucagon are hormones that control the glycemic levels and are secreted in the pancreas by functioning β-cells and α-cells, respectively. Hence, tracking insulin (I) and glucose (G) can provide information about the correlation between their measured concentrations in the plasma and the assumed concentration of functioning β-cells in the pancreas. Previous models have successfully shown a sigmoidal relationship for glucose concentration and activity in the pancreas [51] and this information was used by Topp et al. [26] in their paper that modeled insulin production as a function of β-cells and glucose. Topp's model assumed that insulin and glucose act on a much faster time scale than that of the β-cells. However, they did not distinguish between functioning and dysfunctional β-cells. We assume the existence of two types of β-cells: a functioning class (β_f) that produces insulin at normal levels and a dysfunctional class (β_{nf}) that produces no insulin. The work by DeGaetano et al. [48] provided a nice review of Topp's work. From our perspective, we know the time scale for glucose and insulin are much faster than that of the immune system or β-cells but by distinguishing between functional and dysfunctional β-cells we have introduced non-linearities that cannot be decoupled in a simple fashion as was done in [26]. We also allow for small numbers of dysfunctional β-cells to regain some level of functionality and therefore return to the insulin producing class. Hence, in our model, the insulin secretion rate will depend on glucose concentration and only the functioning β-cells,

$$\frac{dG}{dt} = R_0 - G(Eg_0 + S_i I), \tag{1}$$

$$\frac{dI}{dt} = \frac{\sigma B_f G^2}{\alpha + G^2} - \delta_I I. \tag{2}$$

In (1), R_0 is the net rate of production at zero glucose, Eg_0 is the total glucose effectiveness at zero insulin, and S_i is the insulin sensitivity. Glucose effectiveness is defined as the ability of glucose to stimulate its own uptake and inhibit its own production; insulin's effect on glucose uptake and production is defined as Insulin sensitivity [52, 53]. Bergman et al. [54] provided experimental evidence for this relationship using the glucose clamp technique. The parameter σ represents the rate of insulin secretion due to β_f cells, α represents glucose concentration where the levels reach half saturation, and δ_I is the rate of removal of insulin.

2.2. **β-cells.** We consider two compartments for β-cells. For the functioning β-cells we use the same source term given in [26]. Topp *et al.* assume that new β-cells can be formed by the replication of pre-existing β-cells or by neogenesis, the differentiation of new β-cells from a precursor or progenitor cell [55]. Presently, it is very difficult to quantify rates of neogenesis or of trans-differentiation, the switch from pancreatic ductal cells to β-cells. However, there is a body of research that suggests, albeit indirectly, that these mechanisms make negligible contributions to β-cell mass dynamics except during development and in response to extreme physiological or chemically induced trauma [49, 56, 57, 58]. For these reasons, neogenesis and trans-differentiation are not incorporated into the present model.

The first term in (3) describes the replication, r_1, and apoptotic death, d_0, rates of existing β-cells. In vitro studies show that the percentage of β-cells undergoing replication varies as a nonlinear function of glucose level in the medium [26, 59]. Replication rates for β-cells increase with increasing glucose levels; however, at extreme hyperglycemia ($> 400mg/ml$), β-cell replication may be reduced at a constant rate, r_2 [49]. Apoptotic death has been shown to vary nonlinearly with glucose

[60, 61]. Specifically, increasing the glucose level from very low levels to approximately 110 mg/ml in the medium surrounding cultured β-cells reduced their death rate; however, above 110 mg/ml glucose, the rate of β-cell death either remained low or increased.

In addition to death by apoptosis, cells can be lost from the functioning β-cell pool by losing the ability to produce insulin [62]. This feature was not considered in [26] but is a key feature of our model. Functioning β-cells may lose the ability to produce insulin as a result of $CD4^+$ T cell infiltration and their subsequent production of harmful cytokines and cytotoxins, such as IL-1 and TNF-α leading to iNOS [63]. This process is accounted for in the second term in (3) by assuming pathogenic T cells T_b are directly affecting the β-cells and causing a switch from functional to non-functional. The maximal rate at which this happens is a_1 and k_1 represents the half-saturation constant. With this we have,

$$\frac{dB_f}{dt} = (r_1 G - d_0 - r_2 G^2)B_f - \frac{a_1 T_b B_f}{k_1 + T_b} + \epsilon B_{nf}. \tag{3}$$

An important feature of (3) is the loss of functioning β-cells does not occur at a constant rate; instead, their loss of function and death rate will depend on the presence of pathogenic T cells. Pathogenic T cells.

The observation that actual death/loss of β-cells may occur in phases is considered through the saturation term in (3) which transitions functioning β-cells to the dysfunctional β-cell class as seen in (4). Once β-cells are no longer capable of producing insulin, they either undergo apoptosis or necrosis at an elevated, but glucose independent rate, d_1, or are directly destroyed by cytotoxic T-cells which we account for by the scaling term, γ_T. This term simply increases the rate of removal of cells from this class. In the results that follow, we will show the model's ability to provide clues to the significance of this result. For instance, we will consider the possibility that the β-cells that become dysfunctional may actually, at some future time, be able to reverse this effect and hence begin to produce some levels of insulin. We incorporate this result with a simple linear term ϵB_{nf};

$$\frac{dB_{nf}}{dt} = \frac{a_1 T_b B_f}{k_1 + T_b} - \gamma_T d_1 B_{nf} - \epsilon B_{nf}. \tag{4}$$

Together, (3) and (4) model the rate of change in the total β-cell mass. In this way, the fraction of functioning β-cells is not constant, rather it is dynamically varying.

2.3. **Immune cells.** T cells possess the ability to directly destroy β-cells in a cytotoxic manner and by directly influencing β-cell destruction through the release of cytotoxic molecules such as cytokines and perforin. In type 1 diabetes, there is evidence that when the immune system is unbalanced, favoring islet inflammation and pathogenicity, the system is prone to islet autoimmunity development. This situation can also occur if there is a defect in regulation.

We can test this hypothesis by allowing our model to account for two classes of regulatory T cells: normal regulatory T cells, R, and a second class that represents regulatory T cells that have lost some form of functionality, R_b. Regulatory T cells are a specialized component of the immune system that police the immune system in order to maintain homeostasis and tolerance to self-antigens. The exact way regulatory T cells are derived from precursor T cells is unknown, however it is thought that it is due to their affinity to self peptide MHC complexes. For a nice review of regulatory T cells please see [64]. Hence, regulatory T cells are

responsible for regulating the immune response, specifically controlling any response that is directed at self antigens. We hypothesize in the paper that a change in regulatory T cells leads to an "unregulated" immune response, due to a class of pathogenic T cells (sometimes referred to as effector T cells, Teffs), that affects the characteristics of insulin-producing β-cells by reducing their numbers or changing their functionality. To test this hypothesis we consider two components of the immune response: regulatory T cells and the cells which are being regulated. The regulated cells are considered to be pathogenic T cells, T_b, which have migrated to the pancreas from the thymus and are unresponsive to the regulatory T cells (see Fig. 1). As discussed earlier, there are many possible mechanisms for the introduction of these pathogenic T cells. Our model is focused on the characteristics of β-cells and regulatory T cells, hence we assume that the appearance of pathogenic T cells correlates with the appearance of the islet marker antibodies which will be given by the function $S(t)$ later.

Taking the information above, we are able to generate the next set of equations used to describe the immune response that play a key role in the progression to T1DM. We recognize regulatory T cells (R) and a compartment of pathogenic T cells (T_b) which are considered to be dangerous effector T-cells and become increasingly resistant to control from regulatory T cells. Evidence shows that infiltration of these dangerous pathogenic T cells is gradual initially and directly relates to the two-phase loss of β-cell mass [65];

$$\frac{dT_b}{dt} = S(t) + \frac{a_3 T_b}{k_4 + T_b}\left(1 - \frac{T_b + R + R_b + B_f + B_{nf}}{K_p}\right) - d_3 T_b - \delta T_b R, \tag{5}$$

$$\frac{dR}{dt} = \frac{a_4 R I_2}{k_2 + I_2}\left(1 - \frac{T_b + R + R_b + B_f + B_{nf}}{K_p}\right) - d_2 R - \alpha R. \tag{6}$$

Of note that in (6) the proliferation rate of regulatory T cells depends on the amount of I_2 available in the pancreas, which is assumed to vary according to $\frac{dI_2}{dt} = \frac{\rho_1 I_2 R}{k_4 + R} - \mu I_2$. The rate of production of the pathogenic T cells, however, correlates with the presence of certain types of islet autoantibodies, $S(t)$, that are produced by B cells. K_p represents the carrying capacity in the pancreas of both immune cells and β-cells, d_i is the death rate of immune cells, δ is the rate at which regulatory T cells can kill pathogenic T cells, and the k_i's are the half-saturation constants that control the rate of increase of cells in the pancreas.

$$S(t) = (\alpha_1 H(t - \tau_1) + \alpha_2 H(t - \tau_2) + \alpha_3 H(t - \tau_3) + \alpha_4 H(t - \tau_4))\frac{t^3}{K + t^3}, \tag{7}$$

where $H(\omega)$ is the heaviside function. A unique aspect of our model is the function $S(t)$ which will allow for the inclusion of the autoantibodies ICA, $GAD65$, IAA and $IA2$, which are well known to appear many years before the clinical onset of T1DM [66]. The immune cells produce these autoantibodies against self-antigens in response to the damage of the β-cells. We assume that due to some unknown event, the pathogenic T cells begin to attack the β-cells, which leads to the release of islet autoantibodies, given by τ_i in (7), and an increased level of proliferation of pathogenic T cells. Hence, $S(t)$ represents the hidden interactions that occur between β-cells and pathogenic T cells, without requiring us to introduce more equations into the system. The complexities associated with the islet marker antibodies

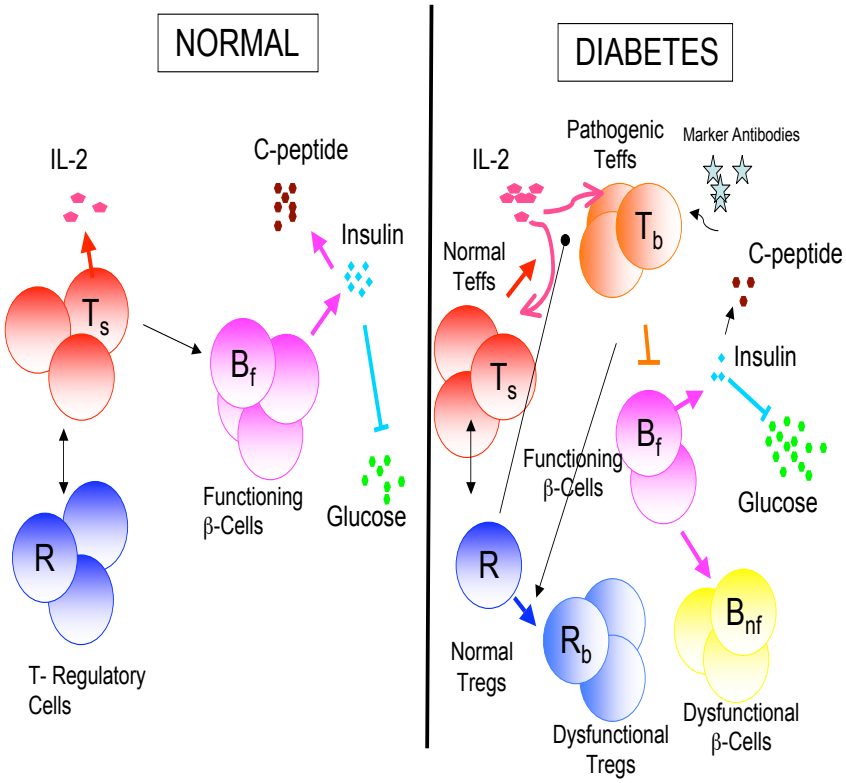

FIGURE 1. Diagram presenting the basic features of our model. The left side shows a normal individual and the right side shows a T1DM patient. For a normal individual there exists a healthy balance between the regulatory T cells, immune cells and functioning β-cells. The functioning β-cells produce insulin which then controls the levels of glucose. IL-2 is produced by the immune cells. The path to T1DM is shown on the right were we introduce two compartments, R_b and T_b, that show the model's ability to track the changes in functionality and concentration of functioning β-cells, B_f, and regulatory T cells, R. We also show the islet autoantibodies which we hypothesis correlates in time with an increase in pathogenic T cells, T_b, that are attacking the functioning β-cells. Notice the resulting changes in the level of glucose and insulin.

are critical for finding a cure to this disease and during the past decade, the molecular characterization and cloning of a number of autoantibodies to islet antigens has allowed major advances in prediction studies [17, 46, 67, 68, 69, 70, 71, 72, 73, 74, 75].

These studies, including ours, have suggested that the use of a combination of humoral immunological markers to these islet antigens, rather than a single test, gives a higher predictive value for T1DM in first degree relatives, and greater sensitivity without significant loss of specificity [17, 68, 70, 73, 74]. The measurement of antibodies to GAD65, IA-2 is now a clear prerequisite in screening for individuals at risk of developing insulin requirement. We found that the presence of two or more of these autoantibodies to islet antigens (such as insulin and/or GAD65 or IA-2, or insulin or ICA) are currently used as entry criteria for intervention trials aimed at preventing Type 1 diabetes [46, 75].

The complexities presented above are enormous and our current model allows us to focus on the dynamics of regulatory T cells and β-cells after the appearance of the marker antibodies. We are working on a new model that allows more specificity for the function $S(t)$ but for now will consider this function to be time-dependent in a manner that allows us to introduce the antibodies in the system at specific times over the course of a patient developing T1DM. We can also use a hyperbolic tangent function which allows for a more continuous dynamic but both functions show similar results. Hence, $S(t)$ allows us to focus on the dynamics of β-cells and regulatory T cells without adding additional complications as to why the auto immunity starts. We are in essence saying that there is some unknown event that leads to pathogenic T cells attacking β-cells and after this event our model can predict what occurs during the progression to T1DM. We are currently focusing on this event in separate work.

Finally, we consider the inclusion of a class of non-functioning regulatory T cells (R_{nf}). Evidence suggests that there is a gradual switch between functioning and non-functioning regulatory T cells [76]. By modeling this class of regulatory T cells we are able to suggest possible pathways for disease progression that have yet to be considered. The rate of transition from functioning to non-functional regulatory T cells is given by $F_1(R, R_b, T_b) = \alpha R$,

$$\frac{dR_b}{dt} = \alpha R - \delta_R R_b. \tag{8}$$

3. **Results.** The results presented here provide evidence of the model's ability to study the dynamics of T1DM. For each figure, we assume specific values for the disease parameters and the results are based on these assumptions. In all cases, we can find and will show variations in these results by simply changing one or two of the key parameters. The simulations were completed using Matlab's ode45 solver.

We first model immunologic fluctuations which may occur during the progression to clinical T1DM. These assumptions may explain the stepwise decline in β-cell mass and function that occurs after the appearance of multiple autoantibodies which are strong predictors of disease development (see Fig. 2). The model assumes all individuals carrying a disease-prone HLA genotype (i.e. DRB1*03-DQB1*0201 (DR3) or DRB1*04-DQB1*0302 (DR4)] will develop a degree of islet autoimmunity. The pathogenic phenotype can be viewed as a spectrum with destructive autoimmunity, loss of β-cell mass, multiple autoantibodies and clinical disease observed at one end, and non-destructive autoimmunity, preservation of β-cell mass, and generally absence of islet autoantibodies, at the opposite end of the spectrum. In the initial phase of the disease, the number of pathogenic T cells is controlled by an increase in number of functional regulatory T cells. As the disease process becomes more prominent, autoreactive effector (pathogenic) T cells that mediate disease exceed

the number of regulatory T cells, which no longer suppress pathogenic autoimmune responses and in turn lose their ability to actively control unwanted immunity even after the onset of pathological manifestations.

3.1. **Relationship between islet autoantibodies, ratio of pathogenic T cells to regulatory T cells, and β-cell mass.** The discovery of islet cell antibodies (ICA) was the prelude to the understanding that type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease [77]. We previously [66] summarized the current evidence for multiple islet autoantibodies as predictive markers for T1DM progression. We incorporated these islet autoantibodies into our model for T1DM to study the dynamics and progression of the disease for individuals considered to be low risk, i.e., present less than three islet autoantibodies, or high risk, i.e., greater than two islet autoantibody markers.

Our results (see Fig. 3 through Fig. 5) show the model's prediction, over a 20-30 year period of time, for the concentration of functioning β-cells and for the ratio of pathogenic T cells to regulatory T cells. In each simulation we allow for the appearance of one islet antibody every five years from the start of our simulation, i.e., in (7) $\tau_1 = 5$ years, $\tau_2 = 10$ years, and $\tau_3 = 15$ years. It must be emphasized that this is just one test case and that we can consider an infinite number of others. For instance, the appearance of certain islet antibodies can occur in as early as 5 months in young kids or over 20 years in the elderly. Each of these scenarios can be tested with our model, by varying τ_i in (7), but we only present the case here where they occur every 5 years. Our initial results, which we vary α_i and K in (7), suggest that the β-cells receive most of their damage during the first attack by the pathogenic T cells, i.e., which coincides with the appearance of the first islet autoantibody. We found roughly a 12% (see Fig. 3) to 28% (see Fig. 5) decrease in the level of functioning β-cells. However, after the appearance of the second antibody we find a smaller reduction in the β-cells. This result is contrary to some current beliefs and by varying the parameter values, we can describe events where the β-cell decrease is more gradual over time, instead of a more rapid reduction, proving the robustness of our model (see Fig. 8.)

What happens next depends on the assumption that either a third islet autoantibody will appear or not. If we assume no more islet autoantibodies appear then the individual is predicted to stay in a pre-diabetic state, i.e., the level of functioning β-cells is decreased but settles to a level that is lower than what is seen in a normal individual (Fig. 3 top left blue line). These results can be dramatically different if we change the values of α_i in (7) and in fact, we can show the biggest decrease in functioning β-cells can occur after the appearance of the second islet autoantibody and not the first (results not shown). When we compared these results with the ratio of pathogenic T cells to regulatory T cells (Fig. 3 top right blue line) we find damped oscillations that occur for a few months after initiation of the pathogenic T cells attack of the β-cells. This leads us to believe that the pathogenic T cells are trying to overtake the system but the regulatory T cells are able to maintain control. Another theory is that an environmental factor, such as a virus, can trigger pathogenic T cells, which can destroy insulin secreting cells. We discuss this more in the future work section.

If we allow for a third attack by pathogenic T cells (shown by the appearance of a third islet autoantibody at 15 years), we find this control is only temporary as seen in Fig. 3 (top right red line) when after a third marker becomes present (high risk) the oscillations become larger and in fact for the first time, the ratio

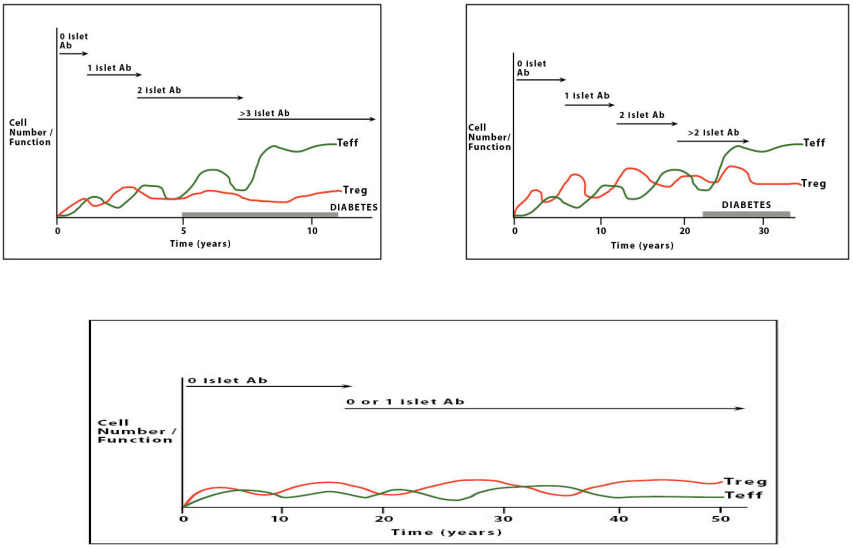

FIGURE 2. During T1DM progression there is an imbalance between the degree of epitope spreading, the cytotoxic potential of autoreactive T cells, the efficiency of regulatory responses and, possibly, the rate of regeneration of β-cells in response to immune-mediated β-cell destruction. These immunologic responses are cyclic and if autoreactive T cells (Teffs or as we refer to them in the text; pathogenic T cells) exceed in number and/or function Tregs or there are functional defects in Tregs (top panel left), which would no longer counteract the cytotoxic potential of Teffs, this leads to β-cell dysfunction and ultimately destruction. This destructive process may take years, as for childhood type 1 diabetes or decades (top panel right), or for instance in Latent Autoimmune Diabetes of the Adulthood (LADA). Islet autoantibodies manufactured by the immune system are directed against one of more of hosts self-proteins and they serve as reliable surrogate predictive markers of disease. The bottom panel shows cyclic variations of Teffs and Tregs in individuals with low risk or no risk of T1DM progression, such as those with single islet autoantibody responses. In this case there is a compensatory regulatory response counteracting effectively the cytotoxic potential of autoreactive T cell responses.

T_b/R becomes greater than one. This result shows comparable dynamics with the experimental evidence presented in Fig. 2 (panel C).

We ran a second test that studies the impact of assuming that the pathway for dysfunctional β-cells to return to a functioning state no longer exists, i.e., $\epsilon = 0$ in (3) and (4). Hence we assume that once a β-cell becomes dysfunctional that they remain dysfunctional. When we run these simulations we find similar results to the above case when we consider a low risk individual (less than 3 islet autoantibodies) Fig. 3 (bottom plots, blue lines). However when we consider a high risk individual we find an interesting result: the time it takes for the β-cells to rapidly decline to zero is only one year instead of three years, however, the pathogenic T cells do not fluctuate as much as the case when $\epsilon > 0$ (see Fig. 3 bottom plots red lines) and in fact show that the disease appears to be more severe but while we would expect the ratio of T_b/R to be greater than in the previous example, we find a non-intuitive result that shows the ratio to be 32% less than the previous case.

As mentioned above, by varying the rates α_i, τ_i, and K we can see quite different dynamics. For instance, in Fig. 3 we found that after the appearance of the third islet autoantibody, the individual will experience a significant reduction of functioning β-cells within a few years. However, this may not be the case in all individuals and if we allow for slight changes in α_i and K we find that the individual can actually maintain some level of functioning β-cells for between 12 years (Fig. 4) to 30 years (Fig. 5) after the third autoantibody is present. During this time, the ratio of pathogenic T cells to regulatory T cells shows some rapid, large amplitude oscillations, with pathogenic T cells exceeding regulatory T cells in number, showing a highly dynamic process between these two immune cells. In fact, we find that even though the β-cells maintain some level of function, the ratio of $\frac{T_b}{R}$ is much larger than the case when the patient experiences a nearly complete annihilation of functioning β-cells.

4. **Describing the number and function of regulatory T cells and β-cells.** Our model allows for the transition of β-cells from functioning to dysfunctional as seen in (3) with the saturation term $\frac{a_1 T_b B_f}{k_1 + T_b}$. In the previous results we allowed for $a_1 = 8$ per day. This term is the source of the dysfunctional β-cells as seen in (4) and can change the model's description. As seen in Fig. 6 our model can account for various declines in functioning β-cells by varying the rate at which they switch over, a_1. In the following graph we provide three simulations that allow for one (Fig. 6 top panel), two (Fig. 6 middle panel) or three islet antibodies (Fig. 6 bottom panel). In each figure we start $a_1 = 8$ per day and then double it and then triple it. As the value for a_1 increases, the level of β-cells begins to decrease and we found that when there are three islet antibodies that if $a_1 > 18$ per day then the level of functioning β-cells drops to zero. Comparing Fig. 3 and Fig. 6 we found similar results showing the decline of β-cells occurs either through an increase of the rate of switch from functioning to dysfunctional or if we keep this rate fixed, through a change in the level of dysfunctional β-cells returning to the functional class. This provides direct evidence for the importance of the rate at which this occurs.

5. **Islet antibodies and describing onset of T1DM.** Finally, we consider applying the model to a generated random set of data points showing the gradual decline of functional β-cells over a 30 year period of time. We were able to keep the model parameters fixed from before and focus on the timing in which the islet

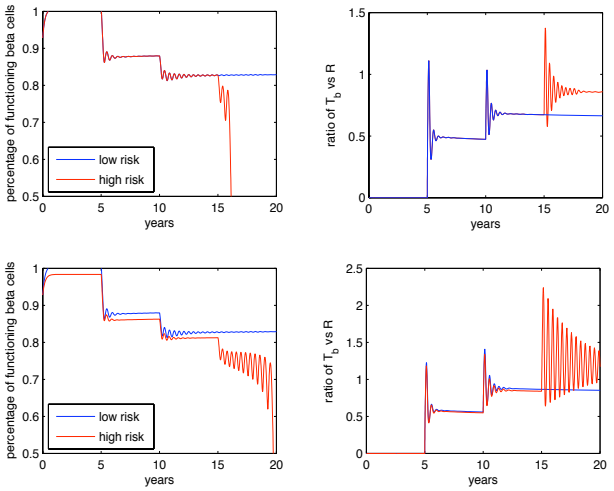

FIGURE 3. Important figure showing the model's capabilities of simulating T1DM results. The mathematical model presented in this paper accounts for the functionality and concentration of regulatory T cells. The top two panels show the decline of functioning β-cells (left) and the ratio of pathogenic T cells to regulatory T cells (right) when we allow for the dysfunctional β-cells to regain some of their functionality ($\epsilon > 0$). What we find is for low risk individuals (≤ 2 islet autoantibodies) that the β-cells decline between $10 - 15\%$ over a 20 year period and the person remains in a pre-diabetic state. However if the person moves to high risk, i.e., > 2 islet autoantibodies, the β-cells begin to significantly decline and with-in three years drop to zero. The right panel shows the ratio of T_b vs R and how the regulatory T cells are trying to control the pathogenic T cells (through the quickly damped oscillations) but become too stressed after the third antibody appears (as seen by the larger oscillations). The bottom set of panels show the same dynamics but when we do not allow for the return of dysfunctional β-cells ($\epsilon = 0$). The significant difference we find is that after the appearance of the third antibody the β-cells decline with-in one year instead of three years but they do so while the regulatory T cells seem to be still controlling the pathogenic T cells (as seen by the ratio of T_b to R being less than one and hence the concentration of R is greater than T_b).

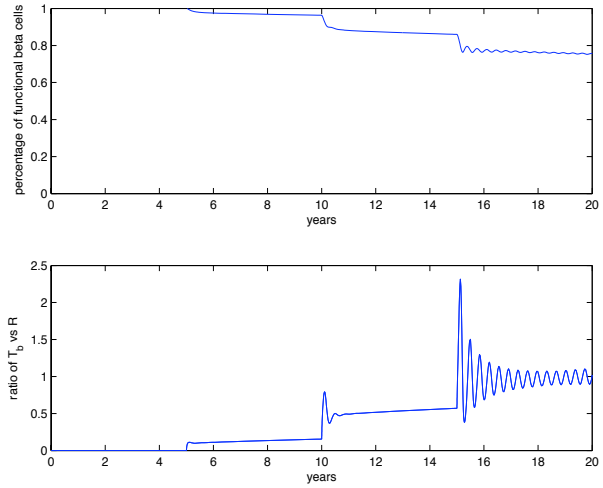

FIGURE 4. In this figure we provide an example that allows for the appearance of three islet autoantibodies but different from Fig. 3 we see that the patient responded with only a 10% decrease in functioning β-cells after the first islet autoantibody appeared, however, after the third autoantibody, the level of functioning β-cells maintains a level that is only reduced by 20% and maintains this level for over 12 years. This dynamic is very different than the one presented in the previous figure. All the model parameters were kept the same except for the ones that control the $S(t)$. In fact, the change in dynamics seen in this figure are due to a doubling of α_i and a two order of magnitude change in K, showing the robustness of our model and the critical need for data to validate our results.

antibodies appear in the pancreas. They appear at 5, 10, 15 and 20 years post start of the simulation, and we are able to describe, using a Monte Carlo algorithm, the level of response needed by the pathogenic T cells in order to fit the data. For this simulation we set $S(t) = (\alpha_1 H(t-1825) + \alpha_2 H(t-3650) + \alpha_3 H(t-5475) + \alpha_4 H(t-7300))\frac{t^3}{K+t^3}$, where the H represents the heaviside (step) function and time is in days. From our data set, which we generated based on evidence about the decline of β-cells taken from the literature [78], we were able to show an increase in the response of pathogenic T cells after each islet antibody entered. As seen in Fig. 7 we used $\alpha_1 = 10$, $\alpha_2 = 50$, $\alpha_3 = 100$, and $\alpha_4 = 150$. With the future application

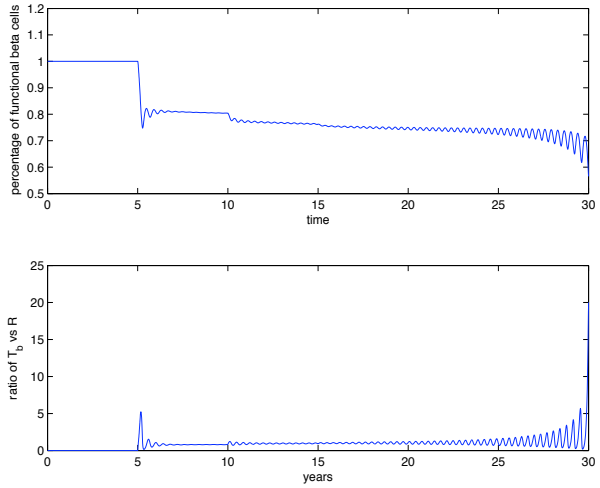

FIGURE 5. Figure similar to Fig. 4, except we change the values of α_i and K by 20%, showing that the functioning β-cells can be predicted by the model to maintain a reduced level for over 20 years after we see the third islet autoantibody. Again, showing the model's robustness to describe a wide variety of dynamics.

of real data, we feel we can make significant contributions to the understanding of T1DM and improve the clarity of the need for future work.

6. **Discussion.** One of the common characteristics of chronic autoimmune disorders, such as lupus, rheumatoid arthritis and T1DM, is their relapsing-remitting nature, which implies a cyclic process of their autoimmune responses. The intensity and duration of cyclic variations of pathogenic immune responses and pro-inflammatory cytokines can cause flare ups of rheumatoid arthritis or T1DM. The fundamental pathophysiology shared by all patients with type 1 diabetes is the progressive loss in the ability of the β-cells in the pancreas to secrete insulin in response to glucose and the progressive decline in β-cell mass. As autoimmunity in type 1 diabetes progresses from initial activation to a chronic state, there is an increase in number of islet autoantigens targeted by T cells and autoantibodies which precede the onset of clinical disease. Multiple antibodies reacting with these autoantigens (i.e., insulin, glutamic acid decarboxylase (GAD65) and the islet antigen IA-2), are detected in the majority of newly diagnosed T1DM patients and their presence is highly predictive of disease progression in otherwise healthy first-degree relatives of T1DM probands. Islet autoantibodies serve as surrogate markers for

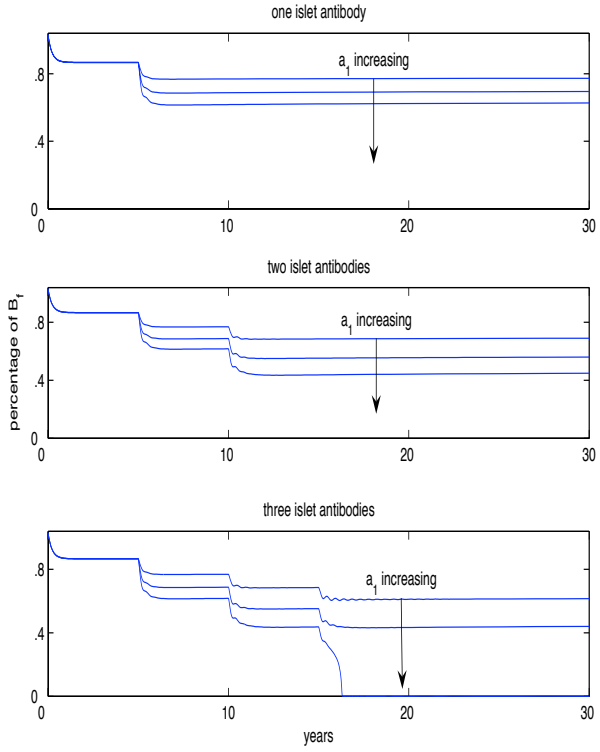

FIGURE 6. Model describing β-cell resilience where the functional form for their transformation to dysfunctional β-cells is given by $\frac{a_1 T_b B_f}{k_1+T_b}$ and hence is considered to be dependent solely on the T cells that have become resistant to regulator T cell responses. The panels allow for one (top) islet autoantibody, two (middle), and three (bottom). In each case we set $a_1 = 8$ per day and then double it and then triple its value. In all cases, the level of functioning β-cells decreases as a_1 increases and in fact, there exists a dramatic drop when we allow for three islet antibodies and let $a_1 > 18$ as seen in the bottom panel.

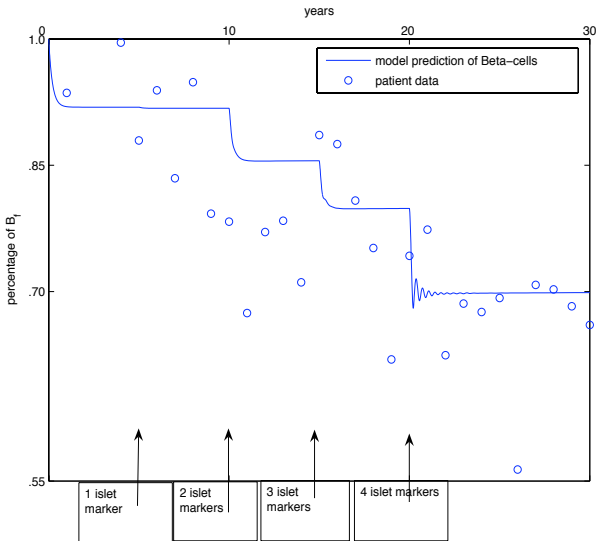

FIGURE 7. Now we consider the predictive ability of the model and show how it can be of use for understanding T1DM. The previous figures have focused on the model's description of changes in β-cell numbers over time. In this figure, we generated a random set of data points that simulates the gradual decline of functional β-cells over a 30 year period of time. Keeping the model parameters fixed from before, we focused on the timing of the appearance of the islet autoantibodies in the pancreas. We assumed they appeared at 5, 10, 15 and 20 years, post start of the simulation, and then described the level of response the pathogenic T cells needed to fit the data. The equation we used was $S(t) = (\alpha_1 H(t-1825) + \alpha_2 H(t-3650) + \alpha_3 H(t-5475) + \alpha_4 H(t-7300))\frac{t^3}{K+t^3}$, where the H represents the heaviside (step) function. From our data set we were able to show an increase in the response of pathogenic T cells after the number of islet autoantibodies increase. As seen in Fig. 7 we used $\alpha_1 = 10$, $\alpha_2 = 50$, $\alpha_3 = 100$, and $\alpha_4 = 150$.

specific autoimmune responses targeting pancreatic β-cells [17, 18, 66]. Although in our armamentarium we have reliable autoantibody markers predicting with accuracy T1DM progression [17, 18], the negative results from the Diabetes Prevention Trial-Type 1 Diabetes Study Group [79] and the European Nicotinamide Diabetes Intervention Trial (ENDIT) Group [80] have for now clouded our vision that effective prevention is around the corner. One reason that could explain these negative

results is that the mechanisms of the disease process prior to diabetes onset are largely unknown.

In the past two decades tremendous progress has been made in the understanding of the genetics, pathophysiology and prediction of the disease. However, there are critical gaps that have yet to be filled. Prompted by an interest in trying to fill some of these gaps, we modeled a few crucial aspects of the disease process. The model that we present is an attempt to address complicated questions about the decline of functioning β-cells, about the ratio of pathogenic T cells to regulatory T cells, and describe the onset of the disease. As seen in Fig. 3 we found in low risk individuals (less than three islet autoantibodies) that the person can have a $10-20\%$ decline in the number of functioning β-cells but still stay in a pre-diabetic state. In fact, we can find declines up to 25% in these pre-diabetic individuals (results not shown). During the pre-diabetic state we also can predict the ratio of pathogenic T cells to regulatory T cells. We found a significant result when allowing dysfunctional β-cells to return to the functioning class. When we assumed $\epsilon > 0$ in (3) we found $\frac{T_b}{R} > 1$ and when the third islet autoantibody appears that the person will have a catastrophic decrease within 3 years of functioning β-cells without intervention. If we do not allow for the return to the functioning class (seen in (3) with $\epsilon = 0$) we see the catastrophic decrease within one year, however, $\frac{T_b}{R} < 1$, which implies the regulatory T cells still out number the pathogenic T cells. A result that is somewhat non-intuitive when compared with Fig. 2 and shows the model's ability to describe dynamics that are not mainstream and may lead to important conjectures that must be tested experimentally.

Another unique feature of our model is the term $\frac{a_1 T_b B_f}{k_1+T_b}$ which allows for a dynamic change in functionality of β-cells over time and hence does not assume the change to be constant. We tested the model's ability to fit a generated random set of data points showing the gradual decline of functional β-cells over a 30 year period of time. The model allows us to focus on certain kinetics associated with the disease and in Fig. 7 we showed how we can use this model to simulate the decay of β-cells in relation to the islet autoantibodies in the pancreas. These results show the potential of our model to make significant contributions to the understanding of T1DM when applied to real clinical data sets.

Albeit evidence indicates that T1DM is the end result of an altered balance between pathogenic T cells and regulatory Tregs, the mechanisms determining this imbalance have not yet been determined. One hypothesis is that the rate of T1DM progression depends on the degree of epitope spreading, the efficiency of regulatory responses and, possibly, the rate of regeneration of β-cells in response to immune-mediated beta cell injury [81]. Treg cells prevent activation of autoreactive T cells in the lymph nodes by limiting their access to dendritic cells and thus their expansion and achievement of effector functions. These activities are largely mediated by thymus-derived natural Tregs. When immune homeostasis is perturbed and inflammation erupts in the tissues, both natural Tregs and cytokine-induced adaptive Tregs traffic to the site of inammation and inhibit the functions of fully differentiated pathogenic effector T cells in the target tissue. If regulatory responses are defective, as postulated in our model, we can find effector T cell responses that outnumber regulatory responses leading to impairment, destruction of β-cell mass and disease onset. However, we can also find disease onset in patients whose regulatory T cells still outnumber the pathogenic T cells, suggesting a more complex implicit dynamic.

Despite significant advances, a simple, scalable, non-toxic, and highly-effective therapeutic strategy that can indefinitely lead to a recovery of β-cell function or mass remains elusive. At least in theory β-cell mass and function could be rescued by blocking the ability to generate pathogenic T cell responses to islet autoantigen(s) thought to signal the beginning stages of the disease, and by either developing Treg-based cellular therapeutics or delete pathogenic T cells in an attempt to suppress autoimmune responses. In vivo potential mechanisms of action with the ultimate goal of safety and efficacy trials in pre-clinical and new-onset Type 1 diabetic patients may be valuable to help design future prevention trials for Type 1 diabetes. T1DM is a chronic autoimmune disease characterized by dysfunction and ultimately destruction of β-cells in the islets of Langerhans. T1DM presents a complex interaction between genetic, immunological, and environmental factors, most of which have yet to be identified. Hence, we proposed in this paper the first model of its kind to study this complex interaction. For instance, while it is known that when the level of β-cell function is no longer sufficient to maintain metabolic homeostasis, the individual is then dependent on endogenous insulin to sustain life, it is not known why or how these β-cells lose function. Also, the fundamental pathophysiology shared by all patients with T1DM is the progressive loss in the ability of the β-cells of the pancreas to secrete insulin in response to glucose. This progressive decline in beta cell function may be secondary to a defect of regulatory T cells.

With this information at hand we have provided the groundwork for the next stage of models to study T1DM. With real connections between experiment and theory, we expect significant advances in our understanding of this disease.

7. **Future studies and limitations.** We have developed a dynamical systems model that describes β-cells, immune cells, cytokines, glucose and insulin. Our current work describes the relationship between functional β-cells and islet autoantibodies. Our results suggest that the timing in which the β-cells switch over from functional to dysfunctional plays a critical role in the model's predictive ability. As seen in (3) we assume the switch is modeled by $\frac{a_1 T_b B_f}{k_1+T_b} + \epsilon B_{nf}$. While we allow for a dynamic change seen in the levels of T_b and B_f, however, it is assumed to occur at a constant rate a_1. We plan to study a switch that is time dependent such that $a_1 = a(t)$.

We should note the limitations of the model at this point. Time series data is scarce for T1DM and we hope that this work will generate interest from the experimental community to collaborate. The model presented here is robust and allows for a wide range of output and hence predictions. However, we have had to estimate a fair number of parameters and we need to find better experimental estimates for these parameters. This will then allow us to focus specifically on the interaction between regulatory T cells and pathogenic T cells and hence lead to a better understanding of the disease.

A second area of interest is focusing more on the regulatory T cells. We assumed that regulatory T cells can switch over to a dysfunctional class and that these cells become unable to control the pathogenic T cells. However, what causes this switch is unknown. We want to expand the model to test specific hypotheses about the causes for the change in regulatory T cells: Is it an imbalance in the regulatory T cells? Is it caused by the migration of pathogenic T cells in the pancreas? or is it a combination of both?

Third, we plan to begin a mathematical study to evaluate the association between infectious diseases and T1DM [82]. Evidence suggests that the recent increase in the incidence of T1DM that cannot be explained by hereditary events leads one to look for environmental causes, such as childhood infectious diseases [83]. Our mathematical model will allow us to study this in detail.

Finally, it is now well established that autoantibodies are some of the most potent risk determinants for Type 1 diabetes with relative risks exceeding 100 [2, 69, 84, 85]. The quintessential model for the application of autoantibody markers in the prediction of a selective immune-mediated tissue damage, is Type 1 diabetes and this concept can be theoretically extended to other chronic autoimmune diseases. For example, several recent studies have suggested that using a combination of autoantibody markers gives a higher predictive value for T1DM [17, 68, 69, 73, 74, 86, 87, 88] . Among the most characterized molecular targets of the T1DM-related autoimmune, such as insulin, the protein tyrosine phosphatase-like molecule IA-2 (or ICA512), the enzyme glutamic acid decarboxylase (GAD, predominantly the 65 kDa isoform) and the recently discovered zinc transporter ZnT8 [89]. Pietropaolo's group has recently found that an antibody response against an epitope localized within the extracellular domain of the neuroendocrine autoantigen IA-2 can predict a rapid progression of T1DM in adolescents as well as young adults (unpublished results). We are working to apply our model to study the pathogenesis of T1DM in young adults. Our model can describe this early and rapid progression as seen in Fig. 8 and with proper connections to experimental data should be able to help in the understanding of this dynamic.

8. **Acknowledgments.** This work was supported by the National Institutes of Health (grants RO1 DK53456, DK56200, NIDDK PA-04-081, MP) and by the University of Michigan Center for Computational Medicine and Biology (CCMB) Pilot Research Program (PN and MP). We gratefully thank the support of the Brehm Coalition.

REFERENCES

[1] T. J. Orchard, J. Dorman, R. Maser, D. Becker, A. Drash, D. Ellis, R. LaPorte and L. Kuller, *Prevalence of complications in IDDM by sex and duration. Pittsburgh epidemiology of diabetes complications study II*, Diabetes, **39** (1990), 1116–1124.

[2] R. LaPorte, M. Matsushima and Y. Chang, *Prevalence and incidence of insulin-dependent diabetes*, In Diabetes in America, NIDDK Publication, **95** (1995), 37–46.

[3] G. Eisenbarth, *Type 1 Diabetes mellitus: a chronic autoimmune disease*, NEJM, **314** (1986), 1360–1368.

[4] Q. Tang and J. Bluestone, *Regulatory T-cell physiology and application to treat autoimmunity*, Immunol. Rev., **212** (2006), 217–237.

[5] T. Brusko, A. Putnam and J. Bluestone, *Human regulatory T cells: role in autoimmune disease and therapeutic opportunities*, Immunol. Rev., **223** (2008), 371–90.

[6] J. Bluestone and R. von Boehmer, *Regulatory T cells*, Semin. Immunol., **18** (2006), 103–110.

[7] J. Todd, N. Walker, J. Cooper, D. Smyth, K. Downes, V. Plagnol, R. Bailey, S. Nejentsev, S. Field, F. Payne, C. Lowe, J. Szeszko, J. Hafler, L. Zeitels, J. Yang, A. Vella, S. Nutland, H. Stevens, H. Schuilenburg and G. Coleman, *Robust associations of four new chromosome regions. from genome-wide analyses of type 1 diabetes*, Nat. Genet., **39** (2007), 857–864.

[8] H. Hakonarson, S. Grant, J. Bradfield, L. Marchand, C. Kim, J. Glessner, R. Grabs, T. Casalunovo, S. Taback, E. Frackelton, M. Lawson, L. Robinson, R. Skraban, Y. Lu, R. Chiavacci, C. Stanley, S. Kirsch, E. Rappaport, J. Orange and D. Monos, *A genome-wide association study identifies KIAA0350 as a type 1 diabetes gene*, Nature, **448** (2007), 591–594.

[9] J. Todd, J. Bell and H. McDevitt, *HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus*, Nature, **329** (1987), 599–604.

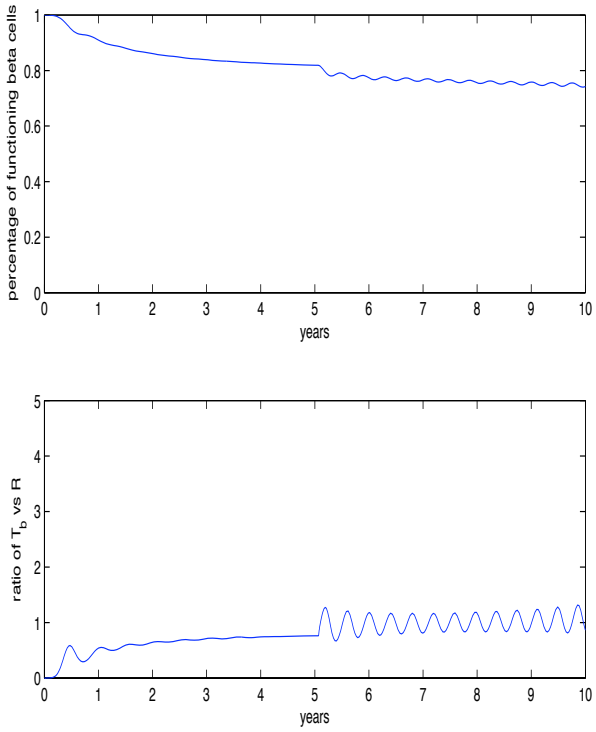

FIGURE 8. With modifications in our function, $S(t)$, we can describe early events in the onset of T1DM in young adults. Top figure shows the gradual decline in functioning β-cells. The initial decline could be due to the subjects genetic disposition to the disease. Once T cell and autoantibody responses occur, we continue to see a decline in the number of functioning β-cells. In conjunction with this, we see the ratio of pathogenic to regulatory T cells (bottom figure) starting to oscillate about 1. With the proper adaption to experimental data, our model can help understand the complex dynamics associated with this disease.

[10] P. Morel, J. Dorman, J. Todd, H. McDevitt and M. Trucco, *Aspartic acid at position 57 of the HLA-DQ beta chain protects against Type I diabetes: A family study*, Proc. Natl. Acad. Sci. USA, **85** (1988), 8111–8115.

[11] C. Greenbaum, D. Schatz, D. Cuthbertson, A. Zeidler, G. Eisenbarth and J. Krischer, *Islet cell antibody-positive relatives with human leukocyte antigen DQA1*0102 DQB1*0602: Identification by the diabetes prevention trial-type 1*, J. Clin. Endocrinol. Metab., **85** (2000), 1255–1260.

[12] M. Atkinson and G. Eisenbarth, *Type 1 Diabetes: New perspectives on disease pathogenesis and treatment*, Lancet, **358** (2001), 221–229.

[13] A. Rossini, *Autoimmune diabetes and the circle of tolerance*, Diabetes, **53** (2004), 267–275.

[14] E. Masteller, Q. Tang and J. Bluestone, *Antigen-specific regulatory T cells – ex vivo expansion and therapeutic potential*, Semin. Immunol., **18** (2006), 103–110.

[15] N. Phillips, N. Giannoukakis and M. Trucco, *Dendritic cell mediated therapy for immunoregulation of type 1 diabetes mellitus*, Pediatr. Endo. Rev., **5** (2008), 873–879.

[16] N. Giannoukakis, B. Phillips and M. Trucco, *Toward a cure for type 1 diabetes mellitus: diabetes-suppressive dendritic cells and beyond*, Pediatr. Diabetes, **9** (2008), 4–13.

[17] C. Verge, R. Gianani, E. Kawasaki, L. Yu, M. Pietropaolo, R. Jackson, P. Chase and G. Eisenbarth, *Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies*, Diabetes, **45** (1996), 926–933.

[18] C. Verge, R. Gianani, E. Kawasaki, L. Yu, M. Pietropaolo, H. Chase and G. S. Eisenbarth, *Number of autoantibodies (against insulin, GAD or ICA12/IA2) rather than particular autoantibody specificities determines risk of type 1 diabetes*, J. Autoimmun., **7** (1996), 379–383.

[19] P. Nelson, J. Mittler and A. Perelson, *Effect of drug efficacy and the eclipse phase of the viral life cycle on estimates of HIV-1 viral dynamic parameters*, J. AIDS, **26** (2001), 405–412.

[20] M. Nowak, S. Bonhoffer, A. Hill, R. Boehme, H. Thomas and H. McDade, *Viral dynamics in hepatitis B virus infection*, Proc. Natl. Acad. Sci. USA, **93** (1996), 4398–4402.

[21] A. Perelson and P. Nelson, *Mathematical models of HIV dynamics in vivo*, SIAM Review, **41** (1999), 3–44.

[22] S. Ciupe, R. Ribeiro, P. Nelson, G. Dusheiko and A. Perelson, *The role of cells refractory to productive infection in acute hepatitis B viral dynamics*, Proc. Natl. Acad. Sci. USA, **104** (2007), 5050–5055.

[23] A. Bauer, T. Jackson and Y. Jiang, *A cell-based model exhibiting branching and anstomosis during tumor-induced angiogenesis*, Biophys. J., **92** (2007), 1–17.

[24] T. Jackson, S. Lubkin, N. Siemers, O. Kerr, P. Senter and J. Murray, *Mathematical and Experimental analysis of localization of anti-tumor antibody enzyme conjugates*, B. J. of Cancer, **80** (1999), 1747–1753.

[25] J. Keener and A. Panfilov, *A biophysical model for defibrillation of cardiac tissue*, Biophys. J., **71** (1996), 1335–1345.

[26] B. Topp, K. Promislow, G. deVries, R. Miura and D. Finegood, *A Model of beta-cell mass, insulin, and glucose kinetics: Pathways to Diabetes*, J. Theor. Biol., **206** (2000), 605–619.

[27] D. Freiesleben, P. Bak, F. Pociot, A. Karlsen and J. Nerup, *Onset of type 1 diabetes: A dynamical instability*, Diabetes, **48** (1999), 1677–1685.

[28] R. Bergman, R. Prager, A. Volund and J. Olefsky, *Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp*, J. Clin. Invest., **79** (1987), 790–800.

[29] R. Bergman, Y. Ider, C. Bowden and C. Cobelli, *Quantitative assessment of insulin sensitivity*, Am. J. Physiol., **236** (1979), E667–E677.

[30] A. DeGaetano and O. Arino, *Mathematical modeling of the intravenous glucose tolerance test*, J. Math. Biol., **40** (2000), 136–168.

[31] J. Li and Y. Kuang, *Analysis of a model of the glucose-insulin regulatory system with two delays*, SIAM J. Appl. Math., **67** (2007), 757–776.

[32] Y. Chen, S. Wang and A. Sherman, *Identifying the targets of the amplifying pathway for insulin secretion in pancreatic beta-cells by kinetic modeling of granule exocytosis*, Biophys. J., **95** (2008), 2226–2241.

[33] J. Li and Y. Kuang, *Systemically modeling the dynamics of plasma insulin in subcutaneous injection of insulin analogues for type 1 diabetes*, Math. Biosci. Eng., **6** (2009), 41–58.

[34] R. Bertram, J. Previte, A. Sherman, T. Kinard and L. Satin, *The phantom burster model for pancreatic beta-cells*, Biophys. J., **79** (2000), 2880–2892.

[35] R. Bertram, L. Satin, M. Zhang, P. Smolen and A. Sherman, *Calcium and glycolysis mediate multiple bursting modes in pancreatic islets*, Biophys. J., **87** (2004), 3074–3087.
[36] C. Nunemaker, R. Bertram, A. Sherman, K. Tsaneva-Atanosova, C. Daniel and L. Satin, *Glucose modulates Ca2+ oscillations in pancreatic islets via ionic and glycolytic mechanisms*, Biophys. J., **91** (2006), 2082–2096.
[37] J. M. Mahaffy and L. Edelstein-Keshet, *Modeling cyclic waves of circulating T cells in autoimmune diabetes*, SIAM J. Appl. Math., **67** (2007), 937–951.
[38] L. Shoda, D. Young, S. Ramanujan, C. Whiting, M. Atkinson, J. Bluestone, G. Eisenbarth, D. Mathis, A. Rossini, S. Campbell, R. Kahn and H. Kreuwel, *A comprehensive review of interventions in the NOD mouse and implications for translation*, Immunity, **23** (2005), 115–126.
[39] X. Wang, Z. He and S. Ghosh, *Investigation of the age-at-onset heterogeneity in type 1 diabetes through mathematical modeling*, Math. Biosci., **203** (2006), 79–99.
[40] Y. Zheng, H. Kreuwel, D. Young, L. Shoda, S. Ramanujan, K. Gadkar, M. Atkinson and C. Whiting, *Applying predictive biosimulation to research in type 1 diabetes*, Ann. N. Y. Acad. Sci., **1103** (2007), 45–62.
[41] M. Pietropaolo, J. Surhigh, P. Nelson and G. Eisenbarth, Primer: immunity and autoimmunity, Diabetes, **57** (2008), 2872–2882.
[42] J. Bach and D. Mathis, *The NOD mouse*, Res. Immunol., **148** (1997), 285–286.
[43] M. Atkinson and E. Leiter, *The NOD mouse model of type 1 diabetes: as good as it gets*, Nat. Med., **5** (1999), 601–604.
[44] A. Rossini, R. Williams, J. Mordes, M. Appel and A. Like, *Spontaneous diabetes in gnotobiotic BB/W rat*, Diabetes, **28** (1979), 1031–1032.
[45] A. Like, A. Butler, R. Williams, M. Appel, E. Weringer and A. Rossini, *Spontaneous autoimmune diabetes mellitus in the BB rat*, Diabetes, **31** (1982), 7–13.
[46] J. Skyler, C. Greenbaum, J. Lachin, E. Leschek, L. Rafkin-Mervis, P. Savage and L. Spain, *Type 1 Diabetes TrialNet–an international collaborative clinical trials network*, Ann. N.Y. Acad. Sci., **1150** (2008), 14–24.
[47] M. von Herrath and G. Nepom, *Animal models of human type 1 diabetes*, Nat. Immunol., **10** (2009), 129–132.
[48] A. De Gaetano, T. Hardy, B. Beck, E. Abu-Raddad, P. Palumbo, J. Bue-Valleskey and N. Porksen, *Mathematical models of diabetes progression*, Am. J. Physiol. Endocrinol. Metab., **295** (2008), E1462–E1479.
[49] D. Finegood, L. Scaglia and S. Bonner-Weir, *Dynamics of beta-cell mass in the growing rat pancreas estimation with a simple mathematical model*, Diabetes, **44** (1995), 249–256.
[50] F. Athanasius, M. Maree, R. Kublik, D. Finegood and L. Edelstein-Keshet, *Modeling the onset of Type 1 diabetes: can impaired macrophage phagocytosis make the difference between health and disease?* Phil. Trans. R. Soc. A., **364** (2006), 1267–1282.
[51] W. Malaisse, F. Malaisse-Lagae and E. McCraw, *Effects of thyroid function upon insulin secretion*, Diabetes, **16** (1967), 643–646.
[52] R. Hoffman, P. Vicini and C. Cobellli, *Comparison of insulin sensitivity and glucos effectiveness determined by the one- and two-compartment-labeled minimal model in late prepubertal children and early adolescents*, Metabolism, **51** (2002), 1582–1586.
[53] K. Daugherty, *Review of Insulin Therapy*, J. Pharm. Pract., **17** (2004), 10–19.
[54] R. Bergman, D. Finegood and M. Ader, *Assessment of insulin sensitivity in vivo*, Endocrine Rev., **6** (1985), 45–58.
[55] G. Weir and S. Bonner-Weir, *Five stages of evolving beta-cell dysfunction during progression to diabetes*, Diabetes, **53** (2004), S16–S21.
[56] A. Fernandes, L. King, Y. Guz, R. Stein, V. Wright and G. Teilelman, *Differentation of new insulin-producing cells is induced by injury in adult pancreatic islets*, Endocrin., **138** (1997), 1750–1762.
[57] S. Bonner-Weir, L. Baxter, G. Schuppin and T. Smith, *A second pathway for regeneration of adult exocrine and endocrine pancreas: A possible recapitulation of embryonic development*, Diabetes, **42** (1993), 1715–1720.
[58] S. Bonner-Weir, A. Inada, S. Yotoh, W. Li, T. Ave, E. Toschi and A. Sharma, *Transdifferentiation of pancreatic ductal cells to endocrine beta-cells*, Biochem. Soc. Trans., **36** (2008), 353–356.
[59] I. Swenne, *The role of glucose in the in-vitro regulation of cell cycle kinetics and proliferation of fetal pancreatic beta-cells*, Diabetes, **31** (1982), 754–760.

[60] M. Hoorens, A. Van de Casteele, G. Kloppel and D. Pipeleers, *Glucose promotes survival of rat pancreatic beta-cells by activating synthesis of proteins which suppress a constitutive apoptotic program*, J. Clin. Invest., **98** (1996), 1568–1574.

[61] B. Efanova, S. Zaitsev, B. Zhivotovsky, M. Kohler, S. Efendic, S. Orrenius and P. Berggren, *Glucose and tolbutamide induce apoptosis in pancreatic beta-cells*, J. Biol. Chem., **273** (1998), 33501–33507.

[62] G. Weir and S. Bonner-Weir, *A dominant role for glucose in beta cell compensation of insulin resistance*, J. Clin. Invest., **117** (2007), 81–83.

[63] A. Jorns, A. Gunther, H. Hendrick, D. Wedekind, M. Tiedge and S. Lenzen, *Immune cell infiltration, cyotkine expression, and beta-cell apoptosis during the development of type 1 Diabetes in the spontaneous diabetic LEW.1AR1/Ztm-iddm Rat*, Diabetes, **54** (2005), 2041–2052.

[64] E. Simpson, *Special regulatory T-cell review: regulation of immune responses - examining the role of T cells*, Immunology, **123** (2008), 13–16.

[65] M. Donath and J. Ehses, *Mechanisms of Beta-cell death in diabetes*, Springer Japan, , 2008

[66] M. Pietropaolo, S. Yu, I. Libman, S. Pietropaolo, K. Riley, R. LaPorte, A. Drash, S. Mazumdar, M. Trucco and D. Becker, *Cytoplasmic islet cell antibodies remain valuable in defining risk of progression to type 1 diabetes in subjects with other islet autoantibodies*, Pediatr. Diabetes, **4** (2005), 184–192.

[67] E. Gale and P. Bingley, *Can we prevent IDDM?* Diabetes, **42** (1993), 213–220.

[68] P. Bingley, M. Christie, E. Bonifacio, R. Bonfanti, M. Shattock, M. Fonte, G. Bottazzo and E. Gale, *Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives*, Diabetes, **43** (1994), 1304–1310.

[69] N. Maclaren, M. Lan, R. Coutant, D. Schatz, J. Silverstein, A. Muir, M. Clare-Salzler, J. She, J. Malone, S. Crockett, S. Schwartz, T. Quattrin, M. DeSilva, P. Vander Vegt, A. Notkins and J. Krischer, *Only multiple autoantibodies to islet cells (ICA), insulin, GAD65, IA-2 and IA-2b predict immune-mediated (Type 1) diabetes in relatives*, J. Autoimmun., **12** (1999), 279–287.

[70] M. Pietropaolo and G. Eisenbarth, *Autoantibodies in human diabetes*, Curr. Dir. Autoimmun., **4** (2001), 252–282.

[71] M. Pietropaolo, D. Becker, R. LaPorte, J. Dorman, S. Riboni, W. Rudert, S. Mazumdar and M. Trucco, *Progression to insulin-requiring diabetes in seronegative prediabetic subjects: The role of two HLA-DQ high risk haplotypes*, Diabetologia, **45** (2002), 66–76.

[72] J. Krischer, D. Cuthbertson, L. Yu, T. Orban, N. Maclaren, R. Jackson, W. Winter, D. Schatz, J. Palmer and G. Eisenbarth, *Screening strategies for the identification of multiple antibody-positive relatives of individuals with type 1 diabetes*, J. Clin. Endocrinol. Metab., **88** (2003), 103–108.

[73] U. Wiest-Ladenburger, R. Hartmann, U. Hartmann, K. Berling, B. Bohm and W. Richter, *Combined analysis and single-step detection of GAD65 and IA2 autoantibodies can replace the histochemical islet cell antibody test*, Diabetes, **46** (1997), 565–571.

[74] M. Pietropaolo, M. Peakman, S. Pietropaolo, M. Zanone, T. Foley, D. Becker and M. Trucco, *Combined analysis of GAD65 and ICA512(IA-2) autoantibodies in organ and non-organ specific autoimmune diseases confers high specificity for insulin-dependent diabetes mellitus*, J. Autoimmun., **11** (1998), 1–10.

[75] J. Skyler, *Prediction and prevention of type 1 diabetes: progress, problems, and prospects*, Clin. Pharmacol. Ther., **81** (2007), 768–771.

[76] D. Homann and M. von Herrath, *Regulatory T cells and type 1 diabetes*, Clin. Immunol., **112** (2004), 202–209.

[77] G. Bottazzo, A. Florin-Christensen and D. Doniach, *Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies*, Lancet, **2** (1974), 1279–1283.

[78] J. Sosenko, J. Krischer, J. Palmer, J. Mahon, C. Cowie, C. Greenbaum, D. Cuthbertson, J. Lachin and J. Skyler, *Diabetes Prevention Trial-Type 1 Study Group. A risk score for type 1 diabetes derived from autoantibody-positive participants in the diabetes prevention trial-type 1*, Diabetes Care, **31** (2008), 528–533.

[79] DPT, *Effects of insulin in relatives of patients with type 1 diabetes mellitus*, NEJM, **346** (2002), 1685–1691.

[80] E. Gale, P. Bingley, C. Emmett and T. Collier, *European Nicotinamide diabetes intervention trial: a randomised controlled trial of intervention before the onset of type 1 diabetes*, Lancet, **20** (2004), 925–931.

[81] M. von Herrath, S. Sanda and K. Herold, *Type 1 diabetes as a relapsing-remitting disease*, Nat. Rev. Immunol., **7** (2007), 988–994.
[82] M. Tenconi, G. Devoti, M. Comelli, M. Pinon, A. Capocchiano, V. Calcaterra, G. Pretti and the Pavia T1DM Registry Group, *Major childhood infectious diseases and other determinants associated with type 1 diabetes: A case-control study*, Acta Diabet., **44** (2006), 14–19.
[83] Z. Laron, *Interplay between heredity and environment in the recent explosion of type 1 childhood diabetes mellitus*, Am. J. Med. Gen., **115** (2002), 4–7.
[84] J. Dorman and others, *Risk factors for insulin-dependent diabetes. In Diabetes in America*, National Institute of Diabetes and Digestive and Kidney Diseases, *1* (1995), 165–177.
[85] T. Palosuo, J. Virtamo, R. Taylor, K. Aho, M. Puurunen and O. Vaarala, *High antibody levels to prothrombin imply a risk of deep venous thrombosis and pulmonary embolism in middle-aged men. A nested case-control study*, Thromb. Haemostasis, **78** (1997), 1178–1182.
[86] H. Barmeier, D. McCulloch, J. Neifing, Warnock, R. Rajotte, J. Palmer and A. Lernmark, *Risk for developing type 1 (insulin-dependent) diabetes mellitus and the presence of islet 64K antibodies*, Diabetologia, **34** (1991), 727–733.
[87] W. Riley, N. Maclaren, J. Krischer, R. Spillar, J. Silverstein, D. Schatz, S. Schwartz, J. Malone, S. Shah, C. Vadheim and J. Rotter, *A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes*, N. Engl. J. Med., **323** (1990), 1167–1172.
[88] A. Ziegler, M. Hummel, M. Schenker and E. Bonifacio, *Autoantibody appearance and risk for development of childhood diabetes in offspring parents with Type 1 diabetes. The 2-year analysis of the German BABYDIAB study*, Diabetes, **48** (1999), 460–468.
[89] J. Wenzlau, K. Juhl, L. Yu, O. Moua, S. Sarkar, P. Gottlieb, M. Rewers, G. Eisenbarth, J. Jensen, H. Davidson and J. Hutton, *The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes*, Proc. Natl. Acad. Sci. USA, **104** (2007), 17040–17045.

9. **Appendix 1.** Complete list of model equations.

$$\frac{dG}{dt} = R_0 - G(Eg_0 + S_i I), \tag{9}$$

$$\frac{dI}{dt} = \frac{\sigma B_f G^2}{\alpha + G^2} - \delta_I I, \tag{10}$$

$$\frac{dB_f}{dt} = (r_1 G - d_0 - r_2 G^2)B_f - \frac{a_1 T_b B_f}{k_1 + T_b} + \epsilon B_{nf}, \tag{11}$$

$$\frac{dB_{nf}}{dt} = \frac{a_1 T_b B_f}{k_1 + T_b} - \gamma_T d_1 B_{nf} - \epsilon B_{nf}, \tag{12}$$

$$\frac{dT_b}{dt} = S(t) + \frac{a_3 T_b}{k_4 + T_b}(1 - \frac{T_b + R + R_b + B_f + B_{nf}}{K_p}) - d_3 T_b - \delta T_b R, \tag{13}$$

$$\frac{dR}{dt} = \frac{a_4 R I_2}{k_2 + I_2}(1 - \frac{T_b + R + R_b + B_f + B_{nf}}{K_p}) - d_2 R - \sigma_r R, \tag{14}$$

$$S(t) = (\alpha_1 H(t - \tau_1) + \alpha_2 H(t - \tau_2) + \alpha_3 H(t - \tau_3) + \alpha_4 H(t - \tau_4))\frac{t^3}{K + t^3}, \tag{15}$$

$$\frac{dR_b}{dt} = \sigma_r R - \delta_R R_b, \tag{16}$$

$$\frac{dI_2}{dt} = \frac{\rho_1 I_2 R}{k_4 + R} - \mu I_2, \tag{17}$$

$$\tag{18}$$

Table 1. Summary of Variables

Variable	Definition	Units	Values	Reference
G	Glucose	mg V^{-1}	$(4-6)$	[26]
I	Insulin	Unit (U) V^{-1}		[26]
B_f	Functioning β-cells	Cells (mg) V^{-1}	$(0-500)$	Estimated
B_{nf}	Non-functioning β-cells	Cells V^{-1}	$B_{nf}(0)=0$	Estimated
T_b	Pathogenic T cells	Cells/(mm^3)	$T_b(0)=0$	Estimated
R	Functioning Tregs	Cells/(mm^3)	$R(0)=100$	Estimated
I_2	Cytokine IL-2	Proteins (IU)/(mm^3)	$I_2(0)=100$	Estimated
R_b	Non-functioning Tregs	Cells/(mm^3)	$R_b(0)=0$	Estimated

Table 2. Summary of parameter values

Parameter	Definition	Units	Values	Reference
R_0	glucose production	[G] day^{-1}	$(500-1000)$	[26]
E_{g0}	glucose effectiveness	day^{-1}	1.44	[26]
S_i	insulin sensitivity	$[I]^{-1}$ day^{-1}	0.72	[26]
σ	Insulin production	U $([B_f]$ day$)^{-1}$	(20 - 50)	[26]
α	half-saturation constant	$[G]^2$	20000	[26]
σ_r	regeneration rate of R	day^{-1}	(0-1)	Estimated
δ_i	death rates	day^{-1}	$(0-1)$	various
δ	killing rate for T_b	([R] day$)^{-1}$	(0-2)	Estimated
r_1	replication rate	(mg [G] day$)^{-1}$	$(8-9)*10^{-4}$	[26]
d_i	death rates	day^{-1}	$(0-1)$	Various
r_2	hyperglycemia rate	(mg [G]$)^{-2}$ day^{-1}	$(0.001-0.01)$	[26]

Table 3. Summary of parameter values

Parameter	Definition	Units	Values	Reference
a_1	rate of switch between B_f and B_{nf}	day^{-1}	(0-5)	Estimated
$k_{1,2}$	Half-saturations constants	Cells	Various	Estimated
k_4	Half-saturation constant	$[I_2]$	Various	Estimated
ϵ	rate of B_{nf} regaining function	day^{-1}	(0-1)	Estimated
γ_T	death factor	unit-less	(0-1)	
γ_R	death rate	day^{-1}	(0-5)	Estimated
a_3	production rate	$[T_b]$ day^{-1}	(0-10)	Estimated
a_4	production rate	day^{-1}	(0-10)	Estimated
K_p	carrying capacity	Cells	$(10^2, 10^5)$	Estimated
δ	control rate	([R] day$)^{-1}$	(0-1)	Estimated
α_i	strength constant	$[T_b]day^{-1}$	(0-1000)	Estimated
ρ_1	rate of IL2 production	day^{-1}	(0-100)	Estimated
μ	death rate for IL-2	$days^{-1}$	(0-1)	Estimated

Received January 16, 2009; Accepted July 10, 2009.

E-mail address: pwn@umich.edu
E-mail address: noahsmit@umich.edu
E-mail address: stanca.ciupe@duke.edu
E-mail address: wzou@med.umich.edu
E-mail address: gomenn@med.umich.edu
E-mail address: maxtp@med.umich.edu