

Perspectives in Diabetes

Onset of Type 1 Diabetes

A Dynamical Instability

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Type 1 diabetes is a disease characterized by progressive loss of β -cell function due to an autoimmune reaction affecting the islets of Langerhans. It is now generally accepted that cytokines are implicated in the pathogenesis of autoimmune diseases. Animal studies have shown that interleukin- 1β , tumor necrosis factor- α , and interferon- γ affect type 1 diabetes development profoundly. It has been suggested that β -cells are destroyed by cytokine-induced free radical formation before cytotoxic T-helper (Th)-lymphocytes and/or autoantibody-mediated cytotoxicity. This hypothesis is known as the "Copenhagen model." We introduce a mathematical model encompassing the various processes within this framework. The model is expressed in rate equations describing the changes in numbers of β -cells, macrophages, and Th-lymphocytes. Being concerned with the earliest events, we explore the conditions necessary to maintain self-sustained β -cell elimination based on the feedback between immune cells and insulin-producing cells. The motivation for this type of analysis becomes clear when we consider the multifactorial and complicated nature of the disease. Indeed, recent research has provided detailed information about the different factors that contribute to the development of the disease, stressing the importance of incorporating these findings into a more general picture. A mathematical formalism allows for a more comprehensive description of the biological problem and can reveal nonintuitive properties of the dynamics. Despite the rather complicated structure of the equations, our main conclusion is simple: onset of type 1 diabetes is due to a collective, dynamical instability, rather than being caused by a single etiological factor. *Diabetes* 48:1677–1685, 1999

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IFN, interferon; IL-1, interleukin- 1β ; Th, T-helper; TNF, tumor necrosis factor. Definitions of mathematical symbols appear in Table 1 and Fig. 3.

Since the first experimental demonstration in 1971 of the role of autoimmunity in type 1 diabetes (1), investigations have focused on elucidating the pathogenesis of the disease. Genetic studies of type 1 diabetes indicate that the susceptibility to the disease is in part inherited, with the major histocompatibility complex on chromosome 6 being particularly important. The number of loci that have been found to either predispose or accelerate the disease development is growing (2,3). Based on the reported concordance rate in monozygotic twins (35–50%) (4,5), it is presumed that environmental factors also participate in the generation of type 1 diabetes. Epidemiological data (6,7) support that several environmental factors may initiate or precipitate the progress of the disease. In particular, interest has been shown in the possible roles played by infectious agents, chemical substances, and nutrient factors (8,9). Considering the prospect of early therapeutic treatment strategies and the identification of individuals with a high risk of developing type 1 diabetes, a fundamental question arises: How does one include all these factors into a succinct description of the onset of insulin-dependent diabetes?

It has often been suggested that the onset follows the occurrence of an environmental triggering event. However, it appears likely that when the triggering event occurs, a collective unbalanced situation between the molecular and cellular mechanisms related to the disease development must already be present. In analogy with the impossibility of starting a forest fire if the trees are wet, the "ignition" of accelerating β -cell destruction is possible only if the dynamics of the system allow the destructive process to propagate.

We focus on the earliest events, addressing the interplay between activated macrophages, T-helper (Th)-lymphocytes, and target cells. We use the language of mathematics to describe the change from a normal stasis to an abnormal situation with accelerating tissue destruction, because mathematics is able to unfold the influence that each of the processes has on the overall dynamical behavior.

The relevant mechanisms are formulated in terms of ordinary, coupled differential equations. A variable M represents the numbers of macrophages, T the numbers of Th-lymphocytes, and A the amount of antigens (Table 1). For an introduction to differential equations and population biology, see Hastings (10). Parameters (a , b , c) give a quantitative measure of the effects that the different mechanisms have on the multitude of cells and antigens present. The signs of the param-

TABLE 1
List of symbols

A , amount of β -cell antigenic proteins in volume.
M , numbers of macrophages in volume.
M_A , numbers of antigen-expressing macrophages in volume.
T , numbers of activated Th-lymphocytes in volume.
\dot{A} , the derivative of A with respect to time, i.e. $\frac{dA}{dt}$.
\dot{M} , the derivative of M with respect to time.
\dot{M}_A , the derivative of M_A with respect to time.
\dot{T} , the derivative of T with respect to time.

a , recruitment rate of macrophages to volume.
b , IL-1-, TNF- α -induced recruitment rate of macrophages.
c , efflux rate of macrophages from volume.
g , uptake rate of β -cell antigens in macrophages.
k , decay rate of activated macrophages.
l , effective rate of IL-1-, TNF- α -induced β -cell damage.
m , clearance rate of free β -cell proteins.
p , effective rate of IFN- γ -induced β -cell damage.
q , effective rate of β -cell damage caused by effector-macrophages.
s , proliferation rate of Th-lymphocytes.
t , death rate of activated Th-lymphocytes.

eters are positive for a process that tends to increase the population, whereas a negative sign corresponds to a mechanism with a limiting effect. One might think of the parameters either as quantitative measures of physical properties originating from different genetic loci—in which case they define the initial type 1 diabetes susceptibility of the individual—or as quantitative measures of environmental factors, in which case they represent the history of the individual.

In practice it is difficult, if not impossible at present, to extract accurate values for the parameters from clinical measurements. Our aim is not primarily to make quantitative predictions, but to illustrate how the many different processes act together to induce the disease. None of these processes could produce the disease alone; indeed, each of them have a natural physiological function. Yet, acting collectively, they form a vicious network leading to destruction of β -cells: the disease is multifactorial.

More specifically, the goal of the mathematical analysis is to describe the dynamics of the components involved and thus to predict the ability of the system to withstand the triggering event without causing further damage to the remaining β -cells. If the system is tolerant to the initial release of β -cell antigenic peptides, the antigens are simply swept away by scavenger cells, and after a short time the islets area will appear unaffected. This behavior is associated with the mathematical notion of stability. In brief, a stable system is robust toward environmental changes, much like the physiological concept of homeostasis. Therefore, any small disturbance away from the steady state—say, the death of few β -cells accompanied by liberation of proteins—will die out, and the long-term behavior remains unaltered. In contrast, an unstable state is sensitive toward small changes, which might have a profound impact on the future state of the system.

For example, consider a ball situated in the bottom of a valley. It is in a stable state: you can give it a kick, but it will return to the same state. In contrast, a ball placed on the top of a hill, or in a saddle point, is in an unstable state: any small kick will cause it to roll downhill. A gradual change of the landscape

around the ball might cause a transition from stability to instability like the one described here.

The quantitative relations among the various parameters control the dynamics. For some values of the parameters, the system is stable; for others, it is unstable. Thus, variation in the parameters representing the various processes may, under some circumstances, lead to dramatic changes in the cell population sizes. With this in mind, it makes sense to view the dynamics in terms of a parameter space, in which each parameter represents a dimension and the state of the system is determined by a point in this space.

Imagine a situation in which all parameters are held constant except one key parameter that controls the overall feedback process. Setting this key parameter equal to zero will render the system tolerant: liberation of β -cell proteins will only transiently activate the immune system. This is in accordance with the fact that only a fraction of school children positive for type 1 diabetes-relevant autoantibodies will ever develop clinical disease (11). By gradually increasing the parameter, hence increasing the feedback, a point will be reached where the process of destruction becomes self-sustained. Any further increase in the key parameter will accelerate the tissue damage. The set of parameters for which the destruction is exactly self-sustained forms a multidimensional surface in the parameter space. Below the surface, the system is termed stable; above the surface, the destruction will propagate through the pancreas.

Figure 1 gives a schematic picture of a parameter space. The stable solutions are separated from the unstable solutions by a surface embedded in this parameter space, and we relate type 1 diabetes onset to a dynamical transition from a stable to an unstable regime. In this context, every parameter represents a degree of freedom, which in principle can be varied individually. Nevertheless, the stability of the system is defined by the collective values of all parameters. A simple, illustrative example of an unstable dynamical system is the familiar situation of a whining loudspeaker that makes noise when sufficient feedback is established through microphone and amplifier.

The parameter space can be a guideline to identify possible intervention strategies based on the following reason-

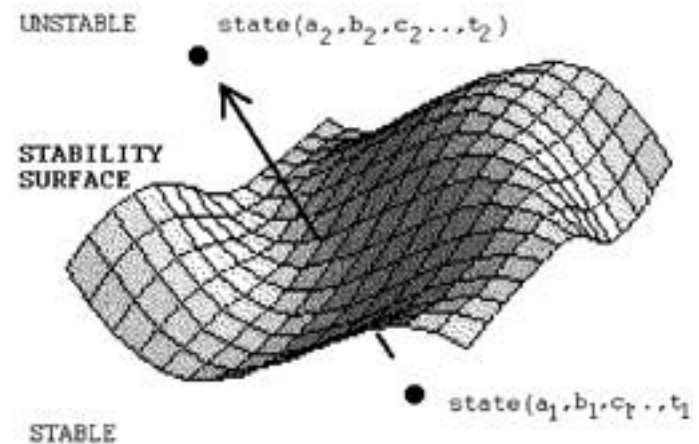


FIG. 1. Illustration of a stability surface. The surface is spanned by the various parameters of the model. During a lifetime, the parameter configuration may change because of various exterior or interior environmental factors. When the surface is traversed, as indicated by the arrow, the system becomes unstable.

ing: any successful early treatment should modify the parameters to reduce the feedback processes. The essential consideration is that the system is brought back to—or prevented from leaving—the stable site of the surface.

The mathematical depiction links type 1 diabetes susceptibility directly to the position of the state on the graph. Presumably, individuals who are genetically predisposed to the disease are “born” with a parameter configuration near the surface. This can be exemplified by the observation that monozygotic twins and HLA-DQ identical siblings to an type 1 diabetic proband have much higher risk of acquiring type 1 diabetes than the risk in the general population (3). Further, in the general population, individuals carrying two HLA-DQ risk alleles have the highest risk of developing type 1 diabetes (12).

The various parameters are not constant during a lifetime, but are thought to vary on time scales much slower than the typical time scale of cellular interactions studied here. Interestingly, susceptibility varies over time: it has been reported that in NOD mice, treatment with tumor necrosis factor (TNF) from the 1st day after birth precipitated diabetes ~4 weeks earlier than in mice treated with phosphate-buffered saline, whereas if treatment was initiated at 4 weeks of age, TNF delayed onset (13).

In short, as a consequence of environmental changes, the path in the parameter space may traverse the stability surface, changing the immunomediated damage from being a local, dampened phenomenon to originating a persistent destruction that is able to propagate through the pancreas islets. Eventually, further activation of other immune cells will accelerate the processes and the person develops overt diabetes.

THE MODEL

Pathogenesis. The clinical manifestation of autoimmune diabetes and complete disappearance of insulin-producing cells in the islets of Langerhans follow years after initiation of the disease. The pathogenesis involves several, discrete events, and each step is subjected to various regulatory mechanisms. Recent research has elucidated that many autoimmune diseases are characterized by an overproduction of cytokines (14,15). In the specific case of type 1 diabetes, several cytokines have been shown to be able to contribute to the pathology, especially interferon- γ (IFN- γ) (16–18) and TNF- α (17), as well as documented effects from *in vitro* and *in vivo* experiments involving interleukin-1 β (IL-1) (19–21). In addition, imbalanced Th-1/Th-2 immune response may contribute to the development of the disease (22,23).

The Copenhagen model of early pathogenesis is described in detail by Nerup et al. (24) and was recently revisited (25). It is shown in its current form in Fig. 2 and described in the corresponding legend.

Mathematical model. We present two simple versions of a mathematical model outlining the interactions between the immune system and the target cells. The first version describes the interactions between macrophages and β -cells; the second is an extension of the simple model, including the effect of Th-lymphocytes. Our aim is not to write down exact equations overloaded with details. Rather, we make a limited number of essential assumptions to investigate the general properties of a cytokine-driven initialization phase and especially to study the part taken by the macrophages.

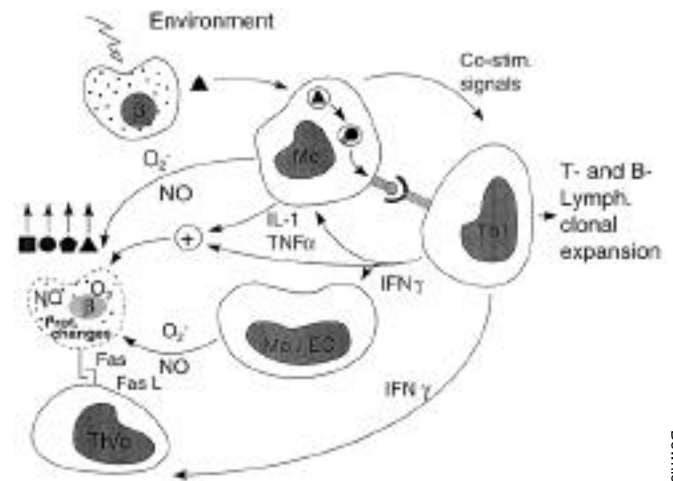


FIG. 2. The Copenhagen model of type 1 diabetes. The model predicts that anything from the external or internal environment that can destroy a β -cell (nutrients? virus? chemicals? IL-1?) will lead to the release of proteins. These proteins will be taken up by residing antigen-presenting cells (APCs), i.e., macrophages, monocytes, and dendritic cells, in the islets; will be processed to antigenic peptides; and, as such, will be presented by major histocompatibility complex (MHC) class II molecules on the cell surface. This activates the APCs to produce and secrete monokines (IL-1, TNF) and costimulatory signal(s), which, if Th-lymphocytes with receptors specifically recognizing the antigenic peptide are present in the islet, induce the transcription of a series of lymphokine genes. One of these, IFN, will feedback-stimulate the APC to increase expression of MHC class II molecules and secretion of IL-1 and TNF. In addition, other cells of the macrophage/monocyte/dendritic cell lineage present in the islet are also induced to secrete monokines. IL-1, potentiated by TNF and IFN, is cytotoxic to β -cells through the induction of free radicals (FRs), e.g., NO, O_2^- formation in the islet. During the inflammatory process, FRs may be induced in both APCs and β -cells. Cytokines further induce changes in expression levels of a multitude of proteins in the target. Among these is induction of Fas expression on the β -cell surface. Interaction between Fas on β -cells and Fas ligand on infiltrating lymphocytes leads to β -cell apoptosis. As part of the β -cell destructive mechanism, β -cell proteins are modified by FRs and, in more antigenic forms, released and presented to the immune system, thereby closing the loop in a self-perpetuating and self-limiting fashion. The magnitude of β -cell destruction will depend on the velocity of the feedback circuit between APCs and the Th-lymphocytes, i.e., on the efficacy of antigen transport/presentation/recognition; on the magnitude and type of cytokine production; and on the capacity of β -cell defense mechanisms during the cytokine exposure.

Consider a small, fixed volume of the pancreas. We will adopt a notation where the various processes are labeled (a), (b), and (c). Their rates are quantified by the corresponding parameters, a , b , c , etc. (see Fig. 3 and Table 1 for list of symbols). The various parameters are not necessarily constant during a lifetime, but are thought to vary on time scales much slower than the typical time scale of cellular interactions studied here.

In the following, we shall use the variable A to denote the amount of free, liberated β -cell antigenic proteins. The variable M represents the numbers of macrophages in the volume, and the variable M_A gives an account of the numbers of antigen-expressing macrophages present. We divide the macrophage population into two groups because only activated cells containing antigenic peptides produce cytokines.

The volume is considered open, so cells may enter or exit the volume. In particular, macrophages enter in a steady inflow rate, indicated in Fig. 3 by (a), and may leave the volume at a rate

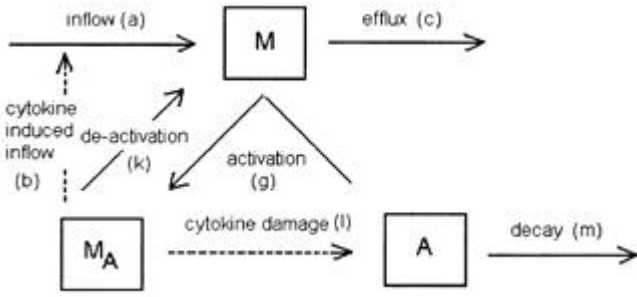


FIG. 3. Schematic representation of the mathematical model.

(c) proportional to their concentration. This assigns a typical time $1/c$ for the macrophages to remain in the volume.

Activated macrophages are formed when antigenic proteins are taken up by the pool of resting cells. This process is named g and is dependent on both the amount of macrophages present and the concentration of antigenic proteins. We assume that the rate of formation is proportional to both quantities. The macrophages exist in active form for a limited time before they rejoin the inactive population, described by the mechanism k . More accurately, we should also include the possibility that these macrophages might die, but we omit this process since a great majority of cells return to the inactive state.

Activated macrophages release cytokines, IL-1 and TNF, that are used as signal molecules during immune responses and will direct other macrophages to enter the volume (b). Summing up, we arrive at the following differential equations:

$$\begin{aligned} \dot{M} &= a + (k + b) M_A - cM - gMA \\ \dot{M}_A &= gMA - kM_A \end{aligned} \quad (1)$$

Here, \dot{M} and \dot{M}_A express the rate of change in numbers of macrophages in time, i.e., $\dot{M} = dM/dt$. The rate is positive if the population grows and negative for a declining population. Simultaneously, the production of cytokines acts as a negative feedback on the remaining β -cells. We assume the rate of death of β -cells, \dot{B} , to be proportional to the numbers of activated macrophages, $\dot{B} = -l_B M_A$, where l_B is a constant. The deaths of insulin-producing cells lead to release of proteins. If, on average, a single cell liberates n proteins, then

$$\dot{A} = -n\dot{B} = lM_A \quad (2)$$

where $l = nl_B$. Note that we do not need to explicitly include the β -cell population in the equations, because the release of proteins is directly related to the numbers of activated macrophages. There is some evidence that some new β -cells are formed to maintain the insulin production (26). This would lower the total death rate but is not crucial to the resulting dynamics.

Proteins (antigens) are taken up by macrophages, monocytes, and dendritic cells residing in the volume (m), implying that free proteins have an average lifetime time of $1/m$. This approximation is validated when many antigens are released and only a fraction of these ends up inside macrophages, the rest being internalized by other kinds of scavenger cells as mentioned above.

Putting all of this together, the model acquires the following form.

$$\begin{aligned} \dot{M} &= a + (k + b) M_A - cM - gMA \\ \dot{M}_A &= gMA - kM_A \\ \dot{A} &= lM_A - mA \end{aligned} \quad (3)$$

It can be seen that the population of macrophages grows with the size of inflow (a, b) and when macrophages are deactivated (g). The population decreases either when cells leave the volume (c) or because they become part of an activation process (g). Macrophages presenting target-cell antigens are formed from their inactive precursors (g) and will return to a resting state (k). Liberation of β -cell proteins is linked to the cytokine production (l), and proteins are cleared from the volume by scavenger cells (m).

Extended mathematical model. The presence of specific Th-lymphocytes is included in the model by adding a variable T to the model (Fig. 4). Th-lymphocytes are induced to proliferate when they recognize antigens on class II major histocompatibility complexes situated on macrophages (s). We assume that the rate of formation is proportional both to the concentration of activated macrophages and to the concentration of Th-lymphocytes. The lymphocytes will decline in absence of stimulation; this mechanism is named t . Thus, we may write

$$\dot{T} = sM_A T - tT \quad (4)$$

Activated Th-lymphocytes secrete IFN- γ , and according to the Copenhagen model, this increases the stress on the remaining β -cells: we name this mechanism p . We assume that the effect on the death rate of the β -cells, and thus the amount of the antigens, A , released, is proportional to the size of the lymphocyte population, T . Besides, Th-lymphocytes are able to restimulate macrophages to enter into effector cells, and this process, q , will depend on the numbers of Th-lymphocytes. These interactions will modify the equation governing the concentration of liberated proteins.

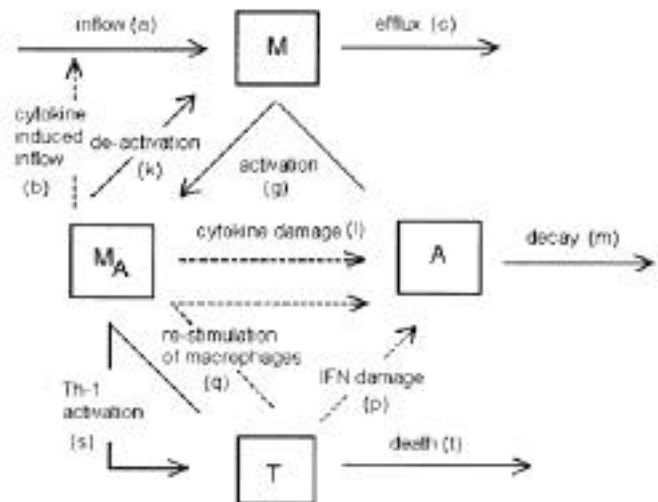


FIG. 4. Schematic representation of the extended mathematical model.

Hence, the simplest system of equations that reproduce the complete Copenhagen model is as follows.

$$\begin{aligned} \dot{M} &= a + (k + b) M_A - cM - gMA \\ \dot{M}_A &= gMA - kM_A \\ \dot{A} &= lM_A + pT + qM_A T - mA \\ \dot{T} &= sM_A T - tT \end{aligned} \tag{5}$$

Recent experimental findings indicate that the binding of Fas ligand on cytotoxic T-lymphocytes to Fas receptors on β -cells will trigger apoptosis in the insulin-producing cells (27,28). The Fas expression on β -cells has been found to be induced by the presence of IL-1, TNF, and IFN (28). The mechanism can easily be included in the model by adding another variable, T_C , representing the numbers of cytotoxic T-lymphocytes. However, stimulation of cytotoxic T-lymphocytes is restricted by the numbers of activated Th-lymphocytes in the volume, and the expression of Fas on β -cells is determined by the simultaneous presence of activated macrophages and Th-lymphocytes.

Although this process might contribute to the killing of β -cells, it is a secondary effect provoked by an activated Th-lymphocyte population. This corresponds well with new experiments on NOD mice (29), in which macrophages were required for activation of cytotoxic T-lymphocytes. Within our picture, this mechanism will not affect the initial instability but acts to intensify the destruction once instability has been reached.

Stability analysis and feedback effect. It is convenient to express the equations in terms of dimensionless variables, because it reduces the parameters to some dimensionless groupings and assigns a characteristic time scale to the model. This procedure is performed in the APPENDIX, and all later equations rely on this rescaling.

Let us for a moment ignore the presence of Th-lymphocytes by setting $T = 0$ in the model, thus reproducing Eq. 3. For some sizes of the populations, we find that $\dot{M} = \dot{M}_A = \dot{A} = 0$. This implies that the numbers of cells and antigens remain constant in time; these states are named steady states (M^* , M_A^* , A^*). One of these is a “healthy” state with a constant concentration of macrophages:

$$M^0 = \frac{1}{c} \quad M_A^0 = 0 \quad A^0 = 0 \tag{6}$$

The state refers to a physiological situation where no antigens are present in the volume. To explore the robustness of this state with respect to some small perturbation, as for instance, liberation of β -cell proteins triggered by the environment, we perform a stability analysis (see APPENDIX).

Several interesting properties can be extracted from the analysis. First, we notice that the stability is not influenced by the activation-dependent inflow of macrophages (b). Second, we are able to write down the condition for the feedback process to be dampened.

The basic feedback effect of the system, f_0 , can be quantified as the number of secondary antigens produced by the primary β -cell damage. Therefore, the system will be unstable when, on average, the death of a single β -cell leads to the

death of more than one cell through the cytokine-driven processes. From the derivations in the APPENDIX, we find

$$f_0 = \frac{gl}{ck} \tag{7}$$

which means that linear stability is guaranteed for $f_0 < 1$. This simple equation shows how four processes, with strengths g , l , c , and k as defined in Table 1, conspire to create an instability. The derivation rests on the assumption that the initial release of antigenic peptides can be considered a small change in the system away from the steady state. Given this situation, each destroyed β -cell, on average, induces the destruction of less than one new target cell.

For $f_0 > 1$, the chain reaction is self-sustained and might initiate a cascade-like response, during which additional autoantigens are being released, and the process can spread to other parts of the target tissue. The stability surface illustrated schematically in Fig. 1 is defined by $f_0 = 1$, and Eq. 7 shows explicitly how this surface depends on the collective behavior of all the effects included in the model. In principle, variation of each parameter may bring about a change from an unstable to a stable regime.

To put the result in a physiological context, we imagine that some nonspecified environmental factor provokes a local outburst of autoantigens at time $t = 0$ in a normal compartment of the islets of Langerhans. The qualitative behavior will roughly be determined by f_0 . Hence, any modification in the efficiency of antigen expression, in the deactivation of macrophages, in the efflux of macrophages, or in the production of cytokines may have profound influence on the overall dynamics. Note that functional inactivation of macrophages ($g = 0$) will shift the system toward a stable regime. This is in agreement with experimental results where inactivation of macrophages in both BB rats (30) and NOD mice (29,31) was shown to prevent diabetes development.

Four numerical simulations of Eq. 3 are shown in Fig. 5. They illustrate the temporal evolution of the variables M , M_A , and A after antigen liberation. All simulations were performed in the stable regime, i.e., $f_0 < 1$, but with varying values of the parameter g , which might be considered as the efficiency of macrophage activation.

There exists a second equilibrium point referring to a condition where the feedback from macrophages to β -cells causes a constant release rate of proteins:

$$\begin{aligned} M^1 &= \frac{k}{gl} & M_A^1 &= \frac{ck - gl}{bgl} \\ A^1 &= \frac{ck - gl}{bg} \end{aligned} \tag{8}$$

For this state to be physically relevant, all population sizes must be non-negative. A comparison of Eq. 7 with Eq. 8 leads to the restriction $f_0 < 1$ if all variables are to be positive.

The second equilibrium point suggests that it is possible to maintain a slow, progressive β -cell elimination without a massive inflow of macrophages. Consequently, the state represents a somewhat “hidden” β -cell elimination. Eventually, such an intermediate state will lead to activation of other parts of the immune system, because of environmental fluctuations or exhaustion of the remaining β -cells.

Type 1 diabetes may roughly be divided into two categories, fast-onset and slow-onset. Thus, type 1 diabetes may

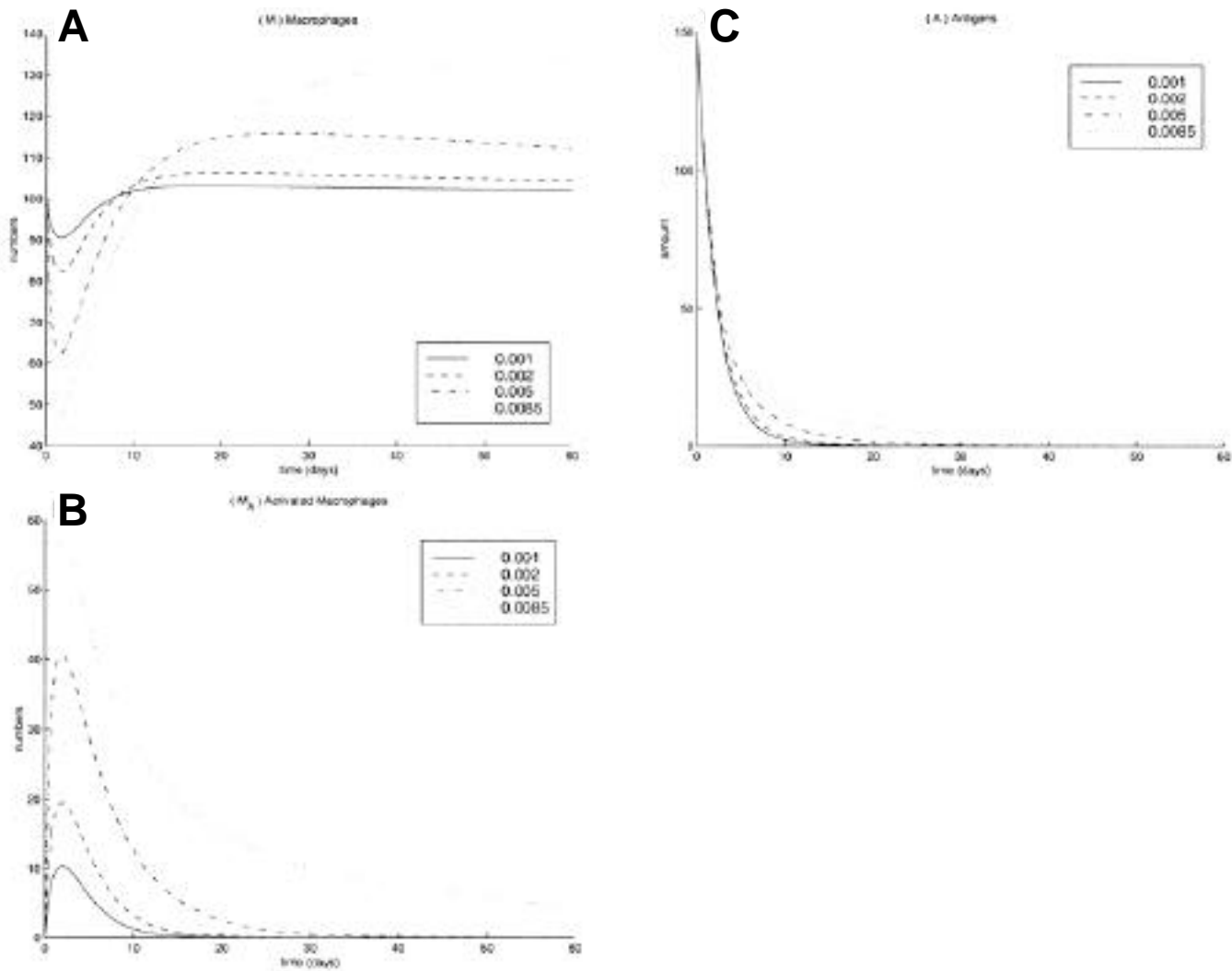


FIG. 5. The graphs show numerical solutions of Eq. 3 and plot the variables M (Fig. 5A), M_A (Fig. 5B), and A (Fig. 5C) as function of time. In all simulations, the initial system at $t = 0$ is in a healthy state, corresponding to Eq. 6. All parameters are held constant in the simulations except g . When the simulation starts, a fixed amount of antigens is released and the response is shown. The number of macrophages initially drops down because of antigen uptake and then increases because of activation-induced inflow and deactivation. The number of activated macrophages grows as antigens are taken up and then declines when the antigens have been swept away. The four different plots are the solutions corresponding to four different values of g (units of per cell \times day): 0.001, 0.002, 0.005, 0.0085. As g increases (see Eq. 7), more feedback is present in the system. As a consequence, the number of transiently activated macrophages in the volume grows, causing more macrophages to enter the volume. In addition, the relaxation time—the time from initial antigen release until clearance—increases. All simulations were performed in the stable regime, i.e., $f_0 < 1$, and illustrate qualitatively the effect of approaching the instability point by variation of one parameter. Once $f_0 > 1$, the stability is lost. However, because we have not included nonlinear terms, all concentrations diverge outside the regime of validity for the equations, and it makes no sense to perform simulations. We can say little quantitatively about what happens in the unstable regime.

develop in the first months of life as well as after a very long period of autoantibody positivity (32). This is further supported by the finding that there is a close-to-linear increase in the type 1 diabetes incidence rate in first-degree relatives with increasing observation time (33). Based on our model, one could speculate that during the progression of slow-onset type 1 diabetes, the system is temporarily caught in a steady state where slow elimination of β -cells occurs.

If we now include the Th-lymphocytes, the system still has the property of reproducing the steady states as discussed above, i.e.:

$$M^0 = \frac{1}{c} \quad M_A^0 = 0 \quad A^0 = 0 \quad T^0 = 0 \quad (9)$$

and

$$\begin{aligned} M^1 &= \frac{k}{gl} & M_A^1 &= \frac{ck - gl}{bgl} \\ A^1 &= \frac{ck - gl}{bg} & T^1 &= 0 \end{aligned} \quad (10)$$

In consequence, the presence of Th-lymphocytes does not modify the existence of a separate macrophage-dependent initialization phase.

Activation of Th-lymphocytes. The third equilibrium point of the model represents the condition where a partly Th-lymphocyte-driven, partly macrophage-induced damage on the target cells exactly maintains a consecutive flow of antigens. This state is

$$M^2 = \frac{s + bt}{cs} \quad M_A^2 = \frac{t}{s}$$

$$A^2 = \frac{ckt}{g(s + bt)}$$

$$T^2 = \frac{t}{ps + qt} \left[\frac{cks}{g(s + bt)} - l \right] \tag{11}$$

We may use this steady state to classify the condition necessary to activate the Th-lymphocytes. The model predicts that the stimulation of the lymphocytes is dependent on the presence of a critical amount of activated macrophages, namely:

$$M_A^{crit} = \frac{t}{s} \tag{12}$$

This reflects the fact that activation of lymphocytes is a highly specific process limited by the affinity of the Th-lymphocyte receptors. If the system is nondampened and numerous macrophages enter the pancreas area so that $M_A > M_A^{crit}$, then the Th-lymphocytes will start to proliferate. For that reason, activation of the Th-lymphocytes is a threshold phenomenon.

The model therefore suggests that a change from a healthy state to an abnormal state with severe tissue damage will be manifest through massive inflow of macrophages followed by invasion of lymphocytes. This is in agreement with the finding that macrophages are the major population of cell infiltrating the pancreatic islets during the early stage of insulinitis in BB rats and NOD mice (29,34–36). Even in humans, clinical type 1 diabetes is seen with macrophages being the only infiltrating cells in the islets (37).

Inclusion of different forms of antigens. The previous model was based on the presence of one single, particular form of antigen. Actually, a multiplicity of islet-cell antigens have been identified in newly diagnosed type 1 diabetes patients (24,38). Once the primary damage has occurred, more autoantigens are released and processed by professional antigen-presenting cells, such as dendritic cells, monocytes, or macrophages. This implies that several distinct types of antigens (A_1, A_2, \dots, A_n) have to be included in the model. In a slightly modified version, populations of activated macrophages and antigens M_A, A , are divided into N compartments.

The N distinct types of antigens give rise to different subpopulations, i.e., $A = \sum_{i=1}^n A_i$ and $M_A = \sum_{i=1}^n M_{Ai}$, respectively. Hence, the total population of antigens and activated macrophages can be written as a sum of the n different antigenic peptides. The resulting system has the form

$$\dot{M} = 1 + (b + k) \sum_{i=1}^n M_{Ai} - cM - gM \sum_{i=1}^n A_i$$

$$\dot{M}_A = gM \sum_{i=1}^n A_i - k \sum_{i=1}^n M_{Ai}$$

$$\dot{A} = l \sum_{i=1}^n M_{Ai} - \sum_{i=1}^n A_i \tag{13}$$

Here, the existence of Th-lymphocytes has been neglected. Because macrophages internalize antigens in a nonspecific way, the uptake is not dependent on the specific structure of the proteins, and all parameters essentially remain unaltered by this procedure. Thus, there is no basic change in the stability criterion, Eq. 7.

As opposed to the macrophages, the specific subpopulations of Th-lymphocytes, T_i , will differ in their rate of proliferation and the production of cytokines according to

the strength of binding between the lymphocyte receptor and the antigens involved. For reasons of simplicity, we assume that a given subpopulation T_i is only able to bind with antigens A_i . The difference in affinity is expressed in the values s_i ; strongly autoreactive subpopulations will have large values of s , whereas weakly autoreactive Th-lymphocytes will acquire small values of s . The variable affinities influence the level of cytokine production in the Th-lymphocytes, and this in turn affects the feedback effects p_i and q_i on the β -cells. These considerations lead to the following system of equations.

$$\dot{M} = 1 + (b + k) \sum_{i=1}^n M_{Ai} - cM - gM \sum_{i=1}^n A_i$$

$$\dot{M}_A = gM \sum_{i=1}^n A_i - k \sum_{i=1}^n M_{Ai}$$

$$\dot{A} = l \sum_{i=1}^n M_{Ai} + \sum_{i=1}^n p_i T_i + \sum_{i=1}^n q_i M_{Ai} T_i - \sum_{i=1}^n A_i$$

$$\dot{T} = \sum_{i=1}^n s_i M_{Ai} T_i - t \sum_{i=1}^n T_i \tag{14}$$

It goes far beyond the scope of the present article to go into the complicated structure of the complete model. However, if many different antigens are present, one could assume the distribution of affinities to be a normal distribution centered around some typical value s_0 and solve the system numerically.

Instead, we note that a model that can simulate the dynamics of individual antigens does not alter the stability criterion Eq. 7, because Eq. 13 and Eq. 3 show similar behavior. Consequently, we conclude that a macrophage-dependent initialization phase is an inherent property of the Copenhagen model and the pathogenesis of type 1 diabetes.

DISCUSSION

In the present article, we have taken a global view of the onset of type 1 diabetes, focusing on the collective effect of several mechanisms that have been identified as being relevant. A similar procedure has been used to study the immune response to persistent viruses (39).

We find the novel mathematical approach well-suited to handle the multifactorial nature of type 1 diabetes, where polygenic susceptibility combined with influence from environmental factors determine the development of the disease. To sum up, we conclude that type 1 diabetes evolves due to a dynamical instability. We found a simple expression for the stability criterion that can be tested against experiments and clinical observations.

According to the model, the onset takes place in a local compartment of the islets of Langerhans and propagates through the pancreas tissue, much like a virus infection in a population. Hence, the destruction of β -cells will be heterogeneous in both time and space. This is in complete agreement with early observations of type 1 diabetes, where β -cells in certain areas are lacking completely, whereas other areas have healthy insulin-producing cells (41). The Copenhagen model predicts that there exists a separate macrophage-dependent initiation phase, and it is therefore worthwhile to clarify experimentally the isolated interactions between the target cells and the macrophages. Besides, this work illustrates the need to perform quantitative measurements of the parameters involved in the pathogenic model.

It is, however, important to make clear that the idea of a dynamical instability is not limited to the specific mecha-

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nisms included in our particular models. Mechanisms can be added or subtracted without changing the main conclusion. The fact that the stability surface involves many different factors indicates that—from a theoretical point of view—many types of interventions might be helpful. As a curiosity, it can be noted that any treatment that affects the parameters has a 50-50 chance of pulling in the right direction toward the stability surface. Perhaps this is the explanation for the empirical observation that more or less random medical treatments may have a probability of curing or delaying onset of complex, multifactorial diseases.

Many details were not included in this simple scenario—for instance, saturation effects or various suppressive mechanisms of macrophage or lymphocyte origin. The mathematical description of the Copenhagen model emphasizes the features of the “aggressive” immune response, i.e., the Th-1 lymphocyte reactivity. After all, in humans, evidence of the Th-2 lymphocyte response is ambiguous and was not incorporated into the model. We also did not consider the presence of autoreactive CD8+ lymphocytes, although inclusion of β-cell reactive CD8+ lymphocytes is possible.

Although the early pathogenesis is very likely more complicated than sketched in this crude model, we are able to reproduce some general characteristics of early phase type 1 diabetes, and the idea of a dynamical instability might well be robust.

APPENDIX

Dimensionless system. Before analyzing the model, it is convenient to express the equations in terms of dimensionless variables. By rescaling time,

$$\bar{t} = tm \tag{15}$$

we use as the basic time-unit the average time (1/m) from when proteins are liberated until they are taken up and processed by various kinds of scavenger cells. Similarly, we rescale the population variables,

$$\begin{aligned} \bar{M} &= \frac{mM}{a} & \bar{M}_A &= \frac{mM_A}{a} \\ \bar{A} &= \frac{mA}{a} & \bar{T} &= \frac{mT}{a} \end{aligned} \tag{16}$$

so talking of “large” or “small” populations now has a definite meaning because the new variables are defined in terms of the numbers of macrophages that enter the volume in an unit of time. Henceforth, we will use the rescaled variables, Eq. 16, although we keep the previous notation *M*, *M_A*, and *A* for matters of convenience. The modified system takes the form

$$\begin{aligned} \dot{\bar{M}} &= 1 + (\bar{b} + \bar{k}) \bar{M}_A - \bar{c}\bar{M} - \bar{g}\bar{M}\bar{A} \\ \dot{\bar{M}}_A &= \bar{g}\bar{M}\bar{A} - \bar{k} \bar{M}_A \\ \dot{\bar{A}} &= \bar{l}\bar{M}_A + \bar{p}\bar{T} + \bar{q}\bar{M}_A\bar{T} - \bar{A} \\ \dot{\bar{T}} &= \bar{s}\bar{M}_A\bar{T} - \bar{l}\bar{T} \end{aligned} \tag{17}$$

The parameters in Eq. 17 have also been changed with respect to *a* and *m*. All linear terms are set proportional to 1/*m*, that is, $\bar{b} = b/m$, etc., and all nonlinear parameters acquire a factor *a/m²*, so that, e.g., $\bar{g} = ag/m^2$. From now on,

all parameters appear in their rescaled form, although we do not write out explicitly \bar{a}, \bar{b} , etc.

Stability analysis. In the following paragraph, we give a detailed description of the stability analysis. In a neighborhood around the steady state, it is possible to determine the stability by considering the corresponding linearized system. This comes about by a Taylor series expansion to first order in Eq. 16. Thus, we rewrite Eq. 17 with *T* = 0 in a vector form

$$\frac{dv}{dt} = \mathbf{m} (v - v_0) \tag{18}$$

where *v* and *v*₀ are vectors $v_0 = (M, M_A, A)$, and $v_0 = (M^0, M_A^0, A^0)$, respectively. **m** is a 3 × 3 matrix with elements *m_{i,j}* given by

$$m_{i,j} = \left. \frac{\partial f_i}{\partial v_j} \right|_{v_j^*} \quad \mathbf{m} = \begin{pmatrix} -c & (b+k) & -g/c \\ 0 & -k & g/c \\ 0 & l & -1 \end{pmatrix} \tag{19}$$

The solutions are determined by a standard procedure. All solutions are calculated by solving the characteristic equation, which in a matrix notation takes the form

$$|\mathbf{m} - r\mathbf{I}| = 0 \tag{20}$$

Here, **I** is the diagonal unit matrix and the bars |...| represent the determinant of the matrix. The characteristic equation is a third-order polynomial in *r*, and the different roots, *r_i*, are called eigenvalues. In general, a solution to a system of linear differential equations can be written as a sum of exponential functions.

$$v_i(t) = \sum_{i=1}^3 C_i \exp(r_i t) \tag{21}$$

The equation *v_i(t)* gives the population sizes in time, i.e., *v_i(t)* = *M(t)*, *M_A(t)*, *A(t)* for *i* = {1,2,3}. The various constants *C_i* are determined from the initial conditions. Notice that the eigenvalues appear in the exponential. Thus, eigenvalues can be used to characterize the qualitative dynamics. In general, the eigenvalues are complex numbers. The dominant behavior is given by the eigenvalue with the largest real part. If the largest real eigenvalue is positive, a small initial disturbance grows exponentially, so the process is amplified. If the real value is negative, the disturbance decays exponentially, and the process is dampened. Following the procedure above, we evaluate the eigenvalues to be

$$\begin{aligned} r_1 &= -c \\ r_{2,3} &= -\frac{1}{2}(k+1) \pm \sqrt{\frac{1}{2}(k-1)^2 + gl/c} \end{aligned} \tag{22}$$

We may notice that the parameter *b* does not appear in the eigenvalues. This implies that the stability is not influenced by the cytokine-driven inflow of macrophages in the volume. From the eigenvalues, we are able to write down the condition for the feedback mechanism to be dampened by demanding the real part of *r_i* to be less than zero, i.e., $\text{Re}(r_i) < 0$.

Defining the basic feedback process, *f₀*, as the numbers of secondary antigens produced by the primary β-cell destruction, we arrive at

$$f_0 = \frac{gl}{ck} \tag{23}$$

where linear stability is guaranteed for $f_0 < 1$. This equation reproduces Eq. 7 in the text.

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