
Banting Lecture 2003

Autoimmune Diabetes and the Circle of Tolerance

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The concept of immunological tolerance is central to our understanding of type 1 diabetes and the development of strategies for its prediction, prevention, and cure. Tolerance simply refers to the absence of an immune response. Most of us are born with an immune system that develops tolerance to all the other systems of our bodies as well as to the things that we eat. It is the loss of immunological tolerance that leads to autoimmunity. And when that autoimmune response directly or indirectly targets the β -cell, type 1 diabetes is the result. In the U.S., 1 in 600 of us loses tolerance to pancreatic β -cells. Interference with T-cell function after the loss of tolerance, as can be achieved with immunosuppressive drugs like cyclosporin, arrests the disease, but the cost in side effects is high. Clearly, stopping the loss of tolerance would be preferable. If we can stop the loss of tolerance, we can prevent the disease. We and many others have investigated both approaches. But what of the people who already have diabetes? For them a separate but related strategy, tolerance induction, is required. Specifically, islet transplantation tolerance induction holds out the promise of being able to cure the disease. This has been the ultimate goal of our laboratory's work for the past two decades. *Diabetes* 53:267-275, 2004

Scientific understanding of the cause of type 1 diabetes began with the discovery of inflammatory insulinitis by von Meyenburg (1). Subsequently, Gepts (2) and others recognized that insulinitis, an inflammatory lymphocyte infiltrate, was specifically associated with the islets of children with diabetes. These discoveries provided the first clue that ketosis-prone diabetes is caused by autoimmunity. The child's β -cells are destroyed by his or her own immune system, specifically by T-cells. The work of Doniach, Bottazzo, and Drexhage (3) then revealed that people with type 1 diabetes circulate antibodies that, instead of targeting some infectious agent, target constituents of the β -cell—even insulin itself. These autoantibodies are often present long before disease onset and can be used to identify persons at risk (4). We now

know that immunosuppressive drugs that interfere with the function of the inflammatory cells involved in these processes can arrest new-onset type 1 diabetes (5).

CAUSES OF TYPE 1 DIABETES

Learning that type 1 diabetes is an autoimmune disorder was a major advance, but even in 2003 the root causes of the disease are incompletely understood, in part because it is complex and multifactorial. Inheritance is non-Mendelian and has been difficult to unravel (6). As is typical of nearly all autoimmune diseases, there is a genetic association with the major histocompatibility complex (MHC). There are also 15 or more additional genetic loci that associate with susceptibility to type 1 diabetes, but only two strong candidate genes have been identified within those loci. One is a variable number of tandem repeats (VNTR) upstream of the insulin; the other is a splice variant of CTLA-4, a regulator of T-cell function (7). In addition to genetics, there is also an environment component to the disorder. Many observations inform us this must be the case. For example, if one of two identical twins develops type 1 diabetes, the odds that the other twin will be affected are only ~50% (8). Another observation implicating the environment is the disturbing increase in the incidence of type 1 diabetes in recent years. The environmental factors implicated include toxins, infections, and diets. But as certain as we are that they must be involved (9), we still, in 2003, do not know for sure which ones are involved or how they work.

THE CIRCLE OF TOLERANCE: PATHOGENESIS AND PREVENTION

Against this background, our laboratory has tried to develop a strategy for both understanding and combating type 1 diabetes. This strategy, outlined in Fig. 1, centers around the concept of immunological tolerance (10). Tolerance simply refers to the absence of an immune response. With rare exceptions, most of us develop immune systems that are tolerant of both the other systems of our bodies and the things that we eat. In the U.S., all but 1 in 600 of us are tolerant to our pancreatic β -cells. It is the loss of immunological tolerance that leads to autoimmunity. When that autoimmune response targets the β -cell, type 1 diabetes is the result. If we interfere with T-cell function after the loss of tolerance, as we can with immunosuppressive drugs like cyclosporine, we can stop the disease. Better than that would be stopping the loss of tolerance. If we can stop the loss of tolerance, we can prevent the

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APC, antigen-presenting cell; DST, donor-specific transfusion; GVHD, graft versus host disease; HSP, heat shock protein; KRV, Kilham rat virus; mAb, monoclonal antibody; MHC, major histocompatibility complex; VNTR, variable number of tandem repeats.

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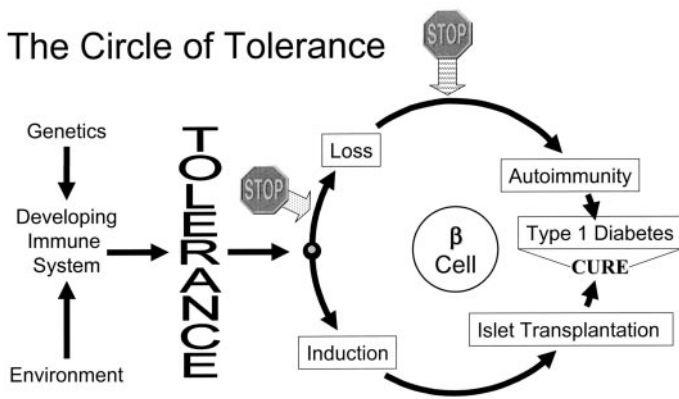


FIG. 1. The circle of tolerance. Tolerance denotes the absence of a detectable, functional immune response in the absence of immunosuppression. It is the loss of immunological tolerance that leads to autoimmunity (61), specifically the autoimmune destruction of β -cells in type 1 diabetes. If we interfere with T-cell function after the loss of tolerance (striped arrow), as we can with immunosuppressive drugs like cyclosporine, we can stop the disease. Better than that would be stopping the loss of tolerance (dotted arrow), thereby preventing the disease. And what of the people who already have diabetes? For them we need a separate but related strategy—tolerance induction (10). If we can induce islet transplantation tolerance, we may be able to cure the disease.

disease. We and many others have looked at both of these strategies.

But what of the people who already have diabetes? For them, as depicted in the lower half of Fig. 1, we need a separate but related strategy—tolerance induction. Specifically, we need to be able to induce transplantation tolerance, and if we can do so we may be able to cure the disease (11).

Models for studying type 1 diabetes. It has been difficult to discern the root causes of type 1 diabetes by studying people with the disease. Fortunately, ethically sound and humane animal research provides answers without putting people at risk (12). Two model systems have provided us with important information on the ways in which tolerance to β -cells can be lost and restored. The BB rat was discovered in Canada in the mid 1970s (13). The nonobese diabetic mouse was discovered in Japan in the early 1980s (14). Both of these animals exhibit selective β -cell destruction in the context of insulinitis. As in humans, the disease is linked genetically to the MHC and is T-cell dependent. As in humans, autoantibodies are present.

Pathogenesis of type 1 diabetes: natural history. The natural history of diabetes in the BB rat is comparable to that observed in humans at both the clinical and cellular levels. That history begins with islets in young animals that are normal in appearance and with respect to insulin secretory capability. In adolescence, progressive inflammatory infiltration of the islets begins and steadily increases in intensity. The infiltrate consists predominantly of T-cells, but macrophages, natural killer cells, natural killer T-cells, and B-cells also participate. Diabetes onset in the BB rat is typically accompanied by ketoacidosis. If the animal is treated with insulin and survives, the inflammatory process recedes, leaving islets devoid of β -cells. Only glucagon and somatostatin cells remain. An important point to make about this and other animal models is that they permit us to assess and potentially to intervene in

the disease at all of these stages—predisposition, pre-diabetes, onset, and clinical illness—something that is impossible to achieve in children at risk for diabetes.

Adoptive transfer. One method for proving that a disease is autoimmune is to use the method of adoptive transfer. Adoptive transfer is an immunological procedure that can demonstrate the capacity of abnormal populations of T-cells to home to islets and cause diabetes. Adoptive transfer of diabetes was first shown using spleen cells removed from diabetic BB rats (15). After injection, these cells caused recipients to develop diabetes with all the characteristics seen in the donor. With the adoptive transfer method as a tool, it was clearly established for the first time that T-cells can mediate autoimmune diabetes. Similar results were obtained in NOD mice, and it was then shown in both BB rats and NOD mice that thymocytes (the precursors of peripheral T-cells) and bone marrow (the precursor tissue of thymocytes) also transfer the disease (13). Sadly, a few unfortunate cancer patients who received bone marrow from donors with type 1 diabetes have also become diabetic (16).

Prevention of type 1 diabetes. With the knowledge that type 1 diabetes in the rat is an autoimmune disease, it was quickly hypothesized that therapies directed against T-cells might prevent it. Antilymphocyte serum and many immunosuppressive drugs were shown to prevent diabetes in both BB rats and NOD mice (17). Immunosuppressive drugs were then tried in children with new-onset diabetes (5), but these agents, which would have had to be taken for a lifetime, were ultimately deemed too toxic (18). We and many others quickly realized that what was needed was something to prevent the disease not by blocking the disease process, but rather by modulating it. In short, we needed to arrest the loss of tolerance (Fig. 1). Again, an early proof of principle in this area came from the BB rat.

Prevention by immunomodulation. The prototype intervention to prevent autoimmune diabetes in the BB rat by immunomodulation was disarmingly simple. It had been discovered that these animals are congenitally deficient in lymphocytes. Simply returning to them a population of histocompatible lymphocytes from a normal donor completely prevented both diabetes and insulinitis (19). The agent of protection appears to be a population of regulatory T-cells that express the $CD4^+ART2^+$ phenotype (20,21). This result eventually came to be viewed as one of the first “real-world” demonstrations of the existence of regulatory T-cells (22,23).

Immunological balance. Transfusion, of course, has not become a therapy for diabetes. The proof of principle has remained valid, however, and contemporary research on stem cell transplantation for treating autoimmune diseases holds out considerable promise (24–26).

Our early transfusion studies gave us a framework for understanding type 1 diabetes in particular and immunological tolerance in general. The gain and loss of tolerance can be viewed in terms of a balance, a concept initially formulated in our studies of the diabetes-prone or BBDP rat and schematized in Fig. 2. The prevention of diabetes in the BBDP rat by transfusion, we believe, represents the restoration of the animal’s immune system to equilibrium and a state of tolerance (27).

What has proven far more interesting in this context,

Immunological Balance in BB Rats

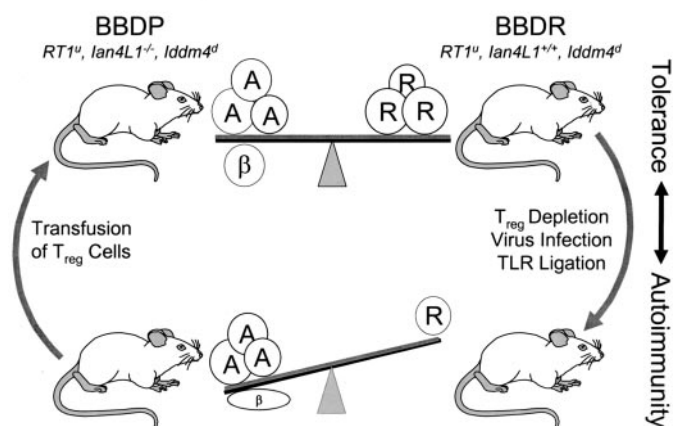


FIG. 2. The balance hypothesis of autoimmune diabetes developed in studies of BBDP and BBDR rats. The lower half of the figure depicts the imbalance between regulatory (R) and autoreactive (A) cell populations associated with insulinitis and the destruction of pancreatic β -cells. The upper half depicts these forces in a state of equilibrium. Both BBDP and BBDR rats share a genetic susceptibility to autoimmunity based on the presence of the *RT1^u* MHC haplotype and the *Iddm4* gene(s) (62). In the case of the BBDR rat, on the left, the presence of an additional mutation in the *Ian4L1* gene causes lymphopenia and an imbalance between autoreactive and regulatory cell populations, leading to diabetes. Restorative transfusions of MHC-compatible lymphocytes restore immunological balance and prevent diabetes. In the case of the BBDR rat, on the right, autoreactive cells are present but do not lead to the expression of diabetes unless the balance is perturbed. Perturbants that lead to diabetes include regulatory cell depletion with anti-ART2.1 mAb; infection with Kilham rat virus, which can decrease regulatory cell numbers; and injection of the TLR3 ligand poly I:C, which may alter the immunological balance by activating APCs (13).

however, has been our analysis of the diabetes-resistant BBDR rat (13). These animals were derived from BBDP forebears. They are not lymphopenic because the breeding process at that time selected against the gene that causes lymphopenia. BBDR rats never become diabetic if housed in a clean environment. If, however, we use a monoclonal antibody (mAb) to deplete the ART2⁺ regulatory T-cells in these animals, we disequilibrate the system and induce diabetes (28). An even more interesting discovery was that a naturally occurring infection, specifically with Kilham rat virus (KRV) can also disequilibrate the system (29).

These linkages of regulatory T-cell loss and viral infection to the pathogenesis of type 1 diabetes in the rat are exciting because they hold out the promise of dissecting the pathways by which comparable perturbants may act on human children.

Virus-induced BBDR rat diabetes. An association between viral infection and autoimmune diabetes in humans has been suspected, but remains unproven (30). A “proof of principle,” however, is available from the BB rat. More than a decade ago, an outbreak of diabetes occurred in the Worcester colony of BBDR rats (29). It was a classic two-peak outbreak and was associated with the appearance of antibody against the KRV virus in the colony. Interestingly, sialodacryoadenitis virus had been present for years without causing diabetes. In general, it appears that naturally occurring infection with KRV leads to diabetes in about 1% of infected rats. The low penetrance is noteworthy given that the animals are inbred and share a common genetic susceptibility profile.

Similar epidemiological data exist for apparent outbreaks of diabetes in human populations, most recently in 1994 in Philadelphia (31), but the BBDR rat has provided an opportunity to do more than document the correlation. We and others have been able to go on to isolate the virus and document its diabetogenicity in controlled circumstances and then to formulate hypotheses about how it might cause diabetes. Initially it was thought that diabetes could be simply due to cytolytic infection of β -cells, as Yoon et al. (32) once observed in a human child, and is known to occur in mice infected with encephalomyocarditis virus (33). But studies by Like and Guberski proved that this is not the case (34). Similarly, KRV does not cause exocrine pancreatitis. Yoon and colleagues hypothesized that diabetes might be the result of molecular mimicry. This hypothesis, in simple terms, holds that in the course of recognizing a viral protein, the immune system recognizes and targets a similar protein on β -cells. The hypothesis was also disconfirmed, however, and Yoon and colleagues suggested that infection of these genetically susceptible animals resulted from something subtler than mimicry (35).

Because alteration of the immunoregulatory environment is an attractive hypothesis for explaining why only a fraction of genetically predisposed children become diabetic, it was appropriate to pursue this hypothesis in the BBDR rat. We infected BBDR rats with either KRV or a very closely homologous virus called H-1. The two viruses are about 98% identical at the level of DNA. We observed that about 40% of animals intentionally infected with KRV (10⁷ PFU) become diabetic 2–4 weeks after infection. In contrast, the highly homologous parvovirus H-1 completely fails to induce diabetes (36). MHC-identical control Wistar Furth (WF) rats do not develop diabetes in response to either virus.

These data generate a new question as to the nature of the difference between KRV and H-1 in the BBDR rat. This question was pursued with additional studies. These revealed that KRV but not H-1 is associated with a decline in the percentage of CD4⁺CD25⁺ regulatory T-cells in the spleens of infected rats (36). This suggests the possibility that there may be virus-specific effects on the immune system that can alter the regulatory balance shown in Fig. 2, converting a genetic predisposition into overt autoimmune diabetes.

“Innate” immunity and type 1 diabetes. The data presented to this point were derived largely from studies of the “adaptive” immune system. The adaptive immune system is responsible for pathogen-specific defense, long-lasting immunity after recovery or vaccination, and involves the MHC and receptors on T-cells. The “innate” immune system, in contrast, provides our first line of defense. It is relatively nonspecific and is activated by bacteria, parasites, viruses, and some chemicals through toll-like receptors (37). There is increasing recognition that innate immune responses may be involved in the expression of autoimmunity (38,39).

We were therefore prompted to study BBDR rats that received not only a viral infection but also a treatment to activate the innate immune response. We observed that the addition of a very brief exposure to poly I:C, which activates innate immunity by binding to toll-like receptor 3

(40), increased the frequency of diabetes to 100%, and accelerated onset. In control studies, infection with H-1 virus failed to induce diabetes with or without the addition of poly I:C (36).

The data provide a tantalizing suggestion as to how some but not all infectious agents may interact with the innate immune system to cause diabetes. The hope, of course, is that these kinds of studies, which obviously cannot be undertaken in children, may help us unravel the puzzling role of viral infection in type 1 diabetes and provide us with a better understanding of how identical twins can be discordant for type 1 diabetes.

Balance, tolerance, and autoimmunity in BB rats. These data and data from many laboratories clearly suggest that an imbalance between autoreactivity and regulation may cause loss of tolerance and autoimmunity. Before leaving this subject, I would like to describe some new discoveries in the BBDP rat that explain how the imbalance comes about in these animals. BBDP rats, as mentioned previously, are severely lymphopenic from birth and they must remain lymphopenic for spontaneous diabetes to occur. Over a decade ago, Jacob et al. (41) identified a locus on chromosome 4 that was associated with this defect. In 2002, Hornum et al. (42) reported that the mutation that causes lymphopenia is a frameshift mutation in the *Ian4L1* gene, a report confirmed by MacMurray et al. (43). The rat *Ian4* protein is found principally if not exclusively in the mitochondria of lymphocytes. In the BBDP rat, this protein is not synthesized. We asked how this mutation causes lymphopenia and diabetes.

Apoptosis and *Ian4L1*. Based on data from our laboratory and the Hagedorn Laboratory in Denmark, we believe that the mutation in *Ian4L1* causes lymphopenia and immunological disequilibrium by inducing apoptosis of T-cells, in particular regulatory T-cells (44). The absence of *Ian4* in T-cells causes dysfunction of mitochondria, with increased levels of stress-inducible chaperonins in the mitochondria and T-cell-specific spontaneous apoptosis (44). Both T-cell activation and caspase 8 inhibition prevent apoptosis, whereas transfection of T-cells with *Ian4*-specific siRNA recapitulated the apoptotic phenotype. The findings establish *Ian4L1* as a novel, tissue-specific regulator of mitochondrial integrity. The arrest of apoptosis in activated T-cells may also explain how BBDP effector T-cells, which are activated to destroy islet tissue, are able to survive and cause diabetes. We would further hypothesize that apoptotic death of the regulatory T-cell population specifically results in disequilibrium of the immune system and autoimmunity in this model system.

Heat shock proteins: a role in diabetes pathogenesis? Although an imbalance between autoreactivity and regulation clearly may cause loss of tolerance and autoimmunity, the imbalance itself does not account for the specificity of the autoimmune process. Why β -cells? The answer, speculatively, may have to do with the nature of secretory tissue in general and heat shock proteins (HSPs) in particular (45,46).

HSPs are found in all cells. There are more than 50 of them, and they account for about 5% of total cellular protein. In stressed cells, the percentage of HSPs may increase to 15 or even 20%. The stressors that increase

HSPs include physical, chemical, and biological processes. The principal functions of HSPs include participation in protein folding and unfolding, protein degradation, and the assembly of multisubunit complexes.

Scientists have speculated on a role for HSPs in diabetes for more than a decade (47,48). Let me now offer you a 2003 view of this speculation. In the β -cell, insulin messenger RNA is transcribed in the nucleus. Insulin protein is then translated from the mRNA in the endoplasmic reticulum, where specific classes of HSPs also reside. HSPs, as I have mentioned above, serve an important role in normal physiologic cell function. In the β -cell, they facilitate the proper folding and three-dimensional conformation of newly synthesized normal insulin. Under conditions of cellular stress, HSPs are upregulated, and one might imagine that the folding of newly synthesized protein may be altered in such a way that the peptide now appears "antigenic." I would like to speculate that the generation of the insulin autoantibodies that are present in type 1 diabetes could be accounted for by this process. The same may occur with respect to other β -cell proteins, thus accounting for the antibodies directed against GAD and IA-2.

The Akita mouse. This of course is speculation. But consider some very recent observations that suggest that HSPs and mal-folded insulin could also be involved in the death of β -cells. These observations come from the Akita mouse (49). Insulin in all mice is encoded by two genes. The insulin 1 gene in the Akita mouse is transcribed in the nucleus and translated in the endoplasmic reticulum, where HSPs aid in the proper folding of the insulin, as we've seen before. However, in the Akita mouse, the insulin 2 gene encodes a cysteine mutation that precludes formation of an essential disulfide bond. This prevents proper folding and processing of this insulin protein. The mutant, mal-folded proinsulin is retained in the endoplasmic reticulum of the pancreatic β -cell, resulting in stress, upregulation of HSPs, and caspase-dependent apoptotic death of the β -cell. The progressive loss of β -cells over time in the Akita mouse results in diabetes (50). I invite readers to speculate as to whether this kind of process may be occurring in the β -cells of children with type 1A and perhaps type 1B diabetes.

THE CIRCLE OF TOLERANCE: TRANSPLANTATION

To this point, we have focused on the upper part of the circle of tolerance (Fig. 1). The available data clearly suggest how genetics and environmental agents can conspire to cause loss of tolerance and type 1 diabetes. They also show that immunomodulation is a viable method for preventing the loss of tolerance. But to aid those individuals who are already diabetic, we need to turn our attention to the lower part of the circle and the ways in which transplantation tolerance might be induced. Good data suggest that transplantation tolerance may hold the key to curing the disease safely. This has been the most recent goal of our laboratory's work (11).

Status of islet transplantation. Segmental pancreas transplantation is a reality, but its benefits are available to only a very few patients and comes with the price of major surgery and lifelong systemic immunosuppression. Islet transplantation has always been the preferred procedure,

and there have been vast improvements in islet transplantation in only a few years. This has been due in part to the introduction of immunosuppressive agents that are less toxic not only to patients but also to their grafts. Thanks to the eximious efforts of Hering, Rajotte, Ricordi, Shapiro, and many others, we now have procedures that generate more islets and better islets. These improvements have enabled us to achieve insulin independence in the majority of persons given two or more islet grafts (51). A few fortunate recipients have now been insulin independent for >3 years, and we are still counting (52).

Our personal experience with the Edmonton protocol at the University of Massachusetts Medical School has been limited, but the results bring home in a personal way both the promise and the vexations of current islet transplantation technology. To date we have treated two patients, each of whom has received two islet grafts. One of the patients is insulin independent, and the other still requires a small amount of exogenous insulin to maintain her very fastidious level of control, but she is gratifyingly free of the debilitating attacks of hypoglycemia that afflicted her for years. This is the promise. Unfortunately, both patients, despite very thoughtful management of their immunosuppressive drugs, have had chronic side effects that are common with these medications. This is part of the downside of current technology.

Islet transplantation: the next step. Where, then, do clinician scientists go next with islet transplantation? I believe that the key is tolerance induction (11). It is a form of therapy that will not require lifelong immunosuppression, will be safe for children, will have minimal side effects, and will have the durability that we all want for islet grafts. The question, of course, is whether we can achieve these goals. The strategy we have developed and have been exploring is the prevention of islet graft destruction by activated alloreactive T-cells.

Alloreactive T-cell activation. Figure 3A summarizes the basic steps involved in the activation of an alloimmune response (10). The principle cell types involved are antigen-presenting cells (APCs) and alloreactive T-cells capable of recognizing alloantigen presented by APCs. The APCs express on their surface MHC molecules and process and then display peptides for evaluation by the immune system. In the case of diabetes, these APCs would present peptides from transplanted allogeneic islets. T-cell receptors recognize these allo-peptides in the context of MHC molecules. APCs also express constitutively the costimulatory molecule CD40, and T-cells express the costimulatory molecule CD28.

When alloreactive T-cells encounter an APC presenting allopeptide, an immune response is initiated. The first step in this response is T-cell activation. After the cognate recognition of allopeptide occurs in step 1, the T-cell then goes on 4–6 h later to express CD154, which binds to CD40. The combination of steps 1 and 2 leads to activation of the APC. The APC then expresses CD80 and CD86, which bind to CD28. With the completion of this third and final step, the alloreactive T-cell becomes activated and capable of destroying an allotransplant like a donated islet of Langerhans.

T-cell costimulation blockade. Knowledge of these three steps in alloreactive T-cell activation enabled us to

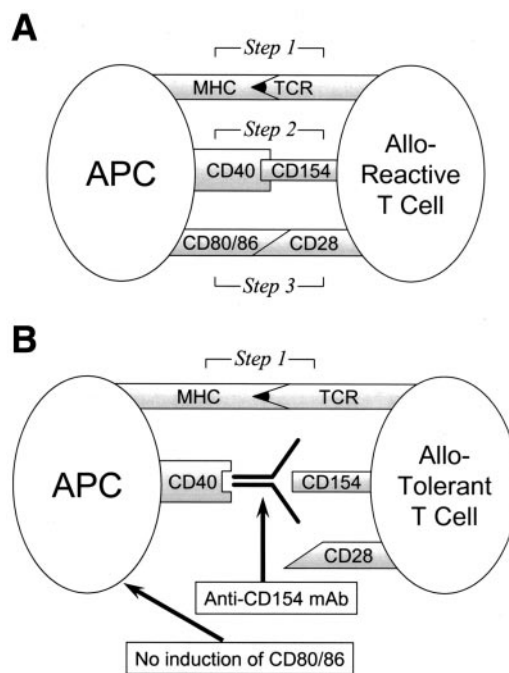


FIG. 3. The basic principles of T-cell activation in response to the presentation of alloantigen and tolerance induction by costimulation blockade. **A:** The process of alloimmune response occurs in three steps. The first is alloantigen recognition: the engagement of the T-cell receptor (TCR) by the MHC-antigen complex on an APC. The antigenic peptide is represented by the black object between the MHC and TCR. Engagement of the TCR then induces CD154 expression on the responding T-cell. This second step, the induction of CD154, permits engagement of CD40 on the APC. Engagement of CD40, in turn, upregulates the costimulatory molecules B7-1 and B7-2 (step 3). As a consequence of increased expression of B7-1/2, the APC reciprocally triggers the T-cell via CD28, a process termed costimulation (63). The activated allospecific T-cell now becomes an effector cell capable of mediating allograft rejection. **B:** Tolerance induction by costimulation blockade (10). Donor origin cells are used as nonprofessional APCs to engage the TcR of alloantigen-specific T-cells. Anti-CD154 mAb prevents engagement of CD40 by CD154 on APCs, thereby preventing the upregulation of B7 costimulatory molecules. This hypothesis predicts allospecific tolerance induction, and permanent islet graft survival should be achieved by the coadministration of a DST and anti-CD154 mAb.

develop a transplantation tolerance protocol based on costimulation blockade. The principle, shown schematically in Fig. 3B, uses a blocking anti-CD154 mAb. The tolerance induction procedure begins with step 1, but it takes place in the presence of blocking anti-CD154 mAb. Step 2 in the activation process does not take place. As a result, APCs do not become activated and do not express CD80 or CD86. The net result of this process is the conversion of the recipient's alloreactive T-cells into tolerant T-cells (10). The actual protocol created to implement this theory uses, in addition to the anti-CD154 mAb, a transfusion of spleen cells from the donor (53). This donor-specific transfusion (DST) in effect prepares the alloreactive system and facilitates the ultimate tolerization of the system.

ISLET ALLOGRAFT TOLERANCE INDUCTION

Figure 4A illustrates the protocol that we now use for islet allograft tolerance induction. Recipients are chemically diabetic C57BL/6 mice that express the $H2^b$ MHC haplotype. Donors are $H2^d$ BALB/c mice. Seven days before transplantation of islets, the prospective recipient receives

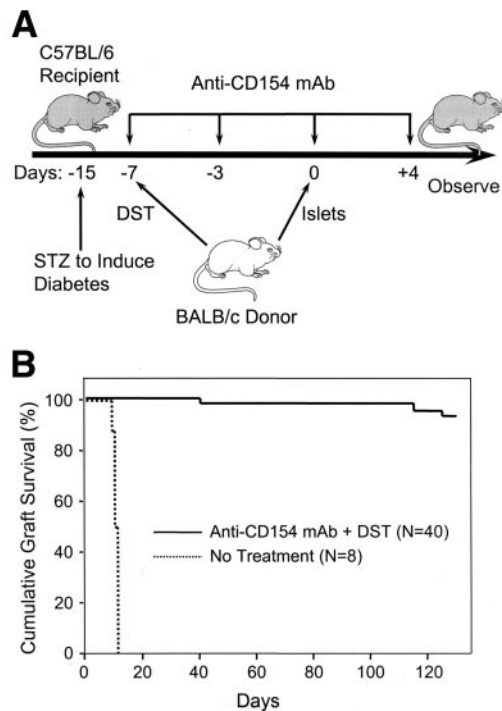


FIG. 4. A: Experimental protocol for inducing islet allograft tolerance (64). Recipients are C57BL/6 mice ($H2^b$) treated with streptozotocin (STZ) to induce diabetes. BALB/c mice ($H2^d$) provide spleen cells for a DST (DST) (10×10^6 spleen cells) on day -7 and an islet graft on day 0. Injections of the hamster anti-mouse MR1 anti-CD154 mAb (0.25 mg per dose) are given immediately before the DST and then on days -3, 0, and +4. No other therapy is given. **B:** Life table showing duration of BALB/c islet allograft survival in C57BL/6 mice treated with DST and anti-CD154 mAb (solid line) or no treatment (dotted line). Data represent a composite of several studies performed in our laboratory.

a DST consisting of BALB/c spleen cells and the first of four doses of anti-CD154 mAb. The third dose is given on the day that islets are transplanted into the renal subcapsular space, and the fourth and last dose is given on the fourth day after grafting. No additional interventions are required.

The life table in the lower half of Fig. 4 illustrates the duration of islet allograft survival that can be achieved. Grafts in untreated diabetic mice are rejected rapidly. In contrast, nearly all islet grafts survive for months in tolerized mice. Survival in the great majority of cases appeared to be indefinite, and we have documented survival for over a year. Given that all the tolerizing interventions ended on day 4 after grafting, the results are of considerable interest.

Mechanisms. Achieving prolonged survival of allografts in treated mice holds out much promise for translation to clinical medicine, but therapeutics effective in small rodents seldom “scale up” to humans without difficulty. To facilitate the potential translation of these observations to the clinic, we have considered it essential to identify the mechanisms that underlie not only the success of the procedure but also its failures.

Our analysis began with the formulation of three questions. First, what is the effect of the procedure on T-cells? We considered anergy, ignorance, regulation, and deletion as possibilities (10). Second, what is the role of the DST? Finally, what is the fate of alloreactive T-cells after costimulatory blockade with and without DST? To address

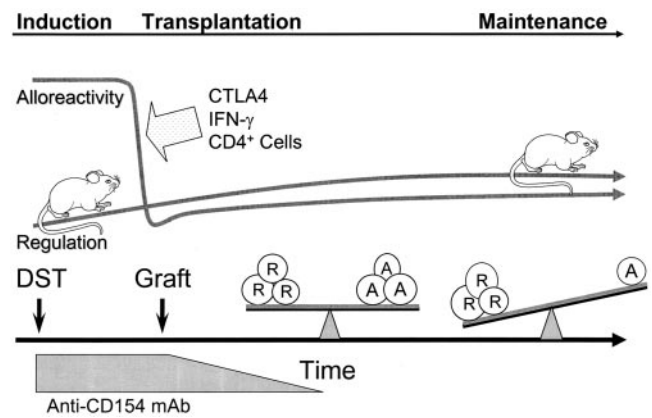


FIG. 5. Stages of transplantation tolerance induction using DST and anti-CD154 mAb (10). Induction with DST and anti-CD154 mAb leads to the deletion of alloreactive T-cells. The process depends on the presence of CD4-positive T-cells, cells that express the costimulatory molecule CTLA4, and the cytokine interferon- γ (55). After transplantation, the graft goes through a transition during which the alloreactive (A) and regulatory (R) forces come into balance. Permanence of grafts, we hypothesize, depends on an equilibrium that stably favors regulation over alloreactivity.

these questions we hypothesized that tolerance based on DST and anti-CD154 mAb leads both to deletion of alloreactive cells and to the generation of regulatory cells.

To test this hypothesis, we devised a method for following alloreactive T-cells in vivo. The method is based on creating “synchimeric” mice (54). Because these were to be studies of the immune system and not diabetes per se, we used skin instead of islet grafts because, among all tissues, skin is most refractory to transplantation. Analysis of these synchimeric mice provided an answer to our initial question. Treatment of mice with a DST and anti-CD154 mAb rapidly leads to deletion of the alloreactive KB5 T-cells (54). The cells remain absent for several weeks and only slowly recover. Skin grafts on control animals were rejected. Grafts on mice treated with DST and anti-CD154 mAb survived for a prolonged period, many of them for nearly a year. Because the alloreactive cells began to recover some weeks after tolerance induction, the results suggest a possible explanation for the eventual loss of the grafts—the reappearance of new alloreactive T-cells generated in the bone marrow and processed in the thymus.

There was something else of note in the results of these studies. Even though the alloreactive T-cells returned in all the mice, grafts were lost in only about half of them. Why did those survive? Extrapolation of the balance hypothesis (Fig. 2) to the realm of transplantation tolerance suggested an answer. We hypothesized that in the mice with surviving grafts, there are populations of CD4⁺ regulatory T-cells, and we found evidence to support that hypothesis. Treatment of tolerized mice with successful grafts with an antibody that depletes CD4-positive cells was associated with activation of CD8⁺ alloreactive KB5 cells and prompt rejection of healthy grafts (54).

Figure 5 summarizes this part of our story. We believe that tolerance induction is a process that progresses through several stages. Induction of tolerance with DST and anti-CD154 mAb leads to the deletion of alloreactive T-cells. It also requires the presence of CD4-positive T-cells, cells that express the costimulatory molecule

CTLA4, and the cytokine interferon- γ (55). After transplantation, the graft goes through a transition phase during which the alloreactive and regulatory forces come into balance. Permanence of grafts, we hypothesize, depends on an equilibrium that stably favors regulation over alloreactivity.

Tolerance and autoimmune disease. All of these data, however, were generated using mice with chemically induced diabetes. Whether tolerance induction might be applicable to patients with autoimmune type 1 diabetes is unclear because this disease represents a special case. In humans, Sutherland et al. (56) has documented that pancreas transplants between identical twins discordant for diabetes fail. In these patients, there was no risk of allorejection, but recurrence of the autoimmune process threatened to destroy the islet β -cell graft. Immunosuppressive therapy had to be given. Accordingly, in the case of type 1 diabetes, grafts must overcome both allorejection and disease recurrence.

To determine if autoimmune diabetes can be cured by costimulation blockade and islet transplantation, we used the NOD mouse model system. We found these animals to be completely resistant to tolerance induction (57). The refractoriness of the autoimmune mouse to curative transplantation was disconcerting, and to address the problem we adopted a different immunological strategy—the induction of central tolerance. Treatment with DST and anti-CD154 mAb induces peripheral tolerance through a process that involves deletion of alloreactive cells and the generation of regulatory cells (10). Central tolerance is different and involves the elimination of autoreactivity by thymic selection. We hypothesized that the generation of central tolerance would succeed where peripheral tolerance induction had failed.

We sought to determine if costimulation blockade would facilitate generation of hematopoietic chimerism without need for the lethal conditioning normally required for bone marrow recipients (58). Recipient mice were either normal BALB/c mice ($H2^d$) or diabetic NOD mice ($H2^g7$). The bone marrow donors were MHC-mismatched C57BL/6 mice ($H2^b$). The recipients were sublethally irradiated, given a bone marrow graft, and then treated with two injections only of anti-CD154 mAb. They were then assayed for the presence of donor C57BL/6 cells and for evidence of graft versus host disease. We confirmed in control mice that chimerism could be achieved with high-dose radiation alone, but at the price of graft versus host disease (GVHD) in all the recipients. In addition, we found that, using costimulation blockade, chimerism could be achieved with lower dose sublethal irradiation and without the development of GVHD. We interpreted the data to suggest that hematopoietic chimerism can thwart the recurrence of autoimmunity and induce donor-specific allotransplantation tolerance.

With this information in hand, we returned to the problem of curing autoimmune diabetes (58). Diabetic NOD mice were given sublethal irradiation, treated with anti-CD154 mAb and bone marrow from C57BL/6 donors, and then transplanted C57BL/6 islets and tested for recurrence of diabetes. Control NOD mice that were not chimeric promptly lost their islet grafts. In contrast, chimeric NOD mice with a C57BL/6 bone marrow allograft could

clearly be cured of their diabetes, with some grafts lasting nearly a year (58). Histological study of successful islet grafts in a chimeric NOD mouse revealed cells that stain positively for both insulin and glucagon and the absence of infiltrating lymphocytes.

Bringing hematopoietic chimerism to the clinic. The translation of these promising results to the clinic, however, will require overcoming significant barriers. Among these are the requirement for preparative conditioning of the recipient. In addition, it is well known that any reduction in the conditioning regimen might necessitate an increase in the number of stem cells required in order to achieve chimerism. This, in turn, could increase in the risk of GVHD. Our laboratory's search for solutions to these concerns has focused on costimulation blockade, adapting our islet and skin graft protocol for use in stem cell transplantation. We hypothesized that the induction of peripheral tolerance by DST plus anti-CD154 mAb treatment would facilitate stem cell engraftment and the generation of hematopoietic chimerism leading to establishment of central tolerance.

The protocol used to test the hypothesis has been published (59). The results showed that mice given both anti-CD154 mAb and a DST before bone marrow transplantation became chimeric and circulated ~10% donor-origin mononuclear cells. In contrast, control mice given only anti-CD154 mAb and no DST before bone marrow all uniformly failed to become chimeric. In additional experiments, we found that that the chimerism in these mice is durable, lasting many months; that it can be generated using several different donor and recipient strain combinations; and that it is capable of supporting donor-specific transplantation tolerance.

Mechanism. We next attempted to discern the underlying mechanisms using the KB5 synchimeric mouse as a bone marrow recipient. In untreated mice the percentage of alloreactive KB5 T-cells is relatively stable. We then studied two groups of mice that did not become chimeric after bone marrow engraftment. One group received anti-CD154 mAb and a bone marrow graft, the other anti-CD154 mAb and a DST. In both cases the result was what we expected. The number of alloreactive peripheral T-cells declined after treatment, but thereafter slowly recovered. In striking contrast, the chimeric mice that were given anti-CD154 mAb, a DST, and bone marrow had no detectable peripheral alloreactive T-cells for months. This, in addition, was also the only group that became chimeric (59). Why did this happen? It appears that intrathymic deletion of the developing alloreactive T-cells occurred, an observation that could have important implications for long-term durability of allografts.

What conclusions can we draw from these data? First, hematopoietic chimerism can be established using peripheral tolerance induction protocols. Second, we can, at least in rodents, obviate any need for myeloablative conditioning. Third, we can achieve permanent chimerism in the absence of GVHD. Fourth, we can generate central transplantation tolerance that supports allogeneic skin transplantation and will surely support islet transplantation. **On the verge of human transplantation tolerance.** All of these data suggest that tolerance induction has exceptional promise for translation to clinical medicine, but we

are not there yet. Still it bears repeating that animal data often do not “scale” well to large animals and humans, and tolerance induction procedures like insulin and BCG (Bacille Calmette Guerin) that worked in mice have failed in children. There is no guarantee that transplantation tolerance will be an exception, and clearly safety issues like inadvertent viral infection at time of tolerance induction need to be addressed (60). Nonetheless, there is much promise and the omens are good. One example that addressed both scalability and safety was a nonhuman primate trial we conducted. In this experiment, cynomolgus monkeys were treated with DST and anti-CD154 mAb and given allogeneic skin grafts in an open, nonisolation facility. These grafts survived long term (A.A.R., unpublished data).

The circle of tolerance. This completes this journey around the circle of tolerance. Learning how it can be lost in BB rats, NOD mice, and other models has given us reason to believe that methods will surely be found to prevent that loss. And for those individuals with type 1 diabetes, those same pathways of tolerance may soon be exploited to generate a rebirth of tolerance, this time for a curative islet graft.

As I conclude, I would like to restate my enduring optimism that the goal of cure and prevention of diabetes is within reach. I hold this belief for a number of reasons. The first relates to the exciting era in which we live. We have the ability to translate basic research to clinical trials. The second relates to advanced technology, including genomics, proteomics, nanotechnology, and siRNA. Third, diabetes research is endowed with committed, honest scientists with a passion for research and a desire to cure and prevent the disease and its complications. Finally, we must recognize that a critical component of diabetes research is the patient. I have had the honor to care for people who suffer with diabetes. These people have provided me and all my fellow clinician scientists with emotional support, encouragement, financial generosity, and willingness to participate in clinical trials. I feel they are the ultimate heroes in the field.

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REFERENCES

1. von Meyenburg JV: Über 'insulinitis' bei diabetes. *Schweiz Med Wochenschr* 24:554–557, 1940
2. Gepts W: Pathology and anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* 14:619–633, 1965
3. Doniach D, Bottazzo GF, Drexhage HA: The autoimmune endocrinopathies. In *Clinical Aspects of Immunology*. Lachmann PJ, Peter K, Eds. Oxford, U.K. Blackwell, 1982, p. 903–937
4. Krischer JP, Cuthbertson DD, Yu LP, Orban T, Maclaren N, Jackson R, Winter WE, Schatz DA, Palmer JP, Eisenbarth GS: Diabetes PT-T screening strategies for the identification of multiple antibody-positive relatives of individuals with type 1 diabetes. *J Clin Endocrinol Metab* 88:103–108, 2003
5. Stiller CR, Dupré J, Gent M, Jenner MF, Keown PA, Laupacis A, Martell R, Rodger NW, von Graffenried B, Wolfe BMJ: Effects of cyclosporine immunosuppression in insulin-dependent diabetes mellitus of recent onset. *Science* 223:1362–1367, 1984
6. Field LL: Genetic linkage and association studies of type 1 diabetes: challenges and rewards. *Diabetologia* 45:21–35, 2002
7. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, Hunter KM, Smith AN, Di Genova G, Herr MH, Dahlman I, Payne F, Smyth D, Lowe C, Twells RC, Howlett S, Healy B, Nutland S, Rance HE, Everett V, Smink LJ, Lam AC, Cordell HJ, Walker NM, Bordin C, Hulme J, Motzo C, Cucca F, Hess JF, Metzker ML, Rogers J, Gregory S, Allahabadia A, Nithiyananthan R, Tuomilehto-Wolf E, Tuomilehto J, Bingley P, Gillespie KM, Undlien DE, Ronningen KS, Guja C, Ionescu-Tirgoviste C, Savage DA, Maxwell AP, Carson DJ, Patterson CC, Franklyn JA, Clayton DG, Peterson LB, Wicker LS, Todd JA, Gough SC: Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423:506–511, 2003
8. Redondo MJ, Yu L, Hawa M, Mackenzie T, Pyke DA, Eisenbarth GS, Leslie, RDG: Heterogeneity of type 1 diabetes: analysis of monozygotic twins in Great Britain and the United States. *Diabetologia* 44:354–362, 2001
9. Laron Z: Interplay between heredity and environment in the recent explosion of type 1 childhood diabetes mellitus. *Am J MedGenet* 115:4–7, 2002
10. Rossini AA, Greiner DL, Mordes JP: Induction of immunological tolerance for transplantation. *Physiol Rev* 79:99–141, 1999
11. Rossini AA, Mordes JP, Greiner DL, Stoff JS: Islet cell transplantation tolerance. *Transplantation* 72:S43–S46, 2001
12. Mordes JP, Serreze DV, Greiner DL, Rossini AA: Animal models of autoimmune diabetes mellitus. In *Diabetes Mellitus. A Fundamental and Clinical Text*. LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippincott Williams & Wilkins, 2004. In press
13. Mordes JP, Bortell R, Groen H, Guberski DL, Rossini AA, Greiner DL: Autoimmune diabetes mellitus in the BB rat. In *Animal Models of Diabetes: A Primer*. Sima AAF, Shafir E, Eds. Amsterdam, Harwood Academic Publishers, 2001, p. 1–41
14. Serreze DV, Leiter EH: Genes and pathways underlying autoimmune diabetes in NOD mice. In *Molecular Pathology of Insulin Dependent Diabetes Mellitus*. Von Herrath MG, Ed. New York, Karger Press, 2001, p. 31–67
15. Koevary S, Rossini AA, Stoller W, Chick W, Williams, RM: Passive transfer of diabetes in the BB/W rat. *Science* 220:727–728, 1983
16. Lampeter EF, Homberg M, Quabeck K, Schaefer UW, Wernet P, Bertrams J, Grosse-Wilde H, Gries FA, Kolb H: Transfer of insulin-dependent diabetes between HLA-identical siblings by bone marrow transplantation. *Lancet* 341:1243–1244, 1993
17. Greiner DL, Rossini AA, Mordes JP: Translating data from animal models into methods for preventing human autoimmune diabetes mellitus: caveat emptor and *primum non nocere*. *Clin Immunol* 100:134–143, 2001
18. Parving HH, Tarnow L, Nielsen FS, Rossing P, Mandrup-Poulsen T, Osterby R, Nerup J: Cyclosporine nephrotoxicity in type 1 diabetic patients: a 7-year follow-up study. *Diabetes Care* 22:478–483, 1999
19. Rossini AA, Mordes JP, Pelletier AM, Like AA: Transfusions of whole blood prevent spontaneous diabetes in the BB/W rat. *Science* 219:975–977, 1983
20. Burstein D, Mordes JP, Greiner DL, Stein D, Nakamura N, Handler ES, Rossini AA: Prevention of diabetes in the BB/Wor rat by a single transfusion of spleen cells: parameters that affect the degree of protection. *Diabetes* 38:24–30, 1989
21. Mordes JP, Gallina DL, Handler ES, Greiner DL, Nakamura N, Pelletier A, Rossini AA: Transfusions enriched for W3/25⁺ helper/inducer T lymphocytes prevent spontaneous diabetes in the BB/W rat. *Diabetologia* 30:22–26, 1987
22. Hu SK, Eardley DD, Cantor H, Gershon RK: Definition of two pathways for generation of suppressor T-cell activity. *Proc Natl Acad Sci U S A* 80:3779–3781, 1983
23. Goodnow CC: Balancing immunity and tolerance: deleting and tuning lymphocyte repertoires. *Proc Natl Acad Sci U S A* 93:2264–2271, 1996
24. Beilhack GF, Scheffold YC, Weissman IL, Taylor C, Jerabek L, Burge MJ, Masek MA, Shizuru JA: Purified allogeneic hematopoietic stem cell transplantation blocks diabetes pathogenesis in NOD mice. *Diabetes* 52:59–68, 2003
25. Chakrabarti S, Handa SK, Bryon RJ, Griffiths MJ, Milligan DW: Will mixed chimerism cure autoimmune diseases after a nonmyeloablative stem cell transplant? *Transplantation* 72:340–342, 2001
26. Lee WY, Oh ES, Min CK, Kim DW, Lee JW, Kang MI, Min WS, Cha BY, Lee KW, Son HY, Kang SK, Kim CC: Changes in autoimmune thyroid disease following allogeneic bone marrow transplantation. *Bone Marrow Transplant* 28:63–66, 2001

27. Mordes JP, Bortell R, Doukas J, Rigby MR, Whalen BJ, Zipris D, Greiner DL, Rossini AA: The BB/Wor rat and the balance hypothesis of autoimmunity. *Diabetes Metab Rev* 2:103–109, 1996
28. Greiner DL, Mordes JP, Handler ES, Angelillo M, Nakamura N, Rossini AA: Depletion of RT6.1⁺ T lymphocytes induces diabetes in resistant Bio-Breeding/Worcester (BB/W) rats. *J Exp Med* 166:461–475, 1987
29. Guberski DL, Thomas VA, Shek WR, Like AA, Handler ES, Rossini AA, Wallace JE, Welsh RM: Induction of type 1 diabetes by Kilham's rat virus in diabetes resistant BB/Wor rats. *Science* 254:1010–1013, 1991
30. Yoon JW, Jun H-S: Role of viruses in the pathogenesis of type 1 diabetes mellitus. In *Diabetes Mellitus: A Fundamental and Clinical Text*. LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippincott Williams & Wilkins, 2000, p. 419–429
31. Petrovsky N, Kyvik KO, Bonnevie-Nielsen V, Beck-Nielsen H, Green A, Harrison LC: Evidence from twins for acquired cellular immune hyperactivity in type 1 diabetes. *Immunology* 106:584–589, 2002
32. Yoon J-W, Austin M, Onodera T, Notkins AL: Virus-induced diabetes mellitus: isolation of a virus from the pancreas of a child with diabetic ketoacidosis. *N Engl J Med* 300:1173–1179, 1979
33. Craighead JE, McLane MF: Diabetes mellitus: induction in mice by encephalomyocarditis virus. *Science* 162:913–914, 1968
34. Brown DW, Welsh RM, Like AA: Infection of peripancreatic lymph nodes but not islets precedes Kilham rat virus-induced diabetes in BB/Wor rats. *J Virol* 67:5873–5878, 1993
35. Chung YH, Jun HS, Son M, Bao M, Bae HY, Kang Y, Yoon JW: Cellular and molecular mechanism for Kilham rat virus-induced autoimmune diabetes in DR-BB rats. *J Immunol* 165:2866–2876, 2000
36. Zipris D, Hillebrands, J-L, Welsh RM, Rozing J, Xie JX, Mordes JP, Greiner DL, Rossini AA: Infections that induce autoimmune diabetes in BBDR rats modulate CD4⁺CD25⁺ T cell populations. *J Immunol* 170:3592–3602, 2003
37. Janeway CA Jr, Medzhitov R: Innate immune recognition. *Annu Rev Immunol* 20:197–216, 2002
38. Bach JF: Mechanisms of disease: the effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 347:911–920, 2002
39. Pasare C, Medzhitov R: Toll pathway-dependent blockade of CD4⁺CD25⁺ T cell-mediated suppression by dendritic cells. *Science* 299:1033–1036, 2003
40. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA: Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 413:732–738, 2001
41. Jacob HJ, Pettersson A, Wilson D, Mao Y, Lernmark Å, Lander ES: Genetic dissection of autoimmune type I diabetes in the BB rat. *Nat Genet* 2:56–60, 1992
42. Hornum L, Rømer J, Markholst H: The diabetes-prone BB rat carries a frameshift mutation in *Ian4*, a positional candidate of *iddm1*. *Diabetes* 51:1972–1979, 2002
43. MacMurray AJ, Moralejo DH, Kwitek AE, Rutledge EA, Van Yserloo B, Gohlke P, Seros SJ, Snyder B, Schaefer J, Bieg S, Jiang JJ, Ettinger RA, Fuller J, Daniels TL, Pettersson A, Orlebeke K, Birren B, Jacob HJ, Lander ES, Lernmark Å: Lymphopenia in the BB rat model of type I diabetes is due to a mutation in a novel immune-associated nucleotide (*lan*)-related gene. *Genome Res* 12:1029–1039, 2002
44. Pandarpurkar M, Wilson-Fritch L, Corvera S, Markholst H, Hornum L, Greiner DL, Mordes JP, Rossini AA, Bortell R: *Ian4* is required for mitochondrial integrity and T cell survival. *Proc Natl Acad Sci U S A* 100:10382–10387, 2003
45. Pockley AG: Heat shock proteins as regulators of the immune response. *Lancet* 362:469–476, 2003
46. Wallin RP, Lundqvist A, More SH, von Bonin A, Kiessling R, Ljunggren HG: Heat-shock proteins as activators of the innate immune system. *Trends Immunol* 23:130–135, 2002
47. Elias D, Markovits D, Reshef T, Van der Zee R, Cohen IR: Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/Lt) mouse by a 65-kDa heat shock protein. *Proc Natl Acad Sci U S A* 87:1576–1580, 1990
48. Bowen-Jones D, Fantes J, Gupta R: Diabetes and heat shock protein. *Nature* 355:119–120, 1992
49. Izumi T, Yokota-Hashimoto H, Zhao SL, Wang A, Halban PA, Takeuchi T: Dominant negative pathogenesis by mutant proinsulin in the Akita diabetic mouse. *Diabetes* 52:409–416, 2003
50. Harding HP, Ron D: Endoplasmic reticulum stress and the development of diabetes: a review. *Diabetes* 51 (Suppl. 3):S455–S461, 2002
51. Shapiro AMJ, Lakey JRT, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238, 2000
52. Shapiro J, Lakey J, Paty B, Senior P, McDonald C, McCready T, Oberholzer J, Kneteman NM, Rajotte R, Bigam D, O'Kelly K, Ryan E: Clinical islet transplantation in Edmonton—three year outcomes and new developments (Abstract). *Diabetes* 52 (Suppl. 1):A66, 2003
53. Parker DC, Greiner DL, Phillips NE, Appel MC, Steele AW, Durie FH, Noelle RJ, Mordes JP, Rossini AA: Survival of mouse pancreatic islet allografts in recipients treated with allogeneic small lymphocytes and antibody to CD40 ligand. *Proc Natl Acad Sci U S A* 92:9560–9564, 1995
54. Iwakoshi NN, Markees TG, Turgeon NA, Thornley T, Cuthbert A, Leif JH, Phillips NE, Mordes JP, Greiner DL, Rossini AA: Skin allograft maintenance in a new synchimeric model system of tolerance. *J Immunol* 167:6623–6630, 2001
55. Markees TG, Phillips NE, Gordon EJ, Noelle RJ, Shultz LD, Mordes JP, Greiner DL, Rossini AA: Long-term survival of skin allografts induced by donor splenocytes and anti-CD154 antibody in thymectomized mice requires CD4⁺ T cells, interferon-gamma, and CTLA4. *J Clin Invest* 101:2446–2455, 1998
56. Sutherland DE, Sibley R, Xu XZ, Michael A, Srikanta AM, Taub F, Najarian J, Goetz FC: Twin-to-twin pancreas transplantation: reversal and reenactment of the pathogenesis of type I diabetes. *Trans Assoc Am Physicians* 97:80–87, 1984
57. Markees TG, Serreze DV, Phillips NE, Sorli CH, Noelle RJ, Woda BA, Greiner DL, Mordes JP, Rossini AA: NOD mice have a generalized defect in their response to transplantation tolerance induction. *Diabetes* 48:967–974, 1999
58. Seung E, Iwakoshi N, Woda BA, Markees TG, Mordes JP, Rossini AA, Greiner DL: Allogeneic hematopoietic chimerism in mice treated with sublethal myeloablation and anti-CD154 antibody: absence of graft-versus-host disease, induction of skin allograft tolerance, and prevention of recurrent autoimmunity in islet-allografted NOD/Lt mice. *Blood* 95:2175–2182, 2000
59. Seung E, Mordes JP, Rossini AA, Greiner DL: Hematopoietic chimerism and central tolerance created by peripheral tolerance induction without myeloablative conditioning. *J Clin Invest* 112:795–808, 2003
60. Welsh RM, Markees TG, Woda BA, Daniels KA, Brehm MA, Mordes JP, Greiner DL, Rossini AA: Virus-induced abrogation of transplantation tolerance induced by donor-specific transfusion and anti-CD154 antibody. *J Virol* 74:2210–2218, 2000
61. Kamradt T, Mitchison NA: Advances in immunology: Tolerance and autoimmunity. *N Engl J Med* 344:655–664, 2001
62. Mordes JP, Leif J, Novak S, DeScipio C, Greiner DL, Blankenhorn EP: The *iddm4* locus segregates with diabetes susceptibility in congenic WF.*iddm4* rats. *Diabetes* 51:3254–3262, 2002
63. Schwartz RH: Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell* 71:1065–1068, 1992
64. Markees TG, Phillips NE, Gordon EJ, Noelle RJ, Mordes JP, Greiner DL, Rossini AA: Improved skin allograft tolerance induced by treatment with donor splenocytes and an extended course of anti-CD154 monoclonal antibody. *Transplant Proc* 30:2444–2446, 1998