Autoimmune disease: Type 1 diabetes

Remodeling rodent models to mimic human type 1 diabetes

Matthias von Herrath[1] and Gerald T. Nepom[2]

[1] Center for Type 1 Diabetes Research, La Jolla Institute for Allergy and Immunology, UCSD, La Jolla, CA, USA
[2] Benaroya Research Institute, Seattle, WA, USA

DOI 10.1002/eji.200939429

Type 1 diabetes (T1D) occurs when the immune system attacks the insulin-producing beta cells located in the islets of Langerhans within the pancreas. Animal models have played a prominent role in developing an understanding of this disease process, through studies of genetic susceptibility, progression of hyperglycemia, and novel approaches to therapy. Here, we critically evaluate the currently available diabetic animal models and their propensity to match and predict disease outcomes in man as well as propose new *in vitro* and *in vivo* systems that may facilitate progress in the T1D field.

Autoimmune diabetes, type 1A (T1D), is a chronic autoimmune disorder, which can be viewed as the result of a summary of events – genetic susceptibility followed by autoimmune activation leading to progressive tissue damage with loss of insulin secretory capacity. Prospects for immune-based therapy underlie a plethora of current clinical trials. One of the key concepts fundamental to a scientific approach to understanding this process in humans is the use of animal models that ideally should mimic crucial aspects of human T1D pathogenesis. Notably, however, we recognize a significant amount of pathogenetic heterogeneity within the human population, which requires attention to the need for appropriate animal models to illuminate relevant aspects of various mechanistic pathways.

For example, genetic susceptibility in humans is based on multiple genes mainly located within the MHC, with a hierarchy of risk associated with specific allelic variants. It also involves a large number of modifier genes, currently estimated at 40, which can alter the risk ratios, even in the presence of the major MHC susceptibility genes [1, 2] Aside from identical twins, therefore, the likelihood that any two diabetic people share all the same genetic susceptibility traits is quite low, and this has implications for using murine models to understand underlying genetic contributions to disease and to therapy.

The stage of autoimmune activation is similarly heterogeneous. We know that the onset of anti-islet autoantibodies is highly variable, ranging from neonatal to adult ages. Even within twin pairs who ultimately become diabetic, the age of onset of autoantibody positivity can be very different; indeed, the precise specificities (*i.e.* targets) of the initial autoimmune response can be to different islet proteins. Similarly, disease progression can be acute, with a short interval between autoantibody positivity and overt hyperglycemia, or very chronic, with more than 10 years of "pre-clinical" autoimmunity [3–5].

These variables undoubtedly impact the therapeutic response of individuals following diagnosis, as well. The length of the diabetic honeymoon period following institution of insulin replacement, and even whether or not there will be a response to immunomodulatory therapy, such as in recent trials with anti-CD3 or glutamic acid decarboxylase (GAD)-alum, shows differences that are currently unexplained [6, 7].

A major challenge for the field, therefore, is to develop animal models of diabetes, which address such issues of diversity, in order to directly inform translational decision-making. An obvious corollary to this concept is that no single animal model is going to adequately address the heterogeneity issues at each of these disease stages.

In spite of these concerns, and an incomplete understanding of T1D development in humans, there is considerable momentum towards the development of novel *in vitro* and *in vivo* models that will aid in the discovery of fundamental mechanistic aspects of T1D and future treatment options. Here, we critically evaluate the currently available diabetic animal models and their propensity to match and predict disease outcomes in man as well as propose new *in vitro* and *in vivo* systems that may facilitate progress in the T1D field.

Translational challenges

There are roughly six major groupings of androdent diabetes models, which offer different perspectives for understanding T1D and different opportunities for translating T1D research:

(i) The non-obese diabetic (NOD) mouse
(ii) Beta-cell damage leading to antigen exposure (*e.g.* streptozoticin or islet-tropic viruses)
(iii) Transgenic "pseudo-self" antigens expressed in the islet (*e.g.* RIP-OVA, RIP-HA, or RIP-LCMV models)
(iv) Humanized mice and mice with human TCR transgenes
(v) Virtual (*in silico*) models based on the NOD mouse
(vi) The diabetes prone lymphopenic BB rat

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

The NOD mouse is the strain used most frequently for *in vivo* studies of T1D. Manifestation of diabetes in the NOD strain is relatively consistent (incidence about 60–80% depending on environmental factors) and onset is within 10–18 wk of age and sensitive to immunomodulation. In fact, over 300 immune manipulations have been reported to prevent or reverse T1D in the NOD mouse model [8]. However, in several important respects, the NOD mouse model has failed to offer translational guidance to human T1D research [9].

One notable example is in the area of biomarker development. Biomarkers are not only critical for understanding disease mechanism, but a necessity for the assessment of treatment safety and efficacy in the patient. Though there are certain metabolic markers, such as peripheral blood c-peptide levels, no reliable immunological biomarkers associated with disease progression post diagnosis or therapeutic outcome have been identified to date. The absence of these biomarkers has hampered optimization of drug efficacy. In particular, in the arena of antigen-specific means to induce tolerance to beta cell proteins (*i.e.* oral or nasal insulin administration or DNA vaccines expressing insulin or GAD) the lack of suitable biomarkers, for example, the precise assessment of islet-cell-specific T-cell responses in PBMC, is unfortunate, because optimization of route, dose, and timing of antigen administration should be optimized in both animal models and humans. Not only have oral or nasal insulin administration studies been affected, but progress with subcutaneous peptide or DNA vaccine interventions have also been slowed, because no immunologic biomarkers are available to date that could serve as a primary trial outcome [10–16]. It is therefore imperative that significant emphasis needs to be placed on identification of immunological elements that can identify disease regression or progression. A recent study of transcript arrays in NOD spleen and lymph node begins to address this issue [17], but monitoring immune components in the peripheral blood will be necessary for improved translation to humans albeit challenging still. Some options include:

Cytokine(s) markers

T1D onset has been reported to be associated with a shift in the cytokine profile of Th2 (*i.e.* IL-10$^+$, IL-4$^+$) towards that of Th1, *i.e.* increased production of IFN-γ or loss of IL-10 secretion [18–20]. Thus, a hypothesis exists that clinical benefits could be realized if the immune system were to be driven back to the Th2 phenotype. If this hypothesis were true, then the Th cytokine expression profile of autoreactive lymphocytes might serve as a biomarker panel that could be used for the interpretation of clinical efficacy and outcome. Indeed, in studies with the anti-CD3 hOKT3gamma1(Ala-Ala) [21] or after *in vitro* (antigen-specific) stimulation of peripheral T cells using the heat shock protein 60 peptide DiaPep277 therapy [22] or proinsulin expressing DNA vaccines, GAD vaccines or peptide therapies [7, 23] an immune deviation such as the one hypothesized above is measured. Though none of these treatments afforded permanent preservation of C-peptide secretion post diagnosis, some of them did delay progression. In addition, the presence or absence of islet antigen-specific IL-10 expressing T cells can be used to discriminate between healthy and diabetic individuals [10] and could maybe act as an indicator of glycemic control at the time of T1D diagnosis [24].

Rationally designed murine models could potentially assist with this linkage between cytokine phenotype and development of disease-relevant biomarkers. For example, studies that drive anti-islet immunity towards specific Th expression profiles, while simultaneously monitoring both serum cytokines and disease progression, and/or studies that evaluate different therapies or therapeutic vaccines in conjunction with serum biomarker profiles as well as clinical outcome, could be useful; however, it will be crucial that the "mouseologists" will in the future assess immune reactivity not only in lymphoid organs but also in peripheral blood, which will allow for easier translation of their findings. A stumbling block for such a strategy is that the frequencies of autoreactive T cells in PBMC is notoriously low, which hampers their detection in both mice and men [25].

Anti-islet autoantibodies

It has been demonstrated by several groups that the most predictive marker for T1D onset is the presence of multiple autoAb (anti-insulin, anti-GAD65, and anti-IA2) in the context of a susceptible HLA genotype [26]. Although these islet-antigen-associated antibodies may facilitate antigen presentation [27, 28], their involvement in human T1D pathogenesis is not yet fully defined [29]. Moreover, serum antibody titers may even be indicative of progressive beta-cell destruction post diagnosis of T1D or drug efficacy [30]. However, none of the intervention trials demonstrating positive outcomes or islet transplantation trials using a non-steroidal immunosuppressive regimens provided evidence for a role of autoAb in predicting T1D disease state or clinical efficacy [7, 22, 31].

The recent observation of additional anti-islet autoantibodies, particularly anti-ZnT8 [32], which improve predictive algorithms, suggests the possibility that there are additional islet-associated antigen specificities that may be informative biomarkers, not yet being studied, particularly for later phases of disease progression and response to therapy. This may be a productive area for the use of retrogenic humanized mice, in which the specificities of diabetes-associated TCR are yet to be defined [33].

Ex vivo auto-reactive T-cell tracking

There are two popular methods that successfully visualize or track autoreactive T cells. One such method, the

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

ELISPOT assay, has the ability to not only quantify the number of T cells present, but also assess antigen specificity and the cytokine expressed by $CD4^+$ or $CD8^+$ T cells as well. The second assay involves the staining of T cells with MHC-peptide multimers followed by FACS analysis or sorting, a technique that can quantify the number of antigen (epitope)-specific T cells as well as enable isolation of a specific population of T cells that could be used for *ex vivo* expansion. The ELISPOT assay can detect relatively small numbers of antigen-specific T cells, an attribute that can identify fluctuations in the population of interest [25, 34]. Although there has been some difficulty in developing functional HLA-class II tetramers with sufficient binding avidity to track autoreactive $CD4^+$ T cells, significant progress has been made [25, 34]. Since both techniques have the ability to measure changes within the antigen-specific T-cell population during clinical trials, one or both could be used to monitor fluctuations in peripheral blood T cells following immune interventions. The use of these techniques has led to the identification of antigen-induced Treg [22, 35, 36] and autoaggressive islet-specific T cells [37, 38]. Two obstacles hamper the use of such assays routinely in clinical trials: one is the lack of their validation post immunotherapy using peripheral blood lymphocytes in animal models, where usually only lymphoid organs, pancreatic islets themselves, or spleens are harvested for T-cell profiling. The other is the need for reliable assays that work on frozen human PBMC, because samples from intervention or prevention trials cannot practically be tested freshly at every site or location.

In addition, with further development of these methodologies and additional ultra-rapid and cost-effective sequencing methods, it will be possible to prove or disprove the hypothesis that in human T1D, diabetogenic TCR usage is oligoclonal within a given individual. If a correlation is found, then the feasibility of tracking autoaggressive T cells with one or few specificities as biomarkers for disease progression or therapeutic success is significantly improved. By introducing a monospecific TCR into NOD mice, early insight into potential pro-diabetogenic (effector) or anti-diabetogenic Treg clones [33] has been achieved. Since there likely will be TcR usage diversity between humans and mice, extrapolating these observations to human T1D becomes difficult. Other possible ways in which to ascertain the pathogenic involvement of specific TCR may be

(i) the comparison of direct tetramer staining profiles of islet sections in both mouse and man and
(ii) the rapid sequencing of TCR present in human insulitis lesions using "next generation" DNA sequencing technology (for example, using freshly frozen and preserved human pancreas specimens obtained through the nPOD consortium (www.jdrfnPOD.org)).

However, the end result may still demonstrate a heterogeneous usage of TCR, thus reducing the practicability of tracing autoaggressive T cells with a single or small number of specificities associated with it.

Clearly, this is an area where humanized mice, such as models in which human T cells are adoptively transferred, or models involving human TCR transgenes, should prove to be informative. It will be important for biomarker studies to include peripheral blood screening as well as the traditional lymph node and spleen studies in order to optimize information for translational applications.

Beta cell mass measurements by in vivo imaging

Though *in vivo* imaging techniques have been fraught with difficulties over the past few years, it remains an approach that is necessary for the direct assessment of the effect of a particular treatment on pancreatic beta cells. Should such a technique be successful, disease kinetics could also be studied, which in turn will have the potential to aid in optimizing T1D disease management. Systems such as magnetic resonance imaging, positron emission tomography, or bioluminescence imaging have been successfully used to detect murine and rat islets in the pancreas *in vivo* [39, 40]. Unfortunately, these techniques have not provided a means to detect islets, let alone beta cells in humans [41]. As *in vivo* imaging has significant potential for clinical use as a means to measure pancreatic beta-cell mass, innovative technologies and scaled-up animal studies are needed to surmount the mouse-human translational barrier.

While there remains a number of scientifically interesting questions to be investigated in the disease-phenotype of the NOD mouse (*e.g.* why is insulin the initiating antigen? Are $CD8^+$ T cells the primary intra-islet effector cells? Why is there a difference between male and female disease incidence?), we must note that these questions are specific to the NOD model, and lead to a focus on just one of the many different pathways that are likely important for understanding human T1D. For a more translational focus, it is necessary to look beyond the NOD mouse to take full advantage of the additional models available. Marker antigens in transgenics, a technique widely used in the late 80s and early 90s to study tolerance, have provided significant insight into the connection between thymic expression, peripheral presentation and cross-presentation, and the interaction of $CD4^+$, $CD8^+$, and B lymphocytes with viral antigens from influenza and LCMV [42] and unrelated proteins (T-Ag, ovalbumin) [43, 44]. These mouse model systems are still being used to this day for testing a variety of tolerance modalities. Even though the autoantigen(s) involved in initializing human T1D are still largely unknown, these types of transgenic models are of significance as they provide insight into the pathogenic pathways that underlie and may prevent beta-cell destruction. If taken a step further, these insights provide a chance to test potential treatments on diverse genetic backgrounds similar to those found in

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

human T1D patients. Also, animals with genetic defects in Foxp3 [45, 46], IL-2 [47], Zap70 [48], FAS [49], and other important tolerance mediators provide interesting insight into the many pathways that modulate autoimmune manifestations. As the mechanistic effects of these genetic defects become better characterized in mice, it will become easier to associate specific genetic defects with disease subsets seen in humans, possibly resulting in the ability to stratify subjects for interventions based on phenotypic variation.

Humanized mice theoretically should help overcome some of the barriers outlined above, but such systems require not only the integration of costimulatory, MHC, and TCR genes, but also consideration of processes such as cell expansion, homing, matrix, and tissue adhesion events. It remains to be seen whether these models can be "sufficiently humanized" to provide significant insight into antigen-driven immunomodulation, where the antigen is presented in the context of humanized MHC and other genes [50, 51], or potentially into the use of biomarkers associated with certain aspects or phases of T1D disease progression. One underdeveloped area for productive study may be the introduction of various non-MHC human diabetes susceptibility genes into mouse strains that carry the NOD $H2^{g7}$ but do not carry the endogenous NOD autoimmunity genes. Should this result in an augmentation of autoimmunity or disease penetrance, molecular pathways could then be linked or associated with genetic profiles.

Finally, the BB rat model has been useful in understanding how lymphopenia can lead to diabetes development. In the BB rat the Lyp gene [52] leads to systemic lymphopenia, which can foster the expansion of autoreactive specificities. Does this occur in human T1D? It is possible that after viral infections or in islet transplant settings [53] lymphopenic situations arise, which support the expansion of autoreactive T cells. In addition, this model has been useful in defining scenarios in which viral infections can trigger type 1 diabetes [54–56].

Models focused on clinical translation

As we previously noted [9], there has been an undue emphasis on the NOD mouse model in T1D experimentation, which constrains progress in areas of translational need. Though the NOD mouse and humans share several susceptibility genes (*i.e.* MHC class II homologues), substantial complexity and heterogeneity still exists in terms of the disease itself as well as in the genetics behind the disease. Despite the significant contributions that the NOD mouse genetics as well as islet pathology have made to our understanding of T1D, it is time for a broader re-evaluation of what is observed in humans and the generation and identification of mouse models most closely associated with human disease. This will, in effect, provide an opportunity to improve translation of results from mouse to man. Here, we describe several potential areas that may facilitate this advancement:

The majority of T1D treatment discoveries in mouse models have not yet translated to viable treatments in man [12, 14, 15, 57–61]. However, the prospect that T1D progression may be blocked by the active stimulation of tolerance induced by (auto)antigen-specific immunization to generate Treg has changed the landscape of possible treatment alternatives. In this instance, treatment is multi-faceted and contingent upon several factors, notably:

(i) The choice of protein or peptide delivery
(ii) The dosage
(iii) The disease stage
(iv) A specific route of administration

Should all four factors be delivered on target, the prospects of tolerance induction and activation of (auto)antigen-specific Treg are encouraging. There are, however, significant barriers to overcome. Addressing all four of these variables systematically will require years of *in vivo* experimentation, with no guarantee that results will be ultimately directly translatable into treatments for humans.

One method by which to gain headway for some of the multi-factorial aspects of immune response occurring in disease onset is through *in silico* biosimulations. This approach has recently been used to test possible hypotheses that may account for the success or failure of T1D therapeutics in NOD mice [62, 63]. There are opportunities to test biosimulation for optimized experimental design and to identify assumptions that are critical for the predicted biological response, thus possibly pointing to new biomarkers. By combining "wet-lab" and "virtual lab" results, investigators may employ more rational and efficient experimental design, which in turn will accelerate the path from basic research to clinical trials.

Using murine T1D disease models that display an acute onset as well as those with more slowly progressive or a relapsing/remitting disease phenotype are potentially valuable for differentiating the advantages of clinical therapeutics at certain stages of human disease presentation [64]. Longitudinal studies in humans indicate that the many of the new-onset T1D subjects have experienced a long period of pre-clinical "silent" autoimmunity. Appropriate animal models for studying this phase of disease will also require non-NOD strains, potentially partially humanized, or perhaps the type of modified NOD mentioned above in which human "resistance genes" slow disease progression.

Tremendous advances have been made with the identification of diabetes susceptibility genes that participate in the immunological response. T-cell modulation models may yield insights into how patients could be stratified for therapeutic intervention, based on susceptibility gene profiling. However, the question that remains is whether the genetic pathways leading to disease are also a possible target for T1D therapeutics. More studies will have to be completed to affirm or disaffirm this. Many pathways act in concert, and we do not know if therapeutic intervention needs to affect several of them at the same time. It may be instructive to use viral-causation models in which determinant spreading leads to the subsequent targeting of islet

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

antigens to evaluate such "downstream" therapies.

To extend and learn from the concept of heterogeneity and facilitate a clearer understanding of B-cell, CD4, and CD8 responses and their roles in T1D disease pathogenesis, expansion of our use of humanized mice to include those that have human islets and TCR/MHC is necessary. And no animal models currently focus on the contribution of macrophages, mast cells, and different dendritic cell subsets during T1D disease pathogenesis and remission.

Though significant advances in beta-cell regeneration and repair have been made, cell survival in the context of antigen-specific autoimmunity needs to be analyzed. Though it is unclear whether islet replication is increased during pathogenesis of T1D, it has been demonstrated in several animal models that inflammatory factors have the potential to enhance beta-cell replication [65]. Quantification of beta-cell turnover remains elusive however as the specific enumeration of apoptotic beta cells as well as those that regenerate is currently unavailable. New models need to be established that follow beta-cell neogenesis [66] while monitoring immunological events in parallel. Similarly, interactions with structural proteins such as extracellular matrix and matricellular interactions or neuronal interactions may modulate islet health and response to injury. *In vitro* and *in vivo* work that explores the contribution of these aspects needs to be conducted.

To evaluate safety of a therapeutic capable of immune-modification, preclinical studies that evaluate the long-term effects of chronic immune modulation in the context of infections or tumor-prone or non-diabetic autoimmune-prone hosts need to be performed. The safety issues associated with therapeutics remain a major determinant of clinical feasibility and speed of clinical trials, and rarely, if ever, are these prospects adequately tested in a "real-life" preclinical model.

Concluding remarks

Although techniques and resources now enable much T1D research to be done directly in human subjects, there remain many important areas such as those suggested in tis *Viewpoint*, which would benefit from in-depth study, in order to optimize research and therapeutic translation (Table 1). These types of animal models would not only have a considerable impact on basic and discovery research, but those that correlate well with novel aspects of the human condition would have a major impact on preclinical drug development. However, just as the need is driven by the dual issues of human heterogeneity and immunologic complexity, so should the choice of preclinical models. No mouse, and that includes the NOD mouse, is a one-size-fits-all system. A broader approach, driven by targeted questions and strategic experimental design, will make immunology's contribution to diabetes research more durable and translational.

Table 1. Challenges in human studies – opportunities for animal models

- Design modalities whereby clinical outcomes can be assessed directly by the non-invasive analysis of pancreata and pancreatic lymph nodes
- Test a wide breadth of dose-dependent variables, including dose and timing in *in vivo* models aligned with experimental therapeutic outcome parameters
- Conduct risk assessment in the context of chronic infection during immunomodulatory therapy
- Assess alternative antigen-specific delivery modalities to induce peripheral tolerance
- Validate biologic-based therapies in multiple model systems so that the contribution of human genetic heterogeneity to therapeutic outcome is also captured

Acknowledgements: This research was funded by JDRF, ADA, NIH and Brehm Coalition.

Conflict of interest: M. van Herrath is on the data safety monitoring board for BayHill Therapeutics, and is a consultant for Genentech, Kirin, and Baxter.

1 **Concannon, P. *et al.***, *N. Engl. J. Med.* 2009. **360**: 1646–1654.
2 **Steck, A. K. *et al.***, *Diabetes* 2009. **58**: 1028–1033.
3 **Hyttinen, V. *et al.***, *Diabetes* 2003. **52**: 1052–1055.
4 **Bonifacio, E. *et al.***, *Diabetes Care* 2004. **27**: 2695–2700.
5 **Redondo, M. J. *et al.***, *Diabetologia* 2001. **44**: 354–362.
6 **Herold, K. C. *et al.***, *N. Engl. J. Med.* 2002. **346**: 1692–1698.
7 **Ludvigsson, J. *et al.***, *N. Engl. J. Med.* 2008. **359**: 1909–1920.
8 **Shoda, L. K. *et al.***, *Immunity* 2005. **23**: 115–126.
9 **von Herrath, M. and Nepom, G. T.**, *Nat. Immunol.* 2009. **10**: 129–132.
10 **Arif, S. *et al.***, *J. Clin. Invest.* 2004. **113**: 451–463.
11 **Ellis, R. J. *et al.***, *Immunology* 2005. **116**: 337–346.
12 **Kupila, A. *et al.***, *Diabetes Metab. Res. Rev.* 2003. **19**: 415–420.
13 **Peakman, M. *et al.***, *J. Clin. Invest.* 1999. **104**: 1449–1457.
14 **Petersen, J. S. *et al.***, *Clin. Exp. Immunol.* 2003. **134**: 38–45.
15 **Pozzilli, P.**, *Diabetes Metab. Res. Rev.* 2002. **18**: 257–259.
16 **Pozzilli, P. *et al.***, *Diabetologia* 2000. **43**: 1000–1004.
17 **Kodama, K. *et al.***, *Clin. Immunol.* 2008. **129**: 195–201.
18 **Wilson, S. B. *et al.***, *Nature* 1998. **391**: 177–181.
19 **Perez, C. *et al.***, *Eur. Cytokine Netw.* 2004. **15**: 364–370.
20 **Ozer, G. *et al.***, *J. Pediatr. Endocrinol. Metab.* 2003. **16**: 203–210.
21 **Herold, K. C. *et al.***, *J. Clin. Invest.* 2003. **111**: 409–418.
22 **Huurman, V. A. *et al.***, *Clin. Exp. Immunol.* 2008. **152**: 488–497.
23 **Azam, A. and Eisenbarth, G. S.**, *Expert Opin. Biol. Ther.* 2004. **4**: 1569–1575.
24 **Sanda, S. *et al.***, *Clin. Immunol.* 2008. **127**: 138–143.
25 **Reijonen, H. *et al.***, *Diabetes* 2002. **51**: 1375–1382.
26 **Pihoker, C. *et al.***, *Diabetes* 2005. **54**: S52–S61.
27 **Zouali, M.**, *Front. Biosci.* 2008. **13**: 4852–4861.

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

28 **Reijonen, H. *et al.***, *Diabetes* 2000. **49**: 1621–1626.
29 **Martin, S. *et al.***, *N. Engl. J. Med.* 2001. **345**: 1036–1040.
30 **Butty, V. *et al.***, *Diabetes* 2008. **57**: 2348–2359.
31 **Herold, K. C. *et al.***, *Diabetes* 2005. **54**: 1763–1769.
32 **Wenzlau, J. M. *et al.***, *Proc. Natl. Acad. Sci. USA* 2007. **104**: 17040–17045.
33 **Burton, A. R. *et al.***, *Diabetes* 2008. **57**: 1321–1330.
34 **Reijonen, H. *et al.***, *Diabetes* 2004. **53**: 1987–1994.
35 **Thrower, S. L. *et al.***, *Clin. Exp. Immunol.* 2009. **155**: 156–165.
36 **Long, S. A. *et al.***, *Eur. J. Immunol.* 2009. **39**: 612–620.
37 **Roep, B. O.**, *Diabetes* 2008. **57**: 1156.
38 **Skowera, A. *et al.***, *J. Clin. Invest.* 2008. **118**: 3390–3402.
39 **Souza, F. *et al.***, *J. Clin. Invest.* 2006. **116**: 1506–1513.
40 **Turvey, S. E. *et al.***, *J. Clin. Invest.* 2005. **115**: 2454–2461.
41 **Martinic, M. M. and von Herrath, M. G.**, *Immunol. Rev.* 2008. **221**: 200–213.
42 **von Herrath, M. G. *et al.***, *Immunity* 1994. **1**: 231–242.
43 **Morgan, D. J. *et al.***, *Proc. Natl. Acad. Sci. USA* 1999. **96**: 3854–3858.
44 **Kurts, C. *et al.***, *J. Exp. Med.* 1997. **186**: 2057–2062.
45 **Appleby, M. W. and Ramsdell, F.**, *Curr. Top. Microbiol. Immunol.* 2008. **321**: 151–168.
46 **Wan, Y. Y. and Flavell, R. A.**, *Nature* 2007. **445**: 766–770.
47 **Shultz, L. D. *et al.***, *Ann. N.Y. Acad. Sci.* 2007. **1103**: 77–89.
48 **Holmdahl, R.**, *Nat. Clin. Pract. Rheumatol.* 2007. **3**: 104–111.
49 **Strasser, A. *et al.***, *Immunity* 2009. **30**: 180–192.
50 **Enee, E. *et al.***, *J. Immunol.* 2008. **180**: 5430–5438.
51 **Serreze, D. V. *et al.***, *Ann. N. Y. Acad. Sci.* 2007. **1103**: 103–111.
52 **Awata, T. *et al.***, *Endocrinology* 1995. **136**: 5731–5735.
53 **Monti, P. *et al.***, *J. Clin. Invest.* 2008. **118**: 1806–1814.
54 **Nair, A. *et al.***, *Ann. N. Y. Acad. Sci.* 2008. **1150**: 139–142.
55 **Zipris, D. *et al.***, *J. Immunol.* 2007. **178**: 693–701.
56 **Zipris, D. *et al.***, *J. Immunol.* 2005. **174**: 131–142.
57 **Gale, E. A. *et al.***, *Lancet* 2004. **363**: 925–931.
58 **Hermitte, L. *et al.***, *Autoimmunity* 1989. **5**: 79–86.
59 **Lampeter, E. F. *et al.***, *Diabetes* 1998. **47**: 980–984.
60 **Piercy, V. *et al.***, *Metabolism* 2000. **49**: 1548–1554.
61 **Yamada, K. *et al.***, *Diabetes* 1982. **31**: 749–753.
62 **Gadkar, K. G. *et al.***, *Ann. N. Y. Acad. Sci.* 2007. **1103**: 63–68.
63 **Wang, X. *et al.***, *Math. Biosci.* 2006. **203**: 79–99.
64 **Rewers, M. *et al.***, *Adv. Exp. Med. Biol.* 2004. **552**: 219–246.
65 **Sherry, N. A. *et al.***, *Diabetes* 2006. **55**: 3238–3245.
66 **Cano, D. A. *et al.***, *Diabetes* 2008. **57**: 958–966.

Correspondence: Dr. Matthias von Herrath, Center for Type 1 Diabetes Research, La Jolla Institute for Allergy and Immunology, UCSD, 9420 Athene Circle, La Jolla, CA 92037, USA
Fax: +1-858-752-6993
e-mail: matthias@liai.org

Additional correspondence:
Dr. Gerald T. Nepom, Benaroya Research Institute, 1201 Ninth Avenue, Seattle, WA 98101, USA
e-mail: nepom@benaroyaresearch.org

Received: 17/3/2009
Revised: 11/5/2009
Accepted: 19/5/2009

Key words: Animal models · Immunotherapy · Pathogenesis · Translation · Type 1 diabetes

Abbreviation: GAD: glutamic acid decarboxylase

See accompanying article:
http://dx.doi.org/10.1002/eji.200939517

The road not taken: A path to curing type 1 diabetes?

Michael J. Haller, Mark A. Atkinson and Desmond A. Schatz

Departments of Pediatrics and Pathology, University of Florida, Gainesville, USA

DOI 10.1002/eji.200939517

The past quarter century has seen a rapid increase in our knowledge about the natural history of autoimmune type 1 diabetes. However, we stand unable to achieve our ultimate goal of preventing or reversing this disease. This *Viewpoint* discusses controversies in current management of type 1 diabetes, the challenges in translating promising studies from mouse models of the disease to humans, hurdles faced in designing optimal prevention and intervention studies, and potential strategies to overcome these obstacles.

"....Two roads diverged in a wood, and I-
I took the one less traveled by,
And that has made all the difference."
-The Road Not Taken
Robert Frost, 1920

Controversies in clinical care

With the discovery of insulin, type 1 diabetes (T1D) was transformed from a uniformly fatal diagnosis to a chronic disease; one characterized by the need for multiple daily insulin injections and sadly, complications driven by long-term hyperglycemia [1]. Undoubtedly, the development of recombinant human insulin, insulin analogues, meters for performing self monitoring of blood glucose, insulin pumps, point of care hemoglobin A1c testing and more recently, continuous glucose monitoring have dramatically improved the quality of life of T1D patients. Still, patients of all ages, and especially children, continue to struggle with the challenges of attempting to maintain normoglycemia [2, 3]. Added to this, the worldwide epidemic of diabetes

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim