

Animal Models of Spontaneous Autoimmune Diabetes: Notes on Their Relevance to the Human Disease

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The spontaneous autoimmune diabetes of the nonobese diabetic mouse and the BioBreeding diabetes-prone rat bears a striking similarity to human type 1 diabetes. For this reason it comes as a disappointment that interventions known to prevent the disease in the animal models proved ineffective in human trials. For an explanation I propose that, although the mechanism of β -cell destruction is the same in the three species, this common mechanism may be the end point of different loss-of-tolerance pathways in different human patients, only two of which are represented by the two animal models. Research strategies to address this problem will require more attention to immune subphenotypes or their corresponding subgenotypes in the human and, possibly, the generation of additional mouse models of spontaneous disease.

Introduction

The aim of the Diabetes Prevention Trial-1 (DPT-1) was to prevent type 1 diabetes (T1D) by administering insulin in a tolerizing context, either orally or parenterally, to antibody-positive relatives. The absence of any observable effect from these interventions [1] was particularly disappointing because both had been proven effective in the two animal models of spontaneous autoimmune diabetes, the nonobese diabetic (NOD) mouse [2] and the BioBreeding diabetes-prone (BBDP) rat [3]. The failure brings renewed interest to the question of how relevant these animal models are to the human disease. There is no simple answer to this question. It is clear that we can learn, and have learned, much that is relevant to human T1D from these models. It is also clear, to most of us, that there are limits to how much we can extrapolate from them. Where these limits are and what their exact nature is, are much more difficult questions whose detailed dissection is outside the scope of this short article; the reader

is referred to a recent review summarizing the immunologic aspects [4]. In this article, I only discuss genetic heterogeneity in the human disease, an aspect that is rarely discussed in this context but which I believe to be of crucial importance in understanding what the animal models can tell us and what they may not tell us.

Of Mice and Rats

To stimulate some thinking, I will reverse the question: Would human T1D be a good model for studying diabetes in the NOD mouse and the BBDP rat? Or, to avoid the complexities of research on humans, a simpler question: is NOD a good model for studying BBDP? And vice versa? This is a simpler question and the ethical constraints of human research are not the most important reason why. More importantly, the animal models represent inbred strains where all individuals are identical genetically and universally homozygous, thus the equivalent of studying one human patient in multiple copies, a patient who is homozygous at every genetic locus.

It is generally accepted that T1D results from the autoimmune destruction of the insulin-producing pancreatic β cells. The presence of autoantibodies [5], dependence on HLA genotype [6], coexistence with other autoimmune diseases, and the scant but compelling autopsy data [7,8] all converge to support the notion that human T1D is essentially due to the same process that destroys the β cells in the animal models: a breakdown in self-tolerance involving cellular immunity, very specific to the β cell and mediated by massive infiltration of the islets of Langerhans by lymphocytes and macrophages (insulinitis). Just as in the NOD mouse and the BBDP rat, human T1D begins at a young age, is associated with anti-islet autoantibodies, and ends with the total or quasi-total destruction of the β -cell mass. Does it follow that the disease has the same etiology? The answer is not automatically yes.

Type 1 diabetes concordance in monozygotic twins approaches 50%, indicating that although it does not explain all, genetic susceptibility is important. In the two animal models, environment does alter frequency and severity of diabetes, but no amount of environmental manipulation can induce the disease in the absence of a susceptible genetic background. Because not a single environmental factor has

been definitely proven as being involved in human T1D, I confine this discussion to genetics. However, the underlying principle (human phenotypic heterogeneity) may be extended to the effects of environmental factors when we know more about them.

Genetically, the NOD mouse, BBDR rat, and human T1D all share a remarkable dependence on genotype at the major histocompatibility complex (MHC) locus as do most autoimmune diseases, because of the importance of the encoded proteins in antigen presentation and their encoding by what is by far the most polymorphic coding sequences in the human genome. The NOD mouse carries a unique haplotype that includes the *H2g⁷* allele at the *I-A* locus (histidine as residue 56 and serine as residue 57, homologous to “diabetogenic” HLA-*DQb* nonaspartic acid at position 57, which explains most of the HLA effect in human T1D) and a null allele at *I-Ea* (homologous to human *DRα*). The corresponding BBDR class II allele is *RT_{1u}*. Congenic studies in both the NOD mouse and BBDR rat clearly show that a diabetes-prone MHC is necessary but not sufficient for diabetes susceptibility which is, again, concordant with the human data: the 10% to 15% of the general population carrying the protective **0602* allele at *DQb* almost never develops diabetes. Conversely, many normal individuals carry **02* and **0302*, the most diabetogenic alleles at the same locus, without ever developing T1D because, presumably, they lack susceptibility alleles at other loci. Although the human situation is complicated by the presence of alleles conferring intermediate risk, it is clear that MHC also plays a permissive role in human T1D but it is not in itself sufficient.

Non-MHC Factors in Autoimmune Diabetes

Other loci, besides MHC, are necessary for diabetes. Of the little that is known about them, it is also clear that this is where important differences between the three models of diabetes exist. The simplest case is that of the BBDR rat, where a striking Mendelian phenotype of lymphopenia is necessary for diabetes, in addition to MHC. The lymphopenia involves *RT6⁺* regulatory T cells, which dominantly prevent diabetes when transferred from the related BBDR (diabetes-resistant) strain. Mendelian inheritance of this trait (*Lyp* locus) has been mapped to a mutation at the *Ian5* gene [9•]. Neither the NOD mouse nor humans with T1D, examined as a group, have such a lymphopenia phenotype. Another important difference between the two animal models is that thymectomy protects the BBDR rat from diabetes whereas, if anything, accelerates it in the NOD mouse. The obvious explanation is that devoid of regulatory cells in the first place, the BBDR rat has nothing to lose and everything to gain from eliminating the source of new and potentially autoreactive T cells. Is this a species difference? The high conservation of immune function and islet structure between at least rat and mouse makes this extremely unlikely. It points to the fact that there is more than one way by which a rat or a mouse (or, for that matter, a

human) can become diabetic. The expected *Ian5* knockout gene should provide valuable information toward testing this.

No equivalently striking immune dysregulation phenotype has been found in either the NOD mouse or human T1D. In the NOD mouse, where experimentation is much easier than in the human, clear abnormalities in T-cell activation [10–14], natural-killer T-cell function [15], and dendritic cell maturation [16] have been described [17]. Unlike the *Lyp* locus, linkage and congenic studies in the NOD mouse are consistent with polygenic inheritance of these abnormalities, mapped to no fewer than 20 loci. This is a situation that more closely approximates the genetics of human T1D, where linkage (sib-pair) analysis suggests at least 17 non-MHC loci (*IDDM2* to *IDDM18*). Of these, only *IDDM2* (the insulin locus) and *IDDM12* (the *CTLA-4* locus) have been multiply confirmed by the gold standard of the transmission disequilibrium test [18,19•] and functionally studied [19•,20,21]. With the possible exception of *CTLA-4* [19•], no confirmed non-MHC loci show synteny between human and mouse. Is this proof of fundamentally different mechanisms in human T1D and NOD mouse diabetes? Not necessarily.

Genetic susceptibility depends on polymorphic DNA sequences between individuals or between inbred strains. It is entirely possible that the exact same functional dysregulation contributes to both human T1D and NOD mouse diabetes, but that a different gene along the signaling chain or physiologic pathway affected is responsible for that dysregulation. This can happen simply because different genes in that system happen to be functionally polymorphic within the human population, versus among the few diabetes-resistant inbred strains against which NOD genetics have been studied.

A more meaningful answer to the question requires examination of the immune phenotype. Within the constraints of human research (essentially limited to examining peripheral blood cells), have NOD-like mouse immune defects been seen in human patients with T1D? An attempt at even summarizing the vast literature on immune abnormalities in humans with T1D is outside the scope of this short report. Much of it dates to one or two decades ago and little of it has subsequently been confirmed and followed up. Even the most convincing examples, where immune dysfunction is shown, demonstrate a large overlap between patients and normal control subjects [22–24]. This is not surprising. NOD mice show consistent differences from other strains because they all share the same genotype (as well as the same environmental determinants). Being much more genetically diverse, human patients need not have such uniformity.

Ultimately, autoimmune disease results from a disruption in the delicate equilibrium the immune system has to maintain between defending against infection and respecting self. Each side in this balance of conflicting priorities is weighed upon by several functional pathways that promote host-defense responses and others that promote self-tolerance. The end point (insulinitis and β -cell destruction) may be arrived at through any combination of disruptions in these pathways.

In the human, this is likely the result of small imbalances from the accumulation of specific combinations of functional alleles, most of which are common and, in isolation, harmless or even advantageous. It is possible that the exact same processes involved in NOD mouse diabetes are also affected by these combinations in all humans with T1D. In that case one would have predicted a much clearer immune dysregulation phenotype approximating that of the NOD mouse, even within the constraints of limiting research to blood cells. The literature to date fails to even remotely make such a case.

Toward Individualized Medicine

Although complex, the picture of human T1D painted by this analysis of the balance of evidence (such as it currently is) is not intractable. It can be systematically attacked if results from patients are examined individually and not as a group in a two-pronged approach.

First, if different immune disturbances account for T1D in different patients, comparing the mean value of any end variable to that of normal control subjects is meaningless. Assuming that a reasonably good proportion of patients have any given disturbance, a more meaningful approach would be to examine the end points looking for a bimodal distribution generated by a small group of outliers that share the same disruption. This will require examining hundreds rather the customary dozens of patients, but T1D is a reasonably common disease, and blood is easy to ethically obtain and is the source of cells where most immune functions can be studied. By this approach, I would be willing to wager that a small percentage of humans with T1D will be found to have the exact same immune pathology as the NOD mouse. A few may even have lymphopenia similar to that of the BBDP rat.

Second, with technologic developments that have increased genotyping throughput exponentially, and the concerted effort of the Type 1 Diabetes Genetics Consortium (<http://www.t1dgc.org/>) to collect DNA from thousands of T1D sibling pairs, the complex non-HLA genetics of T1D do not seem as intractable as in the past. I predict that when the most important T1D loci have been identified and interactions between them defined, several different diabetogenic allele combinations at the non-HLA loci will be defined. This would allow classification of individuals with T1D according to genotypes in a panel of polymorphisms, and these genetic “signatures” can be related back to the functional “signatures” proposed in the previous paragraph.

Such convergence of the two prongs will be the first step toward individualizing the evaluation of preventive strategies, the effectiveness of each of which may differentially depend on the individual’s immune disturbance. Then, and only then, we will know how many human patients with T1D get diabetes for the same reason as the NOD mouse or the BBDP rat and, for those who do not, which of the lessons learned

from the animal models still apply and which may not. With the knowledge of relevant human genetic variations, new human-made models may then be created as needed by “knocking-in” variants with a functional effect mimicking those seen with the human polymorphisms.

Conclusions

The availability of animal models of spontaneous autoimmune diabetes has allowed extensive and in-depth examination of autoimmune phenomena that lead to T1D. For all the breadth and the depth of this research, the genetic uniformity of inbred strains confines the information thus gained to the equivalent of two case studies. In the clinical literature, reports of a successful treatment on only two patients, no matter how well documented biologically and statistically, would carry little credibility. I propose a thorough examination of human subphenotypes of immune disturbance that will examine end-point variables as distributions of individual values rather than means, as a first step toward individualized risk assessment and, eventually, prevention.

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