EDITORIAL

Attacking the source: anti-PDX-1 responses in type 1 diabetes

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Type 1 diabetes (T1D) develops as a consequence of abnormal responses against several self-antigens, eventually leading to the autoimmune attack and destruction of the insulin-producing β cells in the pancreas. In this issue of *Laboratory Investigation*, Li *et al* propose the transcription factor Pancreatic and duodenal homeobox 1 (PDX-1) as a T1D autoantigen by demonstrating autoreactivity to this pancreas-specific protein in both the NOD mouse model and patients with T1D. Because of the known roles of PDX-1 in pancreatic development as well as β cell maintenance and function, targeting of PDX-1 expressing cells may result in the elimination of not only β cells but also the progenitor cells required for regeneration of insulin-producing cells.

Laboratory Investigation (2010) 90, 6–8; doi:10.1038/labinvest.2009.121

Type 1 diabetes (T1D) is a devastatingly difficult disease, and proper therapy requires frequent blood glucose monitoring, a daily multi-injection insulin regimen, and a high level of patient motivation to achieve tight glycemic control. This disease results from responses of an unbalanced immune system against proteins expressed in the insulin secreting pancreatic β cells, leading ultimately to the destruction of these cells, a dearth of insulin secretion, and hyperglycemia. The anti-self or autoimmune responses are against several antigens, including those that are restricted to the β cell as well as some extant in a wide variety of cell types.

The response against the autoantigens in T1D is associated with both aberrant cellular as well as humoral responses. Autoantibodies have been used to predict those at risk for T1D development,^{1–4} yet antibodies are not thought to contribute in the pathology of the disease. Serum positivity for a specific antibody is a marker for T-cell activation to the particular antigen. T-cell responses are seen as predictive of eminent risk as these cells are believed to be the final effectors.⁵

Significant similarities are measured in the antigenic responses comparing patient samples to the NOD mouse. The NOD mouse model is the most well-characterized animal in the study of autoimmune diabetes. Although the majority of

antigens in diabetes were initially identified from patient samples, recent utilization of the NOD model system has led to the discovery of two antigens, glucose-6-phosphatase, catalytic, 2 (G6PC2) and Dystrophin myotonia-protein kinase (DMPK).^{6,7} Studies have since demonstrated that the islet-restricted antigen G6PC2 is an antigen in T1D patients.^{8,9} In this issue of *Laboratory Investigation*, Li *et al*¹⁰ exploit the NOD mouse system to identify Pancreatic and duodenal homeobox 1 (PDX-1) as an autoantigen and extend the findings to show that sera from patients with T1D have circulating autoantibodies to this antigen as well.

Recently, a multidimensional analysis of microarray data for both the abundance and specificity of transcripts to human pancreas ranked the top 'candidate autoantigens' for T1D diabetes.¹¹ Out of the top 68 hits, 14 of the gene products have been reported earlier as autoantigens in T1D. Of interest to this work was the listing of PDX-1 near the top of this list. Li *et al*¹⁰ report that PDX-1 autoantibodies (PAA) were present in roughly half of the NOD females tested. When tested by western blot, sera from both NOD and T1D patients recognize an epitope in the C-terminal portion of PDX-1, whereas human sera recognizes another epitope within amino acids 160–199. Similar to the appearance of insulin autoantibodies (IAA) in the NOD mouse,

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the timing of the humoral response to PDX-1 varied between NOD mice, yet with the small number of animals used in this study it is difficult to determine kinetics of PAA appearance over the spectrum of NOD mice. T cells from NOD mice also responded to the C-terminal epitope, demonstrating the coordinated response between the T and B cells. If these data are corroborated, it would place PAA as relatively equal to IAA for prediction of diabetes in the NOD mouse. Expectantly, forthcoming reports will provide data on kinetics of the anti-PDX-1 antibodies in both female and male mice, as in an earlier study using IAA as a diagnostic marker,¹² as well as include MHC matched controls, such as the NOR mouse strain, for both antibody and T-cell assays. With the tests for recently identified T1D autoantigens rapidly incorporated into clinical laboratories, the validation of PDX-1 should be swift.

During pancreatic development, transcription factors regulate endocrine vs exocrine cell fate. Most of these factors are members of the homeodomain-containing protein (HD) family, such as PDX-1. PDX-1 expression is first detected on embryonic day (E) 8.5 in the part of the gut epithelium destined to develop into the pancreas, and is then highly expressed in the adult β cells.¹³ The role of PDX-1 in development was highlighted in studies showing that targeted mutations of this gene result in agenesis of the pancreas.^{14,15} In addition, a single nucleotide deletion in *Pdx-1* also causes pancreatic agenesis in man.¹⁶ Conditional β cell-specific inactivation of *Pdx-1* during development not only causes early onset diabetes, but also abnormal numbers of other islet cell types.¹⁷ Adult mice with a disruption of the gene in the differentiated β cells failed to maintain glucose homeostasis;¹⁸ a deficiency that correlated with decreased glucose transporter 2 (Glut2) and insulin expression.¹⁸ Thus, in addition to its requirement for pancreatic development, PDX-1 expression is indispensable for retaining the insulin-producing β cell phenotype.

PDX-1 in the maintenance of β cell mass has not been directly demonstrated; nonetheless, a role of PDX-1 has been established for the compensatory expansion of β cell mass in metabolic syndrome.¹⁹ PDX-1 is likely essential for the augmentation of β cell mass after injury resulting from partial pancreatectomy and after injection of streptozotocin in mice, rats, and monkeys.^{20–25} Although recuperation from chemically and surgically mediated β cell damage

requires PDX-1, recovery is not identical for all forms of damage. Mice encoding an FK506-inducible transgene for active caspase 8, the 'PANIC-ATTAC' model, recover from β cell apoptosis and the resulting diabetes using β cell progenitors that were not PDX-1 positive.²⁶ Therefore, for injury models, the cellular source as well as the progenitor cells required for regeneration or recovery remain controversial.^{22,23,27}

The role of PDX-1 in β cell development and recovery of these insulin-producing cells from injury has major ramifications in strategies to cure T1D. What little we know about this process has been gathered from the NOD model. In pre-diabetic NOD mice, inflammation in the pancreatic islets (insulinitis) is associated with β cell proliferation.^{28,29} Indeed, adoptive transfer of splenocytes into immunodeficient NOD-*Scid* mice results in recapitulation of the proliferative process present in NOD mice progressing to spontaneous T1D. Clearly, the replicative capacity of the islet cannot keep pace with the autoimmune destruction and eventually the β cell mass drops below the threshold to maintain euglycemia. A failure to significantly expand the β cell mass after immunotherapy with anti-CD3,^{28,30} suggests either that after T1D onset β cells are significantly dysfunctional through the suppression of PDX-1 expression by hyperglycemia,³¹ the balance of proliferation vs apoptosis is reduced,²⁸ or that the progenitor cells required for the regeneration of the islet β cells are missing. The data presented by Li *et al*¹⁰ may suggest that by targeting PDX-1 the immune system is not only seeking to destroy all β cells but also the potential to create insulin-producing cells. Such a vendetta against the β cells as well as their progenitors should be seriously considered in the development of a strategy for the prevention or reversal of T1D in man.

ACKNOWLEDGEMENT

This work was supported by the Juvenile Diabetes Research Foundation, the National Institutes of Health grant R01 DK74656, and the Sebastian Family Endowment for Diabetes Research.

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