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The Type 1 Diabetes Signature: Hardwired to Trigger Inflammation?

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According to a Vietnamese proverb, brothers and sisters are as close as hands and feet. This proverb is meant to describe siblings' shared origin and resemblance yet also hints at their ability to act independently. Applied to type 1 diabetic (T1D) families, it appears that while an overactive immune system may be a common trait, each family member's body tends to have a particular way of dealing with that imbalance. In this issue of *Diabetes*, Chen et al. (1) reveal that healthy T1D family members share a proinflammatory gene expression signature with their diabetic probands, regardless of HLA-associated risk or autoantibody status. Whether the observed inflammatory state progresses to overt disease, and at what pace, seems to depend on an individual's ability to counteract inflammation.

T1D is a polygenic autoimmune disease that is characterized by innate and adaptive immunity against β-cell components (2). Among the array of susceptibility genes, HLA-associated risk represents the lion's share. It is widely acknowledged that environmental challenges are involved in genetically at-risk subjects' progression toward autoantibody development and clinical onset (3). Monozygotic twins, for instance, show a significant degree of concordance in islet autoimmunity despite great temporal variation, suggesting a putative external trigger (4). At present, no therapy exists that can halt the immune-mediated destruction of β-cells. Some highly anticipated late-stage trials in recently diagnosed patients had disappointing results, most notably with anti-CD3 therapy (5). One reason may be that around the time of diagnosis—when most functional β-cell mass is destroyed—the autoimmune process is very difficult to curb. While preservation of endogenous C-peptide in this population is an end point endorsed by the U.S. Food and Drug Administration and associated with better glycemic control and long-term prognosis (6,7), it stands to reason that prevention of hyperglycemia would be a more desirable objective. Several secondary prevention trials in at-risk subjects are currently under way to test some of the immune modulators that earlier showed unsatisfactory efficacy after diagnosis. A second important conclusion from past clinical trials is that there is substantial disease heterogeneity. Diagnosed patients lose C-peptide at varying rates (8), and despite significant advances in risk stratification among healthy subjects (9), accurate disease prediction remains a challenge. Part of the problem is that we have an incomplete understanding of the underlying immune processes that drive disease prior to and after diagnosis, as well as how these pathways contribute to variability in a genetically diverse population. The new study by Chen et al. now sheds some light on these issues by comparing transcriptional signatures among relatives of T1D patients.

In order to improve on sensitivity, the authors used an indirect approach that assessed transcriptional expression profiles after incubating plasma samples with a "reporter" peripheral blood mononuclear cell population. This methodology inherently restricts the analysis to the secreted, soluble compartment of potential disease-associated factors, thereby leaving differentially expressed intracellular and cell-associated molecules out of the equation. In addition, the responder population is derived from healthy donors, and it therefore is likely that leukocytes derived from T1D patients would carry inherent defects that would lead them to respond differently. Four different cohorts were studied: T1D patients, healthy siblings either with high or low HLA-associated risk, and unrelated healthy control subjects. The expected outcome would be that T1D patients, and possibly their high-risk siblings, would exhibit a proinflammatory signature compared with unrelated control subjects (10). Indeed, the usual suspects—including cytokines, chemokines, and immune

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receptors—were found to be upregulated, even in the low-risk cohort. Remarkably, the low-risk expression profile showed more pronounced inflammation-related transcription compared with the high-risk group. In contrast, the high-risk group showed the most robust expression of factors associated with immune regulation. These results suggest that the immune system of high-risk subjects somehow senses impending autoreactivity and actively attempts to raise a counteracting regulatory response. Longitudinal pattern analysis was performed among T1D progressors and healthy high- or low-risk subjects. The temporal fluctuations in inflammatory genes were inversely correlated with the regulatory expression levels. In turn, this corresponded with the frequency of conventional regulatory T cells as measured by flow cytometry. The only exceptions were the low-risk subjects, in whom no such correlation was observed. In other words, high-risk subjects seem to regulate the "hardwired" proinflammatory response in an age-dependent manner, perhaps explaining why T1D susceptibility declines with age (Fig. 1).

The work by Chen et al. does not offer new insight into which of the differentially regulated molecules could serve

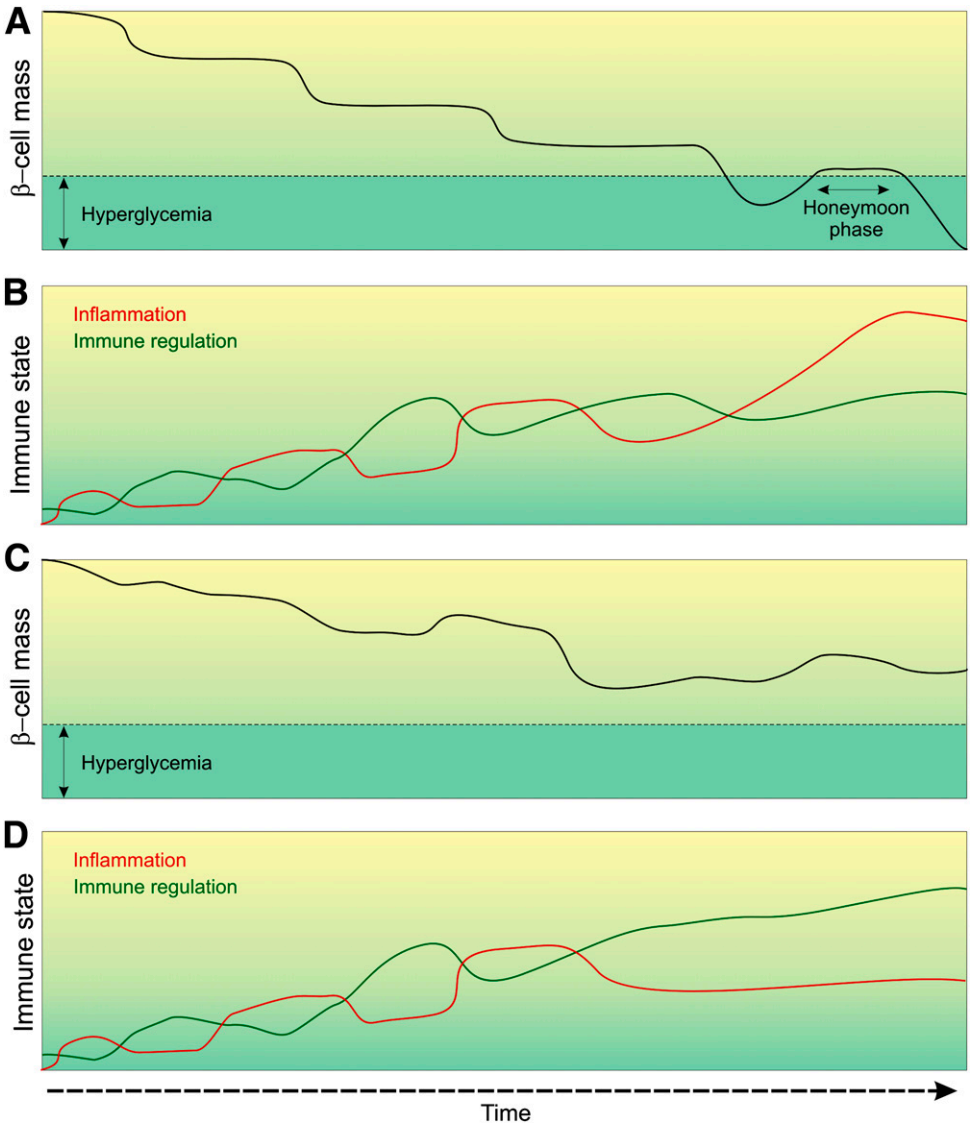

Figure 1—The traditional view of β-cell mass declining in a linear fashion prior to diagnosis, as originally proposed by Eisenbarth (13), may need to be revised to a model with flares and remissions (*A*). Chen et al. (1) elucidate some of the immune profiles that underlie the stepwise loss of β-cell mass prior to diagnosis and may aid in the future prediction of time-dependent risk. Most siblings of diabetic probands will establish a state of low-grade autoinflammation. Whether individuals with a given genetic risk profile will progress to clinical disease, and at what pace, depends on their ability to counterregulate flares of autoimmunity after chance encounters with environmental stimuli. In many high-risk patients, the autoimmune component eventually prevails and β-cell mass drops below the critical threshold (*B*). Nonprogressors, however, gradually generate a regulatory immune compartment that permanently outweighs autoimmunity (*D*). As a consequence, there is only a limited decrease in β-cell mass, allowing for adequate glucose control throughout life (*C*). Successful future prevention trials will depend on the accurate prediction of progressors and could reinforce this default regulatory response.

as pharmacological targets to quell the common inflammatory state prior to diagnosis. Other studies have previously suggested that the interferon or interleukin-1 signaling pathways may be critical disease drivers (11), yet the question remains how to safely intervene, especially in a prevention setting. Moreover, in recently diagnosed patients, interleukin-1 blockade showed no effect, indicating that upregulation does not guarantee success upon blockade (12). The notions that so many important immune genes are differentially regulated and, even more importantly, that they display considerable temporal fluctuation are sobering thoughts for whoever believes that nonbiased transcriptional analysis will one day reveal the magic bullet to cure diabetes. The value of the new study and its methodology, however, lies in its potential to refine risk stratification criteria prior to diagnosis. As the authors acknowledge, measurement of a single or a few cytokines may be uninformative and will certainly fail to detect the rather subtle changes that were identified in the current study. It could be envisioned that future work could identify a discrete set of genes that allows for more granular risk prediction. This would constitute a significant advance because it would facilitate the design and implementation of future primary and secondary prevention trials. Finally, it may be that a defined gene expression array correlates with the efficacy of certain immunotherapies and could ultimately serve as a future surrogate end point in prevention trials, or perhaps in addition to C-peptide in recent-onset trials.

Chen et al. (1) elegantly demonstrate that the immune systems of high-risk people have the inherent ability to counterregulate the diabetogenic autoimmune response. The ambition of future preventive immunotherapies therefore should be to more accurately identify those prone to getting diabetes and lend this endogenous regulatory response a helping hand.

Duality of Interest. K.T.C. and M.G.v.H. are both employed by Novo Nordisk. No other potential conflicts of interest relevant to this article were reported.

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