The Next Big Idea

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Abstract

George S. Eisenbarth will remain in our memories as a brilliant scientist and great collaborator. His quest to discover the cause and prevention of type 1 (autoimmune) diabetes started from building predictive models based on immunogenetic markers. Despite his tremendous contributions to our understanding of the natural history of pre-type 1 diabetes and potential mechanisms, George left us with several big questions to answer before his quest is completed.

Overture

SO, WHAT IS THE NEXT BIG IDEA we should be working on?" was usually the second sentence George would say during our annual faculty evaluation meetings, right after a brief compliment on a great job one had done the previous year. This was his way to move quickly to where he felt comfortable and away from formal evaluation that he disregarded as a potentially antagonizing bureaucratic ritual. He would ask this question on other occasions, especially in late afternoons, seemingly looking for a reward after a hard day's work.

The first time I heard him say that was during our first meeting at the International Diabetes Federation Congress in Sydney, Australia, in 1988. At a press conference, he was interviewed about the early version of his famed model predicting the risk of type 1 diabetes (T1D) using age, insulin autoantibody levels, and insulin response to intravenous glucose.¹ Based on his previous work on immunogenetics of T1D,² George painted a strong picture of T1D being a *genetically determined* disease with a predictable preclinical course.

A few minutes later, I answered questions on behalf of the Diabetes Epidemiology Research International Group, reporting, for the first time, that the incidence of T1D had been rising by 3–5% a year, since the mid-1960s, in most of the 21 ethnic groups from 10 countries that we studied.³ This temporal trend suggested that T1D was an *environmentally determined* disease rising across genetically diverse populations.³ This apparent paradox led to a fascinating conversation (at least from my postdoc point of view), and we agreed that solving the gene–environment puzzle of T1D was a big idea worth pursuing.

We have not talked again until an unlikely Denver, CO, meeting, in September 1991, arranged by Dick Hamman, Chair of Preventive Medicine and Biometrics and my mentor. Dick was part of the search committee for a new executive director of the Barbara Davis Center for Childhood Diabetes

(BDC). Going out of town, he offered me (a fledgling assistant professor) the opportunity to meet with one of the candidates—the famous George Eisenbarth. I doubt that anybody ever read my evaluation of George following that meeting, but this gave me the bragging rights for “hiring” George, rights that truly belong to Dick Krugman (Dean of University of Colorado School of Medicine) and Doug Jones (Chair of Pediatrics).

After a few awkward minutes, George asked me, “So, what is the next big idea we should be working on?” There was a lot to talk about. Four months earlier, Dick Hamman asked me to help renew his National Institutes of Health (NIH) RO1 grant “Colorado IDDM Registry.” In the application submitted in June 1991, I wrote a specific aim proposing a cohort study of genetically high-risk children followed to development of islet cell autoantibodies (ICA) and T1D called the Diabetes Auto-Immunity Study in the Young (DAISY). We proposed to search *prospectively* for potential environmental triggers of T1D in infant first-degree relatives of T1D patients and in genetically high-risk children with no family history of T1D. This was a novel idea that occurred to me during a long flight home from a “brain storming” meeting organized by Hans Akerblom in Helsinki, Finland, in December 1990. At that time, the idea seemed crazy or at least impractical given the low risk of T1D in the general population and low predictive value of human leukocyte antigen (HLA) genotypes. It was incredibly reassuring to hear from George that this could be the next “big idea.” He recommended contacting Henry Erlich, Director of Human Genetics at Roche Molecular, Inc. (Alameda, CA), to find out if a large-scale newborn screening for high-risk HLA genotypes would be feasible.

The NIH RO1 payline stood then, as today, at 7%. The study section liked the idea but was concerned whether we could define the prediabetes phenotype using ICA alone. George’s laboratory, one of the two or three leaders in the field at that time, would solve these concerns immediately. We were elated when he accepted the University of Colorado’s

offer to become BDC Executive Director, in April 1992. In September, he moved his laboratory to Denver and wrote in his support letter:

I will be able to strengthen the ability of the Center to participate in and contribute to the studies Dr. Rewers has outlined. In particular, my laboratory shares an intense interest in the immunologic events leading to the activation of autoimmunity and have developed a series of new assays including the discovery of several new autoantigens which could be applied to samples from this surveillance effort. [...] these studies I believe are essential to the unraveling of the natural history of type I diabetes.

A few months later, DAISY (my first RO1) was funded.^{4,5} Although George and I remained in different departments until late 2000, our collaboration grew stronger every year, leading eventually to 83 joint peer-reviewed articles, including 70 based on the DAISY cohort.

Act 1: DAISY

Immunogenetics

Over the next 20 years, DAISY made major contributions to our understanding of the etiology of T1D. DAISY was first to study the natural history of islet autoantibodies and the risk factors for progression to diabetes in children *without* a family history of T1D where more than 90% of T1D cases occur.⁶ We discovered that among children with identical *HLA-DR,DQ* genotypes, the incidence of islet autoimmunity was dramatically higher in children with, compared with those without, a first-degree relative with diabetes,⁷ pointing to the importance of environmental factors and/or non-HLA Class II genes. DAISY reported the first-ever population-based estimates of the incidence of islet autoimmunity in general population children.⁸ Although islet autoantibodies were found in 3.7% of cord blood samples, they appeared to be maternal of origin and were not predictive of subsequent development of islet autoimmunity.⁹ DAISY demonstrated that over 70% of children expressing multiple islet autoantibodies progress to diabetes in 10 years compared with 15% of those with one autoantibody. Once islet autoimmunity spreads to more than one autoantigen, the progression to T1D is only a matter of time; the rate of progression is linear and hardly influenced by the *HLA-DR,DQ* genotype or family history of T1D. The age of appearance of first autoantibody and the levels of insulin autoantibodies (but not glutamic acid decarboxylase 65 or islet antigen 2) are major determinants of the age of onset of diabetes.¹⁰

DAISY newborn screening of the multiethnic Denver population and comparisons with the local T1D patient population led to novel observations: *DR4,DQB1*0302* haplotypes are more frequent in Hispanics and confer lower T1D risk in this ethnic group than in non-Hispanic whites, whereas *DR3,DQB1*0201* haplotypes are much less frequent but carry similar risk to that in non-Hispanic whites.¹¹ *DRB1*04* subtypes confer additional risk¹² in both groups. In collaboration with Janelle Noble and Henry Erlich, we found *HLA-DPB1*0402* to be protective, even in subjects with the high-risk *DR3/4,DQB1*0302* genotype.¹³ The *HLA-A*0101/0201* genotype increases the risk,¹⁴ whereas the *HLA-B39* allele determines the risk on *HLA-DR8* haplotypes. These discov-

eries have been translated into improved newborn screening protocols.^{15,16}

Many of the DAISY contributions to the genetics of T1D have been led by George and his outstanding Fellows. Akane Ide, Terry Aly, Erin Baschal, and Mohamed Jahromi have explored in the DAISY population additional genetic determinants within the major histocompatibility complex (MHC) region. They found the extreme difference in the risk of islet autoantibodies between *DR3/4,DQB1*0302* siblings sharing both HLA haplotypes by descent with the T1D proband, versus those sharing one or no haplotypes (85% vs. 15% by 12 years of age).¹⁷ The *insulin (INS)*, *tyrosine-protein phosphatase non-receptor type 22 (PTPN22)*, *MHC class I chain-related A (MIC-A)*,¹⁸ *HLA-DRB1*04*, or *HLA-DP* genotypes did not explain this remarkable risk difference. The conserved haplotype *DQ2,DR3,MIC-A5.1,HLA-B8,HLA-Cw7,HLA-A1,D6S2223-177*¹⁹ was the predominant *DR3* haplotype in islet autoimmunity, but it was not differentially transmitted from parents to affected children.^{19–22} An additional major genetic locus determining T1D was identified in linkage with but distinct from high-risk *DR,DQ* alleles.^{17,23–25}

Nearly 50 non-HLA gene variants have been associated with T1D in genome-wide association studies, however, jointly explaining a small part of the familial aggregation not explained by MHC.²⁶ Andrea Steck, George, and I began a systematic evaluation of these markers as predictors of islet autoimmunity and T1D in the DAISY cohort. The *PTPN22 C1858T* polymorphisms further accelerated development of islet autoimmunity and T1D, independently of the *HLA-DR,DQ*,^{27,28} whereas the *CCR5 Δ32* allele was protective. The significant, but relatively minor, role of the *cytotoxic T-lymphocyte A (CTLA)-4-318* and *interferon induced with helicase C domain 1* polymorphism was confirmed, and we have reported novel associations between polymorphisms in interleukin-4R, interleukin-4, and interleukin-13 and islet autoimmunity.²⁹ We discovered that the high-risk phenotype of persistently high level of insulin autoantibodies is strongly ($P < 0.0001$) associated with the *INS-23HphI A/A* genotype conferring higher risk of T1D.¹⁰

Dietary factors

In articles led by Jill Norris, DAISY refuted earlier, highly publicized hypotheses linking T1D to shorter breast-feeding and cow's milk formulas.^{30,31} On the other hand, we discovered that higher ω -3 fatty acid intake and higher ω -3 fatty acid red blood cell membrane levels were associated with lower risk of islet autoimmunity in children at increased genetic risk for T1D.³² However, neither ω -3 fatty acid intake nor levels were associated with progression to T1D in children with islet autoimmunity, suggesting that the protective role of fatty acids may be early in the process.³³ It is interesting that plasma 25-hydroxy-vitamin D levels were not associated with the risk of islet autoimmunity or progression to T1D, nor were there significant interactions between vitamin D levels and polymorphisms in the *vitamin D receptor*, *INS*, *PTPN22*, or *CTLA-4* gene on islet autoimmunity or T1D risk.³⁴ One of the most intriguing findings was increased risk of islet autoimmunity with exposure to cereals (both gluten-free and gluten-containing) before 4 or after 6 months, compared with those exposed in the 4–6-month "window."³¹ The specific causative component of cereals is still unknown. Exposure to

gluten-containing cereals outside this “window” increased the development of celiac disease³⁵ and allergy.³⁶

Infectious agents

With Lars Stene and Janet Snell-Bergeon, we found that children whose mothers reported at least one infection during pregnancy had a significantly *lower* risk of islet autoimmunity compared with other children.³⁷ Older maternal age and complicated delivery predicted islet autoimmunity,³⁸ but cesarean section or neonatal infections did not. More frequent respiratory infections during infancy (but not early daycare attendance or household crowding) reduced the risk of islet autoimmunity.³⁹ Thus, DAISY results concerning pre- and postnatal infections appear to be consistent with the “hygiene hypothesis” linking decreasing number of early childhood infections with autoimmunity.^{40,41} Previously, we found with Patricia Graves that enteroviral infections detected by polymerase chain reaction testing of serum, saliva, and rectal swab samples did not predict islet autoimmunity in high-risk children.⁴² A systematic search for nucleic acids of multiple pathogens in plasma, saliva, throat, and rectal swab samples collected before and after development of islet autoimmunity in 58 DAISY cases and in 107 matched controls detected multiple infections but no associations with T1D (G. Palacios and I. Lipkin, unpublished data, 2010). We were also unable to confirm an association between rotaviral infections and T1D. However, our follow-up study in collaboration with Lars Stene and Heikki Hyöty demonstrated that enteroviral infections may increase the risk of progression from islet autoimmunity to T1D.⁴³ Preliminary virus sequencing showed no evidence for persistence of a specific virus in children progressing to T1D; rather, recurrent infections with unrelated strains may be the final β -cell insult leading to hyperglycemia, in some cases.

Translation to clinical practice

DAISY findings had an immediate and reassuring impact on public perception of potential causes of T1D. It provided evidence that routine immunizations and their timing⁴² as well as breast-feeding duration and age at cow’s milk exposure did not lead to development of islet autoantibodies,^{30,31} confirmed later by prospective studies in Germany⁴⁴ and Finland.⁴⁵ Normal but increasing hemoglobin A1c levels for up to 2 years prior to diagnosis foreshadows progression to T1D⁴⁶—an important observation for potential change in diabetes diagnostic criteria. Children followed by DAISY to diabetes have avoided diabetic ketoacidosis and hospitalization at diagnosis and had higher C-peptide levels and lower blood glucose levels, hemoglobin A1c levels, and insulin doses during the initial year post-diagnosis than community controls.⁴⁷ This indicates preservation of endogenous insulin that may lower the risk of microvascular complications and severe hypoglycemia.⁴⁸

George’s laboratory, in collaboration with DAISY has participated in development and validation of multiple serologic assays for detection of islet autoimmunity,^{49–59} celiac disease,^{60–65} and other autoimmune disorders.^{54,66–72} DAISY has contributed significantly to the “discovery” of celiac disease as a common disease in the U.S. general population^{65,73–76} and special groups.^{77–79} We have extensively studied the relationship between islet autoimmunity and celiac autoim-

munity^{77–81} and begun to disentangle the common mechanisms of islet autoimmunity and celiac, thyroid, adrenal, parietal cell, and rheumatoid autoimmunities.^{68,82,83}

Intermezzo: People, Space, and Money

Looking back, the fascinating research discussions and findings that I have shared with George can be nicely arranged into a string of ideas, grants, and articles. Intermixed with those, there was the other business—running the BDC. I started seeing children with diabetes at the BDC shortly after George’s arrival; over time, he invited me to direct the Center’s Translational Research Unit and, eventually, the Clinical Division—approximately 40 employees. I joined him and John Hutton (Research Division Director) in December of 2000 and immediately faced a triple challenge: (1) to build up the clinical team in order to meet an exponential growth of our patient population, (2) to expand clinical/translational research, and (3) to find enough space to accommodate both. During the following 8 years later, we quadrupled our staff, funding, and space. The Center is now seeing 6,000 children and adults with T1D—among the largest populations anywhere in the world. The talent (Georgeanna Klingensmith, Satish Garg, and Rob Slover, to mention just a few on the clinical side), hard work, and good luck were on our side.

George was a visionary, brilliant scientist, wonderful boss and mentor. The culture he created at the BDC was positive, predictable without a lot of overt conflict. When he took over, the BDC was already famous and successful because of the accomplishments of Peter Chase, Kevin Lafferty, and others, but small by the current standards. His high personal scientific standing and the generous support of the Children’s Diabetes Foundation have been the major drivers of the Center’s exponential growth. George has helped to recruit and retain the best talent by focusing on the big picture, taking risks and responding to opportunities to benefit the BDC. One of them was his bold decision to move to Fitzsimons (now called Anschutz Medical Center) as one of the first units of the Medical School. This paid off well, with a beautiful four-story 110,000 square feet building. We also had a good luck to operate in uniquely favorable research funding environment created by the NIH Special Statutory Funds for Type 1 Diabetes Research (nationally over \$2 billion since, 1998) and the Juvenile Diabetes Research Foundation. The BDC has benefited enormously from their support. More about this fascinating part of our lives can be found in a book recently written by H. Peter Chase and Sue Palandri.⁸⁴

Act 2: TEDDY

The success of DAISY and similar studies has not been lost on the research community. Through a series of international workshops supported by the American Diabetes Association, the Juvenile Diabetes Research Foundation, and the NIH, we were able to convince the NIH to combine our efforts with those of our colleagues in Finland, Germany, Sweden, Seattle, WA, and Augusta, GA/Gainesville, FL.

The Environmental Determinants of Diabetes in the Young (TEDDY) study was initiated in 2002 to identify infectious agents, dietary factors, or other environmental exposures that may trigger or protect against the development of islet autoimmunity and T1D.^{15,85} DAISY had piloted the key components of the protocol for TEDDY. By July 2010, in total, 424,788

newborns have been screened by *HLA-DR,DQ* genotyping to identify children at increased risk for T1D, and 8,677 have been enrolled to be followed four times a year until 4 years of age and twice a year thereafter until 15 years of age.⁸⁶ A successful study outcome should allow better understanding of the etiology and pathogenesis of islet autoimmunity and T1D and the development of new strategies to prevent, delay, or reverse the disease.

George, Liping Yu, and I jump-started the international effort to “harmonize” islet autoimmunity assays⁸⁷ for TEDDY and other consortia. Although his eyes were already set at new ideas and experiments,^{88,89} George remained supportive and active on the TEDDY Immune Markers Committee.

Finale: Primary Prevention of T1D in the General Population

One day, and it is hoped soon, TEDDY will tell us how to modify our children’s environment to stop islet autoimmunity before it even develops. In the meantime, we are getting very close to the point where millions of children could be screened for islet autoantibodies two or three times during the initial 12 years of life and those found positive could be enrolled in prevention trials. George’s work has paved the path to robust, inexpensive autoantibody assays and predictive models. This was one of the big ideas that I promised him to continue work on. Unfortunately, he was not given sufficient time to develop durable intervention that would prevent progression from islet autoimmunity to diabetes, but his belief that insulin is the key autoantigen is being addressed by quite a few groups, and new “big ideas” will undoubtedly follow.

Author Disclosure Statement

No competing financial interests exist.

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