

George S. Eisenbarth: Insulin and Type 1 Diabetes

George S. Eisenbarth, MD, PhD, could be considered a natural conduit in the research of type 1 diabetes (T1D) and the fields of endocrinology and immunology. His recognition of T1D as a chronic autoimmune disease has advanced the field toward prediction, prevention, and clinical trials. His focus on insulin as the key autoantigen in islet autoimmunity unraveled the molecular basis of the insulin autoimmune response, leading to experimental antigen-specific therapies that are presently approaching clinical translation and represent a model approach for the treatment of human T1D and other autoimmune diseases.

Biography—George Eisenbarth was born in 1947, in Brooklyn, New York, to a working-class family of German ancestry. He graduated from Grover Cleveland High School in 1965 and won a Pulitzer Scholarship to Columbia University in New York City. His father feared the scholarship was some sort of scam that would not fully cover tuition, and he had to be convinced to let Eisenbarth enter the program. Eisenbarth obtained a BA degree from Columbia in 1969, followed by his PhD (Physiology-Pharmacology, 1974) and MD (1975) degrees from Duke University Medical School. As a PhD-MD student, Eisenbarth worked under the mentorship of Dr. Harold Lebovitz. According to Dr. Lebovitz, once Eisenbarth joined his laboratory, everyone else started working for him! Eisenbarth did not become a leader over time, he always was one.

Dr. Eisenbarth married his wife Frieda, and they had two children, Stephanie and Stephen, who are both successful in their careers. Nothing gave him more pride than his daughter becoming an immunologist and a productive researcher at Yale University. He was a loving husband, caring father, and proud grandfather. His treasures were family, friends, and his work. Always kind and polite, he was loved and respected by all who knew him and by the many he touched.

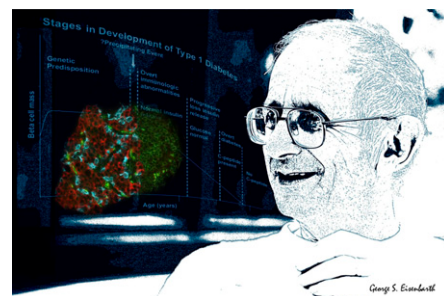
Gifted with an inquisitive and curious mind, he was an avid reader of science,

literature, history, and geology. Never one to waste any time, Dr. Eisenbarth was typically reading a book or an article while walking around, typically with a pen in his mouth to underline important sentences, and listened to audio books in his commute to work. He loved nature and the outdoors—the mountains, skiing, riding horses, and country western dancing. If not busy reading, he would be fixing something around the house. He just never idled.

Dr. Eisenbarth always looked forward, never resting on glory, and was excited about what the future may bring. His natural curiosity was matched with a strong vision for positive change. Despite being a leader to the field, he remained the humblest of human beings. He never wanted to impose anything on anyone, always providing a logical argument to explain a decision. If he had to impose a view, he would reluctantly signify that intention by using the expression “I strongly recommend....” And, as ultimate proof of true intelligence and integrity, he was fully capable of admitting when he was wrong.

He received many awards, including the American Diabetes Association’s Outstanding Scientific Achievement Award (1986), a National Institutes of Health (NIH) Merit Award (1992), the JDRF David Rumbough Scientific Award (1997), the Naomi Berrie Award from Columbia University for Outstanding Achievement in Diabetes Research (2003), and the American Diabetes Association’s Banting Medal for Scientific Achievement (2009). He held faculty positions at Duke University (1979–1982) and the Joslin Diabetes Center (1982–1992), where he founded the Immunology Section, and he was the Executive Director of the Barbara Davis Center for Childhood Diabetes at the University of Colorado (1992–2012).

In 2010, Dr. Eisenbarth was diagnosed with pancreatic cancer. He underwent a total pancreatectomy, which caused him to become insulin-dependent and directly experience the clinical condition he had dedicated his life to eradicate. He fought his battle with cancer with extreme dignity and remained focused on



An evocative rendering of the late George S. Eisenbarth. A pancreatic islet under immune attack against the backdrop of his model of the multiple stages in the natural history of T1D symbolizes the disease process he wanted to defeat.

his work until the very end. In the last written exchange he and Dr. Pugliese had just days before his passing, he expressed gratitude for a plentiful and happy life, his only regrets being not having more time to spend with the grandchildren and not having seen the prevention of T1D achieved. Yet, always the optimist, he believed the prevention of T1D was a goal the next generation of scientists was poised to achieve. To our deepest regret, he died in November 2012, aged only 65 years.

Creating the tools to study

T1D—Early in his career, Dr. Eisenbarth realized that for autoimmune endocrine diseases, and in particular for T1D, prevention and cure would be possible but required identification of key pathogenic mechanisms, knowledge of target autoantigens, and predictive markers. He also recognized that achieving such goals involved the creation of adequate tools such as monoclonal antibodies, cell lines, and biochemical assays. Between 1977 and 1979, Dr. Eisenbarth sought to learn the required skills to achieve these goals by pursuing training at NIH in the laboratory of biochemical genetics led by Dr. Marshall Nirenberg. Also, working with Dr. Anthony Fauci, Dr. Eisenbarth was among the first to produce monoclonal antibodies to neuronal pancreatic islets,

thymic epithelia, and T-cell molecules and apply them in biochemical and clinical research (1–5). He developed biochemical assays (6,7), an activity which continued throughout his career. He outlined his vision and strategies in his first NIH grant application entitled “Characterization of islet cell surface molecules,” submitted from Duke University in 1979. Selected sentences from this application show that—even at this early stage—Dr. Eisenbarth had a clear, long-term strategy:

With these techniques one can derive monoclonal antibodies to cell surface antigens without prior purification of the antigens of interest... which allow subsequent isolation of the antigen recognized by the antibody as well as exploration of an antigen's physiologic function.... Basic to this project is the availability of islet cells (insulinoma) to be used both as immunogens and as targets to assay for the production of anti-cell surface antibodies.... I have adapted and developed semi-automated assays (^{125}I -Protein A radioassay and Cr^{51} cytotoxicity) to measure anti-cell surface antibodies which are performed in 96-well microtiter plates.... Finally, I propose to apply cell surface antibody assays in combination with monoclonal anti-islet cell antibodies to study the anti-islet cell antibodies of normals, diabetics and a group of polyglandular failure patients.

His proposal was clearly innovative and uniquely combined multiple synergistic approaches with both basic science and translational outcomes. Translating his plan, Dr. Eisenbarth developed an assay to measure insulin production and used it to help clone rat insulinoma cell lines (8). He then used the cell lines to demonstrate cytotoxic antibodies to pancreatic islet cells in the serum of T1D patients (9) and to generate several more monoclonal antibodies to islet cell antigens (2,10–12), reporting the generation of human hybridomas producing autoantibodies to islet cells in an article in *Nature* (13). He also conducted fundamental research in the BB rat and identified the T-lymphopenia, which is a phenotypic hallmark of this T1D model (14). Dr. Dan Rotrosen, Director of the Division of Allergy, Immunology, and Transplantation at the National Institute of Allergy and Infectious Diseases, recently noted how Dr. Eisenbarth's productivity from that grant was exceptional and clearly disproportionate to the modest funding it received.

T1D: a chronic autoimmune disease—There are several major areas where Dr. Eisenbarth's subsequent contributions have provided seminal impetus to T1D research: 1) the identification of islet autoantigens, rapidly followed by 2) the development of biochemical assays

to measure autoantibodies and 3) their application for screening and predictive tools in 4) clinical studies, including natural history studies and clinical trials, which he conceived to study genetic and immunological factors as well as β -cell function in relation to disease progression, and 5) the understanding of the molecular mechanisms regulating insulin autoimmunity, which he unequivocally proved to be the critical autoantigen for disease development in the NOD mouse model. These achievements are reviewed below.

Identification of islet autoantigens

By 1983, it was known that patients with T1D and prediabetic subjects had insulin autoantibodies (IAAs), but the antigen(s) targeted by cytoplasmic islet cell antibodies (ICAs) remained unknown. Dr. Eisenbarth produced evidence that ICAs could target a glycolipid containing sialic acid (15,16). Initially through the use of phage libraries, he began a program of autoantigen discovery that over the years led to the identification of new autoantigens such as carboxypeptidase-H (1991), ICA69 (1993), the tyrosine phosphatase-like insulinoma-associated antigen-2 (IA-2, or ICA512) (1995), phogrin (1996), and the zinc transporter 8 (ZnT8) (2007) (17–22).

Development of biochemical assays to measure autoantibodies

Dr. Eisenbarth first engineered a quantitative fluid-phase radioimmunoassay to measure the levels of IAAs (23), which he later evolved to a multiwell plate format for large screenings (24). He recently developed a new IAA assay based on the latest chemiluminescent technology, which affords much improved sensitivity and specificity (25). He also developed biochemical assays for IA-2 (19) and ZnT8 autoantibodies (22). Throughout the years, he promoted and participated in leading roles in standardization workshops (26).

Application of biochemical autoantibody assays as screening and predictive tools in clinical studies

Dr. Eisenbarth's laboratory has measured autoantibodies for countless studies and clinical trials. He applied ICA testing to the screening of first-degree relatives and combined it with intravenous glucose tolerance testing to characterize those at high risk by the presence of autoantibodies and diminished β -cell function (27). He showed an inverse relation of IAA

titers with age of diabetes development in relatives (28–30). He recognized the importance of multiple autoantibodies for disease prediction in relatives, with disease risk being a function of the number of autoantibodies expressed by a subject (31–34). Just recently, he showed how the addition of ZnT8 autoantibodies improves prediction in relatives (35) and was involved in the harmonization of autoantibody assays (36). With Dr. Roberto Gianani, he proved it was feasible to conduct autoantibody screening to identify pancreas organ donors with islet autoimmunity (37) in order to address the lack of access to the pancreas with islet autoimmunity, a major impediment to T1D research. With such data in hand, Dr. Eisenbarth advocated for and supported the formation of the JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD). Through the efforts of Dr. Mark Atkinson and Dr. Pugliese, nPOD now provides pancreata and other tissues from donors with diabetes and islet autoimmunity to over 100 researchers worldwide, at the same time promoting a highly collaborative framework that is leading to an improved appreciation of the human disease and its heterogeneity (38,39).

Natural history studies and clinical trials to study genetic and immunological factors as well as β -cell function in relation to disease progression

Dr. Eisenbarth pioneered the study of first-degree relatives and described a linear loss of C-peptide responses during the prodromic phase preceding diagnosis (40). In 1986, he proposed that T1D was a chronic autoimmune disease in his landmark “Seminars in Medicine” article published in the *New England Journal of Medicine* (41). In that article he presented the conceptual framework of progressive disease stages in T1D's natural history, which remains our reference to this day. He evaluated insulin secretion in children recently diagnosed with T1D, discovering an impaired response to glucose compared with other stimuli, thus describing the existence of a β -cell functional defect (42). He reported increased frequencies of activated T cells in T1D patients (43), which provided the rationale for pilot immunotherapy studies in T1D (44). Further, he helped Dr. David Sutherland in the immunological characterization of diabetes recurrence in twin-to-twin pancreas transplantation, leading to a seminal article

that further established T1D as an autoimmune disease (45). He later described an acquired defect in interleukin (IL)-2 production in T1D patients (46), pointing at a pathway that over 20 years later would emerge as a major player in the disease pathogenesis through the effects of IL-2 on regulatory T cells.

At the Joslin Diabetes Center, he collaborated with Dr. Stuart Soeldner and began studying the genetics of T1D, intrigued by the lessons that could be learned from the study of twins (47,48). He would continue to study twins for the rest of his career. In the early 1990s, recognizing the need for creating a shared resource for genetic studies, he worked with Lee Ducat, Dr. Åke Lernmark, and others to establish a collection of cell lines and DNA samples from T1D families at the Human Biological Data Interchange (HBDI) (49). In the mid-1990s, with Dr. Marian Rewers, Dr. Eisenbarth established DAISY (Diabetes Autoimmunity Study in the Young) to identify at-risk newborns through genetic screening and study the natural history of islet autoimmunity from birth. During the last 20 years, several fundamental observations have been made through the DAISY cohort about genetic, immunological, metabolic, and environmental factors that influence T1D risk and progression (50–55).

He investigated the genetics of T1D in the DAISY, twin (56), and other first-degree relative cohorts, focusing on the effects of genes or risk and disease progression. His studies provided evidence for a contribution to susceptibility from the HLA-DQ locus in a unique family with recombinant haplotypes (57) and described associations of HLA-DR4 and certain HLA-DQ alleles with IAAs (58,59). In studies with Dr. Pugliese and Dr. Carla Greenbaum, he showed that the DR2, DQB1*0602 haplotype afforded protection from T1D, even to autoantibody-positive relatives, which led to an exclusion criteria for prevention trials (60,61). Dr. Eisenbarth also helped to identify susceptibility variants, to define the phenotypic effects of gene variants and their possible relation to disease pathogenesis, and to refine genetic prediction through sophisticated genetic analyses (62–67).

Understanding the molecular mechanisms regulating insulin autoimmunity

Dr. Eisenbarth believed that insulin was a key if not the primary autoantigen in T1D. His studies supported this hypothesis,

initially through the above-mentioned association of IAA levels with age of onset and disease progression (29,30). He showed that oral administration of porcine insulin suppressed diabetes development in NOD mice (68) and thus contributed to the emerging concept of immunological oral tolerance. He then conducted a pilot prevention study, which suggested that subcutaneous insulin administration might delay diabetes onset in at-risk relatives (69). These observations led to the NIH-sponsored multicenter Diabetes Prevention Trial–Type 1 (DPT-1), in which a significant delay was demonstrated in a subset of relatives with IAAs treated with oral insulin (70). A trial to expand these findings is being conducted by Type 1 Diabetes TrialNet, an NIH-sponsored network that conducts clinical trials in T1D. Of note, Dr. Eisenbarth had leading roles in both DPT-1 and TrialNet for two decades and, as such, gave major contributions to most T1D immunotherapy trials.

He later contributed to unravel the genetic basis of insulin autoimmunity through studies of insulin gene polymorphisms and the discovery (with Dr. Pugliese) that insulin is expressed in the thymus, where such polymorphisms control its expression levels with implications for immunological self-tolerance (71). Exploiting and reengineering the NOD mouse in an impressive series of studies, he discovered that the insulin B9–23 epitope is a major target of islet autoimmunity (72–74). With Dr. Maki Nakayama, he showed that this epitope is absolutely essential for diabetes development in the NOD mouse by engineering NOD mice that no longer express the native insulin genes but rather a mutated insulin gene that removes the antigenicity of this epitope and in turn prevents diabetes (75). He then determined that a conserved T-cell receptor (TCR) α chain (TRAV5D-4) is critical for this immune response (76–78) and he engineered TCR transgenic mice to characterize its role (79). Finally, he and Dr. John Kappler discovered that the insulin B9–23 peptide could bind in multiple registers within the antigen binding pocket of the diabetes-associated major histocompatibility complex (MHC) class II molecule (IA-g7) with profound effects on thymic selection and activation of insulin-specific CD4 T cells (80,81); similar mechanisms have been documented by Dr. Emil Unanue's group as well (82). This research was the main focus of Dr. Eisenbarth's 2009 Banting

Lecture (83) given at the American Diabetes Association's 69th Scientific Sessions in New Orleans, Louisiana, where he described the genetic and molecular basis of the pathogenic insulin autoimmune response in the NOD mouse as represented by the key elements and interactions of the trimolecular complex (the TCR, the MHC class II antigen-presenting molecule, and the target insulin peptide; Fig. 1). The mouse IA-g7 molecule is structurally similar to the human HLA class II DQ8 molecule, the primary genetic determinant for T1D; thus, the implications of this work are far-reaching for the development of antigen-specific therapies, a concept he had remarkably envisioned in his seminal article from 1986 (41). With Dr. Aaron Michels and others, Dr. Eisenbarth had been pursuing the development of small molecules to block the activation of insulin-specific T cells and, in turn, prevent diabetes in NOD mice (84). He had outlined and had planned to test several immunological mechanisms that may be modulated by this type of therapeutic manipulation (85). The translation of this effort to man is in progress.

Leading the way toward prevention and cure

—There is no doubt that Dr. George Eisenbarth was a leader in T1D research and made many major contributions to advance the field. His focus on the study of first-degree relatives led to the recognition that T1D is a chronic autoimmune disease. The discovery of several islet autoantigens and the development of many biochemical autoantibody assays, which are used in diagnostics as well as in screening and prediction, make it possible to conduct clinical trials to prevent diabetes. The launches of long-term natural history studies allow for the study of islet autoimmunity from early life. His application of genetics to the study of twins and relatives led to improved predictive models that incorporate genetic factors, pushing toward primary prevention. The discovery of genetic factors and their effects on the pathogenesis of T1D has uncovered pathways that can be therapeutically targeted. The recognition that finding a cure requires studying the human disease and its pathology led him to the concept that organ donors with islet autoimmunity could be identified, and he was instrumental in the creation of the JDRF nPOD program.

Through a lifetime of studies in man and experimental models—many of which he created—Dr. George Eisenbarth

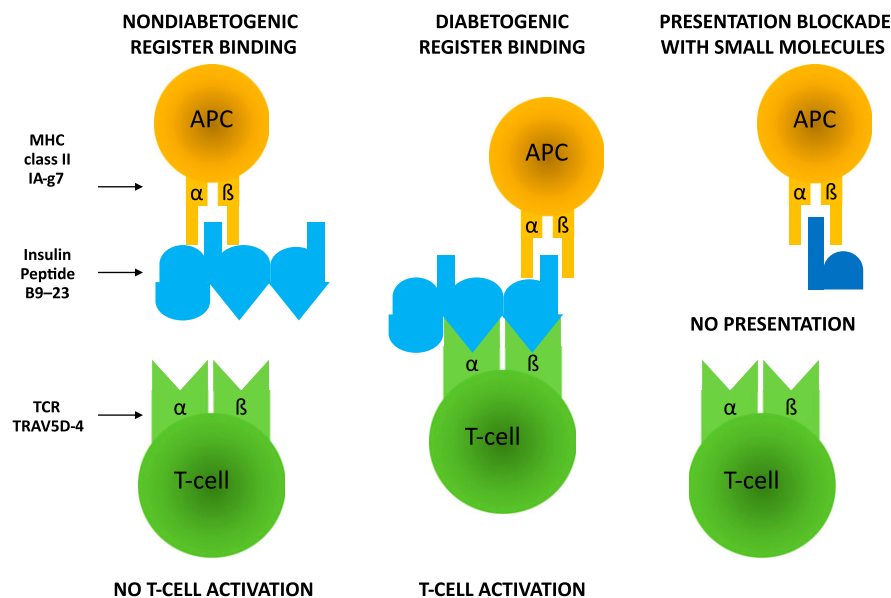


Figure 1—The trimolecular complex—a key target for prevention. The fundamental work of Dr. Eisenbarth is illustrated in this schematic representation of the MHC class II molecule expressed on the surface on antigen-presenting cells (APC), the insulin peptide he proved to be required for diabetes development in the NOD mouse, and a conserved TCR α chain that diabetogenic CD4 T cells use to recognize the MHC-insulin peptide complex. The figure illustrates the concept that the peptide can bind to the MHC in multiple registers. Out of at least four registers, one is considered diabetogenic (middle panel) because it results in recognition of the peptide by insulin-specific autoreactive T cells expressing a conserved TCR α chain, and this is followed by their activation. In this register, peptide binding to the MHC is actually weak, and this is believed to impair the thymic deletion of the autoreactive cells when they first encounter this peptide in the thymus. The left panel models nondiabetogenic binding registers, which fail to result in the presentation of the insulin peptide and T-cell activation. The right panel shows that the use of small molecules could block this interaction and abolish the autoimmune response, with therapeutic benefit in the NOD mouse.

established key features of the insulin autoimmune response and its genetic basis, leading to clinical trials based on insulin as an antigen-specific therapy for the prevention of T1D (in the DPT-1 and Trial-Net networks). In the NOD mouse, he deciphered the molecular rules that govern the interactions of the TCR, the MHC class II antigen-presenting molecule, and the target insulin peptide (the trimolecular complex). Lastly, he had been devising reagents to selectively manipulate that interaction with therapeutic finality and strong translational potential.

Sadly, Dr. Eisenbarth was taken from us before he could see his goal fully accomplished, but he came very close and left a clear path for the next generation to follow. He was an outstanding mentor to more than three generations of diabetes researchers from around the world. He championed openness and collaboration, sharing of ideas and unpublished data with others—even competitors. The collaborative research framework and projects he established or helped others establish represent his legacy, which will continue to impact T1D research for years to come.

He will be remembered for his inquisitive mind and undefeatable logic, unselfishness, kindness, collaborative nature, and integrity—qualities that he devoted to his lifetime goal, the prevention and cure of T1D.

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Acknowledgments—A.P. and J.S.S. have been closely associated with Dr. Eisenbarth for

many years: J.S.S. knew him since he was an MD-PhD student in the 1970s, and A.P. was a postdoctoral fellow with him in the early 1990s. Both had professional and personal relationships with Dr. Eisenbarth, which continued and grew over the years. They regret that space limitations prevented mentioning other important work and naming many of his collaborators.

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