Etiology of Autoimmune Islet Disease: Timing Is Everything

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Life is about timing.
—Carl Lewis

The understanding of autoimmune type 1 diabetes is increasing, and examining etiology separate from pathogenesis has become crucial. The components to explain type 1 diabetes development have been known for some time. The strong association with HLA has been researched for nearly 50 years. Genome-wide association studies added another 60+ non-HLA genetic factors with minor contribution to risk. Insulitis has long been known to be present close to clinical diagnosis. T and B cells recognizing β-cell autoantigens are detectable prior to diagnosis and in newly diagnosed patients. Islet autoantibody tests against four major autoantigens have been standardized and used as biomarkers of islet autoimmunity. However, to clarify the etiology would require attention to time. Etiology may be defined as the cause of a disease (i.e., type 1 diabetes) or abnormal condition (i.e., islet autoimmunity). Timing is everything, as neither the prodrome of islet autoimmunity nor the clinical onset of type 1 diabetes tells us much about the etiology. Rather, the islet autoantibody that appears first and persists would mark the diagnosis of an autoimmune islet disease (AID). Events after the diagnosis of AID would represent the pathogenesis. Several islet autoantibodies without (stage 1) or with impaired glucose tolerance (stage 2) or with symptoms (stage 3) would define the pathogenesis culminating in clinical type 1 diabetes. Etiology would be about the timing of events that take place before the first-appearing islet autoantibody.

The clinical onset and diagnosis of autoimmune (type 1) diabetes (T1D) is the last and final step of a conspicuous prodrome. The progressive loss of a functional β-cell mass has culminated in an inability to keep blood glucose at a normal level. After the diagnosis and disease classification, insulin saves the life of the individual. Insulin, isolated 100 years ago, has saved and changed the lives of millions (1). The ever-continuing upgrading of insulin preparations has continued to improve postdiagnosis care in patients worldwide. Pig and bovine insulins were extracted and purified for some 50 years until recombinant human insulin became available. Presently, human insulin is taken for granted, and, together with insulin pumps and continuous glucose monitoring, diabetes care continues to be improved, although it is far from satisfactory, as indicated by HbA_{1c} measurements in many countries.

Autoimmune Islet Disease

The demarcations "before" and "after" diagnosis are important, as there is little evidence that insulin is efficacious before diagnosis while it is clearly lifesaving after diagnosis (2). In individuals with multiple islet autoantibodies, the eventual clinical diagnosis seems inescapable due to the preceding autoimmune islet disease (AID) (3–5). At present, there is no treatment to halt AID. The secondary prevention trial with parenteral (2) or oral (6) insulin failed, although the latter study reported delayed onset in a group with a high insulin autoantibody (IAA) level (6). The subsequent oral insulin trial by Type 1 Diabetes TrialNet (TrialNet) also failed (7). There were indications of a delay in clinical onset in a subgroup with low first-phase insulin release (7). The CD3 monoclonal antibody teplizumab delayed clinical onset in subgroups of subjects with AID (8). The lack of treatment that would halt AID complicates screening efforts. Well known to all, the World Health Organization does not recommend

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screening for disease unless there is a treatment that would protect the screened from disease. However, screening for T1D risk is advocated as longitudinal follow-up of individuals with multiple islet autoantibodies, as it may alleviate the classical symptoms of T1D with particular emphasis on avoiding the clinical onset of ketoacidosis (9,10). Screening is also advocated to find research subjects eligible for primary or secondary prevention clinical trials.

Treatment of AID

Is it relevant, at this point, to ask when an efficacious treatment of AID will become available? Ever since the early 1980s, when insulin-dependent diabetes mellitus (IDDM) was renamed T1D and non–insulin-dependent diabetes mellitus (NIDDM) was renamed type 2 diabetes, T1D was viewed as an autoimmune disease. The rationale behind renaming was that it would stimulate research of both the etiology and the pathogenesis of both disease entities. The view that T1D was an autoimmune disease was based on the rediscovery of insulitis in 1965 (11), comorbidity with other autoimmune diseases in 1970 (12), and the 1974 *Lancet* publication stating that T1D was associated with HLA (13) as well as with the presence of islet cell antibodies (ICA) (14). The view of the disease as autoimmune was sufficient for initiating open studies of immune-suppressive treatment (15).

The placebo-controlled clinical trials with cyclosporin in newly diagnosed patients with T1D (16,17) showed a transient preservation of residual β-cell function but with significant nephrotoxicity (17,18). Although cyclosporin was abandoned, numerous controlled and uncontrolled clinical studies followed (one of many reviews is in reference 19). Almost every immunosuppressive drug invented (primarily for organ transplantation) has or is likely to be tested in newly diagnosed patients with T1D. So far, most of these drugs or biologicals have been efficacious in transplantation, but they have not alleviated AID. Insulin treatment prevails along with the view that T1D is an autoimmune disease and, therefore, immunosuppression ought to be efficacious.

Etiology of AID

Research in human immunology is becoming more and more sophisticated and informative. Detailed phenotypic and mechanistic studies are now possible along with advanced immunogenetics. Cellular studies are becoming applicable to low-volume human blood samples. The question is what these analyses are really telling us when carried out at different time points when T1D is about to develop or has just been diagnosed. Do sophisticated cellular analyses at the time of clinical diagnosis tell us anything about the etiology of the disease? The answer is likely that circulating immune cells at the time of diagnoses reflect the end stages of a prolonged pathogenic process. Similarly, cellular analyses in subjects who have reached T1D stage 1 (two or more islet autoantibodies, normoglycemia, and no symptoms) (20) may not be suitable to address questions about the etiology of AID. Indeed, little progress has been made toward understanding the etiology of AID, mostly because the timing has not been right.

The key mechanisms that take place from the time of exposure to the appearance of a first islet autoantibody, be it IAA first or GAD antibody (GADA) first (Fig. 1), remains a black box. Indeed, the appearance of a first islet autoantibody, as has been reported for IAA first or GADA first in the BABYDIAB (21), Finish Type 1 Diabetes Prediction and Prevention (DIPP) (22), and The Environmental Determinants of Diabetes in the (TEDDY) (23) studies, illustrates how important timing of samples will be to improve our understanding of the etiology (Table 1). The clinical onset of T1D would seem to be disqualified as a measure of etiology, except for genetic etiology. The designation AID would define a silent prodrome from the time of the appearance of a first islet autoantibody until the clinical onset of diabetes. In other words, the clinical onset of T1D may be viewed as a sequela to AID. As a disease entity, AID would both precede and encompass the recently proposed staging of T1D (20). Stage 1 represents individuals with two or more islet autoantibodies but with normoglycemia, while stage 2 represents individuals with multiple autoantibodies along with dysglycemia but no diabetes symptoms. Stage 3 is islet autoantibodies, diabetes, and symptoms (20). However, AID would also include subjects with one persistent islet autoantibody who may stay that way and still develop diabetes or develop a second autoantibody, or perhaps multiple

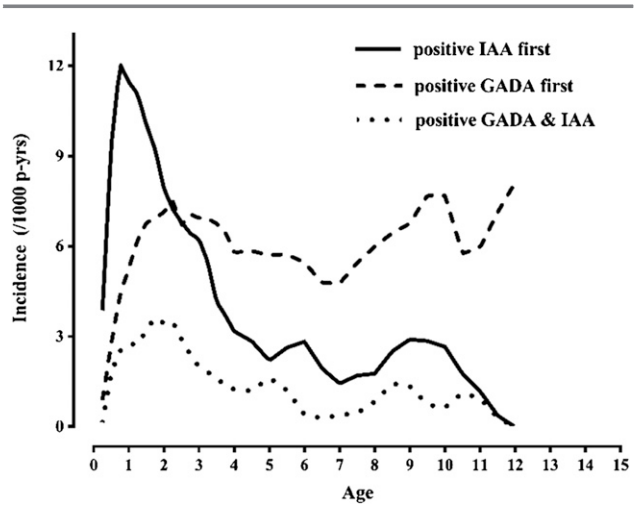

Figure 1—Incidence rate of IAA as the first-appearing islet autoantibody compared with GADA as the first-appearing islet autoantibody and children who had both IAA and GADA as the first-appearing islet autoantibodies. The quarterly blood sampling did not resolve which autoantibody was first when both appeared together. Reprinted with permission from Rewers et al. (63). p-yrs, person-years.

Table 1—Possible approach to dissecting the etiology leading to AID and the pathogenesis of AID resulting in clinical onset of diabetes in subjects at increased genetic risk

	Time	Possible determinants
Etiology		
Exposures	1–10 days	Virus or other infectious exposures Vaccines Unknown triggers
Outcome, cells	5–12 days	NK cells and $CD4^+$ T cells
Outcome, humoral	7–20 days	IAA as first-appearing autoantibody GADA as first-appearing autoantibody
Pathogenesis		
Accelerators	Weeks–years	Second, third, fourth autoantibody
Promoters	Weeks–years	Virus or other infectious exposures Genetic factors, usually non-HLA genes Dietary factors
Outcome		Clinical onset of diabetes

autoantibodies, during follow-up (4). Furthermore, it has been estimated that 70% of children with two or more autoantibodies develop diabetes within 10 years compared with 15% in children with one persistent islet autoantibody (3,4,24). The TEDDY study takes this one step further and reports that the diabetes risk for *1*) children without an islet autoantibody was 0.06 per 100 person-years, *2*) children who reverted from single autoantibodies to autoantibody negative increased to 0.14 per 100 person-years, and *3*) children who remained single-autoantibody positive increased further to 1.8 per 100 person-years (24). These data would support the view that AID is transient and mild (reverters), less aggressive (one persistent autoantibody), and more aggressive (multiple autoantibodies). The pathogenic mechanisms could be set in motion by the same underlying etiological cause, but the subsequent pathogenesis would differ in severity (Table 1).

A textbook example of the human immune response to a fictive virus infection, vaccination, or other exposure is depicted in Fig. 2. While the appearance of a first islet autoantibody is secondary to cellular events required for the development of IgM to be followed by IgG, the timing of events resulting in AID is well illustrated.

AID Is Diagnosed With Islet Autoantibodies

AID is currently diagnosed with islet autoantibody biomarkers. Ever since the first Islet Cell Antibody Workshop in 1985 in Monaco (25) and the identification of autoantigens and their autoantibodies (insulin [IAA], GAD 65 [GADA], IA-2 [IA-2A], and ZnT8 transporter [ZnT8A]) and a variety of assay formats (Table 2), the research community has worked, through the Immunology of Diabetes Workshops, Immunology of Diabetes Society, Diabetes Autoantibody Standardization Program, and Islet Autoantibody Standardization Program (26), to harmonize and standardize the different autoantibody tests.

Currently, attempts are being made for regulatory qualification of islet autoantibodies as enrichment biomarkers for use in T1D prevention clinical trials (27). Once the initiation of AID has been clarified, the prediction would be that other diagnostic criteria and treatment approaches will be worked out and become available. The islet autoantibodies would remain as the biomarkers used to stage T1D (20). The autoantibodies would also remain end point biomarkers of primary prevention, such as in the ongoing Primary Oral Insulin Trial (POInT) (28). If none of the autoantibodies develops, it is unlikely that T1D will materialize. The autoantibodies are also potential qualified biomarkers to be used as end points in secondary prevention trials. A treatment that specifically blocks further production of an islet autoantibody may also block part of the AID pathogenesis and thereby slow down or perhaps even halt AID. AID would represent a biomarker-defined silent and asymptomatic disease, and the autoantibodies would be qualified biomarkers of the etiology of AID.

Cellular Biomarkers of the First-Appearing Islet Autoantibody

Three questions need to be answered when using current islet autoantibody biomarkers. First, at what age does a first islet autoantibody appear? Second, does the first autoantibody come alone, or do multiple autoantibodies appear at the same time? Third, what is the trigger of the first islet autoantibody biomarker? As illustrated in Fig. 2, a trigger may be a factor from the outside, such as a virus that would initiate an immune response leading to the eventual appearance of the islet autoantibody biomarker. The trigger could also be a vaccine, such as Pandemrix, that initiates narcolepsy in HLA-DQB1*06:02 subjects (29). The series of events from the time of exposure to

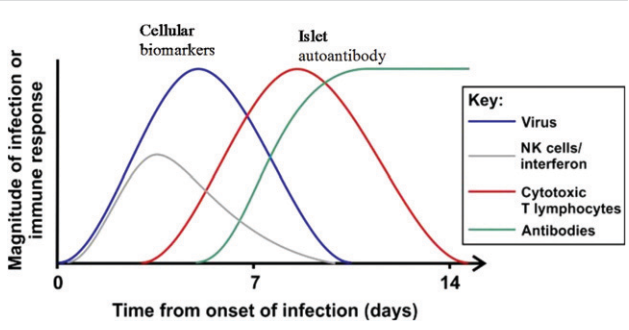

Figure 2—Illustration of the windows of timing of the human immune response after a hypothetical virus infection that potentially would trigger AID.

Table 2—Islet autoantibody biomarkers qualified to define the etiology of AID and autoimmune T1D

Autoantigen	Autoantibody	Abbreviation
(Pre)Proinsulin	Insulin	IAA
Glutamic acid decarboxylase Mr 65K	GAD65	GADA
IA-2	Islet antigen-2	IA-2A
Zn transporter 8	ZnT8	ZnT8A
Tetraspanin-7	TSPAN7	TSPAN7A

the detection of the islet autoantibody biomarkers leaves two windows (Fig. 2). The first window is the cellular reaction to the exposure. Cellular biomarkers will be needed to define the cellular reaction of the adaptive immune response assumed to be the basis for islet autoantibody development. According to the illustration in Fig. 2, the cellular autoimmunity window would be open for a limited 5–10 days. The islet autoantibody window would open up after ?7–10 days after the initial exposure. For example, this timing is consistent with the time it takes for insulin antibodies to appear after insulin therapy has been initiated at the time of clinical diagnosis of T1D (30). Hence, an overlap between cellular and humoral autoimmune responses would seem inevitable. However, direct measures of an environmental factor that triggers an autoimmune cellular reaction will require extraordinary luck or coincidence to time the cellular event. Prolonged shedding of enterovirus B, as described in the TEDDY cohort, prior to the appearance of IAA first may be that type of coincidence (31). During a prolonged shedding of the virus, it cannot be excluded that there is a series of cellular reactions that determines whether neutralizing antibodies are going to be formed or whether autoimmunity is instigated. While neutralizing antibodies are eventually formed to clear the virus, a parallel reaction leads to the formation of autoantibodies. It will be a major challenge in the future to study the initiating autoimmune cellular reactions. At least the studies following children from birth have told us something about the timing and when to look for AID.

In the TEDDY study, it was a rare event that children presented with multiple autoantibodies; it was either IAA first or GADA first (4,23,32). Children being diagnosed with T1D during the first 2 years of life had an increased frequency of infections compared with children diagnosed with T1D at an older age (33). In designing studies to dissect the first appearance of an islet autoantibody, it was necessary to identify the children at birth based on an increased risk for T1D, such as in the BABYDIAB (34), DIPP (35), Diabetes Autoimmunity Study in the Young (DAISY) (36), and Prospective Assessment in Newborns for Diabetes Autoimmunity (PANDA) (37) studies, in order not to miss an event of seroconversion during the first year of life. The challenge then, and in the future, was the number of blood samples that could be obtained during the first year of life. Would it be possible to sample every other week or month? The DIPP (22) and TEDDY (23) studies obtained blood samples every 3 months for up to 4 years of age. In both studies, the incidence rate (23) or proportion of a first islet autoantibody (22) indicated that IAA tended to be the first-appearing autoantibody, with a peak within the first 2 years of life and decreasing thereafter (Fig. 1).

GADA showed up later as a first-appearing autoantibody, but, in contrast to IAA first, the GADA first incidence rate stayed elevated (23,38). The timing to locate the cellular events that precede the first appearing islet autoantibody will represent a major challenge in the future. One approach again will be to obtain blood samples at 3, 6, 9, 12, 15, 18, and 21 months of age from many more children to achieve a sufficient number of events that trigger either IAA first or GADA first. However, the quarterly sampling may have missed environmental events that would trigger a cellular reaction leading to autoimmunity within 10–20 days (Fig. 2). In both DIPP and TEDDY, it was a rare event to observe IA-2A as the first autoantibody (22,23). Alternative approaches may be to collect small capillary blood samples for dried blood spots (39,40) or perhaps to measure the islet autoantibodies in saliva samples (41). Alternatively, studies may focus on GADA first, which occurs in older children at a more or less steady incidence rate (Fig. 1). However, timing is everything, and sampling four times per year or more often will be critical. Furthermore, biomarkers other than autoantibodies will be needed to better time an exposure that triggers a cellular reaction that, within ?7–10 days, leads to the appearance of an islet autoantibody, be it IAA first or GADA first (Fig. 1).

Genetic Etiology of AID

The proposed classification of AID characterized by the persistent presence of one or several islet autoantibodies raises the question of whether the well-known association between HLA and T1D is secondary to a primary association between HLA and AID. As most subjects with two or more islet autoantibodies will move on to develop T1D, it seems a safe assumption that HLA DR-DQ haplotypes associated with T1D will also be associated with AID. It is more complicated to ask the question at the time of T1D diagnosis if the primary association with HLA is with islet autoantibodies rather than with T1D. However, as children were recruited at birth based on T1D risk HLA types, it was possible to test if HLA DR-DQ alleles and haplotypes were associated with the type of the first-appearing islet autoantibody. Highly significant differences in the association between the first autoantibody and HLA were reported in both TEDDY (23,38) and DIPP (42). In both studies, homozygotes for DR3-DQ2 had predominantly GADA as the first autoantibody. IAA first but also GADA first was found in DR3-DQ2/DR4-DQ8 heterozygotes.

There was a strong association between IAA first and DR8-DQ4/DR4-DQ8. In DRB1*04:01-DQ8 but not in DRB1*04:04-DQ8 children, IAA was more often the first autoantibody. As expected, GADA first was also found among DR3-DQ2/DR4-DQ8 subjects. These results support the view that the initiation of AID is dependent on HLA class II. DR-DQ heterodimers may display autoantigen epitopes to initiate the autoimmune response that leads to the appearance of a first islet autoantibody. As discussed previously, the association between HLA and T1D may be secondary to the primary association between HLA and either IAA or GADA as the first-appearing islet autoantibody (23,38,42). This view is supported by the observation that HLA was not associated with the progression from two or more islet autoantibodies to the clinical onset of T1D (4,38,43).

The importance of the observation that HLA may be associated with the first appearing autoantibody, be it HLA DR4-DQ8 with IAA first or DR3-DQ2 with GADA first, has opened up the notion that AID represents two different endotypes (44). The underlying biological mechanism for one endotype, DR4-DQ8 and IAA first, would be antigen presentation following enterovirus (coxsackievirus B1) infection, as revealed in the DIPP study (45), and prolonged shedding of enterovirus B (including coxsackievirus) prior to IAA seroconversion in the TEDDY study (31). The DIPP study measured neutralizing enterovirus antibodies as a proxy for virus exposure, while virus levels in monthly stool samples were determined in the TEDDY study. Therefore, it is reasonable to speculate that coxsackievirus infection represents a trigger of AID, as defined by the appearance of IAA first.

The other endotype would be DR3-DQ2 with GADA first. There was no association between neutralizing coxsackievirus antibodies and GADA first (45). However, there was suggestive association between DR3-DQ2 and GADA first in the TEDDY study with prolonged shedding of mastadenovirus F (31). These observations early in life, that timing is everything, suggest that HLA class II DR, DQ, or both contribute to the mechanism by which a cellular reaction (Fig. 1) would result in both neutralizing virus antibodies and autoantibodies. As the DIPP study found neutralizing virus antibodies in children who developed IAA and the TEDDY study found IAA first to be associated with prolonged shedding of the virus, it is tempting to speculate that the DR4-DQ8 children have a reduced ability to mount an effective immune response to coxsackievirus B1.

The association between HLA class II heterodimers and virus clearance or persistence is well known. DQ2 was associated with viral persistence of hepatitis C and the risk of the autoimmune Sicca syndrome (46), while DRB1*11 or DQB1*03 alleles were associated with an efficient immune response against the virus (47). The response to hepatitis B virus infection as well as to the vaccine was poor in DQ2 individuals, while the response in DRB1*13 and DQ6.2 individuals was strong (48). There are numerous studies of the association between HLA and virus infection or HLA and virus vaccination. Furthermore, attempts have been made to use HLA structural analyses to design virus peptides for vaccine development (49). It has been noted that vaccines against coxsackievirus are already under development, and when these vaccines eventually are used in humans, it will be of importance to vaccinate early in life (50). It needs to be considered if DR4-DQ8 children only should be the target for the vaccine to test if it would be possible to prevent the appearance of IAA as a first-appearing islet autoantibody.

Structural Considerations of HLA DR and DQ in AID

HLA DR and DQ heterodimers provide the mechanism by which antigen peptides are presented to be recognized by the T-cell receptor (TCR) on $CD4^+$ T cells. Foreign proteins taken up and processed to peptides by antigen-presenting cells, such as follicular dendritic cells (Fig. 3), represent a key survival mechanism in the defense of infectious agents. Human survival is dependent on the diversity by which HLA class II heterodimers can engage trimolecular complex with TCR on $CD4^+$ T cells. Once these cells are activated, the signaling cascades will include the generation of both $CD8^+$ T cells and B cells. The latter will turn into antibody-producing cells, both memory B cells and long-lasting antibody-producing plasma cells. Currently there is one major DRA haplotype, although 7 alleles have been reported, to form heterodimers with 322 different alleles of DRB3, DRB4, and DRB5, 2,268 different alleles of DRB1, 95 DQA1 alleles, and 1,295 DQB1 alleles. The number of combinations is immense, but selective pressure from infectious agents in geographically separated or isolated populations has shaped the HLA-DR-DQ genotype distribution. Therefore, it is of interest that the Scandinavian population is dominated by DR3-DQ2, DR4-DQ8, and DQ6.2 haplotypes. It is unclear what survival advantages these haplotypes have had.

In other populations, such as Japan, isolated from the world for centuries, there is a lack of these haplotypes. The incidence rate of T1D is high in the Scandinavian countries and exceedingly low in Japan. Interestingly enough, candidate triggers such as enterovirus are prevalent both in Scandinavia and Japan. Therefore, it is assumed that the DR4-DQ8 haplotype is necessary but not sufficient to trigger the IAA-first endotype. If enterovirus B is the candidate environmental factor, it is noted that the virus infection and prolonged shedding is not sufficient either, since many more DR4-DQ8 children were infected but only a subset went on to develop IAA. Hence, other environmental exposures or other genetic factors may be necessary to mount an autoimmune response leading to IAA first.

A major unanswered question is how a virus infection can result in an immune response that is marked by a

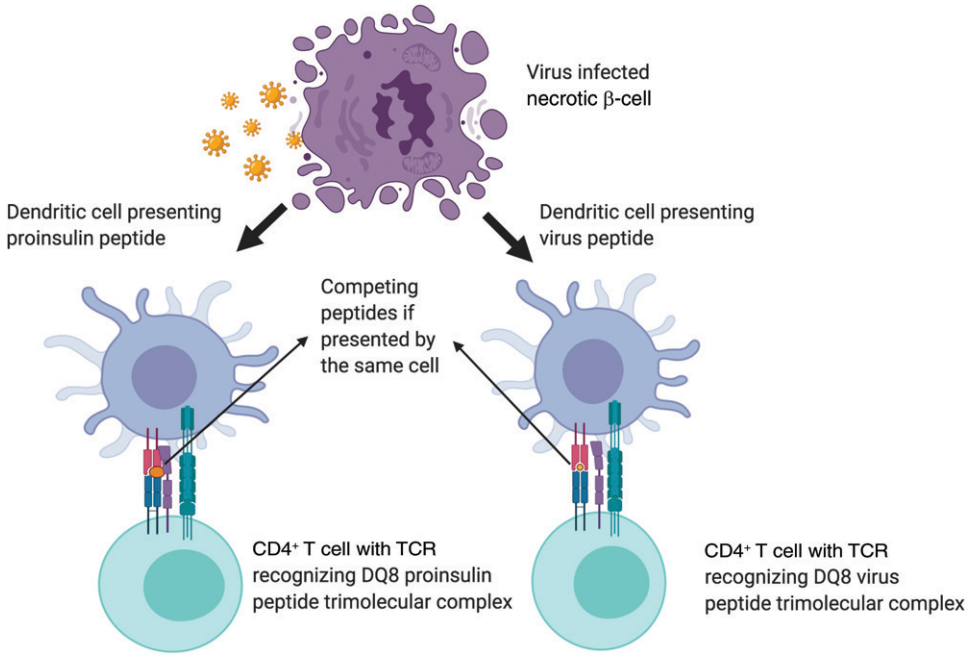

Figure 3—Necrotic or virus lysed pancreatic islet β-cell processed by dendritic cells, resulting in the presentation of autoantigen proinsulin or virus peptides. It is speculated that a proinsulin peptide can compete with and partially displace a virus peptide with similar physicochemical properties. The eventual outcome would be islet autoimmunity due to the appearance of IAA and a poor development of neutralizing virus antibodies, perhaps resulting in prolonged shedding of virus. Image was created with BioRender.com.

first-appearing autoantibody. In narcolepsy triggered by the Pandemrix vaccine in HLA-DQB1*06:02 subjects, it was observed that H1N1 nucleoprotein antibodies had lower affinity in the patients than their HLA-matched control subjects (51). It was suggested that the reduced H1N1 nucleoprotein antibody affinities among patients with childhood narcolepsy show poor protection from the wild-type A/H1N1 virus and possibly increased risk for viral damage.

The observation in the TEDDY study of enterovirus B prolonged shedding may be consistent with a similar phenomenon. It can be speculated that the DR4-DQ8 virus antigen presentation is poor, which may result in a competition with peptides from virus-infected β-cells. B-cell peptides competing with virus peptides may suffice to trigger a cellular immune reaction that generates IAA and low-affinity virus antibodies (Fig. 3).

A way to disentangle whether DR is contributing more than DQ to the above-described reaction may be through high-resolution HLA typing of all DR and DQ alleles by next-generation sequencing (52). Children newly diagnosed with T1D and control subjects were used as a proxy for an etiological triggering event. In contrast to recording the frequency of alleles, we wanted to go a step further first to translate the exon sequences into the expected amino acid residues and then use a recursive organizer, ROR (53), to recursively group sequence variants based on sequence similarities (54). The amino acid residues associated with T1D were identified to reveal their contribution to the pocket structure formed between the α and the β chains of the DR heterodimer. The pocket structure forms the basis for which type of peptide can be uploaded into the pocket to form the trimolecular structure (Fig. 3) that serves as the ligand for the TCR on $CD4^+$ T cells. Understanding these amino acid motifs and the ligand structure should prove useful to begin analyses of possible competition between peptides derived from autoantigens, such as preproinsulin and GAD65, and virus, such as enterovirus B and mastadenovirus F (31). The ideal timing for cellular analyses would be during the time of prolonged shedding. Current technologies of HLA class II heterodimer purification, elution of peptides, and sequencing the eluted peptides by mass spectrometry may reveal a state of competition between autoantigen peptides and virus peptides.

Comparing the frequency of motifs, beyond considering a single amino acid such as the well-known DQ β chain position 57 (Asp and non-Asp) (55), it was found that 11 amino acid residues of DRB1 were strongly associated with T1D. All 11 residues were located in or were adjacent to the peptide-binding groove of DRB1. Similarly, in the less well-studied DRB3, DRB4, and DRB5 heterodimers, there were 15 amino acid residues with T1D association (54). These residues were located in the peptide binding grove and in the β 49–55 homodimerization patch known to interact with the CD4 accessory molecule (54).

In the subsequent analysis of HLA DQ, 45 unique DQ haplotypes were found by a hierarchically organized

haplotype (HOH) association analysis (56). HOH found residues alpha44Q and beta57A to be associated with T1D in the DQ8/9 cluster. Within the DQ2 cluster, HOH analysis found alpha44C and beta135D to share the risk for T1D. The motif "QAD" of alpha44, beta57, and beta135 captured the T1D risk association of HLA DQ8.1. This DQ haplotype has the strongest association with IAA as the first-appearing autoantibody. The risk association between T1D and HLA DQ2.5 was captured by "CAD" of alpha44, beta57, and beta135. These residues are found in and around anchor pockets 1 and 9, as potential TCR contacts, in the areas for CD4 binding and putative homodimer formation (56).

Finally, it is well known that there are DQ haplotypes that are negatively associated with T1D. The best example is HLA-DQB1*06:02, which is negatively associated with the risk for T1D up to about 30 years of age but not thereafter (57) and provides subjects with failed genetic protection against T1D (58). The subsequent analysis revealed seven residues (alphaa1, alpha157, alpha196, beta9, beta30, beta57, and beta70) that were negatively associated with T1D in subjects with HLA DQ4, 5, 6, and 7 haplotypes. The amino acid residue motifs "DAAFYDG," "DAAYHDG," and "DAAYYDR" were strongly negatively associated with T1D (59). The importance of these structures to bind different peptides is illustrated by the observation that a change of a single amino acid residue in the motif "DAAYH**D**G" to "DAAYH**S**G" (D to S at beta57) changed the motif from a strong negative to a neutral association with T1D. These motifs contribute to the physicochemical characteristics of the peptide binding grove and thereby to the type of autoantigen peptide that will bind and compete for binding with exogenous virus peptides (59). A strong preference for virus peptides may be associated with an immune response that generates neutralizing virus antibodies to prevent prolonged shedding of virus and thereby reduce the risk for islet autoimmunity.

Conclusions

The perspective of this type of research is the understanding of the type of peptides that bind these different HLA DR-DQ heterodimers. However, other factors may also contribute, such as genetically determined factors that control the way HLA DR and DQ heterodimers are expressed. Recently, it was reported that the risk for T1D in HLA-DR3 homozygote children was increased significantly by a haplotype of three single-nucleotide polymorphisms within the first intron of HLA-DRA1. These single-nucleotide polymorphisms may represent the expression of quantitative trait loci modulating both HLA DR and DQ expression (60). In islet autoantibody-positive subjects with an increased genetic risk for T1D, cell surface HLA DQ immunofluorescence was reduced compared with that of matched autoantibody-negative control subjects (61). It has also been suggested that the stability of HLA DQ heterodimers on the cell surface contributes to the risk for T1D (62). The above-described physicochemical characteristics of HLA DQ8 and DQ2 perhaps make these molecules more prone to present peptides that break tolerance to allow the first-appearing autoantibody to be either IAA or GADA (Fig. 3). The challenge to future research is to explain how the autoimmune response is related to a prior virus infection and other factors that, together, contribute to a break of the immunological tolerance of self and the initiation of AID that eventually, through pathogenesis, results in T1D.

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