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PATHOGENESIS OF TYPE 1 DIABETES (A PUGLIESE, SECTION EDITOR)

Molecular Interactions Governing Autoantigen Presentation in Type 1 Diabetes

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Abstract Type 1 diabetes is a chronic autoimmune disease resulting from T cell-mediated destruction of insulin-producing beta cells within pancreatic islets. Disease incidence has increased significantly in the last two decades, especially in young children. Type 1 diabetes is now predictable in humans with the measurement of serum islet autoantibodies directed against insulin and beta cell proteins. Knowledge regarding the presentation of insulin and islet antigens to T cells has increased dramatically over the last several years. Here, we review the trimolecular complex in diabetes, which consists of a major histocompatibility molecule, self-peptide, and T cell receptor, with a focus on insulin peptide presentation to T cells. With this increased understanding of how antigens are presented to T cells comes the hope for improved therapies for type 1 diabetes prevention.

Keywords Diabetes · Autoimmunity · HLA · Insulin · T cells

Introduction

Type 1 diabetes (T1D), the immune-mediated form of diabetes, results from the specific T cell-mediated destruction of pancreatic islet beta cells [1••]. The major genetic determinant of T1D resides within the human leukocyte antigen (HLA) complex [2]. HLA genes explain more than 50 % of the genetic risk for disease development [3, 4]. Prior to the onset of clinical disease, which is marked by the presence of hyperglycemia, antibodies directed against insulin and proteins within insulin-producing beta cells are detectable in the serum. These islet autoantibodies make T1D predictable as the presence of two or more autoantibodies directed against insulin, glutamic acid decarboxylase (GAD), islet antigen 2 (IA-2), and zinc transporter 8 (ZnT8) results in a nearly 100 % progression to clinical T1D given enough time [5••]. Despite T1D being a predictable disease, it is not yet preventable. Antigen-specific therapies, especially with insulin preparations, have been utilized to induce tolerance and prevent clinical T1D onset with limited success [6]. Understanding how islet antigens are presented by HLA molecules to activate T cells is vital for improving antigen-specific therapies. We discuss in detail how insulin and other islet antigens are presented by diabetes at-risk HLA molecules to T cells. Utilizing this knowledge, we review the use of these novel peptides as therapies in preclinical studies using animal models of spontaneous autoimmune diabetes.

Trimolecular Complex

T cell activation by an antigen requires three components termed an immunologic synapse or trimolecular complex depicted in Fig. 1 [7, 8]. A major histocompatibility complex (MHC) class II molecule binds a peptide and this peptide/MHC complex is presented on the cell surface of an antigen-presenting cell (dendritic cells, B cells, and macrophages) to interact with a T cell receptor expressed on a CD4 T cell. This
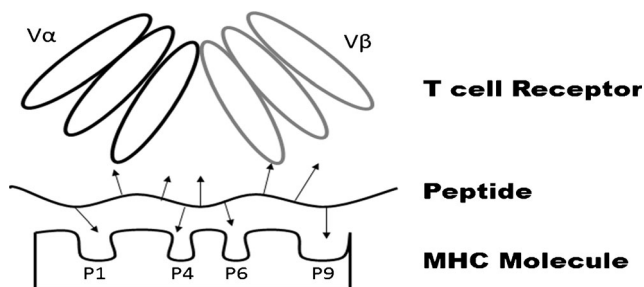

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Fig. 1 The trimolecular complex is consists of the following: (1) major histocompatibility complex class II molecule, (2) peptide, and (3) T cell receptor on a CD4 T cell. The MHC is composed of a binding groove with pockets that can accommodate amino acid side chains (represented as *arrows*) of the peptide. This interaction with distinct structural pockets (pockets 1, 4, 6, and 9) anchors the peptide to the MHC class II molecule. The amino acid side chains of the peptide not binding to the MHC molecule can interact with a T cell receptor, thereby activating a T cell. *P1–P9* denote pockets in the peptide binding groove. *MHC* major histocompatibility complex

occurs normally in adaptive immunity to bacterial and viral antigens. However, in the case of autoimmunity a self-peptide is presented by MHC II to a self or autoreactive T cell.

Major Histocompatibility Complex Class II Molecule

The majority of genetic risk for T1D development is conferred by genes in the HLA complex, which is divided into three regions termed class I, II, and III. Class II genes (termed DQ, DR, and DP) encode MHC class II proteins which are strongly associated with T1D risk. DQ and DR alleles are in close linkage disequilibrium on chromosome 6. Certain haplotypes (alleles on the same chromosome) are predominant in T1D. For example, approximately 90 % of all T1D patients have DR3/DQ2 and/or DR4/DQ8 haplotypes. The DQ8 (DQA1*0301, DQB1*0302) allele is present in 50–60 % of T1D patients and has the highest odds ratio for T1D development in genome-wide association studies with odds ratios ranging from 6.5 to 11 [3, 9]. Interestingly, the polymorphic DQ6 allele (DQB1*0602) provides dominant protection from diabetes development with an odds ratio as low as 0.03 [3, 9]. Understanding the protective role of DQ6 and other T1D resistance mechanisms is an active area of research.

MHC class II molecules are formed from two alpha and two beta chains. The α1 and β1 chains form the peptide binding groove, while the α2 and β2 anchor the MHC II molecule to the cell membrane. These molecules function to present processed antigens as peptides to CD4 T cells. As DQ8 is strongly implicated in T1D pathogenesis, we will focus on the structural characteristics of this molecule. DQ8 has been crystallized with both an insulin peptide and gliadin peptide in the peptide binding groove [10, 11]. Typically nine amino acids of a peptide fill the binding groove. The peptide binding

groove of DQ8 anchors a peptide by binding amino acid side chains in 4 distinct pockets, pockets 1, 4, 6, and 9. Polymorphisms in the DQ α1 and β1 chains shape these pockets. A specific polymorphism in the DQ beta chain at position 57 changes an aspartic acid to valine, leucine, or alanine at pocket 9. Aspartic acid has a negatively charged side chain and this amino acid changes results in the loss of a salt bridge with a conserved arginine residue (positively charged side chain) at position 76 of the DQ8 alpha chain. Because of this, DQ8 favors peptides that have an acidic or negatively charged amino acid side chain (glutamic or aspartic acid) at position 9 [12]. Of note, the diabetes protective DQ6 molecule maintains aspartic acid at β57, allowing for the presentation of a much different peptide repertoire than DQ8. Taken together, subtle structural changes in the binding groove of MHC class II molecules may lead to diabetes by allowing presentation of certain self-peptides, decreasing the affinity by which self-peptides are presented or changing the position of peptide binding.

Self-Peptides

The second component of the trimolecular complex is a peptide or, in the case of autoimmunity, an autoantigen. A number of autoantigens have been described in T1D with almost all of them focused on proteins within beta cells. We will focus primarily on proinsulin/insulin as an autoantigen and several others in the section on posttranslational modification of islet autoantigens.

Insulin as an Autoantigen

Insulin is a major self-antigen for both T and B cells. Insulin autoantibodies are present in up to 90 % of young children that develop islet autoimmunity and eventually T1D [13, 14]. In addition, Pugliese and colleagues showed polymorphisms upstream of the insulin gene, variable number of tandem repeats (VNTR), are associated with levels of insulin expression in the thymus and T1D development risk [15]. It is hypothesized that the amount of insulin message present in the thymus (more VNTR) corresponds to regulation of autoreactive T cells and thus decreased T1D development; less insulin message potentiates the escape of autoreactive T cells from the thymus into peripheral circulation and enhances T1D risk.

Much of our knowledge regarding (pro) insulin peptide presentation in T1D comes from the non-obese diabetic (NOD) mouse model of spontaneous autoimmune diabetes [16]. Identical to humans, MHC class II molecules are necessary for disease development with murine IA^{g7} structurally similar to human DQ8 and DQ2 (both having polymorphisms at β57 resulting in the loss of aspartic acid) [17–19]. A critical amino acid sequence in the B chain of insulin, amino acids 9–

23 (B:9–23), is necessary for T1D development in the NOD mouse. Mutating the insulin gene such that a single amino acid is changed, B16 tyrosine to alanine, abrogates all diabetes development, and mice remain normoglycemic [20, 21]. This striking finding indicates that insulin B:9–23 is pathogenic and the amino acid mutation either leads to: (1) the peptide being unable to bind the murine MHC class II molecule or (2) insulin-specific T cell receptors cannot be activated without B16 tyrosine. Recent investigations suggest the latter to be the case. These studies reveal that B:9–23 can bind in multiple positions or "registers" in the IA^{g7} peptide binding groove. As depicted below in Fig. 2, each register creates a new distinct epitope for T cell recognition even though the peptide is B:9–23 in each case.

Using register trapping techniques, Kappler and colleagues have determined that insulin B:9–23 binds in the unexpected, low affinity, register 3 to murine IA^{g7} [22]. Register 3 is low affinity as B22 arginine would occupy pocket 9, which is a very poor match as arginine has a positively charged side chain and pocket 9 is positively charged (from the polymorphism at $\beta57$). By creating a B:9–23 mimotope, B22 Arg→Glu, binding to register 3 is now favored and this mimotope is able to stimulate T cells approximately 100-fold better than native B:9–23. Of importance, fluorescent IA^{g7} tetramers made with this mimotope detect CD4 T cells in the pancreas and pancreatic lymph nodes of prediabetic NOD mice, suggesting that B:9–23 presented at register 3 is an epitope for autoreactive T cells targeting pancreatic beta cells [23].

We have recently translated these findings to humans, showing distinct patterns of cytokine response to the insulin B chain mimotope in subjects with and without T1D correlated to HLA-DQ genotype. Substituting a glutamic acid for arginine at position B22 of the insulin B:9–23 peptide resulted in robust inflammatory (IFN-γ) responses from new-onset T1D patients more than the native peptide. Unexpectedly, controls without diabetes not only responded with an IFN-γ response to the mimotope but also had strong production of IL10, especially if a non-risk DQ allele was present [24••]. IL10 has anti-inflammatory properties and is associated with regulatory T cells. Most models of autoimmunity indicate that there is a balance of inflammatory and regulatory immune responses. Our data in healthy controls suggests that this balance prevents T1D development.

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MHC Groove Position:      123456789
Register  1        SHL VEA LY LVC G ERG
Register  2        SHLV EAL Y LVCG RG
Register  3        SHLVE ALY L VCGE R G
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Fig. 2 Position or "register" or insulin B chain amino acids 9–23 (B:9–23) binding to murine IA^{g7} or human HLA-DQ8. The amino acids in *red* are contact residues for MHC class II at position 1, 4, 6 and 9, while the remaining amino acids in *black* are potential T cell receptor contacts. Each register creates a distinct epitope for T cell recognition even though the peptide is insulin B:9–23 in each case

Others groups have also documented a protective immune response to insulin and islet antigens in healthy controls. Peakman and colleagues identified epitopes in proinsulin and IA-2 that induce protective IL10 responses in healthy subjects [25]. Recently, Roep and coworkers found protective responses to a modified version of insulin B:30–C:13 that induced inflammatory responses in DQ8-positive T1D subjects and IL10 in some healthy controls [26]. CD4 T cell reactivity to various (pro) insulin epitopes in humans have also been identified from islet-infiltrating T cells [27••], within pancreatic lymph nodes, [28] and in the peripheral blood [29–31]. Insulin-specific T cell responses are not just from CD4 T cells, as it is known that insulin B:10–18 activates CD8 T cells from T1D patients [32].

Posttranslational Modification of Islet Autoantigens

Recent investigations into antigen processing and presentation have discovered that self-antigens may undergo posttranslational modification (PTM) to provide more immunogenic epitopes to activate autoreactive T cells. We will review three PTMs described in autoimmune disorders and examine their roles in T1D: (1) disulfide bond formation, (2) deamidation by tissue transglutaminase, and (3) citrullination by peptidyl arginine deiminase (PAD).

Disulfide bond formation can change the conformation and structure of a protein. Disulfide bonds or bridges are covalent bonds that form between free sulfhydryl (–SH) groups, which are found on the amino acid side chain of cysteine. The first described PTM in T1D comes from an internal disulfide bond within an insulin A-chain peptide, amino acids 1–13 [33]. T cell clones were identified from a child at risk for T1D (presence of insulin autoantibodies) using this peptide but not healthy controls. The cysteine residues at positions A6 and A7 were critical for T cell clone recognition (GIVEQCCTSICSL). Interestingly, the vicinal disulfide bond did not alter binding to HLA-DR4 but instead activated specific T cell receptors.

A very well-studied PTM occurs in celiac disease (gluten sensitivity) in which tissue transglutaminase acts on gluten and gliadin proteins to form highly immunoreactive epitopes for CD4 T cells [34, 35]. Tissue transglutaminase converts glutamine (Gln) residues to glutamic acid (Glu). These modified peptides have increased MHC class II binding affinity as glutamic acid has a negatively charged side chain which is capable of anchoring peptides to pocket 1 or 9 of DQ8 and DQ2. The DQ2 and DQ8 alleles are present in almost all individuals with celiac disease with approximately 90 % of all patients having a DQ2 allele [36]. Of note, about 10 % of all T1D patients have celiac disease as both diseases share at risk DQ alleles [37]. In T1D, a number of islet peptides have been shown to increase binding affinity to DQ8 and T cell reactivity following conversion of Gln to Glu. Notably, the

insulin B:30–C:13 peptide becomes more immunogenic with conversion of both Gln residues to Glu by inducing inflammatory T cell responses in T1D patients [26]. Similarly, there have been epitopes identified within GAD65 that also detect T cells at higher frequencies when Gln is converted to Glu [38]. Chromogranin A is also a target for autoreactive T cells in both murine and human diabetes. In the NOD mouse, islet-infiltrating T cells respond to the WE14 peptide presented by the mouse IA^{g7} in an unusual manner in which the peptide binds in only half the peptide binding groove [39]. It is hypothesized that tissue transglutaminase may act on the peptide to increase immunogenicity [40]. In fact, tissue transglutaminase treated WE14 stimulates T cells from new-onset T1D patients to a greater degree than native or untreated peptides [41].

A final well-studied PTM is the conversion of arginine to citrulline by the PAD enzyme in rheumatoid arthritis [42]. The conversion of arginine to citrulline results in the positively charged arginine side chain becoming neutral, allowing for higher affinity MHC class II binding or forming an epitope for specific T cell receptor activation. PAD is activated with inflammation and creates both citrillunated antibodies (anti-CCP antibodies) [43] and epitopes of vimentin and aggrecan capable of activating T cells when presented by specific MHC class II molecules [44]. In T1D, the conversion of arginine to citrulline within GAD65 creates epitopes that detect autoreactive CD4 T cells at higher frequencies in T1D patients compared to controls [38].
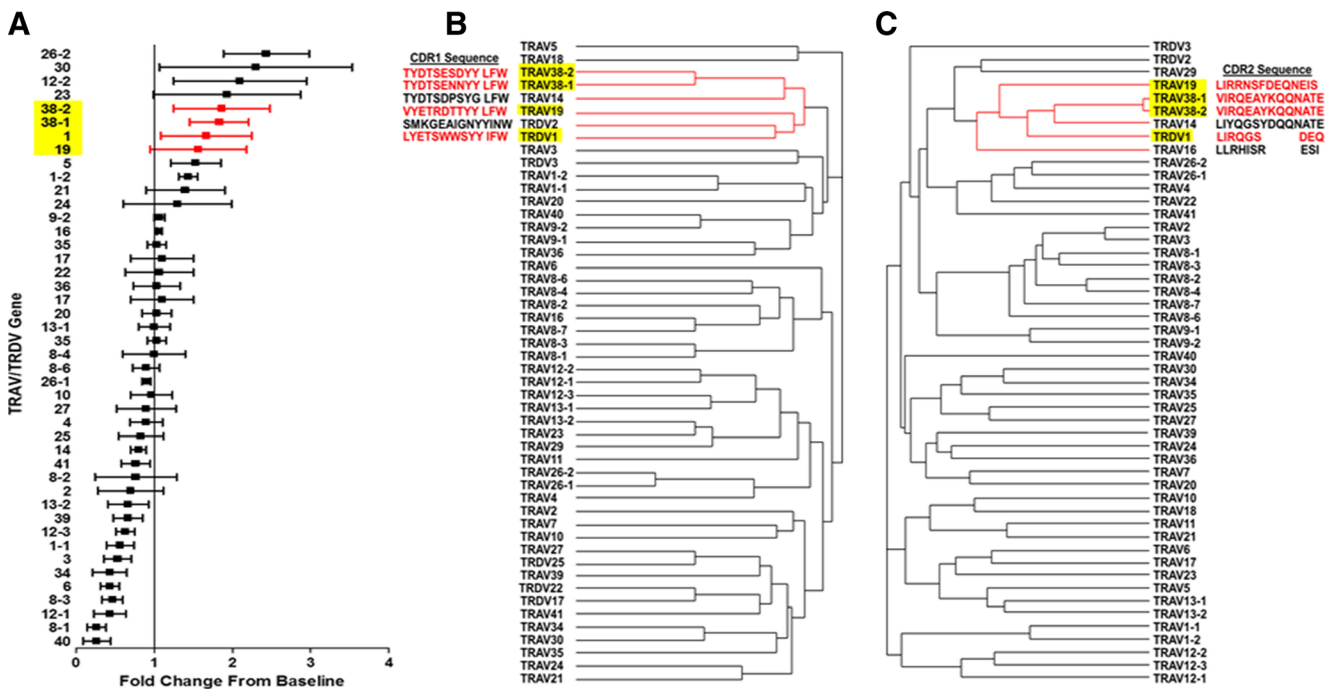
T cell Receptor

The final component of the trimolecular complex is the T cell receptor (TCR). The TCR is composed of alpha and beta chains that interact with a peptide/MHC complex. The alpha and beta chains have both variable (V) and constant regions. Recombination allows for great diversity in TCR sequences such that millions of unique sequences are possible. TCR interaction with peptide/MHC occurs through the binding of complementary determining regions (CDR), three for each alpha and beta chain. It is believed that the germline-encoded CDR1 and CDR2 regions predominantly interact with α helices of the MHC, while CDR3 regions contact the bound peptide [45]. Compared to insulin peptides and MHC molecules, relatively little is known about TCR usage and diversity of autoantigen specific T cells. Again, much of our knowledge stems from the NOD mouse model of spontaneous autoimmune diabetes [46]. In the NOD mouse, about 70 % of TCRs responding to insulin B:9–23 presented by IA^{g7} use a conserved alpha chain component, termed TRAV5D-4 [47]. Interestingly, alpha chains containing TRAV5D-4 pair with many separate beta chains to recognize B:9–23, and are sufficient to induce insulin autoantibodies and diabetes [47–49].

In humans, the ortholog to murine TRAV5D-4 is TRAV13-1. Interestingly, this human alpha chain can induce diabetes in the NOD model when combined with different mouse B:9–23-reactive CDR3 regions [47]. To study human insulin specific TCR usage, we used the insulin B:9–23 mimotope, described in the section on insulin as an autoantigen, to induce proliferation of peripheral blood T cells in three HLA-matched T1D patients all having the DQ8/DR4 and DQ2/DR3 haplotypes [24••]. CD4 T cells were sequenced for TCR alpha chains before and after proliferation. To monitor proliferation, peripheral blood mononuclear cells were labeled with a dye, carboxyfluorescein succinimidyl ester (CFSE), and cells dilute the dye as they divide; the CD4^{+}CFSE^{lo} cells were then sorted and sequenced for TCR alpha chains. As depicted in Fig. 3a, several V alpha genes (TRAV and TRDV) are used by T cells more and less predominantly compared to baseline in all three subjects when stimulated with the insulin B:9–23 mimotope. Analyzing phylogenetic trees of V gene sequences indicates that four of the prevalent V genes (TRAV 38–1, TRAV 38–2, TRAV 19, and TRDV 1) cluster together based upon similarity in CDR1 and CDR2 sequences (Fig. 3b, c). Interestingly, the CDR3 sequences vary in these clustered alpha chains. The V alpha gene skewing and clustering of dominantly used genes based upon CDR1 and CDR2 regions with variable CDR3 sequences is consistent with antigen-specific T cell proliferation and very similar to the findings from the NOD mouse.

In humans, the vast majority of our understanding regarding islet antigen-specific TCR usage comes from the study of T cells in the peripheral blood. However, the pancreatic islets are the target organ in T1D and sequencing TCRs from human islet infiltrating cells is crucial to understand disease pathogenesis. Both the retroperitoneal location and exocrine function of the pancreas make it difficult to obtain tissue for analysis. To address this problem, the Network for Pancreatic Organ Donors with Diabetes (nPOD) has been established to obtain pancreata and other organs (lymph nodes, spleen, and blood) from cadaveric organ donors with T1D and those who are autoantibody positive but without clinical disease [50]. This invaluable network is making the study of human islet infiltrating cells possible with a focus on TCR usage of the islet infiltrating T cells. Notably, an initial study analyzing the TCR repertoire from nPOD obtained pancreas tissue from a T1D patient having the HLA-DR4/DQ8 haplotype shows preferential usage of TRAV13-1 [51].

Antigen Specific Therapy

The use of islet antigens or peptides to induce protective immunity, also called tolerance, is a major focus in the field. Antigen specific therapy is safe and holds the promise to induce regulatory T cells at the site of inflammation. Antigen

Fig. 3 T cell receptor (TCR) V gene skewing in response to an insulin B:9–23 mimotope. **a** Data for Vα gene sequencing before and after stimulation from three T1D subjects all with identical DQ alleles. TCR alpha chain genes were sequenced to identify V gene usage from CD4 T cells prior to and after proliferation to the insulin mimotope. Data are depicted as the fold change (proliferated/baseline) for each V gene and show the mean +/− SEM. **b** Phylogenetic trees of V genes, created using the Clustal-Omega algorithm, based upon similarity in CDR1. **c** CDR2 regions. Four predominant V genes (*highlighted and red*) in the proliferated cells of all 3 patients cluster together based upon similarity in CDR1 and CDR2 sequences

therapy has been attempted at different stages of the disease process: at high genetic risk, islet autoimmunity, and new-onset T1D, unfortunately with minimal success [52]. The most well-studied antigen for therapeutic intervention is insulin having been administered by oral, intranasal, subcutaneous with adjuvant, and intradermal routes [53, 54]. In the Diabetes Prevention Trial-Type 1 (DPT-1), a randomized double-blinded placebo controlled trial was conducted in which at-risk T1D patients ($n=372$) were treated with oral insulin or placebo [55]. Unfortunately, the primary endpoint of delaying progression to clinic T1D was not achieved; however, a post hoc analysis revealed that participants with high levels of insulin autoantibodies had a delay in disease development of about 5 years [56]. The delay was even longer, 10 years, for those with the highest insulin autoantibody levels. A follow-up oral insulin trial through the National Institutes of Health-sponsored Type 1 Diabetes TrialNet is currently enrolling participants with high insulin autoantibody levels in an attempt to delay T1D progression (NCT00419562) [57]. Recently, the results of a small pilot trial using high-dose oral insulin in children at high genetic risk for T1D demonstrated safety and the induction of protective immunity to insulin [58].

While oral insulin is being evaluated to potentially delay T1D onset, our recently gained insights into the molecular presentation of insulin B:9–23 to T cells allows for improved antigen-specific therapies. The insulin B:9–23 mimotope is a strong T cell agonist and has the potential to induce regulatory T cells in preclinical animal studies. Von Boehmer and colleagues used the insulin mimotope to induce tolerance in NOD mice by administering the peptide at a constant low dose through intraperitoneal osmotic pumps [59]. Naïve T cells were converted to Foxp3+ regulatory T cells and NOD diabetes was completely prevented both early and late in the disease course. This was not the case with identical low doses of the native B:9–23 peptide. The mimotope was unable to reverse NOD diabetes after hyperglycemia onset as monotherapy. This study indicates the potential of using a strong T cell agonist to improve the efficacy of antigen specific therapy for T1D intervention.

Conclusions

Type 1 diabetes is predictable and it naturally follows that the disease will be prevented. To date, major prevention efforts using insulin preparations (oral, intranasal, and subcutaneous) have had limited success. Understanding the molecular interactions of islet antigens with MHC and T cell receptors holds promise for improved antigen-specific therapies and bio-markers for disease progression. The register of insulin B:9–23 peptide presentation in murine and human T1D is critical for T cell activation. Utilizing this knowledge for therapeutic

intervention may provide greater efficacy and biomarkers for monitoring therapeutic response. We believe tailoring immune therapy to a specific HLA molecule, autoantigen, and register of islet antigen presentation to autoreactive T cells represents precision medicine that will ultimately allow for T1D prevention.

Compliance with Ethical Standards

Conflict of Interest   Maki Nakayama has a pending patent on Compounds that modulate autoimmunity and methods of using the same.
Kimberly M. Simmons and Aaron W. Michels declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent   All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
•• Of major importance

1.•• Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet. 2014;383:69–82. **Useful review on all aspects of type 1 diabetes over the last 10 years including eitology, pathogeneses, prediction, prevention and treatment**.

2. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. N Engl J Med. 2009;360:1646–54.

3. Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, et al. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. Diabetes. 2008;57:1084–92.

4. Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet. 2009;41:703–7.

5.•• Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013;309:2473–9. **Orignial research indicating that 2 or more serum islet autantibodies leads to clinical T1D development in children from the United States and Europe**.

6. Nanto-Salonen K, Kupila A, Simell S, Siljander H, Salonsaari T, Hekkala A, et al. Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. Lancet. 2008;372:1746–55.

7. Michels AW, Nakayama M. The anti-insulin trimolecular complex in type 1 diabetes. Curr Opin Endocrinol Diabetes Obes. 2010;17:329–34.

8. Michels AW. Targeting the trimolecular complex. Clin Immunol. 2013;149:339–44.

9. Todd JA, Bell JI, McDevitt HO. HLA-DQB gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature. 1987;329:599–604.

10. Lee KH, Wucherpfennig KW, Wiley DC. Structure of a human insulin peptide/HLA-DQ8 complex and susceptibility to type 1 diabetes. Nat Immunol. 2001;2:501–7.

11. Henderson KN, Tye-Din JA, Reid HH, Chen Z, Borg NA, Beissbarth T, et al. A structural and immunological basis for the role of human leukocyte antigen DQ8 in celiac disease. Immunity. 2007;27:23–34.

12. Jones EY, Fugger L, Strominger JL, Siebold C. MHC class II proteins and disease: a structural perspective. Nat Rev Immunol. 2006;6:271–82.

13. Steck AK, Johnson K, Barriga KJ, Miao D, Yu L, Hutton JC, et al. Age of islet autoantibody appearance and mean levels of insulin, but not GAD or IA-2 autoantibodies, predict age of diagnosis of type 1 diabetes: diabetes autoimmunity study in the young. Diabetes Care. 2011;34:1397–9.

14. Steck AK, Vehik K, Bonifacio E, Lernmark A, Ziegler AG, Hagopian WA, et al. Predictors of progression from the appearance of islet autoantibodies to early childhood diabetes: The Environmental Determinants of Diabetes in the Young (TEDDY). Diabetes Care. 2015;38:808–13.

15. Pugliese A, Zeller M, Fernandez A, Zalcberg LJ, Bartlett RJ, Ricordi C, et al. The insulin gene is transcribed in the human thymus and transcription levels correlate with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type I diabetes. Nat Genet. 1997;15:293–7.

16. Liu E, Yu L, Moriyama H, Eisenbarth GS. Animal models of insulin-dependent diabetes. Methods Mol Med. 2004;102:195–212.

17. Latek RR, Suri A, Petzold SJ, Nelson CA, Kanagawa O, Unanue ER, et al. Structural basis of peptide binding and presentation by the type I diabetes-associated MHC class II molecule of NOD mice. Immunity. 2000;12:699–710.

18. Corper AL, Stratmann T, Apostolopoulos V, Scott CA, Garcia KC, Kang AS, et al. A structural framework for deciphering the link between I-Ag7 and autoimmune diabetes. Science. 2000;288:505–11.

19. Suri A, Walters JJ, Gross ML, Unanue ER. Natural peptides selected by diabetogenic DQ8 and murine I-A(g7) molecules show common sequence specificity. J Clin Invest. 2005;115:2268–76.

20. Nakayama M, Abiru N, Moriyama H, Babaya N, Liu E, Miao D, et al. Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. Nature. 2005;435:220–3.

21. Nakayama M, Beilke JN, Jasinski JM, Kobayashi M, Miao D, Li M, et al. Priming and effector dependence on insulin B:9–23 peptide in NOD islet autoimmunity. J Clin Invest. 2007;117:1835–43.

22. Stadinski BD, Zhang L, Crawford F, Marrack P, Eisenbarth GS, Kappler JW. Diabetogenic T cells recognize insulin bound to IAg7 in an unexpected, weakly binding register. Proc Natl Acad Sci U S A. 2010;107:10978–83.

23. Crawford F, Stadinski B, Jin N, Michels A, Nakayama M, Pratt P, et al. Specificity and detection of insulin-reactive CD4+ T cells in type 1 diabetes in the nonobese diabetic (NOD) mouse. Proc Natl Acad Sci U S A. 2011;108:16729–34.

24.•• Nakayama M, McDaniel K, Fitzgerald-Miller L, Kiekhaefer C, Snell-Bergeon JK, Davidson HW, et al. Regulatory vs. inflammatory cytokine T-cell responses to mutated insulin peptides in healthy and type 1 diabetic subjects. Proc Natl Acad Sci U S A. 2015;112:4429–34. **Original research showing CD4 T cell responses to a**

novel insulin B chain mimotope associated with HLA-DQ genotype from new-onset T1D patients and healthy controls.

25. Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, et al. Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. J Clin Invest. 2004;113:451–63.

26. van Lummel M, Duinkerken G, van Veelen PA, de Ru A, Cordfunke R, Zaldumbide A, et al. Posttranslational modification of HLA-DQ binding islet autoantigens in type 1 diabetes. Diabetes. 2014;63:237–47.

27.•• Pathiraja V, Kuehlich JP, Campbell PD, Krishnamurthy B, Loudovaris T, Coates PT, Brodnicki TC, O'Connell PJ, Kedzierska K, Rodda C, et al. Proinsulin specific, HLA-DQ8 and HLA-DQ8 transdimer restricted, CD4+ T cells infiltrate the islets in type 1 diabetes. 2014. **Original research identifying T cell clones from islet infiltrating cells of a pancreas organ donor with type 1 diabetes; about ¼ of the clones responded to proinsulin peptides.**

28. Kent SC, Chen Y, Bregoli L, Clemmings SM, Kenyon NS, Ricordi C, et al. Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. Nature. 2005;435: 224–8.

29. Alleva DG, Crowe PD, Jin L, Kwok WW, Ling N, Gottschalk M, et al. A disease-associated cellular immune response in type 1 diabetics to an immunodominant epitope of insulin. J Clin Invest. 2001;107:173–80.

30. Eerligh P, van Lummel M, Zaldumbide A, Moustakas AK, Duinkerken G, Bondinas G, et al. Functional consequences of HLA-DQ8 homozygosity versus heterozygosity for islet autoimmunity in type 1 diabetes. Genes Immun. 2011;12:415–27.

31. Yang J, Chow IT, Sosinowski T, Torres-Chinn N, Greenbaum CJ, James EA, et al. Autoreactive T cells specific for insulin B:11–23 recognize a low-affinity peptide register in human subjects with autoimmune diabetes. Proc Natl Acad Sci U S A. 2014;111: 14840–5.

32. Pinkse GG, Tysma OH, Bergen CA, Kester MG, Ossendorp F, van Veelen PA, et al. Autoreactive CD8 T cells associated with {beta} cell destruction in type 1 diabetes. Proc Natl Acad Sci U S A. 2005;102:18425–30.

33. Mannering SI, Harrison LC, Williamson NA, Morris JS, Thearle DJ, Jensen KP, et al. The insulin A-chain epitope recognized by human T cells is posttranslationally modified. J Exp Med. 2005;202:1191–7.

34. Shan L, Molberg O, Parrot I, Hausch F, Filiz F, Gray GM, et al. Structural basis for gluten intolerance in celiac sprue. Science. 2002;297:2275–9.

35. Tollefsen S, Arentz-Hansen H, Fleckenstein B, Molberg O, Raki M, Kwok WW, et al. HLA-DQ2 and -DQ8 signatures of gluten T cell epitopes in celiac disease. J Clin Invest. 2006;116:2226–36.

36. Liu E, Lee HS, Aronsson CA, Hagopian WA, Koletzko S, Rewers MJ, et al. Risk of pediatric celiac disease according to HLA haplotype and country. N Engl J Med. 2014;371:42–9.

37. Barker JM. Type 1 diabetes associated autoimmunity: natural history, genetic associations and screening. J Clin Endocrinol Metab. 2006;91:1210–7.

38. McGinty JW, Chow IT, Greenbaum C, Odegard J, Kwok WW, James EA. Recognition of posttranslationally modified GAD65 epitopes in subjects with type 1 diabetes. Diabetes. 2014;63: 3033–40.

39. Stadinski BD, Delong T, Reisdorph N, Reisdorph R, Powell RL, Armstrong M, et al. Chromogranin A is an autoantigen in type 1 diabetes. Nat Immunol. 2010;11:225–31.

40. Delong T, Baker RL, He J, Barbour G, Bradley B, Haskins K. Diabetogenic T-cell clones recognize an altered peptide of chromogranin A. Diabetes. 2012;61:3239–46.

41. Gottlieb PA, Delong T, Baker RL, Fitzgerald-Miller L, Wagner R, Cook G, et al. Chromogranin A is a T cell antigen in human type 1 diabetes. J Autoimmun. 2014;50:38–41.

42. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest. 1998;101:273–81.

43. Goldbach-Mansky R, Lee J, McCoy A, Hoxworth J, Yarboro C, Smolen JS, et al. Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset. Arthritis Res. 2000;2:236–43.

44. Scally SW, Petersen J, Law SC, Dudek NL, Nel HJ, Loh KL, et al. A molecular basis for the association of the HLA-DRB1 locus, citrullination, and rheumatoid arthritis. J Exp Med. 2013;210: 2569–82.

45. Garcia KC, Adams JJ, Feng D, Ely LK. The molecular basis of TCR germline bias for MHC is surprisingly simple. Nat Immunol. 2009;10:143–7.

46. Simone E, Daniel D, Schloot N, Gottlieb P, Babu S, Kawasaki E, et al. T cell receptor restriction of diabetogenic autoimmune NOD T cells. Proc Natl Acad Sci U S A. 1997;94:2518–21.

47. Nakayama M, Castoe T, Sosinowski T, He X, Johnson K, Haskins K, et al. Germline TRAV5D-4 T-cell receptor sequence targets a primary insulin peptide of NOD mice. diabetes. 2012;61:857–65.

48. Kobayashi M, Jasinski J, Liu E, Li M, Miao D, Zhang L, et al. Conserved T cell receptor alpha-chain induces insulin autoantibodies. Proc Natl Acad Sci U S A. 2008;105:10090–4.

49. Zhang L, Jasinski JM, Kobayashi M, Davenport B, Johnson K, Davidson H, et al. Analysis of T cell receptor beta chains that combine with dominant conserved TRAV5D-4*04 anti-insulin B: 9–23 alpha chains. J Autoimmun. 2009;33:42–9.

50. Campbell-Thompson M, Wasserfall C, Kaddis J, Albanese-O'Neill A, Staeva T, Nierras C, et al. Network for Pancreatic Organ Donors with Diabetes (nPOD): developing a tissue biobank for type 1 diabetes. Diabetes Metab Res Rev. 2012;28:608–17.

51. Stumpf M, Landry L, Lau K, Siebert J, Eisenbarth GS, Atkinson M, Larkin J. III, Nakayama M. Analysis of T cell receptor (TCR) sequences in pancreatic tissue from patients with type 1 diabetes (T1D). Keystone Symposia Immunotherapy of Type 1 Diabetes (Abstract)

52. Michels AW, von Herrath M. 2011 update: antigen-specific therapy in type 1 diabetes. Curr Opin Endocrinol Diabetes Obes. 2011;18: 235–40.

53. Skyler JS. Primary and secondary prevention of Type 1 diabetes. Diabet Med. 2013;30:161–9.

54. Simmons KM, Michels AW. Type 1 diabetes: a predictable disease. World J Diabetes. 2015;6:380–90.

55. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med. 2002;346:1685–1691.

56. Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, et al. Effects of oral insulin in relatives of patients with type 1 diabetes: the diabetes prevention trial—type 1. Diabetes Care. 2005;28:1068–76.

57. Skyler JS, Greenbaum CJ, Lachin JM, Leschek E, Rafkin-Mervis L, Savage P, et al. Type 1 Diabetes TrialNet—an international collaborative clinical trials network. Ann N Y Acad Sci. 2008;1150: 14–24.

58. Bonifacio E, Ziegler AG, Klingensmith G, Schober E, Bingley PJ, Rottenkolber M, et al. Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. JAMA. 2015;313:1541–9.

59. Daniel C, Weigmann B, Bronson R, von Boehmer H. Prevention of type 1 diabetes in mice by tolerogenic vaccination with a strong agonist insulin mimetope. J Exp Med. 2011;208:1501–10.