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# The Anti-insulin Trimolecular Complex in Type 1 Diabetes

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## Abstract

**Purpose of Review—**Description of the immunologic components needed for autoimmune diabetes.

**Recent Findings—**The MHC class II molecules are the primary susceptibility genes for many autoimmune diseases, including type 1 diabetes. Understanding of the structural interaction between MHC molecules, antigenic peptides, and T cell receptors (the three components of the trimolecular complex) has increased greatly over the last several years. The components of the anti-insulin trimolecular complex and findings that insulin is a key autoantigen in type 1 diabetes are reviewed.

**Summary—**The anti-insulin trimolecular complex is well defined in the non-obese diabetic mouse model. Insulin and specifically, the amino acid sequence 9 to 23 of the insulin B chain, represents a primary antigenic target for islet autoimmunity in the non-obese diabetic mouse model of type 1 diabetes with a specific mutation of this peptide preventing all diabetes. Initial studies suggest the human homologues of the anti-insulin trimolecular complex may be relevant in human disease.

### Keywords

diabetes; autoimmunity; insulin; human leukocyte antigen; T cell receptor

## Introduction

Type 1A diabetes (T1D) is the immune mediated form of diabetes. The incidence of T1D is increasing dramatically, doubling approximately every 20 years (1). The immunologic mediators of the disease are becoming more defined. The trimolecular complex consists of antigen presenting cells, peptide fragments, and specific T cell receptors that recognize peptide bound to antigen presenting cells, and this trimolecular complex leads to tissue specific targeting in autoimmune diseases. The trimolecular complex for insulin is now well characterized for the non-obese diabetic mouse model of T1D (figure 1). The components of the anti-insulin trimolecular complex in mice and humans will be reviewed.

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## The Major Histocompatability Complex

The major determinant of genetic susceptibility to T1D is conferred by genes in the human leukocyte antigen (HLA) complex, which is divided into three regions: class I, II, and III. Alleles of the class II genes, DQ and DR (and to a lesser extent DP), are the most important determinants of type 1 diabetes. These class II molecules are mainly expressed on antigen presenting cells (macrophages, dendritic cells, and B cells) and present antigens to $CD4^+$ T lymphocytes. Haplotypes (combinations of DR and DQ alleles on the same chromosome) are strongly associated with T1D. Each unique amino acid sequence of DR and DQ is given a number. Since DRA does not vary, haplotypes can be defined by specific DRB, DQA, and DQB alleles. The DR3 and DR4 haplotypes are present in more than 90% of people with T1D possessing one or both of these haplotypes versus 40% of the US population (2). The DR3 haplotype contains the alleles DQA1*0501 and DQB1*0201, while high risk DR4 contains both DQA1*0301 and DQB1*0302. The most extensively studied allele DQ8 (DQA1*0301 and DQB1*0302), present in approximately 60% of type 1 diabetics, has been crystallized accommodating an insulin peptide (3). A polymorphism in the beta chain of DQ8 (substitution of β57 aspartic acid for valine, leucine, or alanine) leads to changes in the peptide binding groove of the molecule (4;5). Subtle structural changes to the binding groove of MHC class II molecules likely leads to diabetes susceptibility by decreasing the affinity by which autoantigens are presented or changing the register of binding (6;7).

Heterozygous DR3/4 individuals have a much higher risk for diabetes development than homozygous DR3/3 and DR4/4 individuals. Recent work by Erlich and coworkers helps explain this phenomenon by analyzing the large family data set of the Type 1 Diabetes Genetics Consortium (8). The T1D risk of DR3/4 individuals is the result of a trans-encoded DQ molecule (DQA and DQB encoded by different chromosomes) that form in DR3/4 individuals, namely DQA1*0501 and DQB1*0302. Trans-encoded DQ8 molecules differ in peptide presentation and T cell recognition of peptides compared to cis-encoded molecules (9;10). Work is being done to identify additional genes contributing to diabetes risk within or linked to the MHC region such as HLA-A24 (11), HLA-B39 (12), and a locus telomeric of the UBD gene several million base pairs away from DR and DQ alleles (13). We believe there are genes to be identified within the MHC that predispose to diabetes risk.

In addition to HLA genes, many genetic loci contributing to diabetes risk have been implicated through genome wide association studies (14), which involves analyzing thousands of single nucleotide polymorphisms from large populations to find alleles associated with a particular disease. While HLA alleles confer the highest risk, multiple non-HLA genetic polymorphisms modify disease risk. One of these non-HLA genes, the group of longer variable number of tandem nucleotide repeats (VNTR) 5′ of the insulin gene, protects against diabetes. The decreased diabetes risk is associated with greater insulin message in the thymus and resultant deletion of autoreactive T cells in the thymus (15;16). Maturing T cells in the thymus that react with autoantigens are deleted and the more insulin that is present in the thymus leads to less insulin reactive T cells.

## Insulin as an autoantigen

Insulin has been focused on as a T1D autoantigen for decades since autoantibodies to this molecule were discovered in patients having T1D, (17) and often precedes the development of other islet autoantibodies (18). Numerous studies have implicated insulin as an autoantigen in human T1D. Pugliese and coworkers demonstrated that polymorphisms upstream of the insulin gene (VNTR) which are associated with levels of insulin expression in the thymus correlate with the risk of T1D development (15). Alleva and colleagues detected T cells that react with the amino acid sequence 9-23 of the insulin B chain (B:9-23) in the peripheral blood of T1D patients but not in HLA-matched normal subjects (19). More recently, Kent and coworkers identified CD4 T cells in the pancreatic lymph nodes of T1D patients that react with the amino acid sequence 1-15 of the insulin A chain (20). Subsequent studies further support that such insulin A:1-15 reactive CD4 T cells are detected in peripheral blood in T1D patients (21;22). In addition, multiple investigators have reported CD8 T cells that react with preproinsulin epitopes (23-25), some of which have been shown to kill beta cells *in vitro* (26) and *in vivo* using a humanized mouse model (27). Given the above evidence, it is likely that the T cell response to insulin or preproinsulin contribute to T1D development of humans.

Much of what we know about autoantigens in T1D comes from the study of the animal model of T1D. The non-obese diabetic (NOD) mouse develops diabetes spontaneously and shares many similarities to the human disease. Wegmann and coworkers first established CD4 T cell clones that react with insulin from pre-diabetic NOD islets (28) and found that the majority of them responds to the insulin B:9-23 peptide, which has an identical amino acid sequence in mouse and human insulin. Also, the structure of human DQ8 is very similar to the mouse MHC class II presenting molecule, I-$A^{g7}$. Both MHC molecules share a polymorphism, the substitution of β57 aspartic acid for a small hydrophobic amino acid. Aspartic acid has a negatively charged side chain that forms a salt bridge with α76 arginine forming pocket 9 of the MHC class II binding groove; disrupting this salt bridge leads to a very basic pocket along the binding groove implicated in diabetes susceptibility (4;6). Several investigators confirmed the existence of CD4 T cells reacting with the insulin B: 9-23 in lymphocytes infiltrating the NOD pancreatic islets although the frequency of such B: 9-23 reactive T cells in the islets differs by studies (29;30). These insulin B:9-23-reactive T cell clones are capable of inducing diabetes when adoptively transferred or transgenically introduced into immuno-compromised recipients (31-33). Furthermore, the administration of the insulin B:9-23 peptide or the altered form of this peptide prevents and delays diabetes onset in NOD mice (34-36). This evidence indicates that the CD4 T cell response to the insulin B:9-23 peptide contributes to islet autoimmunity in NOD mice.

It is still a fundamental question whether insulin is essential for human T1D development. In the NOD mouse model, elimination of the majority of T cells that react with insulin (37;38) but not with glutamic acid decarboxyalse (GAD) (39) or insulin glucose related phosphatase (IGRP) (40) results in protection from diabetes. Conversely, lack of insulin expression in thymic epithelial cells results in aggressive diabetes development even in a diabetes-resistant mouse strain (41). We demonstrated that NOD mice lacking native insulin genes but transgenic for insulin with an alanine mutation at B:16 (B16:A insulin) are completely

protected from diabetes development (42;43). In addition, Krishnamurthy and coworkers demonstrated that the response to IGRP is downstream of that to insulin (40). Thus, it is quite likely that insulin is required to drive anti-islet autoimmunity in the NOD mouse, although this does not necessarily imply that insulin is the only single autoantigen essential for islet cell autoimmunity. What insulin or preproinsulin epitopes are essential for islet autoimmunity? As described above, B:9-23 is the most studied peptide for NOD diabetes. The complete prevention of insulin autoantibodies in insulin knockout NOD mice with B16:A insulin is abrogated when the alanine mutation is converted back to tyrosine which is the native insulin amino acid sequence (44). Further adoptive transfer experiments with CD4 T cells suggest insulin B:9-23 presented by I-$A^{g7}$ is an essential epitope contained in the preproinsulin molecule. Other peptides (e.g. insulin A:7-21 (34), insulin C:15-30 (29), B: 2-17 (29), insulin B:24-C:36 (45;46), and preproinsulin:47-64 (47)) demonstrated as CD4 spontaneous insulin epitopes for NOD diabetes are also being investigated.

Why is the insulin B:9-23 peptide pathogenic? T cells are “educated” not to attack self antigens in the thymus (16;41;48) and also in the peripheral immune organs (49;50). Recently, Unanue and colleagues proposed and demonstrated a novel concept of autoantigen presentation. Namely, the B:9-23 peptide binds to I-$A^{g7}$ with a low affinity, and T cells reacting with such poor binding peptides may escape negative selection in the thymus (47;51). I-$A^{g7}$ presents other autoantigens in an unorthodox manner. Recent work by Haskins and colleagues elucidated a natural epitope of highly diabetogenic T cell clones (BDC-2.5, BDC-10.1, and BDC-5.10.3)(52), a chromogranin peptide, binds to I-$A^{g7}$ in an extremely unusual fashion (53). Another idea is that antigens presented by MHC molecules in a targeted organ (i.e. pancreatic islets) are slightly modified and are different from such “educating” tissues, and as a result, T cells reacting with modified antigens are not eliminated or become tolerant. These antigen modifications include alternative splicing of antigens in the thymus (54) and post-translational modification of antigens in the pancreas (21). Autoantigens including the insulin B:9-23 may rarely be expressed in the proper fashion to eliminate or silence T cells in the thymus and peripheral immune organs, which may facilitate T cell differentiation toward autoreactivity.

## T cell receptors recognizing the insulin peptide-MHC complex

Identifying the types of T cells and the specific T cell receptor (TCR) sequences provides insight into the pathogenesis of the immune destruction of beta cells. Just as the NOD model provided significant insights into insulin as an autoantigen in T1D, the model provides an avenue to understand how TCRs recognize insulin-MHC complexes. T cells recognize antigens via an interaction between a TCR, comprised of a TCR alpha and beta chain, and peptide-MHC complex. Compared to insulin peptides and MHC molecules, we know relatively little about the association of TCRs with T1D. However, cumulative evidence suggests that skewed TCR sequences preferentially respond to specific autoantigens including B:9-23. Alpha and beta chain genes encode up to 100 different variable (TRAV, TRBV) and junctional (TRAJ, TRBJ) segments. Individual T cells assemble TCRs with multiple nucleotides (N region) between these two segments. Millions of TCR sequences are theoretically possible, and approximately 70% of TCRs reacting with B:9-23 in NOD mice utilize a conserved TCR alpha chain sequences containing identical variable (TRAV5D-4)

and junctional (TRAJ53/42) regions (29;55). Two alpha chains that contain these conserved regions but with different N regions can induce insulin autoantibodies *in vivo* when introduced into NOD mice lacking native alpha chains, whereas a beta chain derived from an insulin B:9-23 reactive TCR (12-4.1) does not (56). The current evidence suggests that the conserved alpha chain sequence recognizes the insulin peptide-I-$A^{g7}$ complex by paring with multiple different beta chains, which may predispose to islet autoimmunity. In humans, skewed TCR usage for T cells recognizing insulin A:1-15 and other potential islet antigens have been observed (20;57;58).

A basic question is what makes conserved TCRs target self antigens. Analyzing crystallography of the trimolecular complex is the direct way to understand how TCRs recognize peptide/MHC complexes. Crystallization of a TCR-insulin-MHC complex is yet to be completed. However, five autoimmune CD4 TCRs that react with myelin basic protein (autoantigen for multiple sclerosis) have been solved. All of these structures reveal low binding affinity either between the TCR and peptide-MHC complex or between the peptide and MHC (59-62). Two of the TCRs that have a poor affinity for the peptide-MHC complex bind in an unusual fashion. These binding motifs with low affinity may allow autoreactive T cells to escape selection in the thymus and lead to autoimmune disorders. Spontaneously occurring insulin B:9-23-reactive T cells can be classified into two subsets (33). One subset can only recognize the specific B:9-23 peptide but not insulin, which suggests that the two types of T cells recognize B:9-23 in a different fashion. Thus, both structural and functional studies indicate that unusual and/or weak binding to peptide-MHC complexes may allow T cells to escape thymic selection and become autoreactive T cells.

Are there skewed TCR sequences in the pancreas that target beta cells? A recent study by Vignali and coworkers demonstrates that only T cells recognizing beta cell antigens infiltrate pancreatic islets (63). With the difficulty in accessing the human pancreas, several studies have examined TCR sequences in the peripheral blood. However, Tisch and coworkers demonstrated that TCR sequences recognizing pancreatic antigens in the peripheral blood, which were not diabetogenic, are different from those in pancreatic islets (64). Sequencing TCRs infiltrating pancreatic islets is important. T cells and TCRs from human pancreata and lymph nodes are less well studied than the organs from animal models. The retroperitoneal location of the pancreas makes it one of the most inaccessible organs in the body. Coupled with the facts that islets make up a very small percentage of cells in the pancreas and are surrounded by exocrine cells with proteolytic enzymes, it is difficult to obtain tissue specimens for analysis. The recent creation of a Network for Pancreatic Organ Donors with Diabetes (nPOD) has been established through the Juvenile Diabetes Research Foundation to obtain pancreata and related tissue specimens from cadaveric organ donors with T1D and those who are autoantibody positive but without clinical disease. This project will help answer many remaining questions. How many T cell clones infiltrate the pancreas? What are the TCR sequences of those clones? Does islet cell antigen presentation occur in peripheral lymphoid organs? Do T cells in the peripheral blood correlate with T cells invading the pancreas? Some of these questions have been already studied in the animal models, but answers to these questions will increase our understanding of the immunopathogenesis of human T1D, provide avenues for T cell specific immunotherapies,

and improve assays to detect and measure the functionality of autoreactive T cells in peripheral blood.

## Conclusion

The anti-insulin trimolecular complex has been defined and well studied in animals. The human counterpart is likely critical for diabetes development but further research needs to be done to define the insulin autoantigens and T cell receptors responsible for islet autoimmunity. With increased understanding of this basic immunology, we are hopeful that type 1 diabetes can be prevented and ultimately cured.

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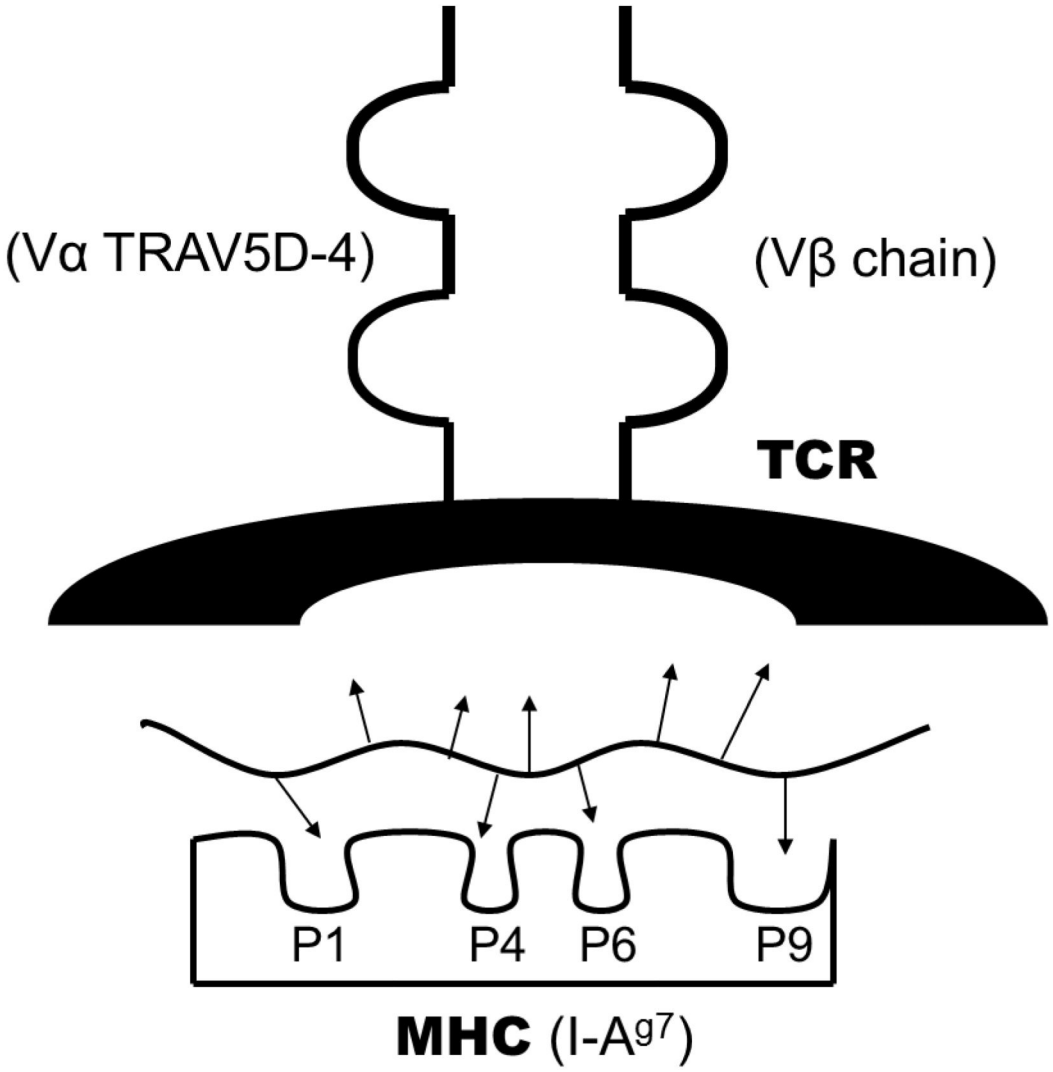


**Figure 1.**

The anti-insulin trimolecular complex is composed of a T cell receptor on CD4 T cells, a peptide, and major histocompatability complex class II molecule. The MHC is composed of a binding groove with pockets that can accommodate amino acid side chains (represented as arrows) of the peptide. The MHC class II binding groove can accompany nine amino acid peptides and contains distinct pockets that interact with peptide side chains. Peptides can have varying affinities for MHC class II molecules depending on the amino acid sequence and alignment in the binding groove. Those peptide side chains not binding to the MHC molecule can interact with the T cell receptor. The components for the non-obese diabetic mouse are denoted in parentheses. MHC = major histocompatability complex, P1-P9 denote pockets, and TCR = T cell receptor.