Molecular Mimicry in Type 1 Diabetes

Immune Cross-Reactivity between Islet Autoantigen and Human Cytomegalovirus but Not Coxsackie Virus

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ABSTRACT: Type 1 diabetes is caused by a T cell–mediated autoimmune destruction of the pancreatic beta cells. Molecular mimicry between viral pathogens and beta cell protein has been a popular theory to explain loss of tolerance in type 1 diabetes. However, functional data in support of this hypothesis have been lacking, presumably because the homologies were defined on the basis of linear similarities in peptide sequences, which ignores the criteria of HLA versus T cell receptor contact residues in peptide epitopes required for T cell recognition. We applied a HLA-binding dedicated peptide microarray analysis using autoreactive T cell clones specific for the autoantigen GAD65 to determine the algorithm of T cell recognition by this given T cell clone. The subsequent database search identified a 100% fit with cytomegalovirus peptide, which was subsequently shown to be recognized by these clonal T cells. However, T cell clones reactive with linear homologies previously described as putative candidates for T cell cross-reactivity between GAD65 and Coxsackie virus peptide were unable to recognize the homologous counterparts.

KEYWORDS: autoreactive T cells; molecular mimicry; microarray; insulin-dependent diabetes mellitus

Viral infections have been associated with the development of the neuroendocrine autoimmune diseases type 1 diabetes and stiff-man syndrome, but the mechanism is unknown. These diseases share glutamic acid decarboxylase GAD65 as a major autoantigen. Antigens of pathogenic microbes that mimic autoantigens are thought to be responsible for the activation of autoreactive T cells.[1] A role of molecular mimicry between GAD65 and Coxsackie virus protein P2C in the pathogenesis of type 1 diabetes mellitus has been suggested.[2] Corecognition of GAD65 and its homologous viral peptide of Coxsackie P2C has been reported on bulk culture level.[3] However, bulk cultures consist of multiple T cells, and an observed cross-reactivity to more

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TABLE 1. Summary of functional consequences of sequence similarities between autoantigen hGAD65 and virus proteins

Source	Position	Peptide sequence	Epitope	Cross-reactivity	Reference
hGAD65	250–273	AMMIARF**K**MF**PEVKEK**GMAA**L**P**RL**	yes	no	Schloot *et al.*[4]
Coxsackie	28–50	FIEWLKV**K**IL**PEVKEK**HEF-**L**S**RL**	yes		
hGAD65	339–352	TVYG**AF**D**PLLA**V**A**D	yes	yes	Hiemstra *et al.*[7]
hCMV	674–687	PYAV**AF**Q**PLLA**Y**A**Y	yes		

than one antigen or peptide might simply reflect T cell proliferation of several T cells with different specificities.

First, we isolated and investigated T cells reactive to GAD65 peptides and homologous peptides of the Coxsackie virus protein P2C from recent-onset type 1 diabetes patients and tested their fine specificity and cytokine production profile. None of four T cell lines reactive to GAD65 peptides (amino acids 247–280) with sequence homology to Coxsackie P2C (amino acids 30–50) cross-reacted to the homologous viral peptide.[4] Two T cell lines corecognized a GAD65 peptide and a Coxsackie P2C peptide. However, the antigen-specific T cell clones from these T cell lines were reacting either with the GAD65 peptide or the Coxsackie P2C peptide using different restriction elements without cross-reacting to the homologous peptide.[4] Our data demonstrate that homologous peptides previously proposed to serve as targets for cross-reactivity indeed are immunogenic. Yet, T cell clones did not cross-react with linear sequence homologies, despite strong T cell responses to individual peptides (TABLE 1).

The hypothesis that sequence homology between GAD65 and Coxsackie B4 virus may lead to T cell cross-reactivity has not been supported by functional evidence at the clonal level thus far. Hence, an association of Coxsackie infection with development of type 1 diabetes could result from direct lytic activity to β cells rather than molecular mimicry. We recently reported a case of acute echovirus infection coinciding with clinical symptoms of juvenile diabetes.[5] Although the viral protein 2C exhibited a sequence similar to that of GAD65, no cross-reactive T cell responses were detected. The patient did not develop antibodies to GAD65 either. Absence of evidence for direct cytolytic action or an indirect effect through molecular mimicry with GAD65 in the present case raises the possibility of another indirect pathway through which enteroviruses can cause diabetes mellitus. It has also been suggested that Coxsackie virus–induced type 1 diabetes is initiated by bystander damage by autoreactive T cells after virus infection.[6]

We then set out to determine the fine specificity of an autoreactive T cell clone reactive with GAD65 and isolated from a prediabetic stiff-man syndrome patient, by screening synthetic peptide libraries dedicated to bind to HLA-DR3, which predisposes to both diseases. A recognition pattern was deduced from the library studies and used for database searching to identify molecular mimics of GAD65.[7–9] A peptide of human cytomegalovirus (hCMV) major DNA-binding protein was identified that stimulated the autoreactive T cell clone. The hCMV-derived epitope can be naturally processed and recognized by GAD65-reactive T cells (TABLE 1).[10]

Clinical onset of type 1 diabetes and SMS has been reported to be accompanied by acute hCMV infection.[11] Recurrent insulitis and autoimmune β cell destruction

in pancreatic allografts presented with a predominant fraction of infiltrating T cells reactive to hCMV.[12] Also, hCMV infection of mice resulted in the generation of autoantibodies directed to the islets of Langerhans.[13] The mechanism by which hCMV infection contributes to neuroendocrine autoimmunity is unknown. hCMV has been shown to infect β cells and neuronal tissue as well as peripheral blood mononuclear cells. Alternatively, systemic hCMV infection could lead to the activation of $CD4^+$ T cells by presentation of hCMV peptide in the context of HLA-class II. Via molecular mimicry these T cells could then cross-react with GAD65 of neural cells, leading to autoimmune disease. We demonstrate that T cells reactive to GAD65 cross-react with a peptide of the human cytomegalovirus major DNA binding protein. This is the first evidence that human cytomegalovirus may be involved in the loss of tolerance to autoantigen GAD65 by a mechanism of molecular mimicry leading to autoimmunity.[10]

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