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Structural Homology of HHV-6 Epitopes as Candidates for Molecular Mimicry Triggers of Type One Diabetes Mellitus Onset

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Abstract

Molecular mimicry is a mechanism by which an infectious agent may trigger an autoimmune response through structural or sequence similarity to self-antigens. Identifying potential molecular mimics is critical to understanding autoimmune disorders, including type 1 diabetes mellitus (T1DM). Human herpesvirus 6B (HHV-6B), a common childhood virus implicated in multiple autoimmune conditions, has been epidemiologically linked to increased risk of T1DM. However, the connection between HHV-6B and T1DM remains unresolved, as HHV-6B epitopes listed in public databases show minimal sequence homology with known T1DM autoantigens. This study explores whether HHV-6B can act as a molecular mimic by assessing structural and electrostatic similarities between HHV-6B-derived and T1DM GAD65-derived epitopes. Epitope peptide structures and their interactions with HLA-DRB5*0101, a class II MHC allele linked to T1DM, were modeled using Boltz-1 and ChimeraX. HHV-6B epitopes studied here demonstrated high structural alignment with T1DM epitopes (RMSD < 1.0 Å) and shared electrostatic surface features. When modeling the epitope complexes with HLA-DRB5*0101, the HHV-6B and T1DM epitopes fit into the same binding region, suggesting a plausible mechanism for T cell cross-reactivity. These findings provide computational evidence that HHV-6B may act as a molecular mimic contributing to autoimmune responses in T1DM genetically susceptible individuals. This study demonstrates that structural modeling is a useful tool for identifying potential mimicry candidates that sequence-based methods may not find, underscoring the importance of integrating structure-based modeling into molecular mimicry prediction pipelines. Future research should explore additional HHV-6B epitopes and incorporate molecular docking to validate their pathogenic potential.

Introduction

An increasing body of evidence implicates molecular mimicry as a contributor to autoimmune diseases (ADs), including type 1 diabetes mellitus (T1DM) [1-4]. Molecular mimicry arises when foreign peptides share similarities with self-antigens, triggering a cross-reactive immune response. Although the etiology of T1DM is not fully understood, it likely involves an interplay of genetic, environmental, dietary, and viral triggers [5-6]. Studying molecular mimicry enhances understanding of T1DM onset and progression, guiding the development of targeted strategies for disease prevention and management.

Human herpesvirus 6 (HHV-6) exhibits a high global prevalence and has been frequently linked to autoimmune diseases, including multiple sclerosis [7-8], celiac disease [9], Hashimoto's thyroiditis [10], and systemic lupus erythematosus [11-12]. Primary infection of HHV-6 affects more than 90% of individuals within the first two years of life, and adult seroprevalence exceeds 95% [13]. Following

primary infection, HHV-6 establishes lifelong latency, and may reactivate and cause further complications to a wide range of cell types [14-15].
First discovered in 1985, HHV-6 was reclassified in 2012 into two distinct species: Human herpesvirus 6A (HHV-6A) and Human herpesvirus 6B (HHV-6B) [16]. The lack of clear distinction between these two variants in existing literature makes etiologic associations difficult to assess, so differentiation between the two, whenever possible, leads to more effective interventions. In the USA, UK and Japan, 97-100% of primary infections by these two viruses were caused by HHV-6B [17-20].
In a previous study, Mistry et al. [21] found that individuals in The Environmental Determinants of Diabetes in the Young (TEDDY) study cohort [22] who experienced a single episode of sixth disease (the clinical presentation of HHV-6B infection), at 12 months of age had a 4.49-fold increased risk of developing T1DM. Paradoxically, no HHV-6B epitopes currently listed in the Immune Epitope Database (IEDB) [23] appear to show meaningful homology with known autoimmune targets [24], including Glutamic Acid Decarboxylase (GAD65), a key autoantigen associated with T1DM onset [25-26].
Bach et al. [25] reported strong reactivity of GAD65-specific T cells toward several epitope candidates, including one from HHV-6. These contradictory findings may be explained because the results of the Bach study are not included in the IEDB. Moreover, the GAD-65 (GAD:248-259 and GAD:246-257) and HHV-6B U2 epitopes in that study exhibit low sequence homology [25]. Nevertheless, structural homology between GAD65 and infectious peptides (such as HHV-6B), even without amino acid similarities, can still produce a cross-reactive response [2,27-29].
The purpose of this study is to evaluate structural homology between the HHV-6B epitope identified in Bach's study and the corresponding GAD65 epitopes, and to assess their binding potential to HLA-DRB5*0101, using Boltz-1 [30] and Chimera-X [31] modeling tools.

Methods

The study by Bach et al. [25] identified candidate mimicry peptides by screening protein databases using epitope motifs derived from GAD65 sequences and performing the corresponding immunological studies. Of interest here, this study focuses on three key epitopes with little apparent sequence homology: GAD:248-259 (epitope 3), GAD:246-257 (epitope 4), and HHV-6 U2 (epitope 7), using the numbering from Table 2 in [25].

Table 1: Bach Epitopes Considered (from Table 2 in Bach et al.)

Identification #	Epitope Name	Sequence
3	GAD65 (248-259)	MYAMMIARFKMF
4	GAD65 (246-257)	SNMYAMMIARFK
7	Human herpesvirus-6 U2	GGVAVVIGRFFG

Boltz-1 [30] is an open-source deep learning model that predicts biomolecular structures and binding sites with accuracy comparable to AlphaFold3 [27]. Boltz-1 uses a diffusion-based generative approach to predict protein structures and potential binding sites based on amino acid sequences provided in FASTA format [32-33].
In this study, Boltz-1 (v.0.4.1) was executed on NVIDIA H100 NVL GPUs (94 GB HBM3 memory) with 64 GB of system RAM per node, using the Granite GPU cluster at the Utah Center for High Performance Computing (https://www.chpc.utah.edu/).
FASTA files were created for the three epitopes considered here (Table 1). The sequence for the HLA-DRB5*0101 molecule, known to bind GAD65-derived epitopes with high affinity [25,34], was obtained from UniProt (https://www.uniprot.org/) and converted into FASTA format. Boltz-1 calculations were performed for all three epitopes, the HLA-DRB5*0101 molecule, and the HLA-DRB5*0101 molecule with each individual epitope.

Boltz-1 generates four structural models per input, but all results presented here are based on the first model, which had the highest accuracy. Visualization, root mean square deviation (RMSD), and electrostatic surface potential calculations were performed with UCSF ChimeraX (v.1.10rc202505210000) [31]. RMSD values were computed using the "matchmaker" tool in ChimeraX, which superimposes protein or nucleic acid structures through pairwise sequence alignment [35]. Electrostatic surface potentials were calculated using ChimeraX's Coulombic Surface Coloring tool.

Results

In all cases selected for analysis, model 0 from Boltz-1 consistently exhibited the highest confidence scores, and these models were used in subsequent analyses.

3.1 Structural Superposition of Isolated Epitopes

Superposition of the structures of the two GAD65 protein epitopes and the HHV-6 epitope revealed a structural match with RMSD values of 0.788 Å and 0.616 Å, respectively, shown in Table 2. These isolated structures each share a coil secondary structure, as depicted in Figure 1.

Table 2: RMSD Calculations between the GAD65 protein epitopes and the HHV-6 epitope.

Epitope #1	Epitope #2	RMSD (Å)	Amino Acid Pairs
4	3	0.674	10
7	3	0.788	11
7	4	0.616	11

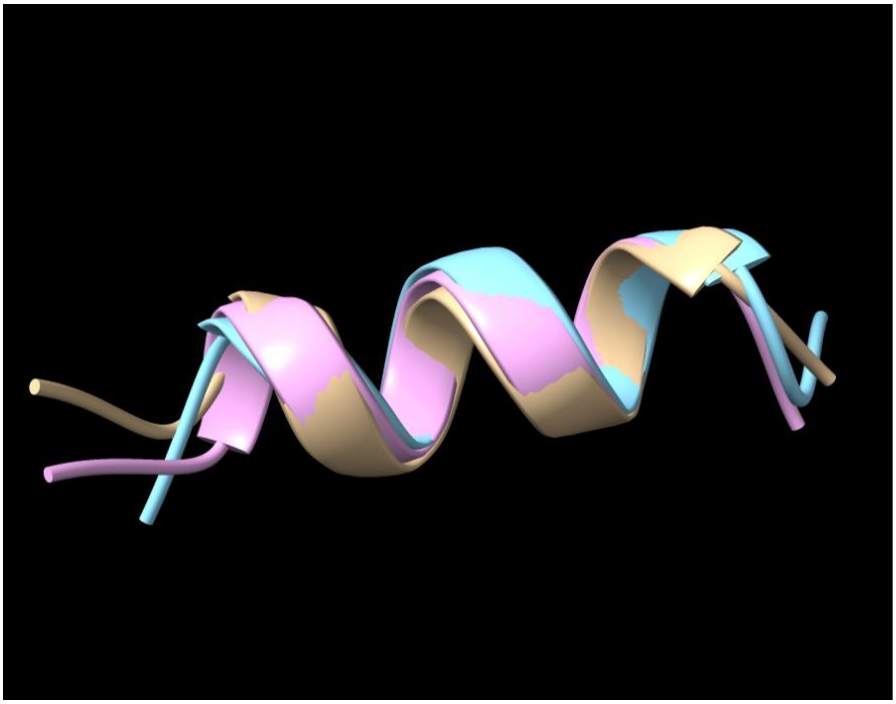

Figure 1: Comparison of the Boltz-1 predicted structures of the isolated epitopes GAD65(248-259) (brown) and GAD65(246-257) (pink) with the HHV-6 (blue) epitope. The corresponding RMSDs are: 0.788 Å and 0.616 Å, respectively. Boltz-1 confidence scores for these isolated epitope structures are 0.55, 0.59, and 0.60.

3.2 Electrostatic Surface Potentials

Electrostatic potential mapping revealed that the epitopes shared similar minimum, mean, and maximum Coulombic values, exhibited in Table 3. These comparable charge distributions indicate that the epitopes will bind to HLA-DRB5*0101 in a related fashion.

Table 3: Coulombic Values of the GAD65 protein epitopes and the HHV-6 epitope in kcal/(mol·*e*) at 298 K.

Epitope	**Minimum**	**Mean**	**Maximum**
3	-11.66	2.47	10.72
4	-10.42	2.43	10.11
7	-11.38	1.19	11.41

3.3 Epitope-HLA Complex Structures

The structural models generated by Boltz-1, for each epitope-MHC (HLA-DRB5*0101) complex are presented in Fig. 2. The conformation of the HLA-DRB5*0101 molecules (grey) does not change significantly upon binding to the epitopes, but all the epitopes show structural reorganization, losing the coil conformation observed in their isolated predicted structures. The binding location of the three epitopes is consistent with existing evidence of binding sites for other well-characterized HLA-epitope complexes [36].

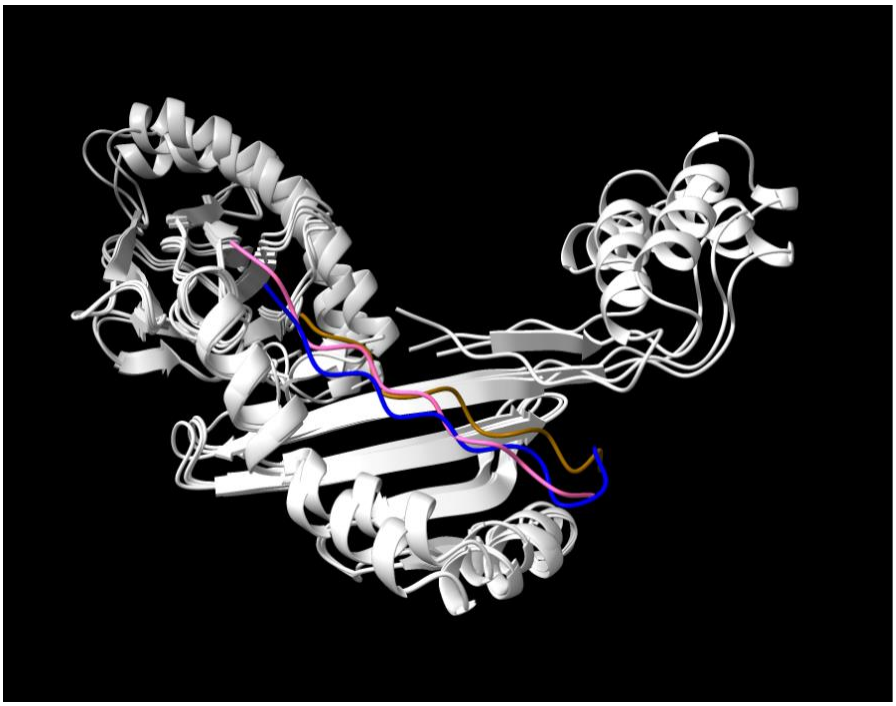

Figure 2: Superimposed conformations of epitopes GAD65(248-259) (brown), GAD65(246-257) (pink), and HHV-6 (blue) bound to HLA-DRB5*0101 (grey). For the predicted structure of these complexes, Boltz-1 confidence scores were 0.732, 0.713 and 0.687, which are higher than the scores for the isolated HLA-DRB5*0101 molecule (0.575) and for the isolated epitopes (see Fig. 1).

Discussion

This study builds on the findings of Bach et al. [25] by demonstrating that peptides with low sequence similarity can nonetheless exhibit significant structural and electrostatic homology, resulting in good candidates for molecular mimicry. Using Boltz-1 and ChimeraX, we show that GAD65-derived and HHV-6B epitopes share similar electrostatic surface potentials, demonstrating that these epitopes may bind with comparable affinity to HLA-DRB5*0101, a class II MHC allele previously linked to T1DM onset [25,34]. This electrostatic resemblance supports the potential for a mechanistic basis for T cell cross-reactivity.

These findings support clinical results derived from the TEDDY [22] cohort, which reported a 4.49-fold increased risk for T1DM following HHV-6B infection in infancy [21]. These findings provide computational evidence supporting HHV-6B as a plausible molecular mimic contributor to T1DM pathogenesis.

The in-silico pipeline developed here can be adapted to identify other molecular mimics based on structural and electrostatic similarity, even without sequence homology. This approach may have wide applicability, given that molecular mimicry has been implicated in the pathogenesis of multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, Sjogren's disease, systemic sclerosis,

and autoimmune hepatitis [2]. HLA-DRB5 molecules have also been linked to multiple sclerosis [37] and immune thrombocytopenia [38], so continuing to investigate HLA-epitope complexes may identify biomarkers and suppressors for autoimmune disease.

Limitations

While Boltz-1 provided reasonable structural predictions for all peptide and complex models, with confidence values ranging from 0.549 to 0.721, it is difficult to evaluate the tool's exact accuracy as there is very little known about Boltz-1 modeling short peptide interactions. Further exploration using established methods for molecular binding and docking is necessary to confirm the biological relevance of Boltz-1 in predicting molecular mimicry.

Conclusion

Using novel bioinformatics tools, this study demonstrates that peptides lacking sequence homology can still exhibit significant structural and electrostatic similarity. These features may enable molecular mimicry, contributing to the onset of autoimmune diseases such as T1DM. Moreover, the results align with previous work that demonstrated that molecular mimicry could occur between structurally similar peptides without significant sequence homology [25].

While previous studies have shown that investigating sequence homology is an efficient and less computationally expensive approach for identifying potential mimics [24,39], and that many candidates showing sequence homology also show structural homology [40], this work highlights that structural modeling is essential for uncovering mimicry candidates that sequence-based methods may not find. These findings emphasize the importance of integrating structure-based modeling into molecular mimicry pipelines, especially in the context of autoimmune disease research.

These results support the hypothesis that HHV-6B infection may contribute to the onset or exacerbation of autoimmune diseases through molecular mimicry. Future studies should investigate additional HHV-6B-derived epitopes using molecular docking to evaluate the generalizability and potential immunogenicity of these mimicry interactions.

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