UL144-derived peptides as modulators of the BTLA/HVEM signaling pathway

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INTRODUCTION

Immune checkpoint blockade is one of the strategies used to treat cancer and autoimmune diseases. One of the immune checkpoint receptors is B and T lymphocyte attenuator (BTLA), which forms a complex with herpes virus entry mediator (HVEM) (Fig. 1A). It is confirmed that the BTLA/HVEM complex plays a negative role in the tumor microenvironment and some autoimmune diseases [1,2].

BTLA also interacts with the human cytomegalovirus glycoprotein UL144 (Fig. 1C), which exhibits noticeable homology to the HVEM protein (Fig. 1B). UL144 binds to a site on BTLA that overlaps with HVEM's binding site, and in consequence is capable of disrupting the interaction of BTLA/HVEM [3].

This study aimed to design and synthesize peptide analogues of the UL144 protein based on the crystal structure of the BTLA/UL144 complex and molecular mechanics/generalized Born surface area (MM/GBSA) analysis conducted for this complex. To determine the inhibitory properties of the peptides, we performed enzyme-linked immunosorbent assays (ELISAs). Besides, we evaluated binding properties of the peptides to BTLA protein with microscale thermophoresis (MST). These results can help determine the therapeutic potential of the examined compounds.

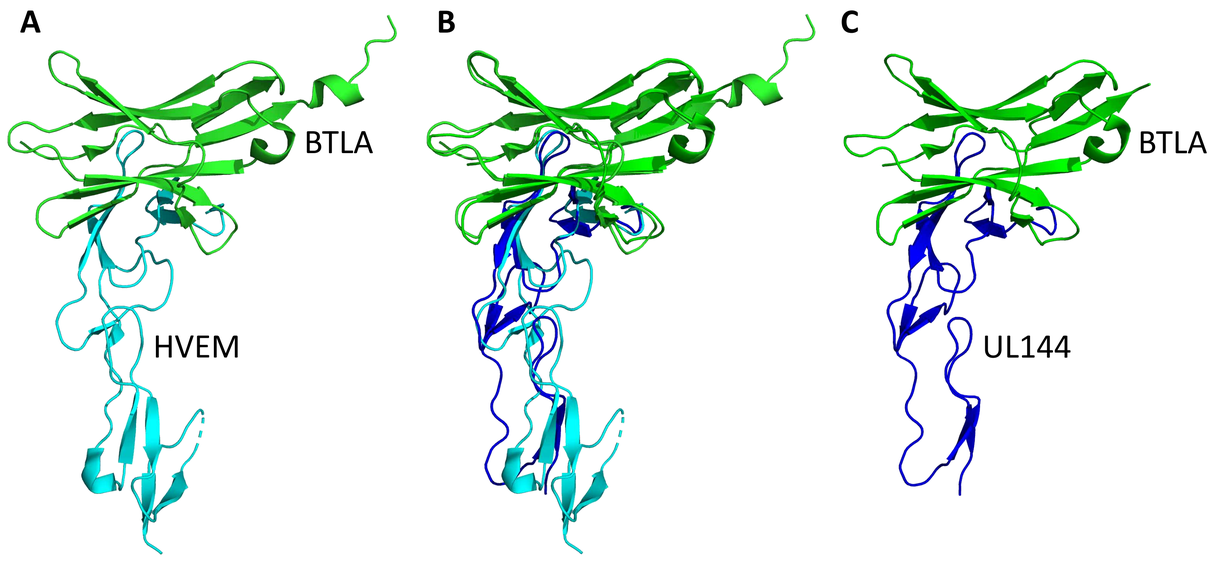

Fig. 1. The crystal structure of (A) BTLA/HVEM complex (PDB code: 2AW2), (B) alignment of both complexes and (C) BTLA/UL144 complex (PDB code: 6NYP).

METHODS AND RESULTS

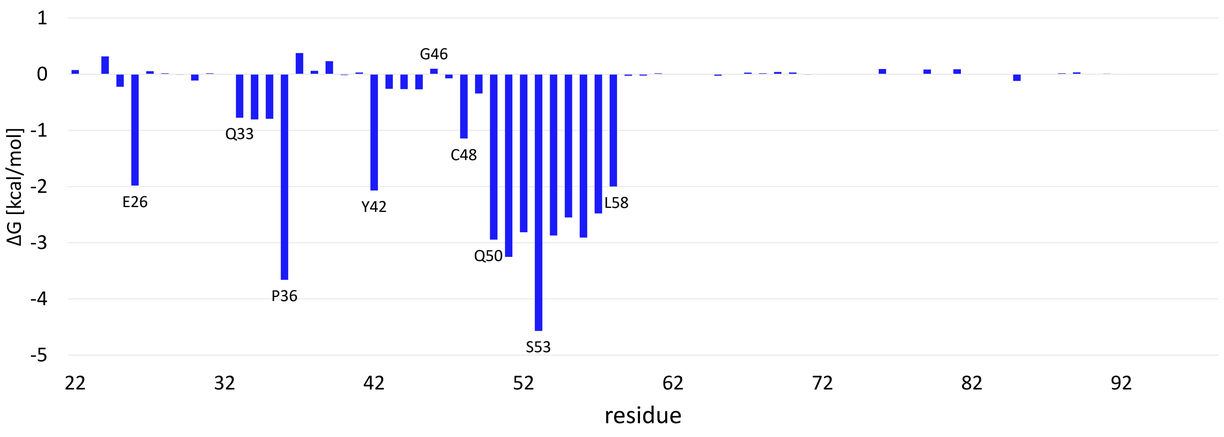

Fig. 2. Representation of the key UL144 amino acid residues involved in the BTLA/UL144 complex formation, determined by per-residue energy decomposition.

1. Design and synthesis of the peptides

To define which amino acid residues of the UL144 protein are crucial for binding to BTLA, we performed the MM/GBSA analysis. The results showed that residues E26, Q33-P36, Y42, C48, and Q50-L58 in UL144 are significant for the interaction with BTLA (Fig. 2). According to Šedý et al., mutation of residue G46 to lysine (G46K) in the UL144 protein significantly increases binding affinity to BTLA, due to a new salt bridge formation [4].

Considering the crystal structure of the BTLA/UL144 complex, MM/GBSA results and reference data, we designed eight peptides containing 0, 1, or 2 disulfide bonds – potential inhibitors of the BTLA/HVEM complex formation (Tab. 1).

The designed peptides were synthesized using solid-phase peptide synthesis (SPPS) and Fmoc/tBu strategy. The synthesis was conducted with an automated microwave peptide synthesizer (Liberty Blue, CEM Corporation). Peptides were then purified with RP-HPLC, oxidized to obtain disulfide bonds (except linear peptides), and purified again.

2. Inhibitory properties of the peptides

To investigate whether the peptides are able to inhibit BTLA/HVEM complex formation, we performed enzyme-linked immunosorbent assays. We used Thermo Scientific™ 96-well NUNC™ Maxisorp™ Immuno Plates for that purpose. First, the BTLA-His protein was immobilized in microplates. After adding the blocking agent (5% BSA), the peptides were added at four different concentrations (300 µM, 150 µM, 75 µM and 37,5 µM) and incubated with BTLA. Unbound peptides were then removed, and HVEM-Fc protein was added. To detect HVEM-Fc, a goat anti-human IgG (H+L)-HRP conjugate and TMB ULTRA substrate (3,3',5,5'-tetramethylbenzidine) were applied.

The results show that all peptides except UL144(K1) have concentration-dependent inhibitory properties towards the BTLA/HVEM complex formation (Fig. 3). The most efficient inhibition display UL144(K2) and UL144(1).

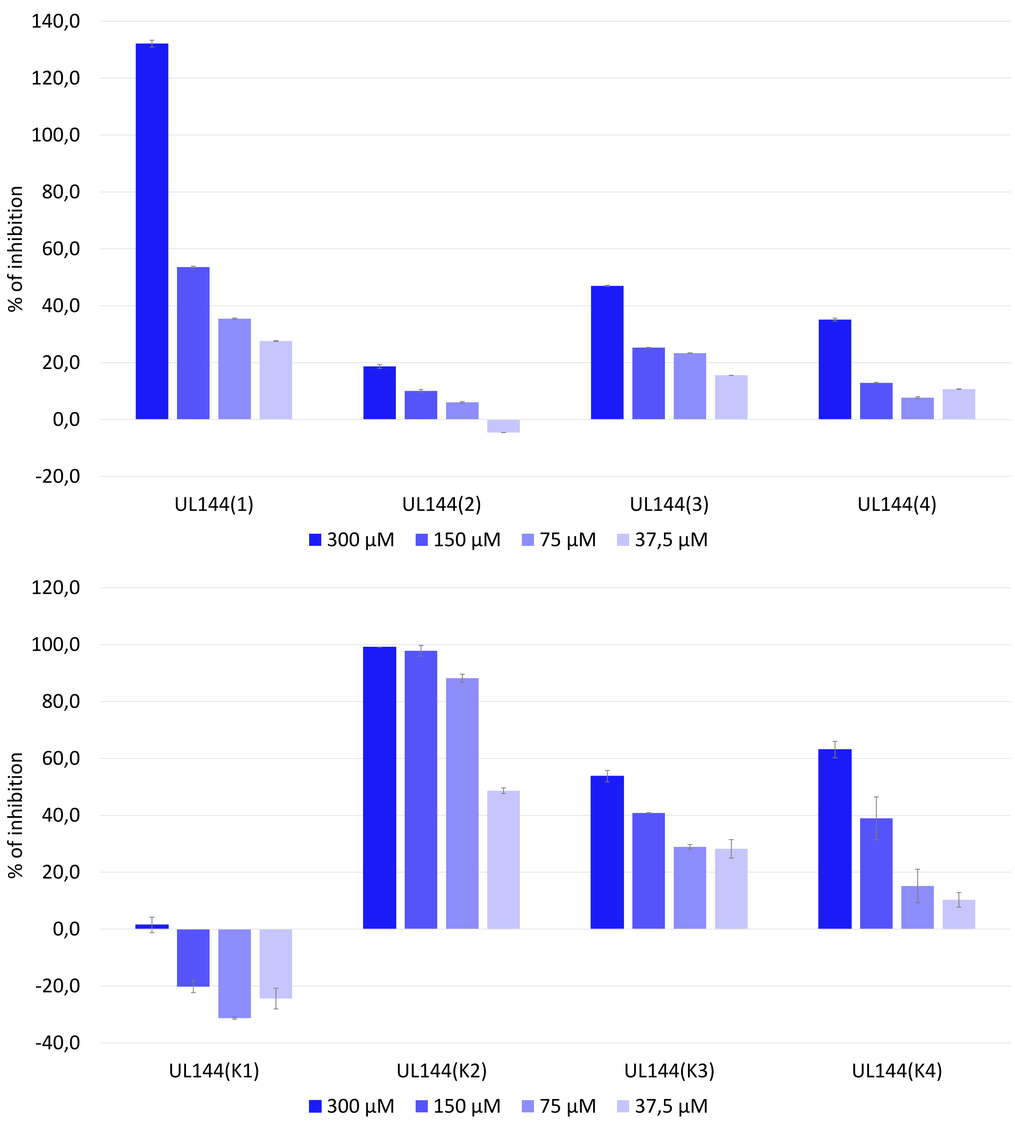

Fig. 3. The inhibitory properties of UL144-derived peptides, obtained from ELISAs.

Tab. 1. Comparison of the designed UL144-derived peptides and their dissociation constants (K_d) obtained with microscale thermophoresis (MST).

Abbreviation	UL144(1)	UL144(2)	UL144(3)	UL144(4)
Disulfide bonds	0	1	1	2
K_d obtained with MST	46,9 µM	500 µM	119 µM	201 µM
Abbreviation	**UL144(K1)**	**UL144(K2)**	**UL144(K3)**	**UL144(K4)**
Disulfide bonds	0	1	1	2
K_d obtained with MST	1,23 mM	15,9 µM	182 µM	236 µM

3. Binding affinity assays

To check the binding affinity of evaluated peptides to BTLA protein, we performed microscale thermophoresis (MST) with Monolith X (Nanotemper). The findings of the assays correspond to ELISAs. Peptides UL144(K2) and UL144(1) have the highest binding affinity to BTLA. Table 1 sums up all the obtained dissociation constants (K_d).

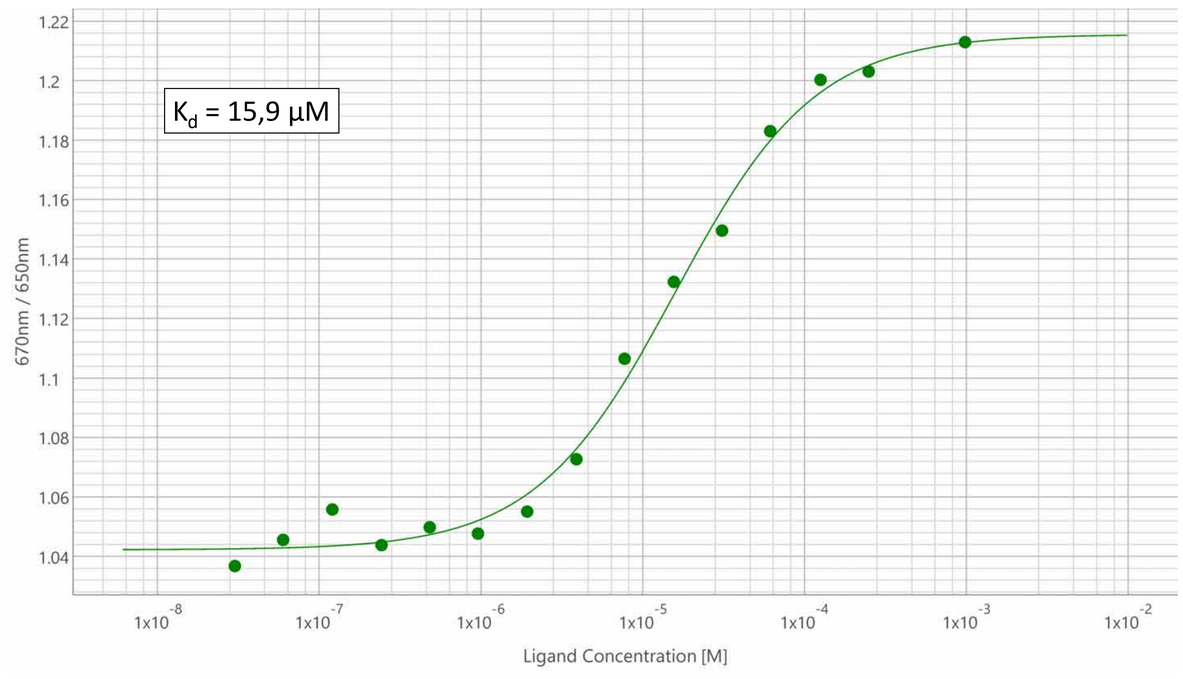

Fig. 4. Dissociation constant (K_d) obtained for UL144(K2) with MST.

CONCLUSIONS

- ✓ BTLA/HVEM protein complex is an interesting molecular target in the field of immune checkpoint blockade.
- ✓ MM-GBSA analysis showed that amino acid residues of the UL144 protein E26, Q33-P36, Y42, C48, and Q50-L58 are crucial for the interaction with BTLA.
- ✓ ELISAs and MST assays showed that UL144(K2) and UL144(1) bind most strongly to the BTLA protein and have the best inhibitory properties.
- ✓ UL144-derived peptides have the potential to become inhibitors of the BTLA/HVEM complex formation, and further investigation, including cellular assays, will determine its therapeutic properties.

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