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Secondary anchor substitutions in an HLA-A*0201-restricted T-cell epitope derived from Her-2/neu

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Abstract

We investigated analogues of GP2 (IISAVVGIL), an HLA-A*0201-restricted T-cell epitope derived from residues 654–662 in the tumor-associated antigen (TAA) Her-2/neu. One limiting factor of GP2 is its poor affinity for HLA-A*0201. Conformational analysis revealed the P5–P7 region in GP2 appears to be linked to the stability of P9 side chain interaction with the MHC molecule. To identify variants of GP2 with enhanced presentation to HLA-A*0201, we tested V6S, V6T, V6Q, G7P, G7F, T6F7, and Q6F7 for their capacity to stabilize cell surface HLA-A*0201 molecules. Of the mono-substituted variants, V6Q and G7F exhibited superior stabilization as compared to GP2. Molecular dynamics simulations suggest the improved binding can be attributed to concerted motions in the central and C-terminal regions of the peptide. These data support the notion that amino acids in HLA-A*0201 epitopes may be inter-dependent. Priming HLA-A*0201 transgenic mice with G7F-loaded syngeneic dendritic cells stimulated mouse T cells to produce a higher level of INFγ than mice immunized with GP2.

Keywords: HLA-A*0201; Tumor vaccine; Altered peptide ligand; Her-2/neu; Molecular dynamics

1. Introduction

The proto-oncogene product Her-2/neu is over-expressed in several tumor types, including ovarian and breast cancers (Slamon et al., 1989). Peptides derived from Her-2/neu can induce CD4 and CD8 T-cell responses (Baxevanis et al., 2005; Disis et al., 1999; Peiper et al., 1997, 1999). IISAVVGIL (denoted GP2), one of several human class I HLA-A*0201-restricted T-cell epitopes identified in Her-2/neu (residues 654–662), is recognized by CD8 lymphocytes infiltrated into malignant breast tissues (Peoples et al., 1995; Yoshino et al., 1994). A limitation of GP2 as a tumor vaccine candidate is its poor affinity for HLA-A*0201 (Rongcun et al., 1999).

Poor MHC binding does not necessarily diminish relevancy of an epitope in the context of tumor vaccination. Selective amino acid replacements in the native sequence can sometime increase antigen presentation and results in more immunogenic peptides. Affinity thresholds of T-cell receptor activation in vitro and/or in vivo may be reached by increasing the number of copies of a given MHC–peptide complex (Abrams and Schlom, 2000; Collins and Frelinger, 1998; Meng and Butterfield, 2002; Meng et al., 2001; Sette et al., 1994a, 1994b; van der Burg et al., 1996), providing that the antigenic surface is recognized by the TAA-reactive T cells. Altered peptide ligands of $MART_{27–35}$ and $gp100_{206–214}$ are examples of successful extension of this strategy to the clinic (Bakker et al., 1995; Parkhurst et al., 1996; Rosenberg et al., 1998). With these in mind, the GP2 sequence may be exploited by identifying variants with improved MHC binding because tolerance to sub-dominant epitopes are purported to be less pronounced than dominant epitopes.

Abbreviations: MD, molecular dynamics; MHC, major histocomptability complex; DC, dendritic cells

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Poor binding of GP2 was unexpected because the sequence possesses appropriate amino acids defined by the binding motif of HLA-A*0201: aliphatic hydrophobic side chains at P2 (peptide positions[1] are abbreviated as P1, P2, P3, etc.) and P9 (Parker et al., 1994; Ruppert et al., 1993), referred as primary anchors. The X-ray structure of GP2 complexed with HLA-A*0201 provided an explanation for the poor binding: the central region of the peptide (P5 and P6) is flexible and lacks stabilizing contacts with residues in the MHC binding cleft (Kuhns et al., 1999). Substitutions at P2 and P9 anchor positions resulted in marginal or no improvement in binding affinity (Sharma et al., 2001). Substituting the valine at P5 with leucine also did not confer improvement in binding (Sharma et al., 2001).

Herein we describe an effort to explore effects of secondary anchor substitutions in the GP2 sequence guided by molecular dynamics (MD), a computational technique used to simulate conformational motion of peptides in the MHC binding groove (Meng et al., 1997, 2000a, 2000b; Rognan et al., 1994). We characterized a panel of GP2 variants substituted at P6 and/or P7 with respect to binding on HLA-A*0201-expressing cells. MD simulations revealed correlated motions between the P5–P7 backbone and the anchor P9 side chain. G7F, a variant with improved binding was shown to induce GP2-specific T cells in HLA-A*0201 transgenic mice.

2. Methods and materials

2.1. Cell line, peptides, and reagents

T2 and JY cells were used to determine the relative strength of the peptides in stabilizing HLA-A2.1 molecules. T2 cells are deficient in the transporter-associated with antigen presentation (TAP) and do not present self-peptides but express "empty" or unstable HLA-A*0201 molecules on the surface (Salter and Cresswell, 1986). The "empty" unstable MHC molecules can be stabilized with addition of exogenous peptides and beta-2 microglobulin (β2m) (Stuber et al., 1994). JY cells express high densities of HLA-A*0201 and were a kind gift of Dr. Lisa Butterfield (University of Pittsburgh). Both cell lines were maintained in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin/fungizone (Life Technologies). Peptides were custom synthesized from Peptron Inc. (South Korea) or EZBiolab (Westfield, IN) with purity greater than 95%.

2.2. T2 stabilization and JY re-constitution assays

Fluorescein isocyanate-labeled β2m (β2m-FITC) was used as an indicator of peptide binding. Human β2m (Sigma–Aldrich, St. Louis, MO) was conjugated with FITC using a labeling kit (Sigma–Aldrich). Reaction products were fractionated using size exclusion columns to remove free FITC molecules and fractions containing β2m-FITC were pooled. Binding of peptides on T2 cells was determined by incubating 5×10^5 cells with peptide (1 μM) and β2m-FITC (1 μg/ml) for 2 h at 37 °C in AIM-V Lymphocyte Media (Life Technologies). JY cells (5×10^5) were first exposed to citrate-phosphate buffers (0.131 M citric acid/0.066 M Na_2HPO_4, pH adjusted to 3.2) prior to adding peptide and β2m-FITC. After extensive washing with cold PBS (0.01% sodium azide) to remove unbound β2m-FITC, cells were analyzed using a Kodak 440 CF Image Station, Beckman Coulter EPICS XL flow cytometer, or a Perkin-Elmer HTS 7000 Bio Assay reader. Statistical analysis was performed using one-way ANOVA.

2.3. Molecular modeling and dynamics

Model construction and calculations were performed using the AMBER89 force field (Pearlman et al., 1991) in the molecular modeling package molecular operating environment (MOE) version 2001.01 (Chemical Computing Group, Montreal, Canada) on a Silicon Graphics Indigo2 workstation. Each simulation was performed at least twice.

Structural models of variant peptides complexed to HLA-A*0201 were built using the X-ray structure of IISAVVGIL/HLA-A*0201 (pdb entry: 1 qri) as the template (Kuhns et al., 1999). Only the peptide and α1 and α2 domains of the MHC were included in the calculations. Amino acid substitutions were implemented by replacing the side chain atoms beyond the Cβ atom in the original residue described previously (Meng et al., 2000a, 2000b). The mutated amino acid was subjected to conjugate gradient minimization until the structure reached a root-mean square gradient of 0.01 Å. This was followed by "soaking" the complex to identify water molecules that can be placed at the MHC–peptide interface. The water molecules were minimized before the entire system was allowed to relax. Following the minimizations, only interior water molecules at the MHC–peptide interface were retained. Two water molecules were added in V6Q/HLA-A*0201 and one water was placed in GP2. No water was added at the interface in G7F/HLA-A*0201. The complexes were then carried out for 200 picosecond (ps) of MD simulations using the NVT ensemble at 300 K. Backbone atoms of the MHC molecule were "fixed" in these simulations. Dielectric constant (ε) of 80 was set to simulate the electrostatics of aqueous environment and a 6.5 Å non-bonded cut off was used in the calculations. Analysis of the MD trajectories was conducted using the "Conformational Geometries" function and "SVL" routines in MOE.

We have previously determined that in performing MD simulations of class I MHC–peptide complexes, it is necessary to include constraints to maintain conserved hydrogen bonds between the peptide termini and the MHC molecule (Meng et al., 1997, 2000a, 2000b). The interactions at the charged peptide termini are conserved in HLA-A*0201–peptide complexes (Bouvier and Wiley, 1994; Madden et al., 1993) and therefore we do not expect the restrains to interfere with interpretation of the trajectories. A similar type of restrain was used in the current study, with an energy penalty placed for distance deviation

[1] Positions in peptide are abbreviated as P1, P2, P3, etc. Specific amino acids in peptide are indicated with one-letter code followed by position in the sequence (e.g. V6, F7). Amino acids of the MHC molecule are represented with three-letter code followed by position in the protein (e.g. Arg-97, Tyr-116).