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A Mechanistic Model for Predicting Cell Surface Presentation of Competing Peptides by MHC Class I Molecules

Denise S. M. Boulanger†, Ruth C. Eccleston2,3†, Andrew Phillips4, Peter V. Coveney2,3, Tim J. Elliott† and Neil Dalchau4*

†These authors have contributed equally to this work.

Major histocompatibility complex-I (MHC-I) molecules play a central role in the immune response to viruses and cancers. They present peptides on the surface of affected cells, for recognition by cytotoxic T cells. Determining which peptides is presented, and in what proportion, has profound implications for developing effective, medical treatments. However, our ability to predict peptide presentation levels is currently limited. Existing prediction algorithms focus primarily on the binding affinity of peptides to MHC-I, and do not predict the relative abundance of individual peptides on the surface of antigen-presenting cells in situ which is a critical parameter for determining the strength and specificity of the ensuing immune response. Here, we develop and experimentally verify a mechanistic model for predicting cell-surface presentation of competing peptides. Our approach explicitly models key steps in the processing of intracellular peptides, incorporating both peptide binding affinity and intracellular peptide abundance. We use the resulting model to predict how the peptide repertoire is modified by interferon-γ, an immune modulator well known to enhance expression of antigen processing and presentation proteins.

Keywords: antigen presentation, major histocompatibility class I, mechanistic model, interferon-γ, peptide competition, abundance

INTRODUCTION

Cellular immunity has a major role in resistance to infection and cancer. CD8 T cells play an important part, by recognizing protein fragments (peptides) that are generated within an infected or cancerous cell and presented on the cell surface by class I major histocompatibility complex (MHC-I) molecules. The recognition of a specific peptide bound to MHC-I, called a peptide–MHC complex (pMHC), is achieved by the T cell receptor (TCR). The abundance of infection- or cancer-specific pMHC complexes on the cell surface is a key factor in the development of an efficient T cell response, where high abundance has been associated with an immunodominance phenomenon in which immune responses focus on only a few of the many potential peptides (1, 2). Other factors influencing the efficiency of the T cell response include the frequency of T cell precursors of a certain specificity (3), and the affinity of the TCR to its multiple target pMHC complexes. In viral infections, additional
Poor correlations between cell surface abundance of pMHC and each of these factors individually have been observed [source protein abundance (25, 30) and peptide affinity (25)]. We hypothesize that these factors need to be appropriately incorporated within a mechanistic model in order to obtain good predictions.

We have previously developed mathematical models that simulate cell surface abundance of multiple peptides bound to MHC-I, at steady-state on the surface of living cells, and incorporate variations in peptide supply and peptide affinity to MHC-I (31, 32). In this context, a high affinity peptide is defined as having a low off-rate, unbinding slowly from MHC-I. The models were used to interpret how tapasin could preferentially select peptides that form stable complexes with MHC-I molecules, and further suggest how MHC haplotypes differ in the extent of their tapasin-mediated selection, some haplotypes have the intrinsic ability to select and assemble with optimal peptides independent of tapasin whereas others are dependent of tapasin to be stably loaded. A key quantitative prediction of the models was that equilibrium cell surface abundance of a given peptide ($P_i$) bound to MHC at the cell surface ($MeP_i$) can be approximated by the following filtering relation:

$$[MeP_i] \approx \frac{g_i}{u_i^\alpha}$$

where $g_i$ is the supply of the peptide via TAP and $u_i$ is the rate of dissociation of the peptide from MHC-I. We found that the exponent $\alpha$ is increased by tapasin, leading to greater filtering of peptides based on their off-rate from MHC. The model has also been used to simulate the competition of peptides for cell surface presentation (32). However, predictions for the direct competition between peptides of known supply and affinity to MHC have so far not been tested in vivo.

In this study, we develop a method for predicting the direct competition of peptides for presentation by MHC-I. We experimentally measure the influence of varying peptide supply on the selection of two competing peptides of different off-rates, and calibrate the peptide filtering relation (Eq. 1) with these data. We also generate model predictions for how competition for surface presentation varies when the competitor peptide off-rate is varied. In doing so, we demonstrate, for the first time, that the filtering relation holds for individual peptides in direct competition with one another and show that the same level of competition can be achieved by a high concentration of a low affinity peptide or a low concentration of a high affinity peptide. We apply this model-based approach to derive a more quantitative understanding of the changes in cell surface abundance of two competing peptides as the antigen-presenting cell is exposed to interferon-$\gamma$ (IFN$\gamma$), a cytokine which has a profound effect on the antigen processing and presentation pathways in infections, autoimmune diseases (33), and cancer.

**MATERIALS AND METHODS**

**Cells**

The B6 fibroblasts cell line was produced from primary ear fibroblasts cells harvested from C57Bl/6 wild-type mice and...
immortalized by transfection with pSV3-neo plasmid (ATCC)
encoding the SV40 T-Ag. RMA-S, TAP2-deficient mouse
T cell line, and the above fibroblasts were cultured in RPMI
1640 (Lonza, Verviers, Belgium) supplemented with 10% FCS
(Globepharm, Guildford, UK), 2 mM glutamine (Lonza), and
50 μM β-mercaptoethanol.

**Fab Antibodies**

Plasmids expressing Fab antibodies specific for ASNENMETM-
H2Db (E10) and SLENFRAY-VH2Db (1C3) were a kind gift
from J. Bennink (34). Rosetta2 (DE3) pLacI competent bacteria
(Novagen) were transformed, grown to OD600 = 1–1.2 and
then induced by the addition of 1 mM isopropyl β-D-thio-
galactoside (IPTG) for 3 hr at 30°C. Proteins were extracted with
BugBuster reagent (Novagen) and applied to a HiTrap Excel
column (GE Healthcare, Uppsala, Sweden). Bound Fabs were
eluted with 250 mM imidazole and then further purified by gel
filtration on a 26/600 Superdex GF column (GE Healthcare).
Purified peak fractions were concentrated using Amicon Ultra-
15 10 kDa cut-off centrifugal concentrator (Merck/Millipore,
Cork, Ireland).

**Generation of 1C3 Chimeric Monoclonal Antibody**

To allow for a more efficient production of the 1C3 reagent we gener-
ated a chimeric monoclonal antibody using the Invivogen pFUSE
system allowing expression in mammalian cells. VL and VH were
amplified from the original plasmid (34) using primers VL_for
(GTCTTGCACCTTGTCAAGAATTCTCCAGCAGTAGT
GACTCAG) and VL_rev (GCATCTGCCCGTTTGATCTCGAG
TTTGATCTCCACTTGGTCC), and VH_for (CCTGCACTT
GTACGAATTTCCGGTAGCTCGGAGCTAGCC) and VH_rev
(GGTGTCTTTTAGCGCTGTAAGGCTTGAGACGGTG
ACCAGG) respectively. VL and VH sequences were inserted
respectively into pFUSe2s-CLig-mk and pFUSeSS-CHig-mG1
(Inovigen, Toulouse, France) using the SIC cloning method
to produce a chimeric monoclonal antibody containing a mouse
IgG1 Fc fragment. Both plasmids were co-transfected into 293F
cells and supernatant was harvested 1 week after transfection,
clarified by centrifugation, and filtered through a 0.22 µm filter.

**Peptides**

Peptides (listed in Table 1) (GL Biochem, Shanghai, China) were
synthesized by fluorenylmethoxycarbonyl chemistry and were
>95% pure by HPLC and mass spectrometry.

**Peptide-Expressing Plasmids**

pSC11 plasmids containing Venus/mCherry-ubiquitin-peptide
cassettes were obtained from J. Bennink. These plasmids were
used in Ref. (35) to generate recombinant vaccinia viruses
expressing the following peptides: SSLENFRAY, PA324-33 and
ASNENMETM
NP366-374. To be able to use those constructs
in transient transfection assays the cassettes were reclustered
into pEGFP-Ub-SIINFEKL (36) (obtained from J. Neefjes). Venus/
mCherry-Ub-peptide cassettes were amplified from the pSC1
plasmids by PCR using primers CGCTAGGCCTACCGTGCG
CAGCATTGGTAGACAGGGCAG and CGCTCCACAGAT
TCCAGCCG containing a NheI and EcoR1 restriction sites
respectively (underlined). The PCR product was amplified using
GoTaq Flexi DNA Polymerase (Promega) to enable ligation into
the pGEM-T vector system (Promega). pGEM-T-cassette plas-
mid sequences were checked by sequencing using SP6 and T7
promoter primers. pGEM-T-cassette and pEGFP-Ub-SIINFEKL
plasmids were then cut with NheI and EcoR1 and the EGFP-
Ub-SIINFEKL cassette was replaced with the Venus/mCherry-
Ub-peptide cassette using the Roche Rapid Ligation Kit (Roche).

**Generation of ASNENMETM Variant Plasmids**

The pVenus-Ub-ASNENMETM construct was used as a template
to generate a series of variants by site-directed mutagenesis using
the QuickChange II Site-Directed Mutagenesis Kit following the
manufacturer’s protocol using the Pfu Ultra enzyme (Agilent).
The list of the variants can be found in Table 1.

**Brefeldin A Decay Assay**

Dissociation of pMHC complexes at the cell surface was assessed
by BFA decay assay as described previously (17). RMA-S cells were
incubated overnight at 26°C to maximize MHC-I surface expres-
sion. After being washed, 5 × 10^5 cells per well of a 96-U-bottom
plate were pulsed, at different time intervals, with peptides (final
concentration of 20 μM) for 1 h at 26°C. After washing with
medium, de novo transport of MHC-I to the cell surface was
blocked by the addition of BFA, and peptide-loaded RMA-S cells
were incubated at 37°C to allow decay of unstable molecules.

<table>
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<th>Peptide</th>
<th>BIMAS score</th>
<th>NetMHC 4.0 (nM)</th>
<th>t1/2 (min)</th>
<th>Off-rates (s⁻¹)</th>
<th>E10 score</th>
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<td>ASNEAMETM</td>
<td>3.4</td>
<td>2208.8</td>
<td>43</td>
<td>2.7 × 10⁻⁴</td>
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<td>ASNENMETT</td>
<td>17</td>
<td>94.6</td>
<td>52</td>
<td>2.2 × 10⁻⁴</td>
<td>14.2</td>
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<td>ASNENMETV</td>
<td>17</td>
<td>13.2</td>
<td>132</td>
<td>8.8 × 10⁻⁵</td>
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<td>ASNENMETL</td>
<td>343</td>
<td>9.6</td>
<td>191</td>
<td>6.1 × 10⁻⁵</td>
<td>11.8</td>
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<tr>
<td>ASNENMETI</td>
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<td>6.6</td>
<td>212</td>
<td>5.4 × 10⁻⁵</td>
<td>13.1</td>
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<td>ASNENMETM</td>
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<td>7.3</td>
<td>223</td>
<td>5.2 × 10⁻⁵</td>
<td>11.5</td>
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<td>ASNENLEMT</td>
<td>411</td>
<td>10</td>
<td>238</td>
<td>4.9 × 10⁻⁵</td>
<td>4.1</td>
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<td>SLENFRAYV</td>
<td>0.5</td>
<td>23.4</td>
<td>408</td>
<td>2.8 × 10⁻⁵</td>
<td>3.8</td>
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<td>ASNENMETM</td>
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<td>3</td>
<td>456</td>
<td>2.5 × 10⁻⁵</td>
<td>8.9</td>
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<tr>
<td>ASNENMETM</td>
<td>1235</td>
<td>3.6</td>
<td>502</td>
<td>2.3 × 10⁻⁵</td>
<td>1.8</td>
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BIMAS score was determined using BIMAS (http://www-bimas.cit.nih.gov/molbio/
BIMAS/IdSwitch) for 9mers binding to H-2Db. Equivalently, analysis was conducted using
NetMHC 4.0, which returns the predicted IC50 of H-2Db binding (nM). Peptide half-
lives (t1/2) were determined experimentally by BFA decay assays and converted into
off-rates as log2(t1/2)/half-life (s). E10 score: E10 Fab binding affinity for all ASN–H2Db
complexes was determined by flow cytometry surface staining of RMA-S cells pulsed
with peptides (Figure S7 in Supplementary Material). The E10 score represents the
MV1000.
analyzed by flow cytometry on a Fortessa X20 flow cytometer (BD, Oxford, UK), and data were analyzed with the Diva software. MFI values were background deducted by subtracting the MFI value obtained in the DMSO control at the last time point. Half-lives and off-rate constants were then determined by fitting the curves using an exponential trend line in the Excel software (Microsoft, USA).

**pMHC Competition Assay**

Fibroblasts were seeded at 2 × 10⁵ cells per 6 cm diameter Petri dish. When IFNγ treatment was applied, 1 µg of mouse IFNγ (Peprotech, Rocky Hill, USA) was added per Petri dish 4 h after seeding. Cells were transfected for the following day with TransIT-LT1 (Mirus, Madison, USA) following the manufacturer’s recommendations using 2.5 µg of each Venus-Ub-peptide and mCherry-Ub-peptide constructs. 1 day after transfection, cells were stained for 45 min on ice with primary reagents, 1C3 hybrid Mab neat supernatant, E10 purified Fab, B22, or Y3 purified Mabs to detect surface pMHC complexes. After washing, cells were incubated for 45 min with AF647-conjugated goat anti-human IgG (used after Fab primary) or goat anti-mouse (after mouse antibodies including 1C3) (Invitrogen/Molecular Probes, Eugene, USA). Flow cytometry was performed using a Fortessa X20 cytometer (BD) and the data were analyzed using FACS Diva software (BD).

**mCherry Calibration**

mCherry flow cytometry calibration beads (Clontech/Takara, USA) were used, as recommended by the manufacturer, to calibrate the amount of mCherry molecules, equivalent to the number of peptide molecules expressed in the transfected cells. Beads were run through the flow cytometer using the same setup as for acquiring cells.

**Quantitation of pMHC Surface Expression by Indirect Immunoﬂuorescence Assay Using Qifikit Calibrator Beads**

The number of SSLENFRAYV-H2Db complexes presented on the cell surface was estimated using Qifikit beads (Dako, Glostrup, Denmark). Beads were treated as recommended by the manufacturer but stained with AF647-conjugated goat anti-mouse IgG (Invitrogen) at the same dilution as used to detect the 1C3 Mab on transfected cells. A calibration curve was drawn by plotting the MFI of the 5 peaks (x-axis) versus the lot-specific numbers of antibody molecules per bead (y-axis). The curve was then used to calculate the number of SSLENFRAYV-H2Db complexes presented on the cell surface expressed in antibody-binding capacity units (number of primary mouse monoclonal antibodies per cell).

**Calibrating the Peptide Filtering Model to Flow Cytometry Data**

The peptide filtering model established in Ref. (32) was adapted to analyze measurements of peptide competition, as illustrated in Figure 2A. The peptide filtering model is described by a system of biochemical interactions, as follows:

- **Synthesis/degradation reactions**:
  \[
  g_r \quad g_u + \gamma \quad 10(g_m + \gamma) \\
  \emptyset \quad P, \quad \emptyset \quad M, \quad \emptyset \quad T \\
  d_r \quad d_m \quad d_T
  \]
  where \( i \) denotes the peptide (e.g., SSLENFRAYV, ASNENMETM, or self) and \( \emptyset \) denotes no molecule. All parameters in the peptide filtering model were taken to be identical to those in the original publication (32), except for the on-rate for ASNENMETM, \( b_{ASN} \), which was allowed to differ from the nominal binding rate that was used for SSLENFRAYV. Also, a new variable \( \gamma \) was used to approximately quantify the increase in MHC-I and tapasin supply in IFNγ-treated cells. The peptide supply rates were defined using fluorescence measurements corresponding to intracellular peptide abundance (\( F_i \)), which were multiplied by scale factors \( f_i \).

\[
g_i = f_i \times F_i
\]

To relate the output of the model to fluorescence measurements corresponding to cell surface presentation (\( H_i \)), model outputs were scaled by peptide-specific scale factors \( h_i \) to give estimates

\[
\hat{H}_i = h_i \times [MeP_i]
\]

All new parameters were inferred by fitting the simulated fluorescence measurements \( \hat{H}_i \) to the experimental fluorescence measurements.

We used the Visual GEC software to perform parameter inference, which uses the domain-specific Language for Biochemical Systems (LBS) for specifying the reaction system, and an adaptive Metropolis–Hastings Markov chain Monte Carlo (MH-MCMC)
algorithm from the Filzbach software. The inference of parameters in LBS models using MH-MCMC is described in the Supplementary Information of Ref. (37), but we provide a short summary here. MH-MCMC enables the calculation of the posterior distribution of the parameter values, given observation data $D$, and some prior belief of the parameter values. We write the posterior distribution as $p(\theta|D)$. The MH-MCMC algorithm uses an iterative stochastic search technique in which parameter sets are sampled in such a way that a Markov chain is formed, with the history of the chain converging to $p(\theta|D)$. At each iteration, the current parameter set $\theta$ is perturbed to generate a new proposed set $\theta^*$. The new parameter set is accepted or rejected based on the ratio of a likelihood function $L(\theta)$ evaluated at each parameter set, such that improvements are always accepted, but lower likelihoods are accepted with some probability. In this way, the chain converges toward and does random walks in regions of high probability mass, avoiding wasting time (computational effort) in regions of lower probability mass. For more details on MCMC algorithms, we recommend (38) in addition to the Filzbach software documentation.

Internally to Visual GEC, the LBS model is simulated as deterministic rate equations, and the simulation output is then related to the experimental data using the log-likelihood function

$$\log L(\theta) = \sum_{k=1}^{N_d} \log p(y_k | \theta), \quad y_k \sim N(x_k, \sigma^2)$$

where the $x_k$ are the model simulations, $N_d$ is the number of measurements, and $\sigma$ is the SD of the measurement error, which is inferred along with the calibration parameters. As Visual GEC expects time-series measurements, the experimental data were specified at a time of 48 h, to enable the peptide filtering model to reach its equilibrium (LBS code is available from the authors upon request). During application of the MH-MCMC algorithm, the calibration parameters are varied, and there is convergence toward values that yield simulation values that are closer to the measured data, thus approximately maximizing the likelihood function.

RESULTS

Surface Presentation of a Target Peptide Decreases With Increasing Amounts of Competitor

To establish how variation in the intracellular abundance of competing peptides influences cell surface presentation, we developed an assay in which intracellular peptide supply and pMHC cell surface abundance could be measured simultaneously (Figure 1A), assay adapted from Ref. (35). Furthermore, this method measures the abundance of peptides actually presented at the surface of living cells and not, as for quantitative high-throughput methods, the abundance of peptides remaining bound to MHC after biochemical purification of pMHC I complexes from cell lysates. Two fluorescent fusion proteins, Venus-ubiquitin-ASNNEMETM (faster off-rate, see Table 1) and mCherry-ubiquitin-SSLENFRAYV (slower off-rate) are co-expressed in fibroblasts. Once expressed in the cytoplasm, the fusion proteins are cleaved by endogenous cytoplasmic ubiquitin hydrolases, releasing the peptides at an equimolecular ratio to their respective fluorescent reporter protein (Figure 1A) that can be quantified by flow cytometry (36) (Figure S1 in Supplementary Material). Using this system, the generation of peptides bypasses the proteasome. After translocation into the ER, peptides compete for loading onto MHC-I molecules and are transported to the cell surface where they can be quantified by flow cytometry (Figure 1A). Both fusion proteins were naturally expressed at a broad range of concentrations after transient transfection, allowing in a single experiment to compare peptide surface presentation in cells expressing low to high levels of both fusion proteins (Figure 1B). To analyze competition between both peptides, cells were partitioned into different gates according to their expression level of mCherry, reporter for the level of expression of SSLENFRAYV, and Venus, reporter for the level of ASNNEMETM (Figure 1B). Competition was then assessed by plotting surface expression of SSLENFRAYV-H2Db as a function of increasing cytoplasmic expression of the SSLENFRAYV target peptide for increasing levels of competing peptide (Venus levels 1–7 in Figure 1B). Figure 1C (top panel) shows that surface expression of the slower off-rate SSLENFRAYV ($2.8 \times 10^{-5}$ s$^{-1}$) decreased as expression of the faster off-rate ASNNEMETM ($5.2 \times 10^{-5}$ s$^{-1}$) increased from P(.,1) to P(.,6). Simultaneously, ASNNEMETM (Figure 1D, top panel) became more abundant on the cell surface.

To determine the order of magnitude of the inferred parameters for our previously published model (32) we quantified the supply of the target peptide ($g_{SSL}$. We considered that the number of SSLENFRAYV target peptides expressed in the cytoplasm was proportional to the number of mCherry molecules, using the simplifying assumptions that all of the fusion proteins are cleaved and that the degradation rate of the fluorescent protein equals the degradation rate of the peptide. This number was determined using mCherry calibration beads (Figure S1 in Supplementary Material) and indicated that transfected cells expressed up to $10^5$ copies of SSLENFRAYV per cell and that surface expression could be detected when cytoplasmic expression approached $10^4$ copies per cell (Figure S1E in Supplementary Material). Venus expression could not be calibrated in the same way due to the lack of available reagents.

Quantification of the Number of Target pMHC Complexes on the Cell Surface

The number of SSLENFRAYV pMHC presented on the cell surface ($MeP_{SSL}$) was then quantified using Qifikit calibration beads, coated with well-defined quantities of monoclonal antibodies mimicking cells with different antigen densities bound to a primary antibody (Figure S1 in Supplementary Material). In untreated cells, up to 80,000 pMHC complexes were presented on the cell surface in the absence of ASNNEMETM competition, and that number was reduced to 10,000 or less in the presence of the highest level of competitor (Figure S1E in Supplementary Material). These values are consistent with the level of expression of abundant peptides measured previously. For example, between 70,000 and 80,000 SIINFEKL–H2Kb complexes were observed on L-Kb cells infected.
FIGURE 1 | Simultaneous measurement of intracellular peptide abundance and cell surface peptide–MHC complex (pMHC). (A) Experimental setup. Fibroblasts were co-transfected with constructs expressing fusion proteins made of a fluorescent protein, ubiquitin, and a peptide. Cytoplasmic ubiquitin hydrolases cleave the fusion proteins, releasing an equimolar ratio of peptide and fluorescent protein. Peptides are transported to the endoplasmic reticulum where they can compete for loading onto major histocompatibility complex-I molecules. Then they migrate to the cell surface where ASNENMETM-H2Db complexes can be detected using E10 Fab and SSLENFRAYV-H2Db using the 1C3 chimeric Mab followed by a secondary antibody conjugated to AF647.

(B) In a single transfection assay, cells were expressing low to high levels of both fusion proteins and were separated in different gates for the purpose of the analysis.

(C) Level of SSLENFRAYV-H2Db surface expression in the presence of increasing amount of competitor. The dark blue curve shows the maximum surface expression as the cytoplasmic level of SSLENFRAYV peptide, represented on the x-axis, increases. The other curves represent the SSLENFRAYV-H2Db surface expression in the presence of different levels of ASNENMETM competitor [top dark blue curve corresponds to gates P(1, 1) to P(8, 1) with no competitor, down to the light blue bottom curve corresponding to gates P(1, 8) to P(8, 8) with the maximum level of competitor] in untreated wild-type cells (top panel) or in IFNγ-treated cells (bottom panel).

(D) Corresponding to ASNENMETM-H2Db surface expression.

Surface Presentation of Two Competing Peptides Is Enhanced in the Presence of IFNγ

IFNγ is known to increase expression of MHC-I, together with chaperones involved in antigen processing and presentation, and plays an important role in inflammatory immune responses to viruses and cancer. However, it is not known whether IFNγ enhances presentation of all peptides or focuses the immune response.
A Calibrated Mechanistic Model Explains Experimental Observations of Peptide Competition

Our previously published model (32) describes the endogenous antigen presentation pathway from the point where peptides are supplied to the ER, through presentation at the cell surface. The model also explicitly describes the interaction between MHC and tapasin, incorporating the binding of peptides to MHC–tapasin complexes that influences peptide loading.

To test the extent to which peptide competition could be predicted based on both peptide affinity and intracellular abundance, we adapted the peptide filtering model of Ref. (32). To relate the model directly to the fluorescence measurements corresponding to intracellular peptide abundance and cell surface abundance in Figure 1, we transformed the experimental data from units of fluorescence into units of molecule numbers using calibration parameters (Figure 2A). For peptide supply, we specified the parameters of the model to be proportional to the intracellular abundance measurements, with proportionality factors $f_{\text{SSL}}$ and $f_{\text{ ASN}}$ (see Materials and Methods). These factors incorporate the conversion from fluorescence units into numbers of peptides, but also implicitly account for any differences in TAP translocation. To compare the model output with the cell surface fluorescence measurements, a similar strategy was used, whereby two scale factors converted from numbers of cell surface pMHC into equivalent measured fluorescence ($h_{\text{SSL}}$ and $h_{\text{ASN}}$, see Materials and Methods). In addition to the target and competitor peptides, we also included a third peptide in the model to represent the presence of self–peptides in the system (Figure 2A), with a pMHC unbinding rate $u_{\text{off}}$, and ER supply rate $g_{\text{est}}$.

We experimentally measured the stability of pMHC complexes by following their decay from the cell surface over time (“BFA decay assay” in Figure 2A), and used these measurements to calculate the corresponding unbinding rates of the peptides from MHC (Table 1; Figure S3 in Supplementary Material). Finally, to incorporate the effect of IFNγ into the model, we specified a new parameter ($\gamma$) that quantifies the increased supply of MHC and tapasin following IFNγ treatment.

To establish estimates for the parameters, we used MCMC parameter inference (see Materials and Methods for further details) applied to two experimental datasets measuring intracellular and cell surface abundance of a target peptide SSLENFRAYV and competitor peptide ASNENMETM. Two repeated peptide competition experiments between SSLENFRAYV and ASNENMETM were used to infer the model calibration parameters. This enabled us to account for inter–experiment variations in the measurement of SSLENFRAYV–H2Db at the cell surface using the 1C3 monoclonal antibody. Accordingly, $h_{\text{SSL}}$ and $\gamma$ were allowed to take on different values between the two experiments, whereas the other parameters were assumed to be invariant. To obtain a good fit to the data, and establish robust estimates of all parameters, we made two additional changes to the setup described thus far: (i) a better fit to the data could be obtained when allowing the peptide on–rate for ASNENMETM to take on a different value from SSLENFRAYV and (ii) we found that both $u_{\text{off}}$ and $g_{\text{est}}$ could not be identified uniquely, so we assumed a value of $u_{\text{off}} = 10^{-4}$ s$^{-1}$, representing a peptide of average affinity for MHC–1, and inferred $g_{\text{est}}$.

The results of the MCMC procedure illustrate only moderate uncertainty in the parameter estimates (Figure S4 in Supplementary Material), and also low pairwise correlation (Figure S5 in Supplementary Material). This suggests that the parameters are well constrained by the data. When parameters are not well constrained by data, there can be flexibility in assigning parameter values, for instance changes in one parameter can be compensated for by changes in another parameter. When this happens, the likelihood function (which quantifies the goodness of fit to the data) will be equally high over a structured region in the parameter space, leading to strong correlation and broad marginal posterior distributions. As such, plots of the pairwise correlations and marginal posterior distributions are commonly used as a diagnostic in Bayesian parameter fitting. Simulation of the resulting maximum likelihood parameter set displayed a reduced presentation of each peptide in response to increasing abundance of the other (Figure 2B; Figure S6 in Supplementary Material), as observed experimentally. Furthermore, our hypothesized increase in MHC–I and tapasin supply in IFNγ-treated cells led to increased simulated presentation of both peptides, and a reduction in the effect of competition (Figures 2D,E), as observed experimentally.

The Model Predicts the Effects of Different Competitors on the Surface Presentation of a Target Peptide

The purpose of the calibrated model is to predict surface presentation of a target peptide in the presence of competitors of different off–rates. In order to test predictions made by the model, surface expression of SSLENFRAYV was measured experimentally in the presence of competitor peptides of different off–rates. Using peptide binding prediction tools (BIMAS and NetMHC 4.0), we selected ASNENMETM variant peptides which should have a range of affinities either lower or higher than the original peptide (Table 1). Off–rates were then determined...
experimentally in brefeldin A decay assays and comparison to the predicted values showed that in this case BIMAS performed better than NetMHC (Table 1). The corresponding plasmids encoding each Venus-Ub-variant were generated and used in competition assays. Surface expression of ASNENMETM and its variants was determined in parallel by staining with E10 Fab (Figures 3C,D). This was only possible for variants of ASNENMETM that were recognized well by E10 (Figure S7 in Supplementary Material; Table 1) and precluded variants with leucine at position 6.

Figure 3 shows the experimentally determined surface presentation of SSLENFRAYV and four measurable ASNENMETM variants...
in the presence (Figures 3B,D) and absence (Figures 3A,C) of IFNγ; as well as the presentation of SSLENFRAYV in the presence of increasing intracellular expression of all nine Venus-Ub-ASN(variant) constructs in the presence (Figure 3F) and absence (Figure 3E) of IFNγ. It shows a trend of increased competition with SSLENFRAYV for H-2Db binding and presentation as the stability of the variants increased, with maximum competition observed in the presence of the slowest off-rate peptides ASIENMETM and ASIENLETM (off-rates of $2.5 \times 10^{-5}$ s$^{-1}$ and $2.3 \times 10^{-5}$ s$^{-1}$ respectively; Table 1; Figures 3A,E). However, the same level of competition could be achieved by a low concentration of a high affinity competitor or by a higher concentration of
Figure 4 | Predicting cell surface presentation between peptide competitors. Using the calibrated model, cell surface presentation was predicted for SSLFRAY competing against variants of the ASNENMET peptide, by simulating with their measured off-rates (Table 1). To control inter-experiment variability, the competition experiments were carried out at the same time as a SSLFRAY-ASNENMET competition experiment. We therefore used the corresponding MHC-I and tapasin supply rates inferred for these experiments during model calibration. The Venus fluorescence intensity can be used to compare corresponding levels of expression of the different ASNENMET variants as they are all expressed in the cytoplasm at the same ratio of 1 peptide per molecule of Venus. We assumed that ASNENMET variants bind to TAP with similar affinities and, therefore, that their rate of ER entry is similar. As the hierarchy of competition follows the hierarchy of peptide stability, this assumption is reasonable. The resulting predictions are superimposed on the corresponding experimental measurements in Figure 4. The model predictions display the same characteristics as the experimental measurements.

In IFN-γ-treated cells, in all cases, surface expression of SSLFRAY was less affected by the competitors than without IFN-γ (Figures 3B,F) and a higher surface expression of the competitors was also observed for the slower off-rate peptides (Figure 3D). IFN-γ did not, however, enhance surface expression of the fast off-rate variants (ASNEMETM, ASNENMETV, and ASNENMETY) which are still unable to reach or remain at the cell surface at detectable levels.

We next sought to determine the predictability of peptide competition, based on a quantitative knowledge of cytoplasmic abundance and off-rate from MHC-I. Using the calibrated peptide filtering model, we predicted the cell surface presentation of SSLFRAY pMHC when competing against variants of

A low affinity competitor, emphasizing the importance of both peptide stability and intracellular abundance in determining cell surface presentation (Figure 3A, black arrow).

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as the data: increasing abundance of the competing variant of ASNENMETM leads to an increase in its own cell surface presentation (Figure S8 in Supplementary Material) but a drop in SSLENFRAYV presentation (Figure 4), and IFNγ treatment increases presentation of both peptides. However, similar to the model output for SSLENFRAYV presentation when competing with ASNENMETM in IFNγ-treated cells (Figure 2D), only a modest reduction in competition could be seen in predictions of competition against ASNENMETM variants (Figure 4).

**Cell Surface Abundance Is Accurately Predicted by a Peptide Competition Metric**

While the full peptide filtering model is demonstrably capable of reproducing and predicting measurements of peptide competition across a range of peptides, the complexity of the model does not offer a simple quantitative explanation. Therefore, we considered whether competition could be explained by the filter relation ([32]; see above), which approximates equilibrium cell surface presentation of a peptide in terms of its supply and off-rate from MHC-I.

To incorporate a contribution from the competitor peptide, we investigated a normalization of the filter relation (Eq. 1) as

\[
\text{MeP}_{\text{ratio}} \approx \frac{\left[P_{\text{SSL}}\right]_{\text{cyt}} / u_{\text{SSL}}^2}{\left[P_{\text{ASN}}\right]_{\text{cyt}} / u_{\text{ASN}}^2 + b_{\text{self}} / u_{\text{self}}^2}
\]

(2)

where SSL denotes the SSLENFRAYV peptide, ASN denotes an ASNENMETM variant, and MeP_{\text{ratio}} represents the ratio of egressed SSL complexes to other complexes (ASN and self).

\[\left[P_1\right]_{\text{cyt}} = f_k F_k\]

denotes the calibrated cytoplasmic abundance of peptide \(k\) [i.e., \(k\) is either SSL or ASN; the scaling factor \(f_k\) converts units of fluorescence \(F_k\) into number of molecules \(\left(P_1\right)_{\text{cyt}}\)]. In this definition (Eq. 2), the absence of competitor peptides leads to surface presentation of SSLENFRAYV being given purely in terms of the background self-peptides.

We calculated the peptide competition metric (Eq. 2) for each peptide competition experiment and compared the output against the corresponding simulations of the peptide filtering model. This established the competition metric to be a good approximation of the equilibrium behavior of the full model, both in the untreated (Figure S9 in Supplementary Material) and IFNγ-treated regimes (Figure S10 in Supplementary Material). Variations in the abundance of the competitor peptides could be almost entirely accounted for by the metric, which can be seen in Figures S9 and S10 in Supplementary Material as the different colored traces (corresponding to different levels of competitor abundance) collapsed onto a consistent relationship between the metric and model-predicted cell surface abundance of SSLENFRAYV-H2Db.

We then applied the peptide competition metric directly to the experimental observations, without using the model. Accordingly, we calculated

\[
F_{\text{ratio}} \approx \frac{F_{\text{SSL}} / u_{\text{SSL}}^2}{F_{\text{ASN}} / u_{\text{ASN}}^2}
\]

(3)

where \(F_{\text{SSL}}\) and \(F_{\text{ASN}}\) represent measurements of intracellular peptide abundance (fluorescence intensity units). Unlike for MeP_{\text{ratio}}, the metric does not include a contribution from self-peptides, as in general this quantity would not be available.

We compared \(F_{\text{ratio}}\) with measurements of cell surface abundance of SSLENFRAYV (Figure 5). We found that variations in peptide abundance could be predicted with high accuracy for untreated cells (−IFNγ; Figure 5), though the variations in the abundance of ASNENMETV peptide were only partially accounted for (Figure 5C). By contrast, the peptide competition metric was less accurate for IFNγ-treated cells (Figure S11 in Supplementary Material). This is reflected in the Pearson correlation scores (compare Figure 5 with Figure S11 in Supplementary Material). Overall, we found that differences in competitor abundance are not as well captured by \(F_{\text{ratio}}\) as they are by MeP_{\text{ratio}} (Figure S10 in Supplementary Material). In particular, \(F_{\text{ratio}}\) over-approximates surface presentation of SSLENFRAYV when competitor peptide abundance is low. As the same over-approximation was not seen in the comparison of MeP_{\text{ratio}} and simulated surface abundance of SSLENFRAYV (+IFNγ; Figure S10 in Supplementary Material), our interpretation is that this is due to \(F_{\text{ratio}}\) not incorporating the potential impact of self-peptides. At low competitor abundance, self-peptide availability will be more important, leading to a loss of accuracy of \(F_{\text{ratio}}\). Nevertheless, we have found that very simple formulae can largely predict semi-quantitatively how the presentation of a given peptide will be reduced by the increased abundance of a competitor peptide.

**DISCUSSION**

We have developed a mathematical model based on known cellular mechanisms which, despite including only three components (MHC, peptide, and tapasin), can predict pMHC surface expression under physiological conditions, given some knowledge of the intracellular abundance of peptides. The abundance of specific pMHC on antigen-presenting cells can determine both the intensity of the primary CTL (cytotoxic T lymphocyte) response to that pMHC and also the susceptibility of target cells bearing the pMHC to killing by those CTL. At present there is no predictive model for estimating the rate of CTL killing as a function of pMHC abundance. Current high-throughput methods for detecting MHC-I bound peptides have advanced significantly over the past decade and have given rise to better algorithms for estimating whether a particular peptide is likely to be presented as a pMHC. Nevertheless, the estimates are not quantitative and depend on the ability to detect specific peptides following stringent purification of pMHC prior to peptide extraction during which time peptides are progressively lost according to their individual dissociation rate constants. Our model relates intracellular peptide abundance to cell surface abundance via the intracellular process of chaperone assisted peptide editing of MHC-I, which occurs in the face of competition between millions of peptides for binding to the same MHC-I. Such conditions are likely to be especially important, for example, during viral infection where viral epitopes need to compete with a vast number of self-peptides, or when trying to generate an immune response against a polytype vaccine (multiple epitopes artificially joined into a single polypeptide, possibly being expressed from large virus vectors generating themselves many other viral epitopes), or against cancer neo-epitopes competing against a multitude of more abundant or higher affinity self-peptides.
Thus, we developed a competition assay that allowed us to measure surface abundance of a target peptide in the presence of increasing amount of competitor peptides of different off-rates. Our results highlighted the importance of the abundance of competing peptides, as indeed the same level of competition could be achieved by a low abundance of a high affinity competitor or a high abundance of a low affinity peptide. Based on these experimental data, we calibrated our model of MHC-I presentation, which was originally developed to explain how peptide optimization differs between MHC-I alleles (32). Here, we found that this same model was able to predict surface expression of a target peptide in the presence of competitors of different off-rates (Figure 4). Furthermore, we found that simple peptide competition metrics (Eqs 2 and 3), based on the filter relation (Eq. 1), could quantify the impact of increasing intracellular competitor abundance on target peptide surface presentation (Figure 5). This is the first time that the filter relation has been experimentally tested on competing peptides. The present study provides justification for its use in vaccine design, for instance, to assess the relative merits of increasing peptide abundance or improving peptide stability to achieve a desired level of cell surface presentation, using a simple calculation.

We were also able to predict pMHC surface expression when MHC-I and tapasin supply increased following IFNγ treatment, by extending the basic model to incorporate a factor quantifying the extent of IFNγ upregulation of MHC-I and tapasin. IFNγ is well known to stimulate the immune system, increasing expression of MHC-I heavy chain, β2-microglobulin, subunits of the immunoproteasome (MECL1, LMP2, and LMP7), TAP, tapasin, the ER aminopeptidase associated with antigen processing (ERAAP) (41) and the tapasin-related protein (TAPBPR); it is produced as part of the immune response against viruses. IFNγ also plays a critical function in cancer immunosurveillance (42) as it can be secreted in the tumor micro-environment, determining the inflammatory status of the tumor (43) and influencing tumor prognosis. For many years, IFNγ has been used in the clinic as an immunostimulant within immunotherapy regimes although the details of how it works were poorly understood. Those studies resulted in variable outcomes, sometimes detrimental to the patients (44). In the current study, as both the target and competitor peptides were generated from cleavage by cytoplasmic Ub-hydrolases, we were able to bypass the effect of IFNγ on the proteasome [IFNγ induces a switch from constitutive to immunoproteasome with different cleavage specificity that can
be observed at the level of the immunopeptidome (45) and only
consider the effect on the antigen presentation machinery. We
have confirmed in our system an increase of MHC-I and tapasin
expression after treatment with IFNγ and observed an increase
in surface expression of both competing peptides. The level of
competition of the SSLENFRAYV peptide was reduced in the
presence of IFNγ, presumably due to the increased level of avail-
able MHC-I molecules. This is in line with observations made
by others, for example, (33) showed that after treating NIT-1
insulinoma cells with IFNγ, presentation of the high affinity
JAK-1.S53–63 (SYFPEITHI) peptide by H-2Kb was increased from
~2,000 to ~15,000 copies per cell; whereas, the lower affinity
IGRF.206–214 peptide (VYLKTNVFK), barely detectable at 1 copy
per cell in untreated cells, reached 25 copies per cell after IFNγ
treatment. The differential enhancement of presentation by IFNγ
(7.5- or 25-fold increase respectively) did not merely follow the
fivefold increase in overall H-2Kd surface expression. This means
that the peptide repertoire presented by β-cells shows subtle dif-
f erences under inflammatory conditions even though the length
or binding affinity of peptides presented by either H-2Kd or
H-2Db did not change. In other words, cytokine treatment did
not bias toward high-affinity ligands. In this instance such a subtle
change in the peptide repertoire might be related to the transition
from benign to destructive insulitis.

Likewise, IFNγ can also have a dramatic effect in cancer
immunology. The tumor environment is likely to progress from an
inflammatory surrounding with active T cells producing IFNγ, to
a non-inflammatory environment populated by regulatory T cells
and exhausted T cells that have stopped producing the cytokine.
Tumor cells can also evolve immune escape mechanisms blocking
the IFNγ pathway. It would be, therefore, beneficial to be able
to predict CD8+ T cell targets that are likely to be presented on
tumor cells in both absence and presence of IFNγ.

We showed in our study that the surface expression of the lower
affinity peptide was enhanced in the presence of IFNγ. This could
result in a CD8+ T cell response to develop against a broader range
of peptides as priming, activation of a T cell at first encounter with
its target PMHC, only occurs above a threshold antigen dose (1).
As a result, competition between different specificity CD8+ T cells
resulting in immunodominance, occurring very early on during
the immune response (46), might be altered in the presence of IFNγ.

Our observations of increased intracellular abundance
enhancing cell surface presentation of low affinity peptides might
explain why in other systems some low affinity peptides (that lie
outside the 500 nM cut-off often used to define MHC-I-binding)
can be presented efficiently and are able to induce strong CD8+
T cell responses. For example, the two immunodominant epitopes
from the transplantable murine tumor CT26 have half-lives of 60
and 20 min (H-2Ld binding SPSSYVYHQF and H-2Dd binding
GGPESFYCASW respectively (47), despite there being almost
500 neo-epitopes generated from point mutations that are con-
sidered likely to be immunogenic as they have an IEDB percentile
rank less than or equal to 1 (48, Table S3 in Supplementary
Material). Both of these peptides originate from the highly
abundant gp70 retrovirus envelope protein encoded by a gene
located in a CT26 tetraploid region and transcribed at high copy
number (48). It seems, therefore, that lower affinity peptides with
moderate half-lives can still be presented in sufficient abundance
at the cell surface to induce immunodominance. Interestingly,
strong CTL responses to the GGPESFYCASW-H2Dd complex
correlate with anti-tumor efficacy in immunotherapeutic settings
such as Tregs depletion (47) and anti-CD1 checkpoint blockade
(G. Sugiyarto, personal communication).

Mechanistic modeling has the advantage of incorporating
knowledge of the antigen processing pathway and of being
modifiable as that understanding grows. For example, although
the current model incorporates the functions only of MHC-I and
tapasin, extensions of our model could incorporate the func-
tion of ERAAP antigen processing, calreticulin (which recycles
empty MHC-I from ERGIC to ER), and the emerging function of
TAPBPR (which also functions as a peptide editor in connection
with the UDP-glucose-glycoprotein glucosyltransferase (49)).
Our model could also be used to simulate peptide presentation
in tumor escape mutants with altered expression of: (i) some of
the immunoproteasome sub-units (LMP2 and LMP7) that would
maintain a "non-inflammatory" peptide even in the presence
of IFNγ; (ii) proteins involved in the IFNγ signaling pathway
(50) also resulting in the presentation of a "non-inflammatory"
immunopeptidome within a pro-inflammatory tumor micro-
environment; (iii) mutations affecting the expression of antigen
processing and presentation molecules, including TAP, tapasin,
and ERAAP. These mutations would result in a modification of
the peptide repertoire presented by MHC-I molecules and also
in a drastic reduction of the pMHC surface expression level. Low
tapasin expression has been shown to correlate with low T cell
infiltration and poor prognosis in colorectal cancer (51) together
with the loss of presentation of some, normally, immunodomi-
nant CTL epitopes (17, 52, 53). However, tapasin expression can
also have a negative impact on the presentation of other immuno-
dominant epitopes such as MUC1 which is revealed when tapasin
expression in tapasin deleted cells precludes the experimental
measurement of cell surface peptide abundance in these condi-
tions. Modeling, however, could be used to predict shifts in the
immunopeptidome resulting from the selective downregulation
or loss of tapasin from cancer cells and may, therefore, help
to guide the selection of anti-cancer vaccines or other therapies.

While we have observed how intracellular peptide abundance
can influence cell surface presentation in a direct assay, the chal-
lenge will be to test our approach at the whole immunopeptidome
level. However, the validation of such a model is restricted by the
experimental methods available today: in particular, limitations
on the biochemical isolation of peptides recovered from MHC-I
mean that around 90% of the immunopeptidome is lost prior to
analysis (27). Also, only a quantitative immunopeptidome gener-
ated from elution of surface pMHC would allow to demonstrate
the benefit of our model including tapasin filtering versus the
use of algorithms based purely on the amino acid sequence of
the peptide. Another limitation is that our model requires prior
quantification of intracellular peptide abundance for each peptide
to produce a prediction of the cell surface presentation profile.
Prediction of an entire cell surface peptide repertoire would,
therefore, require high-throughput measurements of protein
expression and turnover by quantitative proteomics (SILAC) (55),
or measurements of transcription levels or protein translation rates, combined with proteasomal cleavage (56) and TAP binding (57) predictions. The dynamical modeling approach that we advocate (26, 58) has the advantage of encoding mechanistic hypotheses, which should enable us to also predict how peptide presentation changes under a range of genetic or physiological perturbations to the antigen presentation machinery.

AUTHOR CONTRIBUTIONS

DB conducted experimental work and RE conducted modeling work. AP, PC, and ND advised on modeling, and TE advised on experiments. The study was designed by TE, DB, ND, and AP. All authors wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fmmu.2018.01538/full#supplementary-material.

REFERENCES


Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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