RESEARCH ARTICLE

Viral infection causes a shift in the self peptide repertoire presented by human MHC class I molecules

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Purpose: MHC class I presentation of peptides allows T cells to survey the cytoplasmic protein milieu of host cells. During infection, presentation of self peptides is, in part, replaced by presentation of microbial peptides. However, little is known about the self peptides presented during infection, despite the fact that microbial infections alter host cell gene expression patterns and protein metabolism.
Experimental design: The self peptide repertoire presented by HLA-A*01;01, HLA-A*02;01, HLA-B*07;02, HLA-B*35;01, and HLA-B*45;01 (where HLA is human leukocyte antigen) was determined by tandem MS before and after vaccinia virus infection.
Results: We observed a profound alteration in the self peptide repertoire with hundreds of self peptides uniquely presented after infection for which we have coined the term "self peptidome shift." The fraction of novel self peptides presented following infection varied for different HLA class I molecules. A large part (approximately 40%) of the self peptidome shift arose from peptides derived from type I interferon-inducible genes, consistent with cellular responses to viral infection. Interestingly, approximately 12% of self peptides presented after infection showed allelic variation when searched against approximately 300 human genomes.
Conclusion and clinical relevance: Self peptidome shift in a clinical transplant setting could result in alloreactivity by presenting new self peptides in the context of infection-induced inflammation.

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1 Introduction

MHC class I restricted antigen processing and presentation inform T cells as to the internal state of the cell by binding cytoplasmic peptides and presenting them at the cell surface [1–3]. Under homeostatic conditions, these peptides are derived from self proteins and their presentation signifies normal cellular operations and as such is ignored by

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Abbreviations: **BLAST**, basic local alignment search tool; **FDR**, false discovery rate; **GVHD**, graft versus host disease; **HIV**, human immunodeficiency virus; **HLA**, human leukocyte antigen; **INV**, influenza virus; **MeV**, measles virus; **pMHC**, peptide/MHC complexes; **VACV**, vaccinia virus

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Clinical Relevance

MHC-encoded class I molecules present peptides derived from cellular proteins to CD8, informing them of a cell's *milieu intérieur* (cellular homeostasis). Cellular homeostasis is altered under a variety of stressed conditions, including during microbial infections. While there were hints that infected cells altered the presentation of self peptide repertoire, this notion was not fully explored. Herein, through mass spectrometric analysis of thousands of self peptides isolated from HLA class I molecules (the largest study of its kind to date), we observed a dramatic shift in the self peptide repertoires presented after infection of HeLa cells with VACV. Of significant clinical importance was the revelation that a fraction of the self peptides were derived from tumor-specific antigens. Furthermore, the self peptides uniquely presented after infection contained variants of such peptides—called allopeptides—within the human population. Approximately 12% of the self peptides uniquely presented after infection were potential allopeptides, the recognition of which can result in GVHD or transplant rejection. The proportion of allopeptides was very similar to the reported rate of transplant complication and failure (approximately 10%). Our study suggests that deep sequencing and proteomics analyses of self peptides may enhance the success of clinical transplant outcome and tumor immunotherapies.

self-educated T cells. During an infection however, microbial peptides are processed and fed into this antigen presentation pathway alerting the immune system to the presence of a pathogen [4]. Activation of an innate immune response to the pathogen creates an inflammatory milieu that provides additional signals to the T cell, triggering its full activation.

Self peptide presentation at the immunological synapse contributes to T-cell activation by lowering the activation threshold [5–7]. Since self peptides are continuously present at the immunological synapse, T cells strongly recognizing self peptide/MHC complexes (pMHC) must be deleted during thymic education to prevent the development of autoimmune disease [8]. However, T-cell positive selection requires weak recognition of self pMHC complexes [9]. Peripheral self pMHC recognition in the absence of inflammation leads to tolerization [10]. Conversely, self peptide recognition in the context of the inflammatory stimulus emerging from a microbial infection can lead to activation of weakly self-reactive T cells and the development of autoimmune disease, for example, diabetes, multiple sclerosis, and polymyositis [11, 12]. Therefore, optimal peripheral T-cell activation requires a combination of inflammatory signals, nonself pMHC recognition, and low affinity self pMHC recognition to fully activate T cells.

Even though T cells strongly reactive to self pMHC are deleted during development, self peptides can act as minor histocompatibility antigens in the context of allograft transplantation if the genes encoding self peptides show allelic variation (i.e., when DNA sequences for the same gene differ between two or more individuals) within the human population [13–16]. Presentation of peptides containing allelic differences, termed allopeptides, by the human leukocyte antigen (HLA) of donor-transplanted tissue cells can activate recipient T cells, leading to graft rejection. Alternatively, donor T cells may recognize recipient allopeptides, leading to graft versus host disease (GVHD) even in a HLA-matched bone marrow transplant. Since immunosuppressive drugs given to the otherwise healthy transplant recipient suppress inflammation, allopeptide recognition by T cells should lead to tolerance. Nonetheless, once the graft has been accepted and the immunosuppressive drugs are withdrawn, subsequent infections would incite inflammatory conditions. Indeed, viral infections occur after transplantation in approximately 10–60% of immunosuppressed patients, leading to adverse effects on the host and/or transplanted organ [17, 18]. Recognition of new allopeptides that T cells have not been tolerized against, for example, those that are not presented during homeostatic conditions, could result in T-cell activation and immunopathology [19]. Hence, alterations in self peptides under inflammatory conditions can be detrimental to transplanted tissues/organs.

Despite their importance, little is known regarding the nature of the self peptide repertoire (peptidome) displayed during infection. Small-scale studies have reported minor changes in the self peptidome displayed by HLA-A*02;01 and HLA-B*07;02 after human immunodeficiency virus (HIV [20]), influenza virus (INV [21]), and measles virus (MeV [22]) infections. Herein, we use a large-scale proteomics approach to study the dynamics of self peptides presented by five major HLA class I molecules, HLA-A*01;01, HLA-A*02;01, HLA-B*07;02, HLA-B*35;01, and HLA-B*45;01 before and after vaccinia virus (VACV) infection. In contrast to earlier studies (18–20), we observed a profound shift in the self peptidomes uniquely displayed by the five HLA class I molecules studied herein after VACV infection. The newly presented self peptides did not derive from any specific chromosomal region. A fraction (approximately 40%) of them represented peptides derived from type I interferon-induced genes—consistent with the activation of cellular antiviral pathways—but also included other unrelated peptides, suggesting a global change in cellular protein metabolism in response to infection. Furthermore, population analyses of self peptides presented after

infection revealed that a significant number of peptides were derived from proteins containing allelic variation(s). The frequency (approximately 12%) of allelic variation was similar to the rate of complications reported for transplants between HLA-matched pairs [23, 24]. This changing repertoire may provide a possible mechanism for the initiation of allograft rejection or GVHD. Hence, sequencing of the transplant donor and recipient transcriptomes/proteomes could help uncover potential allopeptides that can complicate allograft outcomes [15, 16, 25–32].

2 Materials and methods

2.1 Viruses

The Western Reserve strain of VACV (ATCC, VR-119) was grown in and titrated with BSC-40 cells as previously described [33].

2.2 Large-scale cell culture and VACV infection

Soluble HLA class I (sA1.1, sA2.1, sB7.2, sB35.1, and sB45.1) production and harvest were used as described previously [34]. Briefly, approximately 1×10^9 viable cells were inoculated with VACV (MOI 0.1). Supernatants containing approximately 0.3–4.2 mg/L sA1, sA2, sB7, sB35, or sB45 were collected at 24, 48, and 72 h post inoculation.

2.3 Isolation and fractionation of class I associated peptides

sA1, sA2, sB7, sB35, and sB45 were affinity purified using W6/32-bound protein A Sepharose (GE Healthcare). Class I associated peptide elution, separation, and reversed-phase HPLC purification were all performed as previously described [35].

2.4 MS sequencing of eluted peptides

Lyophilized fractions were resuspended in 0.1% formic acid and subjected to reversed-phase microcapillary LC-nanoESI-MS/MS analysis using an Agilent 1100 binary HPLC pump and a linear trap quadropole (LTQ) linear ion trap mass spectrometer 2.2 (Thermofisher). A fritless, microcapillary column (100-μm inner diameter) was packed with 10 cm of 5-μm C_{18} reversed-phase material (Synergi 4u Hydro RP80a, Phenomenex) as previously described [36]. RPC-fractionated peptides were loaded onto the column equilibrated in buffer A (0.1% formic acid, 5% ACN) using an LC Packing's autosampler. Flow splitting was used to reduce the HPLC flow rate from 200 to 0.3 μL/min as previously described [36, 37]. Peptides from the microcapillary column were eluted directly into the linear ion LTQ mass spectrometer equipped with a microelectrospray source (James Hill Instrument Service). Peptides were eluted using a 60-min linear gradient from 0 to 60% buffer B (0.1% formic acid, 80% ACN) at a flow rate of 0.3 μL/min. During the gradient, the eluted ions were analyzed by one full precursor MS scan (400–2000 m/z) followed by five MS/MS scans of the five most abundant ions detected in the precursor MS scan while operating under dynamic exclusion. Sixty-five percent of peaks were identified in replicate samples by this analysis. The program extractms2 was used to generate the peak list and identify +1 or multiply charged precursor ions from the native MS data file [38]. Tandem spectra were searched with no protease specificity using SEQUEST-PVM [39] against a concatenated Human RefSeq protein database, released May 2005 (28 818 entries), Vaccinia WR Copenhagen Uniprot protein database (760 entries), or a merged human and vaccinia FASTA database of protein sequences [40]. For multiply charged precursor ions ($z \geq +2$), an independent search was performed on both the +2 and +3 mass of the parent ion. A weighted scoring matrix was used to select the most likely charge state of multiply charged precursor ions as previously described [41, 42]. SEQUEST search results were imported into Bioinformatic Graphical Comparative Analysis Tools (BIGCAT) and analyzed as previously described [33, 41]. Xcorr threshold of 1.5 for charge state 1, 2.0 for charge state 2, and 2.5 for charge state 3 and above was used to filter the peptides. This resulted in a false discovery rate (FDR) of 3.6%, which was calculated as the percentage of the number of peptide hits from the reversed database in the total number of peptides in the filtered list (FDR = number of reverse peptides/total number of peptides × 100).

2.5 Bioinformatics

Searches for publicly reported CD8 T-cell epitopes and potential epitopes identified by algorithms were conducted through the Immune Epitope Database (http://www.iedb.org/). Peptide conservation among other *Orthopoxviridae* members was performed using basic local alignment search tool (BLAST) search (NCBI). Self peptides were searched against the human proteome and nucleotide databases using the PAM30 matrix with the BLAST search program restricting the searches with the entrez criterion txid9606[orgn] to specify *Homo sapiens*. Genes encoding these peptide sequences were verified to be present in the HeLa genome by searching the translated HeLa Cell Genome Sequencing Studies (phs000640.v2.p1) database at the Database of Genotypes and Phenotypes (dbGaP), Bethesda, MD: National Center for Biotechnology Information, National Library of Medicine [43, 44]. Proteins were classified by functional class using the Panther Database version 9.0 [45, 46]. Innate immune responsive proteins were identified using the Interferome database [47]. So also, self peptides derived from known or potential oncogenes products were identified by BLAST search against the TSGene database [48]. Tissue expression of proteins was determined using the Tissue-specific Gene Expression and Regulation (TiGER) database [49].

2.6 Analysis

Microsoft Excel and PowerPoint, Prism GraphPad and Adobe Photoshop were used to analyze data and generate graphs and figures.

3 Results

3.1 VACV infection induces a profound self peptidome shift

To determine whether the presentation of self peptides changes after VACV infection, which is known to alter host protein metabolism [50–55], HeLa cells expressing secreted HLA-A*01;01, HLA-A*02;01, HLA-B*07;02, HLA-B*35;01, and HLA-B*45;01 [34] molecules were infected with VACV. Days 1, 2 and3 post infection, soluble class I molecules were affinity purified from culture supernatants of infected cells or uninfected controls. The associated peptides were acid-eluted and fractionated by reversed-phase chromatography, as described previously [56]. Each of the resulting 150 fractions were individually subjected to mass analyses by 2D HPLC in line with ESI-MS/MS. Peptide mass spectra so obtained were compared against both VACV and human proteomes to determine their origin and confirm sequence. MS data revealed the processing and presentation of numerous peptides derived from VACV, confirming infection of the HeLa cells (Fig. 1, Table 1, and Supporting Information Table 1; [33]).

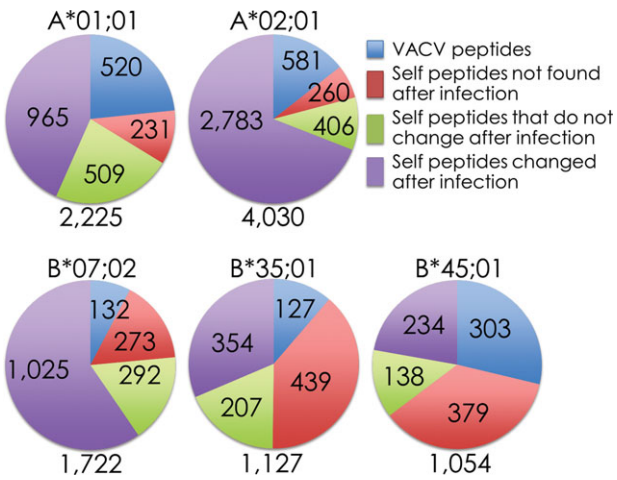

Figure 1. Numerous unique self and viral peptides are presented after VACV infection. MS was used to sequence peptides eluted from the indicated soluble HLA class I molecules. Comparison of the pre- and postinfection samples identified a significant fraction of viral peptides (blue) presented after infection. In addition, large alterations in the self peptidome were observed with some peptides disappearing (red), unchanged (green), or newly presented (purple) after infection. The total number of peptides sequenced for each HLA class I molecule is annotated under each chart.

Comparative proteome searches identified 520 A*01;01-, 581 A*02;01-, 132 B*07;02-, 127 B*35;01-, and 303 B*45;01-associated VACV-derived peptides that were presented only upon infection; of those, 34 A*01;01-, 109 A*02;01-, 65 B*07;02-, 68 B*35;01-, 49 B*45;01-restricted peptide sequences correlated with the MS/MS spectra's fragment ion data with high confidence (see Section 2). Similar to prior reports, these naturally processed and presented peptides were derived from all functional and kinetic classes of VACV proteins (Table 1, Supporting Information Table 1; [33, 57]).

To identify self peptides, MS/MS spectra of ligands eluted from the same class I preparation from which VACV peptides were identified were searched against a Human RefSeq protein database (release May 2005; 28 818 entries). These searches returned 1705 A*01;01-, 3,449 A*02;01-, 1,590 B*07;02-, 1,000 B*35;01-, and 751 B*45;01-associated peptide sequences derived from the human proteome (Figs. 1 and 2). However, since these peptides were eluted from HeLa cells, we ascertained whether the peptides identified by MS/MS analysis were contained within proteins encoded by the HeLa genome. For this, the HeLa Cell Genome Sequencing Studies database (phs000640.v2.p1; Database of Genotypes and Phenotypes [dbGaP], National Center for Biotechnology Information, National Library of Medicine [43, 44]) was searched. Indeed, the peptide sequences reported herein were derived from proteins encoded by the HeLa genome (Table 2 and Supporting Information Table 2).

In order to determine the reproducibility of peptide identification, peptides presented by HLA-A*02;01 were eluted from two independently infected cell samples and sequenced by MS/MS. Replicate analyses of the self peptides eluted from sA2.1 molecules expressed by VACV-infected cells revealed that approximately 60–80% of the HLA-bound peptides were presented at the same time points in both experiments (Supporting Information Table 3).

Comparisons of the self peptides sequenced before and during infection revealed that all five HLA class I molecules investigated here presented numerous novel self peptide sequences after VACV infection. Ranging from approximately 30 to 80% of the sequenced peptides, we identified 965 A*01;01-associated (approximately 56%), 2783 A*02;01-associated (approximately 80%), 1025 B*07;02-associated (approximately 64%), 354 B*35;01-associated (approximately 35%), and 234 B*45;01-associated (approximately 30%) human peptide sequences that were presented solely after infection. Some of the new self peptides were presented stably throughout the course of the infection, while others (majority) were presented in a kinetic fashion: 1, 2, or 3 days post infection (Fig. 2), likely reflecting the kinetics of protein expression from which a given peptide was derived. These data, in conjunction with the high reproducibility of the self peptides between replicate experiments, indicate that VACV infection induced a self peptidome shift that was much more profound than those induced by INV, MeV, and HIV infections [20–22].

Table 1. Characterization of HLA-B*35;01-restricted VACV-derived determinants presented during active infection

VACCC ORF[a)]	Sequence	Prior reports[b)]	Cn[c)]	DPI[d)]	No. of hits[e)]	Function[f)]	Temporal Exp[g)]	VARV[h)]	MONPV	ECTV
$A4L_{83-91}$	VPTATPAPI	[33]	1.5598	1,3	10	S	E/L	NSH[i)]	NSH	NSH
$A8R_{25-34}$	TPMIKENSGF		1.717	1	3	T	I/E	S8I		
$A10L_{111-119}$	NPIINTHSF	[133]	1.5417	3	1	S	L			S8N
$A10L_{457-465}$	FPRKDKSIM		1.5653	3	1	S	L			
$A10L_{853-862}$	RPKILSMINY		1.8534	1	7	S	L			
$A11R_{298-306}$	SPVLNIVLF		1.5163	1	7	O	L			
$A16L_{250-259}$	YPKSNSGDKY	[133]	1.8503	1	3	S	L			
$A17L_{91-100}$	LPLTSLVITY	[133]	2.9936	1,3	8	S	L			
$A18R_{52-61}$	SPSVKTSLVF		1.505	3	1	T	E		S3C	
$A18R_{237-245}$	TPRPANRIY		1.6559	1,3	2	T	E	A5S		
$A20R_{4-12}$	LPVIFLPIF		1.9299	3	2	R	E	NSH		NSH
$A20R_{162-170}$	IPKYLEIEI	[33]	1.8813	1	1	R	E	K3N	K3N	
$A21L_{99-107}$	IPGFARSCY		1.5227	3	1	S	L	A5T		I1L;A5V
$A24R_{663-671}$	FPAEFRDGY		1.9133	1,3	2	T	E			
$A24R_{1002-1010}$	KPYASKVFF	[33]	1.924	3	1	T	E		A4E	
$A32L_{1103-1111}$	IPISDYTGY		2.0188	1,3	10	T	E			
$A37R_{129-138}$	IPSKRLVTSF		1.6865	1	1	U	I/E	NSH		NSH
$A37R_{240-248}$	VPIKEQILY		2.2542	1	1	U	I/E	NSH	V1L	NSH
$A39R_{394-403}$	MPQMKKILKM		1.9143	1	1	E/V	L	NSH	NSH	M1I;Q3R
$A44L_{326-335}$	SPIFDVDVAF		1.5898	1	1	E/V	I/E	NSH		
$A51R_{94-102}$	TPTGVYNYF		1.6732	1	1	U	E			
$A55R_{213-221}$	SPQVIKSLY		1.838	1,3	4	E/V	E	NSH	NSH	
$B3R_{39-48}$	IPSTVKTNLY	[133]	1.8943	1	2	U	I/E	I1M		
$B8R_{70-78}$	FPKNDFVSF	[33];[133]	2.237	1	2	E/V	E			K3N
$B8R_{104-112}$	PPTVTLTEY		1.6996	3	1	E/V	E		T5R	
$B8R_{158-167}$	EPVTYDIDDY	[33]	1.5432	3	1	E/V	E	D6N		T4I
$B9R_{10-19}$	FPSIIYSMSI		1.5804	3	1	U	E/L	NSH		NSH
$B12R_{52-60}$	KPLLSEIRF		1.7458	1	1	U	I/E	I4M;R8N		K1R
$B16R_{4-12}$	LPVIFLSIF		1.5123	1	1	E/V	L	NSH	S7P	NSH
$B16R_{52-61}$	NPTQSDSGIY		1.5403	1	1	E/V	L			S5T
$B16R_{76-84}$	IPIDNGSNM		1.6	1	4	E/V	L	G6C;S7N	P2Q	S7N
$B17L_{181-190}$	APLPGNVLVY	[33]	1.8017	1,3	5	U	E	L3Y	NSH	
$C2L_{337-345}$	LPNLITPRY		2.4458	3	2	E/V	E			
$C9L_{130-138}$	IPTCSNIQY		1.5769	1,2,3	3	U	E/L	NSH		NSH
$D1R_{475-483}$	VPIKFIAEF		1.5121	1	1	T	E			
$D4R_{181-189}$	HPAARDRQF		1.6429	3	1	R	E		R7H	R7H
$D4R_{186-194}$	SPVTTIVGY		1.7325	1,3	6	R	E			
$D8L_{160-169}$	LPSKLDYFTY	[33]	1.8428	3	1	S	L	T9K	K4T	K4T
$D11L_{185-194}$	TPIVNSVQEF		1.6056	1,3	5	T	L			I3V
$D11L_{506-514}$	MPTVDEDLF		1.606	1	1	T	L			
$D12L_{34-43}$	LPSLEYGANY	[133]	1.6592	3	3	T	E			
$D13L_{160-168}$	TPFDVEDTF	[133]	2.2957	1	8	O	L			
$E1L_{10-18}$	FPNITLKII	[133]	1.9468	1,3	5	T	E		F1L	F1L
$E3L_{117-125}$	NPVTVINEY		2.2336	1,2,3	21	E/V	I/E	V5I		V5I
$E8R_{233-241}$	DPVLMFLLF		1.736	3	2	S	E/L			
$E9L_{488-496}$	LPQSMVFEY		1.5988	3	2	R	E			
$E9L_{526-534}$	FPYEGGKVF	[33]	1.8446	3	2	R	E			
$F1L_{162-170}$	NPVKTIKMF		1.7642	3	2	U	E	K4E	K4E	K4E
$F2L_{26-35}$	SPGAAGYDLY	[33]	1.5367	1,2,3	4	R	E	G3Y		NSH
$F3L_{435-443}$	YPRDNPELI		1.5905	3	1	E/V	E	P2Q		
$G2R_{1-9}$	MPFRDLILF		2.2046	1	6	T	E			
$G9R_{69-77}$	GPGGLSALL	[33]	1.5564	3	1	S	L			G4N
$H1L_{133-142}$	SPMLYFLYVY		1.6313	3	2	T	E/L			
$H2R_{141-150}$	DPSAQQFCQY		1.585	3	2	S	L			
$H4L_{636-645}$	EPTDASLKNF		1.8267	3	1	T	L	N9Q	E1K	
$H6R_{156-164}$	SPDEIVIKF		1.7386	3	3	T	E/L	E4K		
$I1L_{53-62}$	IPVDLVKSSF	[133]	2.9981	1,3	3	S	L			
$I4L_{670-678}$	LPEDIKRVY		2.1136	3	1	R	E			

(*Continued*)

Table 1. Continued

VACCC ORF[a)]	Sequence	Prior reports[b)]	Cn[c)]	DPI[d)]	No. of hits[e)]	Function[f)]	Temporal Exp[g)]	VARV[h)]	MONPV	ECTV
I6L$_{159\text{-}167}$	IPMSIISFF	[134]	1.6008	1,3	5	U	E/L		M3I	
I7L$_{153\text{-}161}$	NPKVVKMKI		1.5407	3	1	S	L			
I12L$_{15\text{-}23}$	SPEDDLTDF		1.6251	3	1	U	N/A		P2Q	
J6R$_{177\text{-}185}$	WPLLEIHQY		2.5974	1	1	T	E			
L3L$_{291\text{-}299}$	VPKEDYYFI		2.2064	1	1	T	L			
L4R$_{37\text{-}45}$	FPRSMLSIF	[33];[135]	1.9615	1	1	T	L			
L5R$_{10\text{-}18}$	NPVFIEPTF		1.6253	3	2	S	L			
N2L$_{147\text{-}155}$	KPVYSYVLY		1.5179	1,3	3	O	I/E		V3I	V3I
O1L$_{4\text{-}12}$	YPEFARKAL		1.543	1	2	U	I/E			
O1L$_{549\text{-}557}$	IPITDSLSF		1.6041	1,3	2	U	I/E	D5E		

a)ORFs and location of epitopes are defined based on Copenhagen reference strain (VACCC, ID 10249).
b)Prior reports according to immune epitope database (IEDB; www.iedb.org); blank, this study.
c)Correlation coefficient represents the number of peak identities determined between the theoretically and experimentally derived spectra for a given parent ion normalized to the charge state of the peptide.
d)Days post infection of HeLa cultures with VACV at which the peptide was identified.
e)Total number of times a given peptide sequence was identified by MS.
f)Protein function according to ([5]); S, structural (virion membrane and core); T, transcription; E/V, evasion/virulence; O, other; U, unknown; P, pseudogenes.
g)Temporality of expression (shortened) according to ([6]): IE, immediate early; E; early; E/L, early/late; L, late; N/A, unidentified.
h)Peptide homologies were identified using Netblast (blastcl3 at www.ncbi.nlm.nih.gov) using the following taxonomy id: VARV, variola virus, 10255; ECTV, ectromelia virus, 12643; MONPV, monkeypox virus, 10244.
i)Amino acid changes for homologous poxviral epitopes; NSH, no significant homology; blank, conserved sequences with 100 % homology.
Cn, correlation coefficient.

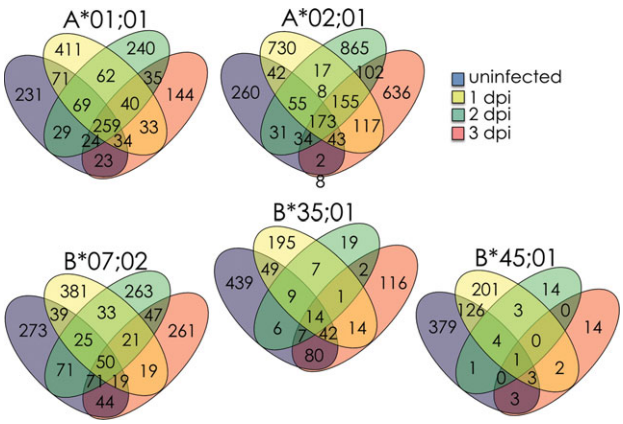

Figure 2. Self peptidome shift after infection with VACV WR strain. The peptides presented by the indicated soluble HLA class I molecules were sequenced by MS. The number of self peptides (correlation coefficient [Cn] > 1.5) detected 0, 1, 2, or 3 days after infection (dpi) are reported in the single or overlapping regions for each HLA allele. The total number of peptides (Cn > 1.5) sequenced is reported as n = number under each day.

3.2 Characterization of the self peptidome shift reveals that VACV infection upregulates type I IFN-regulated and other cellular pathways

We previously reported that about a fifth of the VACV peptides presented by HLA class I molecules were recognized during a natural infection [56]. We reasoned that the nonantigenic VACV peptides may have close sequence homology to the self peptides that are uniquely presented by infected cells. To narrow the analysis of these potentially homologous peptides, focus was laid on 169 A*01;01-, 309 A*02;01-, 157 B*07;02-, 71 B*35;01-, and 107 B*45;01-associated peptide sequences that correlated with the MS/MS spectra's fragment ion data with high confidence (see Section 2). These peptides were further interrogated to determine the mechanism(s) that induced the presentation of altered self peptidomes (Table 2 and Supporting Information Table 2). In initial analyses, self peptides were compared against the VACV proteome to determine whether self peptides uniquely presented after infection had similarity to VACV peptides. On average only about 4.7% (range 2.5–8.2%) of the self peptides were ≥66.6% identical, that is, less than three amino acid changes, when compared to potential VACV peptides (Table 2 and Supporting Information Table 2). Hence, there was very little sequence similarity between self and viral peptides presented after infection.

The presentation of unique self peptides after infection had been reported to result from the activation of multiple cellular pathways [20, 22]. Therefore, we classified the proteins from which these unique infection-induced self peptides were derived based upon their cellular functions by searching the Panther database [45, 46]. This analysis revealed that the proteins, from which self peptides uniquely presented after infection were derived, were distributed among multiple functional categories (Fig. 3 and Supporting Information Table 2). There was little alteration in the proportion of each functional protein family after VACV infection compared with uninfected samples (Supporting Information Fig. 1), suggesting

Table 2. Characterization of HLA-B*35;01-restricted self peptides presented during active infection

Protein	Peptide sequences[a)]	Prior reports[b)]	Interferon responsive[c)]	Cn[d)]	DPI[e)]	No. of hits[f)]	Oncogene status[g)]	VACV[h)]
AHSA1	SPEELYRVF			3.6857	1,3	4		
AIM1	LPDNSLKVF		4	2.2947	3	2	Candidate	K7D;V8G;F9Y[i)]
APEH	VPFKQGMEY			2.5126	1	2		
ARHGEF18	LPSGVGPEY		1	2.3883	1	2		
ARPC4	KPVEGYDISF			3.4382	3	4		
ATP5F1	VPVPPLPEY		1	2.4713	3	7		
ATP6V1B2	HPIPDLTGY		2	2.6049	1	2		
BLMH	KPLFNMEDKI			2.1907	1	1		
CANX	APPSSPKVTY		1	2.5506	3	1		
CAPN1	LPIKDGKLVF			2.2217	3	1		
CCT4	HPTIISESF	[137]		2.2973	1	2		H1Y;I5F;S8T
CTNNA1	NPVQALSEF			2.6248	1	3		
DDOST	FPDKPITQY		1	2.8022	1,2	7		
DDX21	SPPKDVESY			2.4713	3	2		
DDX50	SPPQDVESY			2.8877	2,3	5		
DEK	FPFEKGSVQY		2	2.6723	1,3	3	Known	
DNAJC13	LPVARFLKY		1	2.0127	3	1		
EEF1G	FPAGKVPAF			2.6279	1,3	5		
EEF2	LPSPVTAQKY		3	3.8636	3	4		
	LPVNESFGF			2.275	1	3		
EFHD2	NPYTEFKEF			2.7636	3	1		
ERH	NPNSPSITY	[138]		3.0901	1,2,3	15		
FH	MPTPVIKAF		2	2.8021	1	6	Known*	
FLNA	VPASLPVEF		2	2.0951	3	2	Candidate	
GLS	DPRLKECMDM		16	2.1995	1	1		
GOT2	LPIGGLAEF			2.1101	1	5		
HDGF	FPYEESKEKF		1	2.9516	3	5		
HPRT1	IPDKFVVGY			3.1871	1,3	7		
HSPA8	IPTKQTQTF		1	2.4455	3	2		T6N;Q7F
	QPGVLIQVY			2.2033	3	1		K4R;Q5K;Q7R
ILF2	KPAPDETSF			2.1768	1	1		
ISOC1	IPVIVTEQY		1	2.4097	1	2		
LGALS3	FPFESGKPF			2.2508	1	1		
LTA4H	VPYEKGFAL		4	2.4068	3	3		
MPI	RPVEEIVTF		1	2.4651	3	4	Candidate	
MTHFD1	TPVPGGVGPM		1	2.5411	1	3		
MYO1C	APVGGHILSY			2.3905	3	2		
MYO1G	DPIGGHIHSY			2.6728	3	4		
NARG1	TPLEEAIKF			2.0495	1	1	Candidate	
NDUFS2	LPYFDRLDY			2.1946	3	1		
NIT2	IPEEDAGKLY			2.7275	1	1		
NONO	RPSGKGIVEF		1	2.4937	2,3	7		
NUP210	FPAPAKAVVY		7	2.0796	3	2	Known	
PABPC1	VPNPVINPY		2	3.4778	1,2	2	Candidate	
PDCD6IP	FPQPPQQSY			2.1958	1	1	Candidate	
PLEC1	LPTEEQRVY			2.171	3	2		
PPA2	EPMNPIKQY			2.9655	3	1		
PRPF8	SPIPFPPLSY		2	2.7818	1,3	17		
PSMD7	LPINHQIIY			2.6684	3	2	Candidate	
RAD23A	FPVAGQKLIY			2.7215	3	2		
RAD23B	FPEGLVIQAY		1	2.2462	1	1		
RPL15	RPVPKGATY		3	2.725	3	2		
RPN1	APDELHYTY			2.1773	1,3	2		
SFRS2IP	LPADVQNYY			2.6707	1	2	Known	
SLC25A6	IPKEQGVLSF		7	2.2361	1	1		
SPTBN1	YPNVNIHNF		1	2.5082	3	2		
SRRM2	SPRVPLSAY			2.2579	3	2		
STIP1	NPFNMPNLY			2.2604	1	1		

(*Continued*)

Table 2. Continued

Protein	Peptide sequences[a)]	Prior reports[b)]	Interferon responsive[c)]	Cn[d)]	DPI[e)]	No. of hits[f)]	Oncogene status[g)]	VACV[h)]
SYNCRIP	DPYYGYEDF		1	3.1554	3	3	Candidate	
SYTL3	RPDGTLNSF			2.1761	1	1		G4S;T5E;F9S
TMOD3	IPIPTLKDF		1	2.6011	1	3		
TMPO	TPFKGGTLF		2	2.7071	1,3	8		
	FPEISTRPPL			2.6193	3	6		
TOP2A	LPVKGFRSY		2	2.2794	1	1		
TUBB3	YPDRIMNTF		1	2.6326	3	1		
UBE2L3	YPFKPPKITF			2.0431	1	1		
VCP	YPVEHPDKF			2.9714	1	4		

a)Potential peptides were determined to be derived from proteins encoded by the HeLa genome.
b)Prior reports according to immune epitope database (IEDB; www.iedb.org); blank, this study.
c)Number of entries reporting type I interferon responsiveness for the protein.
d)Correlation coefficient represents the number of peak identities determined between the theoretically and experimentally derived spectra for a given parent ion normalized to the charge state of the peptide.
e)Days post infection of HeLa cultures with VACV at which the peptide was identified.
f)Total number of times a given peptide sequence was identified by MS.
g)Peptides derived from proteins that represent known or potential oncogenes; * mutated self peptide.
h)Amino acid substitutions compared with VACV proteome. Only amino acid substitutions from sequences >66% identical are annotated. Blank, no significant homology.
i)Amino acid changes for homologous VACV epitopes; blank, no significant homology.
Cn, correlation coefficient.

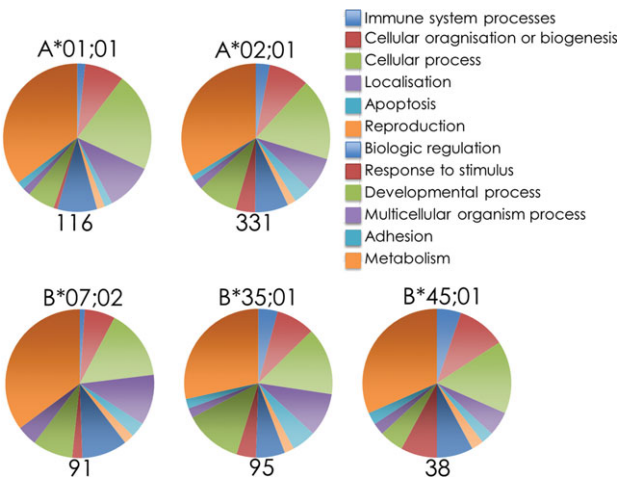

Figure 3. Proteins from which newly presented peptides are derived represent numerous functional families. Panther GO Biological Process identification of the proteins from which self peptides uniquely presented during VACV infection of HeLa cells are derived. Proteins were searched using the Panther database and presented as the proportion of peptides allocated to each functional classifications.

that no particular functional family was induced in response to infection.

HeLa cells respond to infection with a type I interferon response by upregulating internal innate sensors and mediators to defend against infection [58]. Therefore, we specifically sought to identify whether interferon responsive proteins were upregulated following VACV infection by searching the Interferome database for proteins known to be responsive to type I interferons [47]. Approximately, 40% of the self peptides uniquely presented after infection were attributed to type I interferon signaling (Table 2 and Supporting Information Table 2). Hence, the remaining 60% of the newly presented peptides were derived from host proteins other than those directly responsive to interferon signaling (Fig. 3). These proteins are either uniquely expressed post infection or are differentially processed by interferon-induced immunoproteasome in infected cells—compared to the standard proteasomes of uninfected controls. Therefore, alterations in host cell metabolism, possibly through interferon signaling, might play a significant role in the presentation of an infection-induced self peptidome.

Recent drafts of the human proteome revealed that proteins routinely expressed by cells were encoded by genes distributed across all chromosomes, but rarely, if at all, from chromosome 21 and not at all from the Y chromosome [25, 26]. Because our analyses so far indicated very little preference for protein families from which the infection-induced self peptides are derived, we mapped the chromosomal location of the genes that encode proteins from which newly presented peptides were derived in order to identify a particular chromosomal region containing the majority of newly presented peptides. The control of this region would perhaps point to a possible mechanism for this presentation. Infection-induced self peptides identified herein were derived from proteins encoded by genes distributed across all chromosomes with no concerted enrichment evident at any particular region of the human genome (gray bars, Fig. 4 and Supporting Information Fig. 2). Similar to the reported proteome maps, only a few peptides were derived from proteins encoded from chromosome 21, and from chromosomes 20

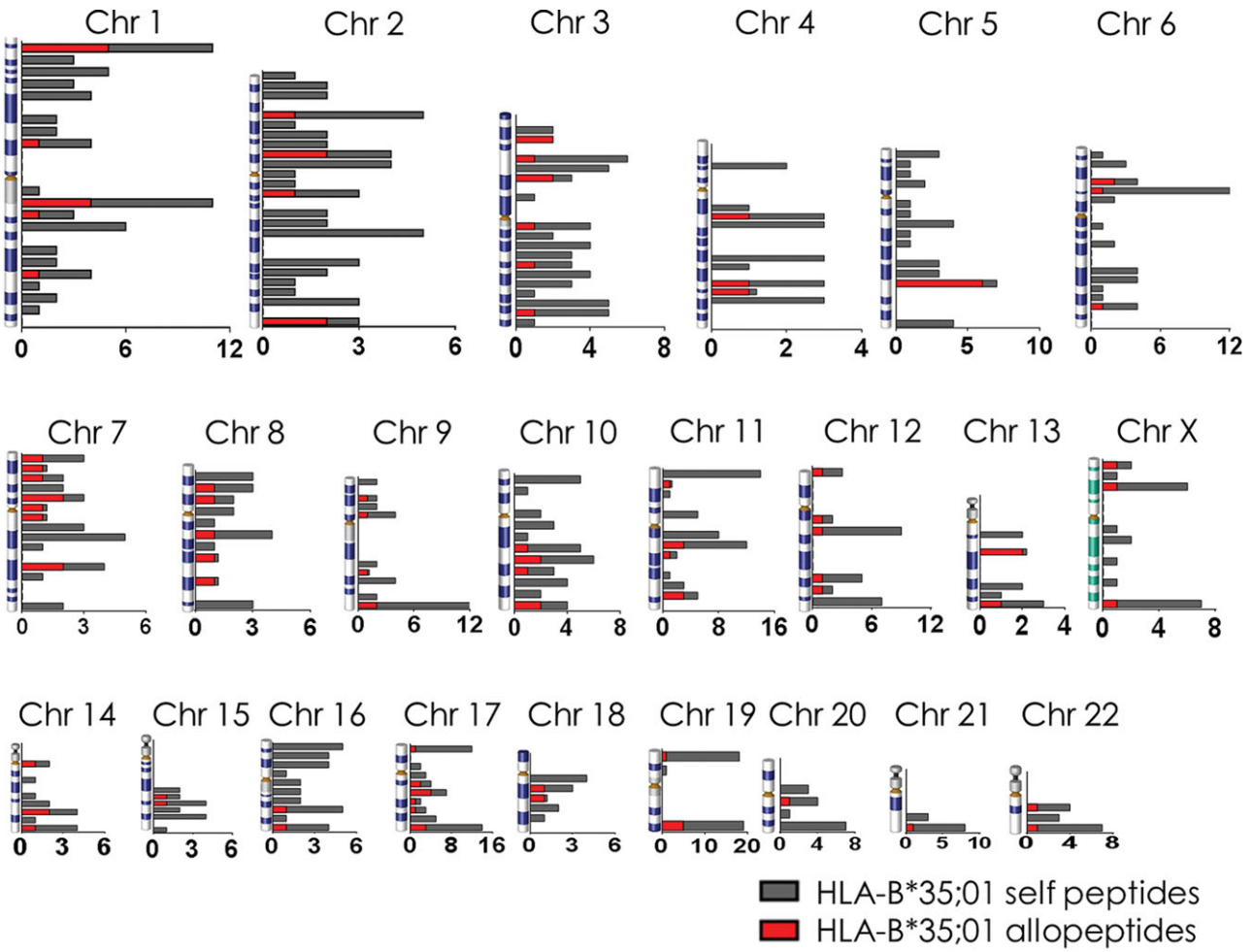

Figure 4. Peptides presented after infection by HLA-B*35;01 are derived from proteins encoded by genes dispersed across the chromosomes. The number of proteins, from which peptides presented only after infection were derived, is enumerated for HLA-B*35;01 (gray bars). Due to size limitations, the locations of each peptide were grouped according to major banding patterns for each chromosome (e.g., 1p36) along the vertical axis (as in [26]). Note the scale of the *x*-axis varies for each chromosome. The number of proteins containing allopeptides in the human population is enumerated for each location (red bars).

and 22 as well. No proteins were identified as encoded from the Y chromosome; this is, consistent with the source HeLa cells being derived from a female subject [59]. Collectively, these data suggested that the shift in self peptide presentation by HLA class I molecules post VACV infection represented a global change in the overall protein metabolism of the cell and not a specific response to the infection.

3.3 Several peptides uniquely presented after infection are derived from oncogenes

Proteomics and proteogenomics approaches have recently identified a few neoepitopes derived from tumor-specific antigens [56, 60–64]. The success of these approaches was predicated on the knowledge of the tumor "mutome"—a collection of mutations within the tumor cell under study—encoded by nonsynonymous single nucleotide polymorphisms revealed by exome and/or transcriptome analyses [57, 60–64]. As HeLa cells were originally isolated from a cervical tumor, we determined whether the collection of self peptides presented by the five HLA class I molecules under study here were derived from the HeLa "mutome." Therefore, the proteins from which self peptides were derived were searched against the TSGene Database containing 184 tumor samples, including 28 cervical cancers [48]. On average, approximately 10% of self peptides were derived from proteins that are known or potential oncogenes (Table 2 and Supporting Information Table 2). Self peptides derived from known oncogene products or candidate cancer proteins were then searched against the TSGene Database using BLAST to specifically identify self peptides that matched the mutated cancer protein sequence. We identified 16 HLA-A*01;01-, 34 HLA-A*02;01-, 13 HLA-B*07;02-, 22 HLA-B*35;01-, and 3 HLA-B*45;01-restricted self peptide sequences that match known cancer associated mutations (* in Table 2 and Supporting Information Table 2). Hence, a direct analysis of the five self peptidomes

revealed that a fraction of the self peptides presented by class I molecules were derived from HeLa mutome.

3.4 Self peptidome presented after infection contains allopeptides

T-cell activation by peptides induced for presentation under inflammatory conditions may be of great significance for HLA-matched transplant recipients responding to infections. In this regard, it is noteworthy that immunosuppressed transplant recipients are susceptible to cytomegalovirus infections [65–68]. This infection is known to induce acute allograft rejection [67, 68] and GVHD [65, 66]. These adverse outcomes would be further augmented if new peptides presented during infection contained allelic differences between the donor and recipient tissues as in allopeptides. Hence, recipient T cells would not be tolerized to such allopeptides. Therefore, in order to determine whether the self peptidomes presented after infection contains allopeptides, BLAST searches of the peptide sequences were performed against translated sequences from 297 human genomes. This analysis revealed variations of one to two amino acids in approximately 12% (range 9.6—13.2%) of the peptides newly presented after infection (Table 3; Supporting Information Table 4; red bars in Fig. 4 and Supporting Information Fig. 2). This percentage is well above the FDR calculated and presented in Section 2, suggesting the observed variability may be biologically relevant. In comparison, only about 2.7% (range 2.3–3.4%) of the self peptides presented before infection displayed variation among the human population. This is less than the FDR, suggesting that the presentation of allopeptides in uninfected cells may be an artifact of incorrect peptide identification. The percentage of allopeptides presented after infection (12%) is similar to previous reports of variations in human MHC class I associated peptides arising from genetic polymorphisms in the population [69].

We noted that the frequency of allopeptides (12%) was similar to the reported rates of unsuccessful transplants (10%), suggesting a possible relationship between allopeptides and transplantation success. However, the presentation of allopeptides would only affect clinical outcomes if those peptides are presented by cells in transplanted tissues. Search of the TiGER tissue expression database revealed that 75% of the proteins from which the allopeptides were derived are expressed at variable levels in commonly transplanted tissues such as the liver, heart, lung, kidney, and colon ([49]; Fig. 5 and Supporting Information Fig. 3).

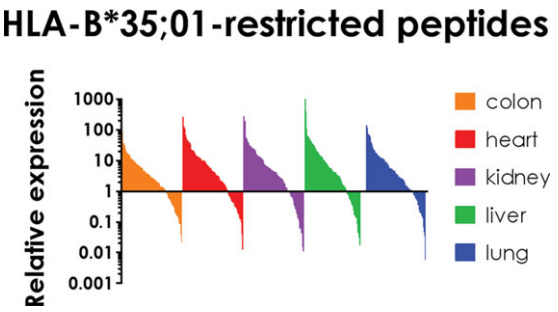

Figure 5. Proteins from which newly presented HLA-B*35;01-restricted allopeptides are derived are expressed in transplanted tissues. Proteins expressed by the colon, heart, kidney, liver, and lungs were identified by searching the TiGER database of tissue expression. This database consists of expression data from microarray, real-time PCR, and proteomic studies and is reported as a fold change compared with housekeeping genes/proteins used in each respective assay. These proteins are variably expressed in the tissue; yet, all tissues express some proteins from which new peptides were derived after infection.

4 Discussion

Cumulatively, we have shown a profound shift in self peptides presented by MHC class I molecules after infection of HeLa cells with VACV. Depending on the HLA allele, the new self peptides represented between 30 and 80% of all peptides presented during infection. They were derived from proteins encoded by genes belonging to multiple cellular functional families and were broadly distributed across chromosomes. We did note that approximately 40% of the proteins from which peptides were derived were responsive to type I interferon signaling, which may play at least some role in the generation of these peptides uniquely presented after infection. Critically, a subset of the altered peptides contained allelic variations within the human population and the proteins from which they were derived were expressed in commonly transplanted tissues, suggesting the potential to negatively affect the outcome of clinical tissue/organ allografts.

This study significantly extends previous reports of self peptides that are uniquely presented by HLA-A*02;01 and HLA-B*07;02 during INV, MeV, and HIV infections [20–22]. While these previous studies reported that only 20 self peptides were uniquely presented during infection, here, we report that more than 1000 peptides were presented by five different HLA class I molecules solely after infection with VACV, the largest study of its kind to date. Herein, we identified four of the 20 peptides uniquely presented after infection with INV [21], three of the 15 peptides uniquely presented after infection with HIV [20], and neither of the two type I interferon-induced peptides presented after MeV infection [22]. Despite analysis of infected HeLa, similar to previous studies, we observed that two orders of magnitude greater number of peptides represented within the altered self peptidome than in the previous studies [20–22]. Perhaps the limited numbers of the total self peptides reported from past studies to be uniquely presented after infection with HIV, INV and measles was insufficient to detect a larger peptidome shift [20–22]. Critically however, alterations in the self peptidome have now been observed for five different HLA class I molecules **(HLA-A*02;01 and HLA-B*07;02)** and in response to multiple infections: HIV, INV, MeV, and VACV.

Table 3. Potential allopeptides presented by HLA-B*35;01 after infection with VACV

Gene	Variants			VACV[c)]
	A[a)]	B[b)]	C[b)]	
PABPC1	VPNPVINPY	VPHPVINPY		
CNOT8	FPSIYDVKY	SPSIYDVKY	FPVIYDVKY	
UPP1	FPALFGDVKF	FPALFGDVKV	LPAMFGDVKF	
LOC441837	DPFIDLNYM	DPFIDLKYM		
LOC387820	SPEDIKKAY	SPEEIKKAY		
ACTB	APEEHPVLL	APEEHPILL		
CNOT7	FPVIYDVKY	FPSIYDVKY		
USP11	TPARDYNNSY	TPARDYSNSS		
UBE2D2	YPFKPPKVAF	YPFKPPKVTF		
C1D	YPVEIHEYL	YPVEIHDYL		
ADAR	NPISGLLEY	NPVSGLLEY		
DDX50	SPPQDVESY	SPPQDIESY		
ITM2B	DPANIVHDF	DPADIIHDF		
DDOST	FPDKPITQY	FPDKPITQV	FPDKRITQY	
KIAA0828	GPFKPNYYRY	GPFKPDHYRY		
CCND1	TPHDFIEHF	TPHDFIEHI		
HNRPD	TPEEKIREYF	ATEEKIREYF		
MLSTD2	NPFHWGEVEY	NPFHWGEVGM		
THRAP1	KPINKSEHL	KPVNKSEHL		
EFHD2	NPYTEFKEF	NPYTEFPEF		
CCT6A	HPRIITEGF	HPRIIAEGF		
BACH1	SPEPGQRTF	PEPGQRTF*		
HSPA4L	APFSKVITF	APFSKVLTF		
NIT2	IPEEDAGKLY	IPEEDAGKLD		
RPL15	RPVPKGATY	HPVPKGATY	RPVPKGVTY	
OTUB1	FPEGSEPKVY	FPEGSEPQVY		
ROR2	FPELGGGHAY	FPELNGGHSY		
ARGBP2	FPISYVEKL	FPISYVEKP		
MYO1G	DPIGGHIHSY	DPIGGHINNY		
DEK	FPFEKGSVQY	FPFEKGSAQY		
UBE2E1	YPFKPPKVTF	YPFKPPKITF		
KIF1A	IPQLCEDLF	IPQLCEELF		
TUBB3	YPDRIMNTF	YPDRIINTF		
CTNNA1	NPVQALSEF	SPVQALSEF		
SF3B4	RPITVSYAF	HPITVSYAF		
ATP6V1B2	HPIPDLTGY	HPIPDLTGF		
C20orf172	HPIHQGITEL	HPIHQGITEV		
ADAR	NPVGGLLEY	NPVSGLLEY		
MTHFD1	TPVPGGVGPM	TPVSGGVGPM		
SMARCA2	APSVVKISY	APSVVKVSY		
KIAA1374	TPYPAILHEY	PPYPAILHEY		
SPTBN1	YPNVNIHNF	YPNVNVHNF		
KIAA1102	SPLGGERPF	SPLGGQRPF		
UBAP2	NPYPGDVTKF	NPYSGDVTKF		
SMARCA5	APFHQLRISY	APFHQLRIQY		
NT5C	FPEEPHVPL	SPEEPHVPL		
DIAPH1	NPVSWVQTF	NPVSWVESF		
CTPS	RPIKPSPPY	RPMKPSPPY		
HSPA8	IPTKQTQTF	IPTKQTQIF		T6N;Q7F K4R;Q5K;Q7R
SUHW2	NPIVLLSDF	NPIVLLSNF		
CP	FPRTPGIWL	FPRTPGLWL		
MYO1C	APVGGHILSY	IPVGGHIISY		
UBP1	SPWPDAPTAY	SPWPDASTAY		
DHX38	TPLPTPSYKY	TPLPAPSYKY		
LPP	YPVTGPKKTY	CPVTSPKKTY		
FLJ10706	LPLWQHISF	SPLWQHIGF		
FHL2	KPITTGGVTY	MPITTGGVTY		
TTF1	FPFRDIFYY	FPSRDIFYY		
UBE2D1	YPFKPPKIAF	YPFKPPKITF		
C6orf150	VPRIQLEEY	VPRIQLEDF		
HDHD4	KPAPSIFYY	LPAPSIFYY		
KIF21A	HPNNVVSVKY	HPNNVVSIKY		
LOC441032	HPGQISAGY	HPGQISSGY		
AHCY	GPFKPDHYRY	CPFKPDHYRY		
FLJ14827	APHTNGPQDL	VPHTNGPQDL		
BNIP2	MPESSQPNY	TPESSQPNY		
UBE2V2	LPQPPEGQTY	LPQPPEGQCY		
TUSC4	HPTLGPKITY	HLTLGPKITY		

(*Continued*)

Table 3. Continued

Gene	Variants			VACV[c)]
	A[a)]	B[b)]	C[b)]	
NME2	RPFFPGLVKY	RPFFAGLVKY		
TCP1	HPTSVISGY	HPTSVISSY		
FLNB	IPYLPITNF	VPYLPITNF		
LOC220717	VPHSIINGY	VPHSIIDGY		
PSMC4	LPLTHFELY	LPVTHFELY		
ZNF581	SPCPQPLAF	SPCPQPLPF		
TFCP2L2	LPLNIQVDTY	LPLNIQIDTY		
TOP2A	LPVKGFRSY	LPVNGFRSY		
PHCA	YPWLRGLGY	YPWLRGLGI		
ASCC3L1	RPVPLEQTY	RPVPLERTY		
SLC25A6	IPKEQGVLSF	IPKDQGVLSF		
LOC391387	HPWKVMPDL	HPWEVMPDL		
LOC339077	LPKLEKAARL	LPKLERAARL		
HSPA8	QPGVLIQVY	QPGVFIQVY		
SUHW1	NPIVLLSNF	NPIVLLSDF		
VIL2	FPWSEIRNI	FPWNEIRNI		
ING1	LPIDPNEPTY	MPVDPNEPTY		
FLJ14803	HPKYPDGKTF	HPKYRDGKTF		
LOC391387	HPWKVMPDLY	HPWEVMPDLY		
GAPD	APSADAPMF	TPSADAPMF		
CARD8	HPHPEDIKF	RPHPEDIKF	HRHPEDIKF	
NACA	SPASDTYIVF	SPASDTYVVF		
TPMT	DPTKHPGPPF	DPTKHAGPPF		
LOC391062	RPNSNGSQFF	PNTNGSQFF		
ANXA7	YPQPPSQSY	YPQPPSQSI		
RYR1	SPHEQEIKFF	SPRDQEIKFF		
ADIPOR2	APLQEKVVF	PPLQEKVVF		
FLJ10774	IPWTVSEQF	IPWTVSEQV		
ALB	VPQVSTPTL	VPEVSTPTL		
TIP120A	GPLVSKVKEY	GPLVVKVKEY		
PTPRS	WPDHGVPEY	WPDHGVPEH		
GRK5	KPENILLDDY	KPENILLDDH		E3D;D8F;Y10S
ENO1	SPDQLADLY	SPNQLADLY		
ARHGEF1	VPVPPNVAF	PPVPPNVAF		
SORCS3	SPVHCLLPF	SPVHCLLPQ	DPVHCLLPY	
MAGI1	KPGEGLGMY	KPSEGLGMY		
ITGB4	RPLQGYSVEY	RPLQGYSVAS		
HLA-C	HPLSDHEATL	HPISDHEATL		
SLC17A6	MPLAGILVQY	MPLAGVLVQY		
UBE2L3	YPFKPPKITF	YPFKPPKVTF		
PIK3C2B	LPQLVQALKY	LSQLVQALKY		
CDC42	FPSEYVPTVF	FPGEYVPTVF		
ProSAPiP1	DPGRDPLLAF	DPGKEPLLAF		
AATF	LPQPDVFPLF	LPQPDVFPVF		
RER1	LPTKQNEEF	YPTKQNEEF	LPTIQNEEF	
GRK5	SPDYWGLGCL	SPDWWGLGCL		
PACS2	LPIAEAMLTY	LPVAEAMLT		
NDUFB9	FPDSPGGTSY	FPDSPRGTSY		
LIPC	QPGCHFLELY	QPGCHSLELY		
HNRPK	FPNTETNGEF	FPNTETSGEF		
DHRS4	SPSPGFSPY	IPSPGFSPY		
METAP2	FPKGQECEY	FPKGQESEY		
SMOC1	RPLPGTSTRY	RPIPGTSTRY		
DHX40	MPDHVIPEI	MPDHVIPQF		
PREI3	TPKECPAIDY	TPKECRAIDY		
PCDHB5	APETVVAVF	SPETVVAVF		
SMARCE1	MPSTPGFVGY	MPSTPSFVGC		
DSC3	IPCSMQENSL	IPCSMLENSL		
RHOC	FPEVYVPTVF	FPEEYVPTVF		
NOTCH1	CPPGFTGSY	CPPGFTGDY		
GLCCI1	CPDKNKVNF	CPDKNKVHF		
BIRC5	DPIGPGTVAY	DPIGPGTVA		
SMURF2	NPYYGLFQY	NPYYGLFEY		
MEN1	APDPPGGLTY	APDPHGGLTY		
NUP210L	SPLTPGLAIY	KSLTPGLAIY		
ING5	MPVDPNEPTY	LPIDPNEPTY		
JUP	SPVESVLFY	SPVDSVLFY		
UBE2D2	SPYQGGVFF	RPYQGGVFF		
GPNMB	GPQLMEVTVY	GPQFMEVTVY		

(*Continued*)

Table 3. Continued

Gene	Variants			VACV[c)]
	A[a)]	B[b)]	C[b)]	
PURB	LPAQGLIEF	LPAQGMIEF		
CPA4	LPVANPDGY	LPVTNPDGY		
LOC440059	CPIMDLTLY	CPIMDLTL		
CDADC1	LPDANTDFY	LPDANTDLY		
TINP1	TPQGAVPAY	IPQGAVPAY		
IFI16	MPPSTPSSSF	MPPTTPSSSF		
AATF	LPQPDVFPL	LPQPDVFPVF		
FMNL3	DPSVTRKKF	DPAVTRKKF		
B4GALT6	APGIANTYLF	APGIVNTYLF		
GPR	VPVVVVFLFL	IPVVVIFLFL		
ATXN10	HPDKKIVAY	CPDKKIVAY		

*Corresponding amino acid is deleted in variant.
a)Peptide sequence identified by MS.
b)Alternate peptide sequences identified by BLAST search.
c)Amino acid substitutions compared with VACV proteome. Only amino acid substitutions from sequences >66% identical are annotated. Blank, no significant homology.

Despite a common core of host proteins responsive to infection [70], there is little overlap among other host proteins that are involved in cellular responses to VACV, MeV, HIV, and INV infection [71–79]. This may, in part, explain the differences in the self peptidome shift reported here for VACV infection compared with HIV, MeV, and INV [20–22]. In addition, VACV encodes more than 200 proteins [80, 81], including a number of proteins that alter cellular functions and immune response [79, 82–89]. In comparison, HIV genome encodes 15 proteins [90], INV genome encodes 17 proteins [91], and MeV genome encodes eight proteins [92]. The large number of proteins encoded by VACV many result in substantially different effects on the host cell protein metabolism compared with the limited host range factors of HIV, INV, and MeV.

In addition to virus-induced changes in host cell metabolism, 40% of peptides uniquely presented after infection were derived from proteins responsive to host interferon signaling. Although not as effective as type II interferon, type I interferons have also been reported to induce immunoproteasomes [93–100], which in turn alters the processing of self and viral peptides for presentation by MHC class I molecules [101]. In response to both types I and II interferon signaling, the metabolism of HeLa cells is altered [102–109]. Although it remains unknown whether VACV infection leads to upregulation and function of immunoproteasomes, the presentation of newly processed peptides represents a change in intracellular protein turnover that will include antigen processing by the immunoproteasome. It is also possible that the extent of self peptidome shift might vary between different viral infections and, if so, the mechanism underlying this process could be of interest for future studies. For example, whether levels of cellular interferon response correlate with the magnitude of the self peptidome shift for different classes of viruses.

In recent years, much attention has been directed to the production of unique peptides through alternate translation pathways [110–117]. These cryptic peptides are produced from translation of alternate reading frames [118–122], readthrough into the untranslated region [123], and frame shift mutations [124–126]. The peptides uniquely presented during VACV infection reported herein map to protein coding regions; however, it is possible that these proteins are generated through these alternate translation mechanisms. Determining whether such peptides are generated by translation of alternate reading frames or through mutations occurring during translation will require further analyses as infection [114, 115, 120] and perhaps interferon signaling may accentuate this process.

Infection of HeLa cells was confirmed by the identification of VACV-derived peptides presented by all HLA molecules studied. Viral peptides presented by HLA-A*02;01 and HLA-B*07;02 were shown previously to contain a subset of peptides that elicit protective immune responses in HLA class I transgenic mice [33]. With similar characterization, a subset of the HLA-A*01;01-, HLA-B*35;01-, and HLA-B*45;01-restricted peptides reported here may also represent CD8 T cell epitopes useful for the development of next-generation vaccines.

Examination of self peptides presented by the five different HLA class I molecules revealed that each HLA molecule had a different propensity to present an altered self peptidome after infection. The greatest variety of newly presented peptides was observed with HLA-A*02;01 with 80.7% of the total peptides sequenced presented only after infection. HLA-B*07;02 and HLA-A*01;01 had an intermediate shift in the peptidome with 64.4 and 56.6%, respectively, of total peptides presented only after infection. The presentation of unique self peptides after infection was less striking for HLA-B*35;01 (35.4%) and HLA-B*45;01 (31.2%). It remains to be determined whether this variability among HLA class I molecules to present a shifted peptide repertoire after infection has a biological consequence, for example, whether it correlates with the susceptibility to allograft rejection.

Biologically, the presentation of self peptides by MHC class I molecules annotates the internal state of the cell. Our data indicated that VACV infection profoundly impacted the cellular metabolism and, hence, the altered state of the cell. Significantly, a fraction of the self peptides presented

by class I molecules were derived from HeLa mutome. This finding implies that the knowledge of the self peptidomes of noncancer and cancer cells from the same individual can reveal neoepitopes that can be targeted by tumor-specific T cells. Considering the finding that viral infections can alter the presentation of self peptides, cancer therapies based on oncolytic viruses can coax the tumor cell to display neoepitopes that are coded by genes induced by viral infections [127, 128].

Of clinical concern is whether T cells recognize the peptides from the shifted self peptidome. Aire-regulated peptide expression in the thymus is thought to lead to the presentation of self peptides derived from proteins ordinarily not expressed by the thymus [129, 130]. However, if these peptides are presented only after infection and are not presented during T-cell development, self-reactive T cells may persist in the periphery. Following infection, T cells may be activated under an inflammatory environment causing additional immunopathology. Recognition of cross-reactive peptides seems to be dependent upon only selected amino acid residues that vary for each particular T cell receptor [131]. Similarities within five amino acids between self and foreign peptides are sufficient to induce cross-reactive T cells after infection, potentially resulting in autoimmunity [132]. Yet, identification of these peptides proves to be difficult, as no single immunologic property is able to computationally predict immunogenic peptides [56].

Our results may help to explain the complication and approximately 10% failure rate of transplantation even among HLA-matched allograft recipients. Of the peptides that were identified to be uniquely presented after infection, approximately 12% displayed allelic variation among the human population. These potential allopeptides in transplanted tissues would remain innocuous until the recipient acquired an infection. The presentation of new peptides during the inflammatory conditions generated in response to infection may result in T-cell activation and recognition of these potential alloepitopes. Again, if certain HLA class I molecules are less likely to present novel peptides after infection, patients that express them would be less susceptible to GVHD or allograft rejection. Analysis of the proteomes of donor and recipient transplant patient may identify these allopeptides prior to committing to the organ transplant. Following the recent publication of drafts of the human proteome, these comparisons may become more commonplace, leading to better proteomic matches for organ transplants, preventing allopeptide presentation after infection, and ultimately more successful transplants [25–28].

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5 References

[1] Hickman, H. D., Luis, A. D., Buchli, R., Few, S. R. et al., Toward a definition of self: proteomic evaluation of the class I peptide repertoire. *J. Immunol.* 2004, *172*, 2944–2952.

[2] Perreault, C., The origin and role of MHC class I-associated self-peptides. *Prog. Mol. Biol. Transl. Sci.* 2010, *92*, 41–60.

[3] de Verteuil, D., Granados, D. P., Thibault, P., Perreault, C., Origin and plasticity of MHC I-associated self peptides. *Autoimmun. Rev.* 2012, *11*, 627–635.

[4] Yewdell, J. W., Bennink, J. R., Hosaka, Y., Cells process exogenous proteins for recognition by cytotoxic T lymphocytes. *Science* 1988, *239*, 637–640.

[5] Yachi, P. P., Ampudia, J., Gascoigne, N. R., Zal, T., Nonstimulatory peptides contribute to antigen-induced CD8-T cell receptor interaction at the immunological synapse. *Nat. Immunol.* 2005, *6*, 785–792.

[6] Yachi, P. P., Lotz, C., Ampudia, J., Gascoigne, N. R., T cell activation enhancement by endogenous pMHC acts for both weak and strong agonists but varies with differentiation state. *J. Exp. Med.* 2007, *204*, 2747–2757.

[7] Anikeeva, N., Lebedeva, T., Clapp, A. R., Goldman, E. R. et al., Quantum dot/peptide-MHC biosensors reveal strong CD8-dependent cooperation between self and viral antigens that augment the T cell response. *Proc. Natl. Acad. Sci. USA* 2006, *103*, 16846–16851.

[8] Zinkernagel, R. M., Callahan, G. N., Klein, J., Dennert, G., Cytotoxic T cells learn specificity for self H-2 during differentiation in the thymus. *Nature* 1978, *271*, 251–253.

[9] Adamopoulou, E., Tenzer, S., Hillen, N., Klug, P. et al., Exploring the MHC-peptide matrix of central tolerance in the human thymus. *Nat. Commun.* 2013, *4*, 2039. doi: 10.1038/ncomms3039

[10] Matzinger, P., Zamoyska, R., Waldmann, H., Self tolerance is H-2-restricted. *Nature* 1984, *308*, 738–741.

[11] Fissolo, N., Haag, S., de Graaf, K. L., Drews, O. et al., Naturally presented peptides on major histocompatibility complex I and II molecules eluted from central nervous system of multiple sclerosis patients. *Mol. Cell. Proteomics* 2009, *8*, 2090–2101.

[12] van der Werf, N., Kroese, F. G., Rozing, J., Hillebrands, J. L., Viral infections as potential triggers of type 1 diabetes. *Diabetes Metab. Res. Rev.* 2007, *23*, 169–183.

[13] Macdonald, W. A., Chen, Z., Gras, S., Archbold, J. K. et al., T cell allorecognition via molecular mimicry. *Immunity* 2009, *31*, 897–908.

[14] Bharat, A., Mohanakumar, T., Allopeptides and the alloimmune response. *Cell. Immunol.* 2007, *248*, 31–43.

[15] Spencer, C. T., Gilchuk, P., Dragovic, S. M., Joyce, S., Minor histocompatibility antigens: presentation principles, recognition logic and the potential for a healing hand. *Curr. Opin. Organ Transplant.* 2010, *15*, 512–525.

[16] Spencer, C. T., Joyce, S., Know thyself: variations in self peptidomes and their immunologic consequences. *Am. Soc. Histocompat. Immunogenet. Quart* 2012, *36*, 28–36.

[17] Weikert, B. C., Blumberg, E. A., Viral infection after renal transplantation: surveillance and management. *Clin. J. Am. Soc. Nephrol.* 2008, *3*, S76–S86.

[18] Martin-Gandul, C., Mueller, N. J., Pascual, M., Manuel, O., The impact of infection on chronic allograft dysfunction and allograft survival after solid organ transplantation. *Am. J. Transplant.* 2015. doi: 10.1111/ajt.13486

[19] Ohashi, P. S., Oehen, S., Buerki, K., Pircher, H. et al., Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell* 1991, *65*, 305–317.

[20] Hickman, H. D., Luis, A. D., Bardet, W., Buchli, R. et al., Cutting edge: class I presentation of host peptides following HIV infection. *J. Immunol.* 2003, *171*, 22–26.

[21] Wahl, A., Schafer, F., Bardet, W., Hildebrand, W. H., HLA class I molecules reflect an altered host proteome after influenza virus infection. *Hum. Immunol.* 2010, *71*, 14–22.

[22] Herberts, C. A., van Gaans-van den Brink, J., van der Heeft, E. D., van Wijk, M. et al., Autoreactivity against induced or upregulated abundant self-peptides in HLA-A*0201 following measles virus infection. *Hum. Immunol.* 2003, *64*, 44–55.

[23] Dierselhuis, M., Goulmy, E., The relevance of minor histocompatibility antigens in solid organ transplantation. *Curr. Opin. Organ Transplant.* 2009, *14*, 419–425.

[24] Gratwohl, A., Dohler, B., Stern, M., Opelz, G., H-Y as a minor histocompatibility antigen in kidney transplantation: a retrospective cohort study. *Lancet* 2008, *372*, 49–53.

[25] Kim, M. S., Pinto, S. M., Getnet, D., Nirujogi, R. S. et al., A draft map of the human proteome. *Nature* 2014, *509*, 575–581.

[26] Wilhelm, M., Schlegl, J., Hahne, H., Moghaddas Gholami, A. et al., Mass-spectrometry-based draft of the human proteome. *Nature* 2014, *509*, 582–587.

[27] Spierings, E., Kim, Y. H., Hendriks, M., Borst, E. et al., Multicenter analyses demonstrate significant clinical effects of minor histocompatibility antigens on GvHD and GvL after HLA-matched related and unrelated hematopoietic stem cell transplantation. *Biol. Blood Marrow Transplant.* 2013, *19*, 1244–1253.

[28] Spierings, E., Goulmy, E., Minor histocompatibility antigen typing by DNA sequencing for clinical practice in hematopoietic stem-cell transplantation. *Methods Mol. Biol.* 2012, *882*, 509–530.

[29] Dierselhuis, M. P., Spierings, E., Drabbels, J., Hendriks, M. et al., Minor H antigen matches and mismatches are equally distributed among recipients with or without complications after HLA identical sibling renal transplantation. *Tissue Antigens* 2013, *82*, 312–316.

[30] van der Torren, C. R., van Hensbergen, Y., Luther, S., Aghai, Z. et al., Possible role of minor h antigens in the persistence of donor chimerism after stem cell transplantation; relevance for sustained leukemia remission. *PLoS ONE* 2015, *10*, e0119595.

[31] Vogt, M. H., van den Muijsenberg, J. W., Goulmy, E., Spierings, E. et al., The DBY gene codes for an HLA-DQ5-restricted human male-specific minor histocompatibility antigen involved in graft-versus-host disease. *Blood* 2002, *99*, 3027–3032.

[32] Spierings, E., Vermeulen, C. J., Vogt, M. H., Doerner, L. E. et al., Identification of HLA class II-restricted H-Y-specific T-helper epitope evoking CD4+ T-helper cells in H-Y-mismatched transplantation. *Lancet* 2003, *362*, 610–615.

[33] Gilchuk, P., Spencer, C. T., Conant, S. B., Hill, T. et al., Discovering naturally processed antigenic determinants that confer protective T cell immunity. *J. Clin. Invest.* 2013, *123*, 1976–1987.

[34] Prilliman, K., Lindsey, M., Zuo, Y., Jackson, K. W. et al., Large-scale production of class I bound peptides: assigning a signature to HLA-B*1501. *Immunogenetics* 1997, *45*, 379–385.

[35] Joyce, S., Tabaczewski, P., Angeletti, R. H., Nathenson, S. G. et al., A nonpolymorphic major histocompatibility complex class Ib molecule binds a large array of diverse self-peptides. *J. Exp. Med.* 1994, *179*, 579–588.

[36] Link, A. J., LaBaer, J., Cold Spring Harbor Laboratory Press, *Proteomics: A Cold Spring Harbor Laboratory Course Manual*, Vol. *viii* 2009, 228 p.

[37] Link, A. J., Jennings, J. L., Washburn, M. P., Analysis of protein composition using multidimensional chromatography and mass spectrometry. *Curr. Protoc. Protein Sci.* 2004, Chapter *23*, Unit 23.1.

[38] Gerbasi, V. R., Link, A. J., The myotonic dystrophy type 2 protein ZNF9 is part of an ITAF complex that promotes cap-independent translation. *Mol. Cell. Proteomics* 2007, *6*, 1049–1058.

[39] Sadygov, R. G., Eng, J., Durr, E., Saraf, A. et al., Code developments to improve the efficiency of automated MS/MS spectra interpretation. *J. Proteome Res.* 2002, *1*, 211–215.

[40] Eng, J. K., Mccormack, A. L., Yates, J. R., An approach to correlate tandem mass-spectral data of peptides with amino-acid-sequences in a protein database. *J. Am. Soc. Mass Spectrom.* 1994, *5*, 976–989.

[41] McAfee, K. J., Duncan, D. T., Assink, M., Link, A. J., Analyzing proteomes and protein function using graphical comparative analysis of tandem mass spectrometry results. *Mol. Cell. Proteomics* 2006, *5*, 1497–1513.

[42] Link, A. J., Eng, J., Schieltz, D. M., Carmack, E. et al., Direct analysis of protein complexes using mass spectrometry. *Nat. Biotechnol.* 1999, *17*, 676–682.

[43] Mailman, M. D., Feolo, M., Jin, Y., Kimura, M. et al., The NCBI dbGaP database of genotypes and phenotypes. *Nat. Genet.* 2007, *39*, 1181–1186.

[44] Tryka, K. A., Hao, L., Sturcke, A., Jin, Y. et al., NCBI's database of genotypes and phenotypes: dbGaP. *Nucleic Acids Res.* 2014, *42*, D975–D979.

[45] Mi, H., Muruganujan, A., Thomas, P. D., PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. *Nucleic Acids Res.* 2013, *41*, D377–D386.

[46] Mi, H., Muruganujan, A., Casagrande, J. T., Thomas, P. D., Large-scale gene function analysis with the PANTHER classification system. *Nat. Protocols* 2013, *8*, 1551–1566.

[47] Rusinova, I., Forster, S., Yu, S., Kannan, A. et al., INTERFEROME v2.0: an updated database of annotated interferon-regulated genes. *Nucleic Acids Res.* 2013, *41*, D1040–D1046.

[48] Zhao, M., Sun, J., Zhao, Z., TSGene: a web resource for tumor suppressor genes. *Nucleic Acids Res.* 2013, *41*, D970–D976.

[49] Liu, X., Yu, X., Zack, D. J., Zhu, H. et al., TiGER: a database for tissue-specific gene expression and regulation. *BMC Bioinform.* 2008, *9*:271. doi: 10.1186/1471-2105-9-271

[50] Rice, A. P., Roberts, B. E., Vaccinia virus induces cellular mRNA degradation. *J. Virol.* 1983, *47*, 529–539.

[51] Pedley, S., Cooper, R. J., The inhibition of HeLa cell RNA synthesis following infection with vaccinia virus. *J. Gen. Virol.* 1984, *65*, 1687–1697.

[52] Becker, Y., Joklik, W. K., Messenger RNA in cells Infected with vaccinia virus. *Proc. Natl. Acad. Sci.* 1964, *51*, 577–585.

[53] Bablanian, R., Esteban, M., Baxt, B., Sonnabend, J. A., Studies on the mechanisms of vaccinia virus cytopathic effects. *J. Gen. Virol.* 1978, *39*, 391–402.

[54] Bablanian, R., Coppola, G., Scribani, S., Esteban, M., Inhibition of protein synthesis by vaccinia virus: IV. The role of low-molecular-weight viral RNA in the inhibition of protein synthesis. *Virology* 1981, *112*, 13–24.

[55] Guerra, S., Lopez-Fernandez, L. A., Pascual-Montano, A., Munoz, M. et al., Cellular gene expression survey of vaccinia virus infection of human HeLa cells. *J. Virol.* 2003, *77*, 6493–6506.

[56] Gilchuk, P., Hill, T. M., Wilson, J. T., Joyce, S., Discovering protective CD8 T cell epitopes—no single immunologic property predicts it! *Curr. Opin. Immunol.* 2015, *34*, 43–51.

[57] Oseroff, C., Kos, F., Bui, H. H., Peters, B. et al., HLA class I-restricted responses to vaccinia recognize a broad array of proteins mainly involved in virulence and viral gene regulation. *Proc. Natl. Acad. Sci. USA* 2005, *102*, 13980–13985.

[58] Kim, O., Sun, Y., Lai, F. W., Song, C. et al., Modulation of type I interferon induction by porcine reproductive and respiratory syndrome virus and degradation of CREB-binding protein by non-structural protein 1 in MARC-145 and HeLa cells. *Virology* 2010, *402*, 315–326.

[59] Skloot, R., The Immortal Life of Henrietta Lacks, Broadway Books, New York, NY. Vol. *xiv*, 2011, 381 p.

[60] Gubin, M. M., Artyomov, M. N., Mardis, E. R., Schreiber, R. D., Tumor neoantigens: building a framework for personalized cancer immunotherapy. *J. Clin. Invest.* 2015, 1–9.

[61] Gubin, M. M., Zhang, X., Schuster, H., Caron, E. et al., Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* 2014, *515*, 577–581.

[62] Yadav, M., Jhunjhunwala, S., Phung, Q. T., Lupardus, P. et al., Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. *Nature* 2014, *515*, 572–576.

[63] Kowalewski, D. J., Schuster, H., Backert, L., Berlin, C. et al., HLA ligandome analysis identifies the underlying specificities of spontaneous antileukemia immune responses in chronic lymphocytic leukemia (CLL). *Proc. Natl. Acad. Sci. USA* 2015, *112*, E166–E175.

[64] Duan, F., Duitama, J., Al Seesi, S., Ayres, C. M. et al., Genomic and bioinformatic profiling of mutational neoepitopes reveals new rules to predict anticancer immunogenicity. *J. Exp. Med.* 2014, *211*, 2231–2248.

[65] Cantoni, N., Hirsch, H. H., Khanna, N., Gerull, S. et al., Evidence for a bidirectional relationship between cytomegalovirus replication and acute graft-versus-host disease. *Biol. Blood Marrow Transplant.* 2010, *16*, 1309–1314.

[66] McCarthy, A. L., Malik Peiris, J. S., Taylor, C. E., Green, M. A. et al., Increase in severity of graft versus host disease by cytomegalovirus. *J. Clin. Pathol.* 1992, *45*, 542–544.

[67] Beam, E., Razonable, R., Cytomegalovirus in solid organ transplantation: epidemiology, prevention, and treatment. *Curr. Infect. Dis. Rep.* 2012, *14*, 633–641.

[68] Linares, L., Sanclemente, G., Cervera, C., Hoyo, I. et al., Influence of cytomegalovirus disease in outcome of solid organ transplant patients. *Transplant. Proc.* 2011, *43*, 2145–2148.

[69] Granados, D. P., Sriranganadane, D., Daouda, T., Zieger, A. et al., Impact of genomic polymorphisms on the repertoire of human MHC class I-associated peptides. *Nat. Commun.* 2014, *5*:3600. doi: 10.1038/ncomms4600

[70] Jenner, R. G., Young, R. A., Insights into host responses against pathogens from transcriptional profiling. *Nat. Rev. Microbiol.* 2005, *3*, 281–294.

[71] Konig, R., Stertz, S., Zhou, Y., Inoue, A. et al., Human host factors required for influenza virus replication. *Nature* 2010, *463*, 813–817.

[72] Karlas, A., Machuy, N., Shin, Y., Pleissner, K.-P. et al., Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. *Nature* 2010, *463*, 818–822.

[73] Brass, A. L., Huang, I. C., Benita, Y., John, S. P. et al., The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, west Nile virus, and dengue virus. *Cell* 2009, *139*, 1243–1254.

[74] Zhou, H., Xu, M., Huang, Q., Gates, A. T. et al., Genome-scale RNAi screen for host factors required for HIV replication. *Cell Host Microbe* 2008, *4*, 495–504.

[75] König, R., Zhou, Y., Elleder, D., Diamond, T. L. et al., Global analysis of host-pathogen interactions that regulate early-stage HIV-1 replication. *Cell* 2008, *135*, 49–60.

[76] Beard, P. M., Griffiths, S. J., Gonzalez, O., Haga, I. R. et al., A loss of function analysis of host factors influencing *vaccinia virus*replication by RNA interference. *PLoS ONE* 2014, *9*, e98431.

[77] Brass, A. L., Dykxhoorn, D. M., Benita, Y., Yan, N. et al., Identification of host proteins required for HIV infection through a functional genomic screen. *Science* 2008, *319*, 921–926.

[78] Guerra, S., López-Fernández, L. A., Pascual-Montano, A., Muñoz, M. et al., Cellular gene expression survey of vaccinia virus infection of human HeLa cells. *J. Virol.* 2003, *77*, 6493–6506.

[79] Assarsson, E., Greenbaum, J. A., Sundström, M., Schaffer, L. et al., Kinetic analysis of a complete poxvirus transcriptome reveals an immediate-early class of genes. *Proc. Natl. Acad. Sci.* 2008, *105*, 2140–2145.

[80] Chou, W., Ngo, T., Gershon, P. D., An overview of the vaccinia virus infectome: a survey of the proteins of the poxvirus-infected cell. *J. Virol.* 2012, *86*, 1487–1499.

[81] Chung, C.-S., Chen, C.-H., Ho, M.-Y., Huang, C.-Y. et al., Vaccinia virus proteome: identification of proteins in vaccinia virus intracellular mature virion particles. *J. Virol.* 2006, *80*, 2127–2140.

[82] Barber, G. N., Host defense, viruses and apoptosis. *Cell Death Diff.* 2001, *8*, 113–126.

[83] Durbin, J. E., Hackenmiller, R., Simon, M. C., Levy, D. E., Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. *Cell* 1996, *84*, 443–450.

[84] Meraz, M. A., White, J. M., Sheehan, K. C., Bach, E. A. et al., Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. *Cell* 1996, *84*, 431–442.

[85] Symons, J. A., Alcamí, A., Smith, G. L., Vaccinia virus encodes a soluble type I interferon receptor of novel structure and broad species specificity. *Cell* 1995, *81*, 551–560.

[86] Smith, G. L., Symons, J. A., Khanna, A., Vanderplasschen, A. et al., Vaccinia virus immune evasion. *Immunol. Rev.* 1997, *159*, 137–154.

[87] Kirwan, S., Merriam, D., Barsby, N., McKinnon, A. et al., Vaccinia virus modulation of natural killer cell function by direct infection. *Virology* 2006, *347*, 75–87.

[88] Smith, G. L., Symons, J. A., Alcami, A., Immune modulation by proteins secreted from cells infected by vaccinia virus. *Arch. Virol. Suppl.* 1999, *15*, 111–129.

[89] Moss, B., Poxvirus cell entry: how many proteins does it take? *Viruses* 2012, *4*, 688–707.

[90] Watts, J. M., Dang, K. K., Gorelick, R. J., Leonard, C. W. et al., Architecture and secondary structure of an entire HIV-1 RNA genome. *Nature* 2009, *460*, 711–716.

[91] Dubois, J., Terrier, O., Rosa-Calatrava, M., Influenza viruses and mRNA splicing: doing more with less. *MBio* 2014, *5*(3):e00070-14. doi: 10.1128/mBio.00070-14

[92] Bankamp, B., Takeda, M., Zhang, Y., Xu, W. et al., Genetic characterization of measles vaccine strains. *J. Infect. Dis.* 2011, *204*, S533–S548.

[93] McCarthy, M. K., Weinberg, J. B., The immunoproteasome and viral infection: a complex regulator of inflammation. *Front. Microbiol.* 2015, *6*:21. doi: 10.3389/fmicb.2015.00021

[94] Shin, E. C., Seifert, U., Kato, T., Rice, C. M. et al., Virus-induced type I IFN stimulates generation of immunoproteasomes at the site of infection. *J Clin Invest* 2006, *116*, 3006–3014.

[95] Freudenburg, W., Gautam, M., Chakraborty, P., James, J. et al., Reduction in ATP levels triggers immunoproteasome activation by the 11S (PA28) regulator during early antiviral response mediated by IFNbeta in mouse pancreatic beta-cells. *PLoS ONE* 2013, *8*, e52408.

[96] Freudenburg, W., Gautam, M., Chakraborty, P., James, J. et al., Immunoproteasome activation during early antiviral response in mouse pancreatic beta-cells: new insights into auto-antigen generation in type I diabetes? *J. Clin. Cell Immunol.* 2013, *4*, 141.

[97] Fabunmi, R. P., Wigley, W. C., Thomas, P. J., DeMartino, G. N., Interferon gamma regulates accumulation of the proteasome activator PA28 and immunoproteasomes at nuclear PML bodies. *J. Cell Sci.* 2001, *114*, 29–36.

[98] Nathan, James A., Spinnenhirn, V., Schmidtke, G., Basler, M. et al., Immuno- and constitutive proteasomes do not differ in their abilities to degrade ubiquitinated proteins. *Cell* 2013, *152*, 1184–1194.

[99] Robek, M. D., Garcia, M. L., Boyd, B. S., Chisari, F. V., Role of immunoproteasome catalytic subunits in the immune response to hepatitis B virus. *J. Virol.* 2007, *81*, 483–491.

[100] Kuzina, E. S., Chernolovskaya, E. L., Kudriaeva, A. A., Zenkova, M. A. et al., Immunoproteasome enhances intracellular proteolysis of myelin basic protein. *Dokl. Biochem. Biophys.* 2013, *453*, 300–303.

[101] Kincaid, E. Z., Che, J. W., York, I., Escobar, H. et al., Mice completely lacking immunoproteasomes show major changes in antigen presentation. *Nat. Immunol.* 2012, *13*, 129–135.

[102] Shaw, A. C., Rossel Larsen, M., Roepstorff, P., Justesen, J. et al., Mapping and identification of interferon gamma-regulated HeLa cell proteins separated by immobilized pH gradient two-dimensional gel electrophoresis. *Electrophoresis* 1999, *20*, 984–993.

[103] Shirey, K. A., Jung, J. Y., Maeder, G. S., Carlin, J. M., Up-regulation of IFN-gamma receptor expression by proinflammatory cytokines influences IDO activation in epithelial cells. *J. Interferon Cytokine Res.* 2006, *26*, 53–62.

[104] Zhang, H. M., Yuan, J., Cheung, P., Chau, D. et al., Gamma interferon-inducible protein 10 induces HeLa cell apoptosis through a p53-dependent pathway initiated by suppression of human papillomavirus type 18 E6 and E7 expression. *Mol. Cell. Biol.* 2005, *25*, 6247–6258.

[105] Yang, W., Tan, J., Liu, R., Cui, X. et al., Interferon-γ upregulates expression of IFP35 gene in HeLa cells via interferon regulatory factor-1. *PLoS ONE* 2012, *7*, e50932.

[106] Lizard, G., Chignol, M. C., Chardonnet, Y., Schmitt, D., Differences of reactivity to interferon gamma in HeLa and CaSki cells: a combined immunocytochemical and flow-cytometric study. *J. Cancer Res. Clin. Oncol.* 1996, *122*, 223–230.

[107] Muller, U., Steinhoff, U., Reis, L. F., Hemmi, S. et al., Functional role of type I and type II interferons in antiviral defense. *Science* 1994, *264*, 1918–1921.

[108] Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H. et al., How cells respond to interferons. *Annu. Rev. Biochem.* 1998, *67*, 227–264.

[109] Zhou, A., Hassel, B. A., Silverman, R. H., Expression cloning of 2–5A-dependent RNAase: a uniquely regulated mediator of interferon action. *Cell* 1993, *72*, 753–765.

[110] Schwab, S. R., Li, K. C., Kang, C., Shastri, N., Constitutive display of cryptic translation products by MHC class I molecules. *Science* 2003, *301*, 1367–1371.

[111] Weinzierl, A. O., Maurer, D., Altenberend, F., Schneiderhan-Marra, N. et al., A cryptic vascular endothelial growth factor T-cell epitope: identification and characterization by mass spectrometry and T-cell assays. *Cancer Res.* 2008, *68*, 2447–2454.

[112] Andersen, R. S., Andersen, S. R., Hjortso, M. D., Lyngaa, R. et al., High frequency of T cells specific for cryptic epitopes in melanoma patients. *Oncoimmunology* 2013, *2*, e25374.

[113] Li, C., Goudy, K., Hirsch, M., Asokan, A. et al., Cellular immune response to cryptic epitopes during therapeutic gene transfer. *Proc. Natl. Acad. Sci. USA* 2009, *106*, 10770–10774.

[114] Rutkowski, M. R., Ho, O., Green, W. R., Defining the mechanism(s) of protection by cytolytic CD8 T cells against a cryptic epitope derived from a retroviral alternative reading frame. *Virology* 2009, *390*, 228–238.

[115] Cardinaud, S., Consiglieri, G., Bouziat, R., Urrutia, A. et al., CTL escape mediated by proteasomal destruction of an HIV-1 cryptic epitope. *PLoS Pathog.* 2011, *7*, e1002049.

[116] Shastri, N., Nguyen, V., Gonzalez, F., Major histocompatibility class I molecules can present cryptic translation products to T-cells. *J. Biol. Chem.* 1995, *270*, 1088–1091.

[117] Malarkannan, S., Afkarian, M., Shastri, N., A rare cryptic translation product is presented by Kb major histocompatibility complex class I molecule to alloreactive T cells. *J. Exp. Med.* 1995, *182*, 1739–1750.

[118] Schwab, S. R., Shugart, J. A., Horng, T., Malarkannan, S. et al., Unanticipated antigens: translation initiation at CUG with leucine. *PLoS Biol.* 2004, *2*, e366.

[119] Malarkannan, S., Horng, T., Shih, P. P., Schwab, S. et al., Presentation of out-of-frame peptide/MHC class I complexes by a novel translation initiation mechanism. *Immunity* 1999, *10*, 681–690.

[120] Cardinaud, S., Moris, A., Fevrier, M., Rohrlich, P. S. et al., Identification of cryptic MHC I-restricted epitopes encoded by HIV-1 alternative reading frames. *J. Exp. Med.* 2004, *199*, 1053–1063.

[121] Starck, S. R., Ow, Y., Jiang, V., Tokuyama, M. et al., A distinct translation initiation mechanism generates cryptic peptides for immune surveillance. *PLoS ONE* 2008, *3*, e3460.

[122] Starck, S. R., Jiang, V., Pavon-Eternod, M., Prasad, S. et al., Leucine-tRNA initiates at CUG start codons for protein synthesis and presentation by MHC class I. *Science* 2012, *336*, 1719–1723.

[123] Goodenough, E., Robinson, T. M., Zook, M. B., Flanigan, K. M. et al., Cryptic MHC class I-binding peptides are revealed by aminoglycoside-induced stop codon read-through into the 3′ UTR. *Proc. Natl. Acad. Sci. USA* 2014, *111*, 5670–5675.

[124] Garrison, K. E., Champiat, S., York, V. A., Agrawal, A. T. et al., Transcriptional errors in human immunodeficiency virus type 1 generate targets for T-cell responses. *Clin. Vaccine Immunol.* 2009, *16*, 1369–1371.

[125] Zook, M. B., Howard, M. T., Sinnathamby, G., Atkins, J. F. et al., Epitopes derived by incidental translational frameshifting give rise to a protective CTL response. *J. Immunol.* 2006, *176*, 6928–6934.

[126] Garbe, Y., Maletzki, C., Linnebacher, M., An MSI tumor specific frameshift mutation in a coding microsatellite of MSH3 encodes for HLA-A0201-restricted CD8+ cytotoxic T cell epitopes. *PLoS ONE* 2011, *6*, e26517.

[127] Wold, W. S., Toth, K., Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. *Curr. Gene Ther.* 2013, *13*, 421–433.

[128] Knipe, D. M., Howley, P. M., Fields virology, Vol. *2*, 2013. Lippincott Williams & Wilkins, Philedelphia, PA.

[129] Coutinho, A., Caramalho, I., Seixas, E., Demengeot, J., Thymic commitment of regulatory T cells is a pathway of TCR-dependent selection that isolates repertoires undergoing positive or negative selection. *Curr. Top. Microbiol. Immunol.* 2005, *293*, 43–71.

[130] Park, Y., Moon, Y., Chung, H. Y., AIRE-1 (autoimmune regulator type 1) as a regulator of the thymic induction of negative selection. *Ann. NY Acad. Sci.* 2003, *1005*, 431–435.

[131] Birnbaum, M. E., Mendoza, J. L., Sethi, D. K., Dong, S. et al., Deconstructing the peptide-MHC specificity of T cell recognition. *Cell* 2014, *157*, 1073–1087.

[132] Nelson, Ryan W., Beisang, D., Tubo, Noah J., Dileepan, T. et al., T cell receptor cross-reactivity between similar foreign and self peptides influences naive cell population size and autoimmunity. *Immunity* 2015, *42*, 95–107.

[133] Moutaftsi, M., Peters, B., Pasquetto, V., Tscharke, D. C. et al., A consensus epitope prediction approach identifies the breadth of murine T(CD8+)-cell responses to vaccinia virus. *Nat. Biotechnol.* 2006, *24*, 817–819.

[134] Oseroff, C., Peters, B., Pasquetto, V., Moutaftsi, M. et al., Dissociation between epitope hierarchy and immunoprevalence in CD8 responses to vaccinia virus western reserve. *J. Immunol.* 2008, *180*, 7193–7202.

[135] Moise, L., McMurry, J. A., Buus, S., Frey, S. et al., In silico-accelerated identification of conserved and immunogenic variola/vaccinia T-cell epitopes. *Vaccine* 2009, *27*, 6471–6479.

[136] Assarsson, E., Sidney, J., Oseroff, C., Pasquetto, V. et al., A quantitative analysis of the variables affecting the repertoire of T cell specificities recognized after vaccinia virus infection. *J. Immunol.* 2007, *178*, 7890–7901.

[137] Schittenhelm, R. B., Dudek, N. L., Croft, N. P., Ramarathinam, S. H. et al., A comprehensive analysis of constitutive naturally processed and presented HLA-C*04:01 (Cw4)-specific peptides. *Tissue Antigens* 2014, *83*, 174–179.

[138] Barber, L. D., Percival, L., Arnett, K. L., Gumperz, J. E. et al., Polymorphism in the alpha 1 helix of the HLA-B heavy chain can have an overriding influence on peptide-binding specificity. *J. Immunol.* 1997, *158*, 1660–1669.

