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

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Abstract

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, -A*02;01, -B*07;02, -B*35;01 and -B*45;01 was determined by mass spectrometry 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 (~40%) of the self peptidome shift was composed of peptides derived from type I interferon-inducible genes, consistent with cellular responses to viral infection. Interestingly, ~12% of self peptides presented after infection showed allelic variation when searched against ~300 human genomes.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Conclusion and clinical relevance—Self peptidome shift in a clinical transplant setting could result in alloreactivity by presenting new self peptides in context of infection-induced inflammation.

Keywords

infection; minor histocompatibility; peptidome; self peptides; transplantation

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]. During homeostasis, these peptides are derived from self proteins and their presentation signifies normal cellular operations and as such is ignored by self-educated T cells. However, during infection, 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, e.g., diabetes, multiple sclerosis and polymyositis [11, 12]. Therefore, optimal peripheral T cell activation requires a combination of inflammatory signals, non-self 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 variant alleles) within the human population [13-16]. Presentation of peptides containing allelic differences, termed allopeptides, by the 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 ~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, e.g., 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 little change 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, HLAA*01;01, -A*02;01, -B*07;02, -B*35;01, -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 (~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 (~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].

Materials and Methods

Viruses

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

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 as described previously [34]. Briefly, ~1×10^9 viable cells were inoculated with VACV (MOI 0.1). Supernatants containing ~0.3—4.2mg/L sA1, sA2, sB7, sB35 or sB45 were collected at 24, 48, and 72 hrs post inoculation.

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].

Mass spectrometry 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 an 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% acetonitrile) using a LCPacking's autosampler. Flow splitting was used to reduce the HPLC flow rate from 200μl/min to 0.3μl/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% acetonitrile) at a flow rate of 0.3μl/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. 65% of peaks were identified in replicate samples by this analysis. The program extractms2 was used to generate the ASCII peak list and identify +1 or multiply charged precursor ions from the native mass spectrometry data file [38]. Tandem spectra were searched with no protease specificity using SEQUEST-PVM [39] against a concatenated Human RefSeq protein database release 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).

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 amongst other *Orthopoxviridae* members was performed using 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].

Analysis

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

Results

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, A*02;01, B*07;02, B*35;01 and B*45;01, [34] molecules were infected with VACV. One, two and three days 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. Mass spectrometry data revealed the processing and presentation of numerous peptides derived from VACV, confirming infection of the HeLa cells (Figure 1, Table 1 and Supplemental Table 1; [33]).

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 Materials and Methods). Similar to prior reports, these naturally processed and presented peptides were derived from all functional and kinetic classes of VACV proteins (Table 1, Supplemental 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 1,705 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 (Figure 1 and Figure 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 Supplementary 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 ~60-80% of the HLA-bound peptides were presented at the same time points in both experiments (Supplemental 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 ~30 to ~80% of the sequenced peptides, we identified 965 A*01;01- (~56%), 2,783 A*02;01- (~80%), 1,025 B*07;02- (~64%), 354 B*35;01- (~35%), and 234 B*45;01 (~30%)-associated 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: one, two or three days post infection (Figure 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].

Characterization of the self peptidome shift reveals that VACV infection up-regulates 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 Materials and Methods). These peptides were further interrogated to determine the mechanism(s) that induced the presentation of altered self peptidomes (Table 2 and Supplemental 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, i.e., less than three amino acid changes, when compared to potential VACV peptides (Table 2 and Supplemental 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 amongst multiple functional categories (Figure 3 and Supplemental Table 2). There was little alteration in the proportion of each functional protein family after VACV infection compared with uninfected samples (Supplemental Figure 1), suggesting that no particular functional family was induced in response to infection.

HeLa cells respond to infection with a type I interferon response by up regulating internal innate sensors and mediators to defend against infection [58]. Therefore, we specifically sought to identify whether interferon responsive proteins were up regulated 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 Supplemental Table 2) suggesting that the remaining 60% of the newly presented peptides were derived from host proteins other than those directly responsive to interferon signaling (Figure 3). These proteins are either uniquely expressed post infection or are differentially process by interferon-induced immunoproteasome in infected cells, — compared to the resting proteasome 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]. As well 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. The 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 (grey bars, Figure 4 and Supplemental Figure 2). Similar to the reported proteome maps, few peptides were derived from proteins encoded from chromosome 21, but chromosomes 20 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.

Several peptides uniquely presented after infection are derived from oncogenes

Proteomics and proteogenomics approaches have recently identified a few neo-epitopes 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 non-synonymous 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, ~10% of self peptides were derived from proteins that are known or potential oncogenes (Table 2 and Supplemental 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 HLAB*35;01-, and 3 HLA-B*45;01-restricted self peptide sequences that match known cancer associated mutations (* in Table 2 and Supplemental 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.

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 ~12% (range 9.6%—13.2%) of the peptides newly presented after infection (Table 3; Supplemental Table 4; red bars in Figure 4 and Supplemental Figure 2). This percentage is well above the false discovery rate calculated and presented in Materials and Methods 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 amongst the human population. This is less than the false discovery rate 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]; Figure 5 and Supplemental Figure 3).

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—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 ~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 the presentation of only 20 self peptides uniquely presented during infection, here, we report over 1,000 peptides presented by five different HLA class I molecules solely after infection with VACV, the largest study of its kind to date. Herein, we identified 4 of the 20 peptides uniquely presented after infection with INV [21], 3 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 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 and in response to multiple infections (HLA-A*02;01 and HLA-B*07;02): HIV, INV, MeV and VACV.

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 over 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 8 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 type I and type 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 correlates 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], read-through 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. Those viral peptides presented by HLA-A*02;01 and -B*07;02 were shown 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-, -B*35;01-, and -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 -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 -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, e.g., 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 non-cancer and cancer cells from the same individual can reveal neo-epitopes 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 neo-epitopes 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 select 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 ~10% failure rate of transplantation even amongst HLA-matched allograft recipients. Of the peptides that were identified to be uniquely presented after infection, ~12% displayed allelic variation amongst 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 allo-epitopes. 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].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BLAST	basic local alignment search tool
Cn	Correlation coefficient
GVHD	graft versus host disease
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
INV	influenza virus
MeV	measles virus
MPXV	monkeypox virus

VACV	vaccinia virus
VARV	variola virus

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Statement of 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. Whilst 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 vaccinia virus. Of significant clinical import 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 graft-versus-host-disease or transplant rejection. The proportion of allopeptides was very similar to the reported rate of transplant complication and failure (~10%). Our study suggests that deep sequencing and proteomics analyses of self peptides may enhance the success of clinical transplant outcome and tumor immunotherapies.

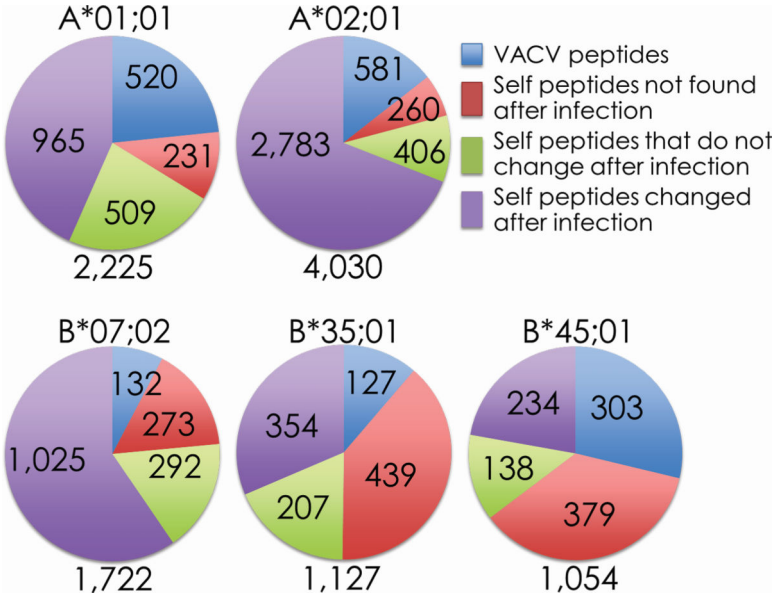

Figure 1. Numerous unique self and viral peptides are presented after VACV infection

Mass spectrometry was used to sequence peptides eluted from the indicated soluble HLA class I molecules. Comparison of the pre- and post-infection 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.

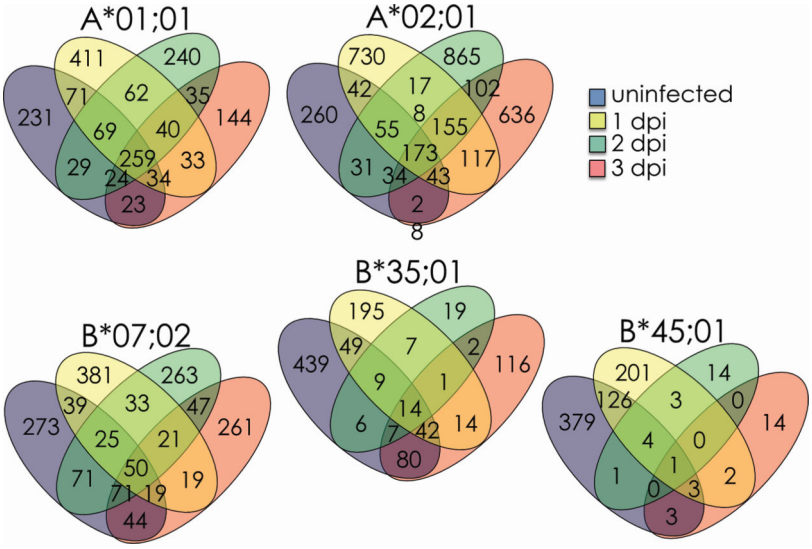

Figure 2. Self peptidome shift after infection with VACV WR strain

The peptides presented by the indicated soluble HLA class I molecules were sequenced by mass spectrometry. The number of self peptides (Cn>1.5) detected 0, 1, 2 or 3 days after infection 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=# under each day.

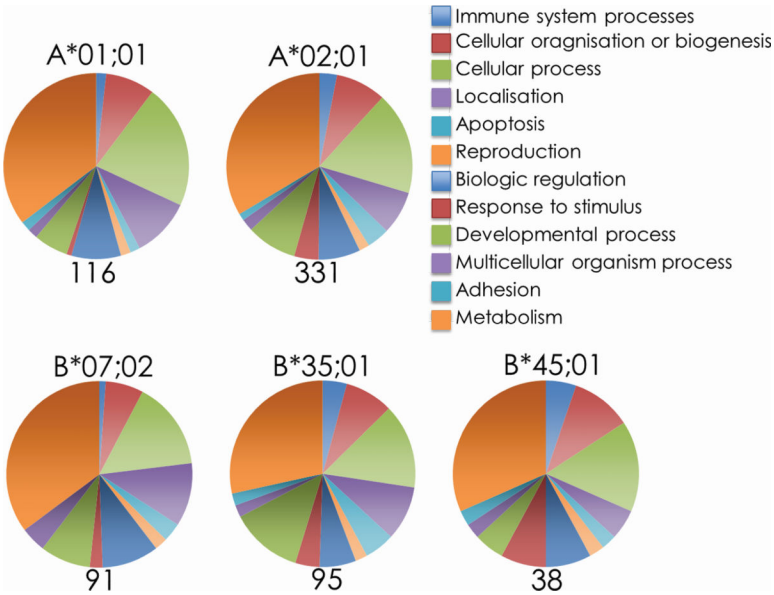

Figure 3. Proteins from which newly presented peptides are derived represent numerous functional families

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.

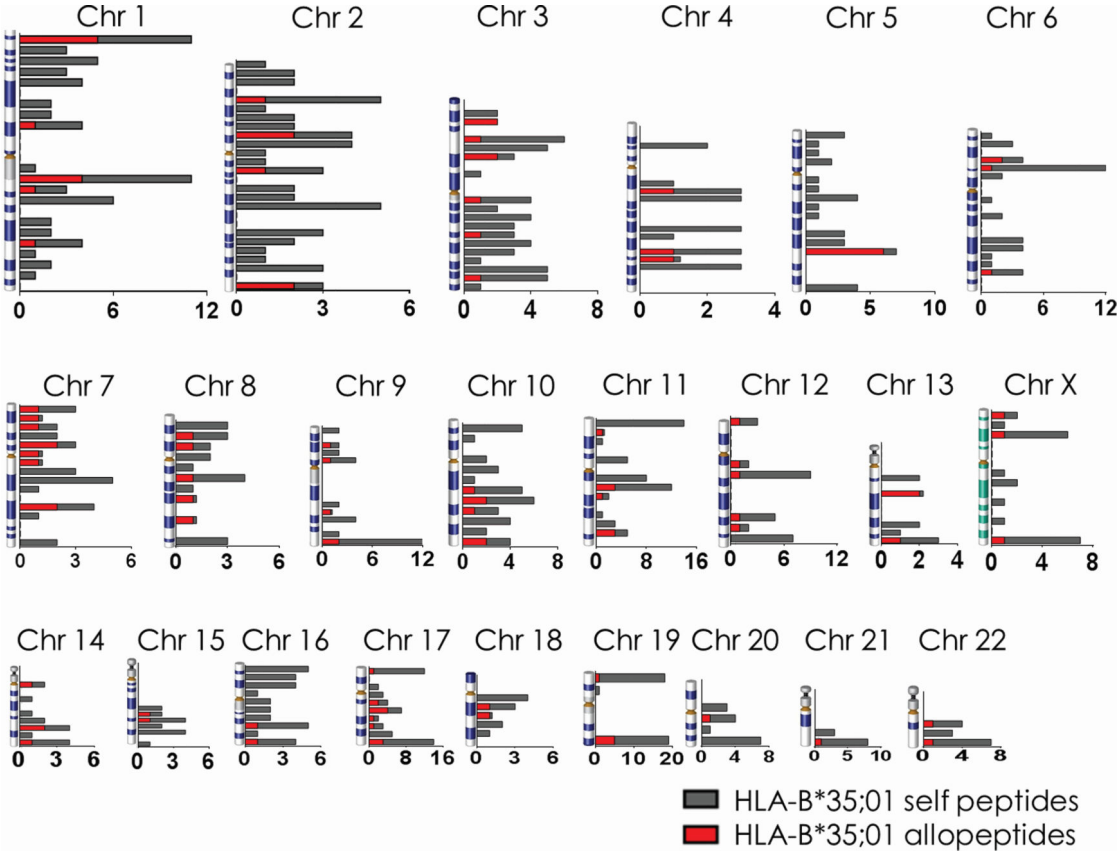

Figure 4. Peptides presented after infection by HLA-B*35;01 are derived from proteins encoded by genes dispersed across the chromosomes

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 reference [26]). Note the scale of the x-axis varies for each chromosome. The number of proteins containing allopeptides in the human population are enumerated for each location (red bars).

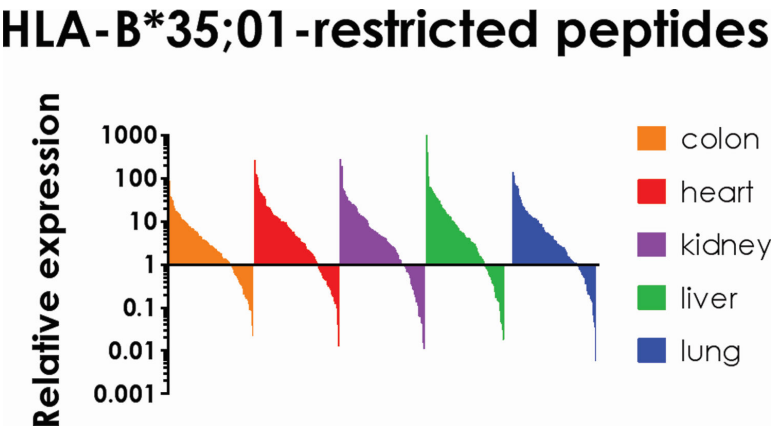

Figure 5. Proteins from which newly presented HLA-B*35;01-restricted allopeptides are derived are expressed in transplanted tissues

Proteins expressed by the colon, heart, kidney, liver and lung 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.

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]	#Hits[e]	Function[f]	Temporal Exp[g]	VARV[h]	MONPV	ECTV
A4L$_{83-91}$	VPTATPAPI	[1]	1.5598	1,3	10	S	E/L	NSH	NSH	NSH
A8R$_{25-34}$	TPMIKENSGF		1.717	1	3	T	I/E	S8I		
A10L$_{111-119}$	NPIINTHSF	[2]	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	[2]	1.8503	1	3	S	L			
A17L$_{91-100}$	LPLTSLVITY	[2]	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	[1]	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	[1]	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	[2]	1.8943	1	2	U	I/E	I1M		
B8R$_{70-78}$	FPKNDFVSF	[1] ; [2]	2.237	1	2	E/V	E			K3N

VACCC ORF[a]	Sequence	Prior reports[b]	Cn[c]	DPI[d]	#Hits[e]	Function[f]	Temporal Exp[g]	VARV[h]	MONPV	ECTV
$B8R_{104-112}$	PPTVTLTEY		1.6996	3	1	E/V	E		T5R	
$B8R_{158-167}$	EPVTYDIDDY	[1]	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	[1]	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	[1]	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	[2]	1.6592	3	3	T	E			
$D13L_{160-168}$	TPFDVEDTF	[2]	2.2957	1	8	0	L			
$E1L_{10-18}$	FPNITLKII	[2]	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	[1]	1.8446	3	2	R	E			
$F1L_{162-170}$	NPVKTIKMF		1.7642	3	2	U	E	K4E	K4E	K4E
$F2L_{26-35}$	SPGAAGYDLY	[1]	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			

VACCC ORF[a]	Sequence	Prior reports[b]	Cn[c]	DPI[d]	#Hits[e]	Function[f]	Temporal Exp[g]	VARV[h]	MONPV	ECTV
$G9R_{69-77}$	GPGGLSALL	[1]	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	[2]	2.9981	1,3	3	S	L			
$I4L_{670-678}$	LPEDIKRVY		2.1136	3	1	R	E			
$I6L_{159-167}$	IPMSIISFF	[3]	1.6008	1,3	5	U	E/L		M3I	
$I7L_{153-161}$	NPKVVKMKI		1.5407	3	1	S	L			
$I12L_{15-23}$	SPEDDLTDF		1.6251	3	1	U	N/A		P2Q	
$J6R_{177-185}$	WPLLEIHQY		2.5974	1	1	T	E			
$L3L_{291-299}$	VPKEDYYFI		2.2064	1	1	T	L			
$L4R_{37-45}$	FPRSMLSIF	[1] ; [4]	1.9615	1	1	T	L			
$L5R_{10-18}$	NPVFIEPTF		1.6253	3	2	S	L			
$N2L_{147-155}$	KPVYSYVLY		1.5179	1,3	3	0	I/E		V3I	V3I
$O1L_{4-12}$	YPEFARKAL		1.543	1	2	U	I/E			
$O1L_{549-557}$	IPITDSLSF		1.6041	1,3	2	U	I/E	D5E		

[a] Open reading frames (ORF) and location of epitopes are defined based on Copenhagen reference strain (VACCC, ID 10249)

[b] Prior reports according to immune epitope data base (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 mass spectrometry

[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

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]	# Hits[f]	Oncogene status[g]	VACV[h]
AHSA1	SPEELYRVF			3.6857	1,3	4		
AIM1	LPDNSLKVF		4	2.2947	3	2	candidate	KVF7DGY[i]
APEH	VPFKQGMEY				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		1	2		
BLMH	KPLFNMEDKI			2.1907	1	1		
CANX	APPSSPKVTY		1	2.5506	3	1		
CAPN1	LPIKDGKLVF			2.2217	3	1		
CCT4	HPTIISESF	[1]		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	4		
	LPVNESFGF			2.275	1	3		
EFHD2	NPYTEFKEF			2.7636	3	1		
ERH	NPNSPSITY	[2]		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		3	5		
HPRT1	IPDKFVVGY			3.1871	1,3	7		
HSPA8	IPTKQTQTF		1	2.4455	3	2		TQ6NF K4R;Q5K; Q7R
	QPGVLIQVY			2.2033	3	1		
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	

Protein	Peptide sequences[a]	Prior Reports[b]	Interferon Responsive[c]	Cn[d]	DPI[e]	# Hits[f]	Oncogene status[g]	VACV[h]
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		
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 data base (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 mass spectrometry

[g] Peptides derived from proteins that represent known or potential oncogenes

[*] indicates 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

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		

Gene	Variants			VACV[c]
	A[a]	B[b]	C[b]	
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		TQ6NF 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		
NME2	RPFFPGLVKY	RPFFAGLVKY		
TCP1	HPTSVISGY	HPTSVISSY		
FLNB	IPYLPITNF	VPYLPITNF		
LOC220717	VPHSIINGY	VPHSIIDGY		

Gene	Variants			VACV[c]
	A[a]	B[b]	C[b]	
PSMC4	LPLTHFELY	LPVTHFELY		
ZNF581	SPCPQPLAF	SPCPQPLPF		
TFCP2L2	LPLNIQVDTY	LPLNIQIDTY		
TOP2A	LPVKGFRSY	LPVNGFRSY		
PHCA	YPWLRGLGY	YPWLRGLGI		
ASCC3L1	RPVPLEQTY	RPVPLERTY		
SLC25A6	IPKEQGVLS F	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	
MAGI 1	KPGEGLGMY	KPSEGLGMY		
ITGB4	RPLQGYSVEY	RPLQGYSVAS		
HLA-C	HPLSDHEATL	HPISDHEATL		
SLC17A6	MPLAGILVQY	MPLAGVLVQY		
UBE2L3	YPFKPPKITF	YPFKPPKVTF		
PIK3C2B	LPQLVQALKY	LSQLVQALKY		
CDC42	FPSEYVPTVF	FPGEYVPTVF		

Gene	Variants			VACV[c]
	A[a]	B[b]	C[b]	
ProSAPi P1	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		
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		

[*] Indicates corresponding amino acid is deleted in variant

[a] Peptide sequence identified by mass spectrometry

[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.