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Title

Immunological evasion of immediate-early varicella zoster virus proteins.

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Abstract

The varicella zoster virus (VZV) causes the childhood disease commonly known as chickenpox and can later in life reactivate as herpes zoster. The adaptive immune system is known to play an important role in suppressing VZV reactivation. A central aspect of this system is the presentation of VZV-derived peptides by the major histocompatibility complex (MHC) proteins. Here, we investigate if key VZV proteins have evolved their amino acid sequence to avoid presentation by MHC based on predictive models of MHC-peptide affinity. This study shows that the immediate-early proteins of all characterized VZV strains are profoundly depleted for high-affinity MHC-I-restricted epitopes. The same depletion can be found in its closest animal analog, the simian varicella virus. Further orthology analysis towards other herpes viruses suggests that the protein amino acid frequency is one of the primary drivers of targeted epitope depletion.

Herpes Zoster (HZ) occurs as the result of reactivation of varicella zoster virus (VZV), which is known to latently persist in the absolute majority of adults across the world. The adaptive cellular immune system is known to play an important role in suppressing VZV reactivation. In several HLA (human leukocyte antigen) association studies, a correlation between persisting HZ (postherpetic neuralgia (PHN)), and the patient's HLA haplotype have been found (Sato-Takeda et al. 2004; Sumiyama et al. 2008; Meysman et al. 2015). VZV is known to have several mechanisms for evading the adaptive immune system, such as interference with the expression of the major histocompatibility complex (MHC) proteins (Abendroth et al. 2010), coded in humans by the HLA genes. As is evident from the known HLA associations, VZV is unable to evade the adaptive immune system entirely. Indeed several key immunogenic VZV epitopes have been characterized (van der Heiden et al. 2009) and high MHC class I affinity for VZV-derived peptides has been associated with reduced VZV complication risk (Meysman et al. 2015). However several VZV proteins seem to show decreased affinity across different HLA alleles, whether or not they were associated with increased risk of VZV complication (Meysman et al. 2015). In other words, some VZV proteins seem strongly depleted for MHC class I high-affinity peptides, compared to what could be expected if high-affinity peptides were distributed randomly across the VZV proteome. Depletion of peptide epitopes in key proteins may be a successful strategy to conceal VZV presence from the host immune system in certain stages of the viral cycle, reducing the number of immune epitopes while working as another immune response evasion mechanism employed by VZV. However the singular study into this epitope depletion only checked four HLA alleles for a single VZV strain, without any accompanying statistical analysis if this depletion was in fact significant across multiple HLA alleles, without a thorough functional investigation of the putative depleted proteins and without determining if any depletion is caused by the primary protein structure, the amino acid sequence or simply the amino acid content. Here we performed a thorough investigation into this phenomenon in different strains of VZV and other herpes viruses for a large number of common HLA alleles. This was done by means of computational analyses of viral peptide affinity predictions for the human MHC-I molecules.

Each viral reference genome was processed by NetCTLpan v1.1 (Stranzl et al. 2010), transforming each protein sequence into a collection of all possible 9-amino-acid-long peptide fragments, potentially presented on MHC class I molecules on infected cells. Simulating the natural course of immunopresentation by MHC-I molecules, the affinity of all 9-mers was predicted for the 30 most common HLA alleles reported for the US donor registry (Gragert et al. 2013), producing 30 lists of 34 947 peptides with predicted affinity scores. The top 10% highest affinity peptides for each HLA allele were then retained and binned according to their source proteins. This resulted in a list of protein-specific epitope frequency, in which every viral protein is associated with the number of high-affinity peptides found in it, compared to the total number of 9-mers in a protein – i.e. ORF0 with 16 high-affinity peptides out of total 121 9-mers, ORF1 with 20 out of 100, ORF2 with 17 out of 213, and so on. The full list of high-affinity peptide distributions can be found in Online Resource 1. As can be expected, there is a clear relationship between the size of the protein and the number of

high affinity peptides, with larger proteins containing more than smaller ones, as described in Online Resource 2. It is thus more useful to analyze these numbers as frequencies, or by using statistical enrichment models. The distribution of the high-affinity peptides among the viral proteins for each HLA allele was analyzed using a hypergeometric cumulative distribution function, to determine if certain VZV proteins are under-represented for high-affinity peptide content (i.e. have a number of high-affinity peptides that is significantly lower than is expected for a protein of its size based on the provided background). This approach has the further advantage that it uses the high affinity peptide distribution across the viral proteome as the background for statistical enrichment, instead of unrelated or synthetic protein sequences. The results from this analysis are shown in figure 1.

A second enrichment analysis was used to determine which proteins were consistently depleted across the 30 alleles which showed that protein products of VZV ORFs 4, 9, 32, 61, 62 and 63 are, indeed, significantly depleted in the number of high-affinity peptides across many alleles. The same proteins were depleted in varicella strains Dumas, Varivax and DQ457052, indicating that the sequence differences delimiting VZV strains are insufficient to contribute to this phenomenon.

Simian varicella virus (SVV) as the closest VZV analogue, but also a non-human virus, was similarly tested for its distribution of HLA high-affinity peptides. In this manner, SVV ORFs 9, 61, 62 and 63 were found to be depleted, mostly corresponding to the pattern of depletion found in the human virus. The homology of the SVV and VZV proteins in question was confirmed using OrthoMCL v2.0.9 (Li et al. 2003). The smaller number of proteins flagged as depleted for SVV could potentially indicate that SVV is less fit to conceal its presence from the human immune system. Further using the same method, several proteins in alpha- (HSV1, HSV2), beta- (CMV, HHV6 and 7) and gamma-herpesviruses (EBV, KSHV) were found to be similarly depleted for high-affinity MHC class I peptides. No less than five proteins were depleted in each herpesvirus of any of three subfamilies, as shown in table 1. Furthermore, the homolog of VZV ORF62, HSV1 protein RS1 also showed depletion. However, this trend was not present in all homologous proteins, as most homologs were not consistently depleted across several viruses.

To determine if different amino acid composition was a possible cause of this phenomenon, the amino acid frequencies in pairs of homologous proteins with different binding preferences in the same set of HLA alleles were compared. Presence of large, nonpolar, hydrophobic amino acids such as methionine, phenylalanine, leucine, and polar hydrophilic large tyrosine, tended to uniformly increase the number of high-affinity epitopes in homologous proteins. In general, it appears that a larger molecular volume and a higher hydrophobicity of amino acids positively contribute to the ability of peptides to be bound by MHC class I molecules, as previously shown (Calis et al. 2013). Random sequence permutations of the proteins retaining the same overall amino acid frequency were predicted to have a similar number of high affinity peptides. This indicates that the amino acid frequency, and not specific sequence composition, is one of the primary drivers behind the divergence in the occurrence of immunogenic epitopes in the homologous proteins of

these viruses.

A certain functional similarity was observed in epitope-depleted proteins. Of six proteins which are known to be transcribed during latent phase of VZV infection, three - IE4, IE62 and IE63 - were found to be depleted of high-affinity peptides. These proteins are known as "immediate early", and are involved in earliest stages of infection/re-activation and provide transactivator functionality for genes transcribed in later stages of viral cycle. The low number of high-affinity epitopes in immediate-early genes of other herpesviruses was noted before (Vider-Shalit et al. 2007; Vider-Shalit et al. 2009), however VZV was never analyzed from this perspective. The presence of an evolutionary incentive to reduce the number of epitopes in immediate early genes was also hypothesized by others (Vider-Shalit et al. 2007). By delaying detection to later stages of infection, the virus prevents early immune-induced destruction of a host cell and increases the probability of budding. Interestingly, the observed distinction in the number of epitopes between early and late genes was mentioned to not hold for animal analogues of human viruses (Vider-Shalit et al. 2009) - however the similar pattern of epitope depletion observed in SVV and VZV may suggest otherwise. In addition, as shown in Online Resource 3, the depletion of the immediately early genes in VZV persists even when tested against mouse MHC variants. This further supports the observation that this depletion is not specific towards human MHC variants, but that the lower frequency of large, non-polar, hydrophobic amino acids may allow VZV immediately early genes to escape a broad spectrum of MHC variants, even beyond those of its host.

In this work, we explored the phenomenon of high-affinity epitope depletion in the VZV "immediate early" proteins. Our findings support the hypothesis that a selective evolutionary pressure exerted by the immune system on a slow-evolving DNA virus could drive an adaptation strategy of epitope evasion. This evasive epitope depletion persisted throughout human and simian varicella viruses. This may indicate that this evasion mechanism precedes the divergence of these pathogens.

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Figure legends

Figure 1: Heat map showing the depletion probability for high-affinity peptides in each VZV protein (rows) for the 30 most common HLA (MHC class I) alleles (columns). Reported depletion P-values are Bonferroni-corrected where lower numbers signify a more significant degree of depletion for high-affinity peptides in the protein.

Tables

Table 1: High affinity MHC I epitope -depleted proteins in the *herpesviridae* family.

Name and clade	Abbrev.	Proteins found depleted of high-affinity peptides
Human herpesvirus type 1 (α)	HSV1	RS1 (<i>ICP4 family</i>), RL2 (<i>ICP0 family</i>), US11, UL36, UL54
Human herpesvirus type 2 (α)	HSV2	RS1 (<i>ICP4 family</i>), RL2 (<i>ICP0 family</i>), US1, US11, UL36, UL49, UL54
Varicella zoster virus (α)	VZV	ORF4, ORF9, ORF32, ORF61 (<i>ICP0 family</i>), ORF62/71, ORF63/70
Epstein-Barr virus (γ)	EBV	EBNA-1, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-2, EBNA-LP, BLLF1, BPLF1, BKRF4
Human Cytomegalovirus (β)	CMV	UL13, UL32, UL48, UL69, UL80/80.5, UL99, UL112, UL122, UL135
Human herpesvirus type 6 (β)	HHV6	DR3, B1, U11, U79, U86, U90, U95, B9
Human herpesvirus type 7 (β)	HHV7	U11, U53, U79, U86, U90, U100
Kaposi's sarcoma-associated virus (γ)	KSHV	KCP, ORF45, ORF50, ORF52, K9, vIRF-2, vIRF-4, ORF60, ORF64, ORF72
Simian varicella virus (α)	SVV	ORF9, ORF61, ORF62/71, ORF63/70

Online resources

Online Resource 1 - High affinity peptide counts for VZV Dumas strain.xls

A raw data table containing the high affinity peptide counts for each VZV Dumas ORF and each HLA allele as analyzed in this study. Furthermore it contains the uncorrected and corrected P-values for epitope depletion for each combination.

Online Resource 2 - Analysis of epitope counts for VZV Dumas.pdf

A detailed analysis on the high affinity counts for VZV Dumas with respect to their distribution among VZV genes for HLA-A*02:01. It contains figure S2.1, which shows the strong relationship between high affinity peptide count per ORF and ORF length, and figure S2.2, which shows the difference in high affinity peptides counts for the different VZV ORFs.

Online Resource 3 - Peptide depletion for mouse MHC variants.pdf

A repeat of the VZV depletion analysis described in the main manuscript but for common mouse MHC variants instead of human MHC variants. Figure S3.1 shows a similar depletion pattern to Figure 1 for these mouse MHC variants.