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5 **The roseoloviruses downregulate the protein tyrosine phosphatase PTPRC (CD45)**
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40 *Running title: Roseolovirus downregulation of CD45*
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47 **Abstract**

48 Like all herpesviruses, the roseoloviruses (HHV6A, -6B, and -7) establish lifelong
49 infection within their host, requiring these viruses to evade host anti-viral responses.
50 One common host-evasion strategy is the downregulation of host-encoded, surface-
51 expressed glycoproteins. Roseoloviruses have been shown to evade host the host
52 immune response by downregulating NK-activating ligands, MHC class I, and the
53 TCR/CD3 complex. To more globally identify glycoproteins that are differentially
54 expressed on the surface of HHV6A-infected cells, we performed cell surface capture of
55 N-linked glycoproteins present on the surface of T cells infected with HHV6A, and
56 compared these to proteins present on the surface of uninfected T cells. We found that
57 the protein tyrosine phosphatase CD45 is downregulated in T cells infected with
58 HHV6A. We also demonstrated that CD45 is similarly downregulated in cells infected
59 with HHV-7. CD45 is essential for signaling through the T cell receptor and as such, is
60 necessary for developing a fully functional immune response. Interestingly, the closely
61 related β -herpesviruses human cytomegalovirus (HCMV) and murine cytomegalovirus
62 (MCMV) have also separately evolved unique mechanisms to target CD45. While
63 HCMV and MCMV target CD45 signaling and trafficking, HHV6A acts to downregulate
64 CD45 transcripts.

65 **Importance**

66 Human herpesviruses-6 and -7 infect essentially 100% of the world's population before
67 the age of 5 and then remain latent or persistent in their host throughout life. As such,
68 these viruses are among the most pervasive and stealthy of all viruses. Host immune

69 cells rely on the presence of surface-expressed proteins to identify and target virus-
70 infected cells. Here, we investigated the changes that occur to proteins expressed on
71 the cell surface of T cells after infection with human herpesvirus-6A. We discovered
72 that HHV-6A infection results in a reduction of CD45 on the surface of infected cells.
73 Targeting of CD45 may prevent activation of these virus-infected T cells, possibly
74 lengthening the life of the infected T cell so that it can harbor latent virus.

75 **Introduction**

76 Human Herpesvirus-6A (HHV6A) is a human-specific, T cell-tropic β -herpesvirus that is
77 most closely related to the other members of the roseolovirus genus, HHV-6B and HHV-
78 7, as well as human cytomegalovirus (HCMV). Primary infections with HHV-6 and -7
79 usually occur before the age of three and are often characterized by a high fever (1, 2).
80 HHV-6 and -7 infect over 90% of the population, and like other herpesviruses, HHV-6
81 and -7 remain latent or establish lifelong infections in their hosts. As such, these viruses
82 are among the most pervasive and stealthy of all viruses; they must necessarily excel at
83 escaping immune detection throughout the life of the host, yet little is known about how
84 these viruses so successfully escape host defenses.

85 The ability of host immune cells to detect virus-infected cells is largely dependent on
86 interactions between proteins expressed on the surface of immune cells and those
87 expressed on the surface of their targets. Viruses have necessarily evolved to alter the
88 expression of these cell-surface-expressed proteins to evade detection by the host
89 immune system. The herpesviruses, particularly the cytomegaloviruses, have long been
90 known to employ devious strategies to interfere with expression of host-encoded
91 surface-expressed proteins. For example, most herpesviruses, including HHV-6 and -7,
92 interfere with antigen presentation by downregulating class I major histocompatibility
93 complex (MHC) molecules from the surface of infected cells (4-13). Additionally, HCMV,
94 the most closely-related virus to the roseoloviruses, encodes at least 5 different gene
95 products and a miRNA that all participate in downregulation of natural killer (NK)-
96 activating ligands, all in an effort to prevent natural killer cells from identifying and killing
97 infected cells (14-18). We have found that HHV6A, -6B, and -7 each encode a single

98 gene product, U21, that acts as a multifunctional transmembrane glycoprotein to
99 downregulate not only multiple NK activating ligands, but also most class I MHC alleles
100 (12, 19-21).

101 Herpesviruses also target surface-expressed proteins to hinder the ability of immune
102 cells to perform their effector function. For example, herpesviruses inhibit T cell function
103 through the downregulation of co-stimulatory ligands like B7 and CD40, and
104 upregulation of co-inhibitory ligands such as PD-L1 and galectin-9 (22-32).

105 Herpesviruses also downregulate adhesion molecules, such as I-CAM, V-CAM, PECAM
106 and ALCAM, which can physically disrupt formation of the immune synapse and inhibit
107 T cell activation (33-37).

108 Since most herpesviruses are not T cell-tropic, changes in surface expression occur in
109 infected cells, and infected cells then exert their immunosuppressive effects through
110 interaction with uninfected T cells. But what happens to the surface of a T cell when it is
111 infected with a herpesvirus? The T cell-tropic roseoloviruses allow us to explore and
112 learn from alterations to the glycoprotein landscape on the surface of herpesvirus-
113 infected T cells. Herein, we applied a mass spectrometry strategy to identify and
114 quantify N-linked glycoproteins on the cell surface of HHV6A-infected T cells to yield the
115 first insights into how virus infection induces alterations in the cell surface landscape of
116 host cells. We discovered that the protein tyrosine phosphatase CD45 (PTPRC) is
117 downregulated from the surface of HHV6A-infected T cells. CD45 is expressed on the
118 surface of all nucleated cells of hematopoietic origin, where its activity is critical for the
119 proper function of immune cells (reviewed in (39-42)). The phosphatase activity of CD45
120 is required for successful signaling through the TCR (43-45), and as such, the

121 downregulation of CD45 could be an attractive strategy for viruses to impair T cell
122 signaling.

123 **Results**

124 *Cell-surface capture of CD45 in HHV6A-infected T cells*

125 To identify host-encoded surface-expressed proteins targeted by HHV6A, we performed
126 cell surface capture (CSC), a chemoproteomic approach for the specific identification of
127 cell surface N-glycoproteins (46-50). Using CSC and bioinformatic analysis tools, we
128 compared N-linked glycoproteins identified on the surface of T cells infected with
129 HHV6A to those identified on the surface of uninfected T cells (3, 38). To minimize
130 biological variability, we performed CSC in HHV6A-infected cell line. HHV6A infects
131 only a small number of cultured cell lines, and for these studies, we used JJhan cells, a
132 CD4⁺ T cell line variant of Jurkat cells (51).

133 We identified 605 cell surface N-linked glycoproteins on the surface of infected and
134 uninfected JJhan cells, including 594 human and 11 viral proteins. Strikingly, we
135 identified 47 unique peptides derived from the protein tyrosine phosphatase PTPRC
136 (CD45), spanning all 22 N-linked glycosylation sites (Fig. 1a). CD45-derived peptides
137 were consistently less abundant on the surface of HHV6A-infected JJhan cells across
138 multiple biological and technical replicates (Fig. 1b-d), suggesting that CD45 is
139 downregulated from the surface of HHV6A-infected JJhan cells.

140 *Localization and steady-state expression of CD45 in HHV6A-infected T cells*

141 To independently validate the
 142 downregulation of CD45
 143 observed in our mass
 144 spectrometry experiments, we
 145 examined surface-expression of
 146 CD45 in HHV6A-infected cells by
 147 flow cytometry. We infected
 148 JJhan cells with a recombinant,
 149 bacterial artificial chromosome
 150 (BAC)-derived HHV6A virus
 151 encoding soluble green
 152 fluorescent protein (GFP), which
 153 allowed us to identify actively-
 154 infected cells (52). In uninfected
 155 JJhan cells, we observed a single
 156 population of CD45-expressing
 157 cells, while in HHV6A-infected
 158 JJhan cells, we observed
 159 reduced surface expression of CD45 (Fig. 2a), consistent with our mass spectrometry
 160 finding that the presence of CD45 is diminished on the surface of HHV6A-infected cells.
 161 CD45 is expressed as multiple different isoforms in human cells (53-55). To determine
 162 whether all isoforms of CD45 are downregulated in HHV6A-infected JJhan cells, we
 163 labeled cells with antibodies directed against the CD45 isoforms commonly expressed

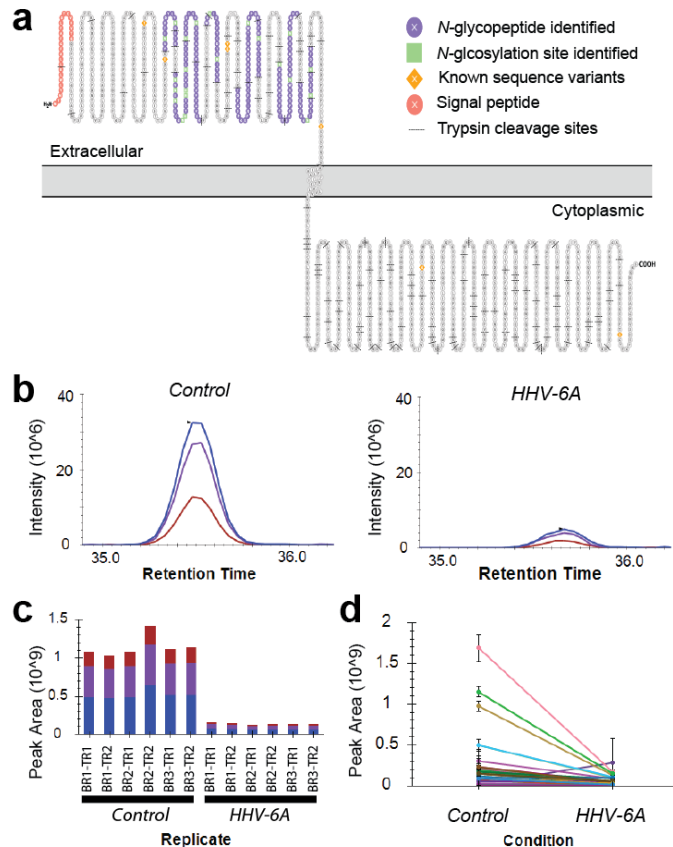


Figure 1. Mass spectrometry data for CD45. (a) Graphical representation of CD45 protein with extracellular N-linked glycopeptides identified by CSC indicated. This approach specifically enriches and identifies N-glycopeptides from the extracellular domain of cell surface proteins. Image generated using Protter (3). (b-d) Label-free quantitation data for CD45. Representative peaks of a peptide (YAnITVDYLYNK, where n is the site of N-glycosylation) detected across individual replicate analyses (b) and three replicate analyses (including technical replicates) (c) for each sample group, showing CD45 is more abundant in control compared to infected cells. Each color (blue, purple, and red) are associated with the peak area from the [M]+0, [M]+1, and [M]+2 isotopic peaks, respectively. [M] is the monoisotopic mass. (d) Summary of peak areas for all 67 peptide observations for CD45 across all technical and biological replicates. Each line is a different peptide. BR: biological replicate; TR: technical replicate. Results shown in b-d were generated using Skyline (38).

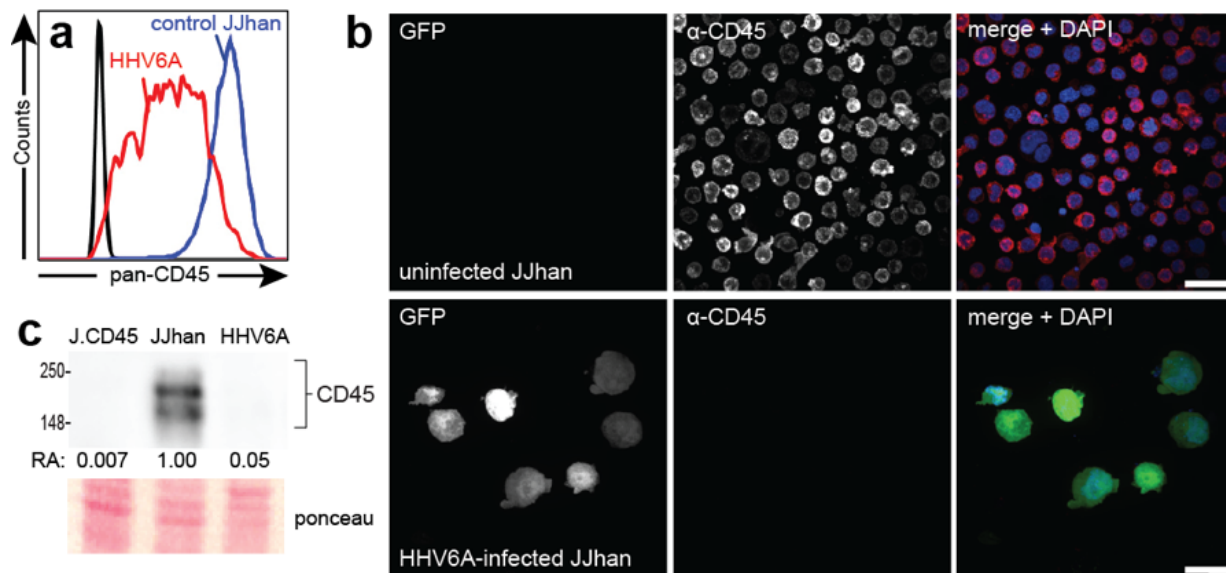


Figure 2. CD45 is downregulated in HHV6A-infected cells. (a) Flow cytometric analysis of HHV6A-infected JJhan cells (red) and uninfected JJhan cells (blue). HHV6A-infected cells were gated on as GFP⁺. Live cells were labeled with an antibody directed against CD45. (b) Confocal immunofluorescence microscopy images showing uninfected JJhan cells (top panels) and HHV6A-infected (GFP⁺) JJhan cells (bottom panels) that were fixed, permeabilized, and labeled with an antibody directed against CD45. Images were taken at 100X magnification and are shown as maximum intensity projections. Scale bar = 20 μ m. (c) Immunoblot analysis of CD45 in whole cell lysates generated from J.CD45 cells (negative control), uninfected JJhan cells, and HHV6A-infected JJhan cells. Protein was normalized to total protein for each lane. RA : relative abundance. Abundance is calculated relative to uninfected JJhan cells. Ponceau shows total protein loaded per lane.

164 in Jurkat cells, the parental cell line of JJhan cells (56). CD45-RO, -RB, and -RC were
165 all downregulated in HHV6A-infected JJhan cells relative to uninfected JJhan cells (Fig.
166 S1), suggesting the downregulation of CD45 from the surface HHV6A-infected JJhan
167 cells is not specific to any one CD45 isoform.

168 Viruses often reroute the intracellular trafficking of surface-expressed proteins from the
169 cell surface to alter host cell biology. For example, the HHV6A U24 gene product
170 downregulates the T-cell receptor (TCR)/CD3 by stimulating endocytosis of the receptor
171 resulting in relocalization of TCR/CD3 from the cell surface to endosomes, and U21
172 reroutes class I MHC molecules to lysosomes (13, 57). We therefore sought to
173 determine whether the reduction in surface-expressed CD45 observed in HHV6A-
174 infected cells was the result of a redistribution of CD45 within the cell. To examine the
175 localization of CD45, we performed immunofluorescence microscopy of permeabilized

176 cells labeled with an antibody directed against human CD45. As expected, CD45 was
177 localized to the plasma membrane in uninfected JJhan T cells. In HHV6A-infected
178 JJhan cells, however, we observed a striking disappearance of CD45 labeling ([Fig. 2b](#)).

179 To better quantify the downregulation of CD45 in HHV6A-infected JJhan cells, we next
180 examined steady-state levels of CD45 protein by immunoblot analysis of whole-cell
181 lysates generated from uninfected or HHV6A-infected JJhan cells. Consistent with our
182 immunofluorescence microscopy data ([Fig. 2b](#)), immunoblot analysis showed a 95%
183 reduction in CD45 protein in HHV6A-infected JJhan cells relative to uninfected JJhan
184 cells ([Fig. 2c](#)). Taken together, these results demonstrate that CD45 is not only
185 downregulated from the surface of HHV6A-infected JJhan cells but is also depleted
186 from HHV6A-infected cells.

187 *CD45 expression in HHV7-infected T cells*

188 The HHV6A genome is almost entirely co-linear with the two other roseolovirus
189 genomes HHV-6B and HHV-7 (58, 59). As such, we reasoned that HHV-6B and HHV-7
190 infection may also result in CD45 downregulation. To determine whether CD45 is
191 downregulated in HHV7-infected cells, we evaluated the localization of CD45 in HHV7-
192 infected SupT1 cells by immunofluorescence microscopy. Since a recombinant GFP-
193 containing BAC containing the coding sequence of HHV-7 is not yet available, we
194 identified HHV7-infected cells using an antibody directed against the U21 gene product
195 from HHV-7 ([Fig. 3](#), green). Similar to cells infected with HHV6A ([Fig. 2b](#)), HHV7-
196 infected SupT1 cells showed reduced CD45 labeling ([Fig. 3](#), bottom panels as

197 compared to
198 uninfected
199 SupT1 cells
200 (Fig. 3, top
201 panels),
202 suggesting that
203 the ability to
204 downregulate
205 CD45 is
206 common to all
207 roseoloviruses.

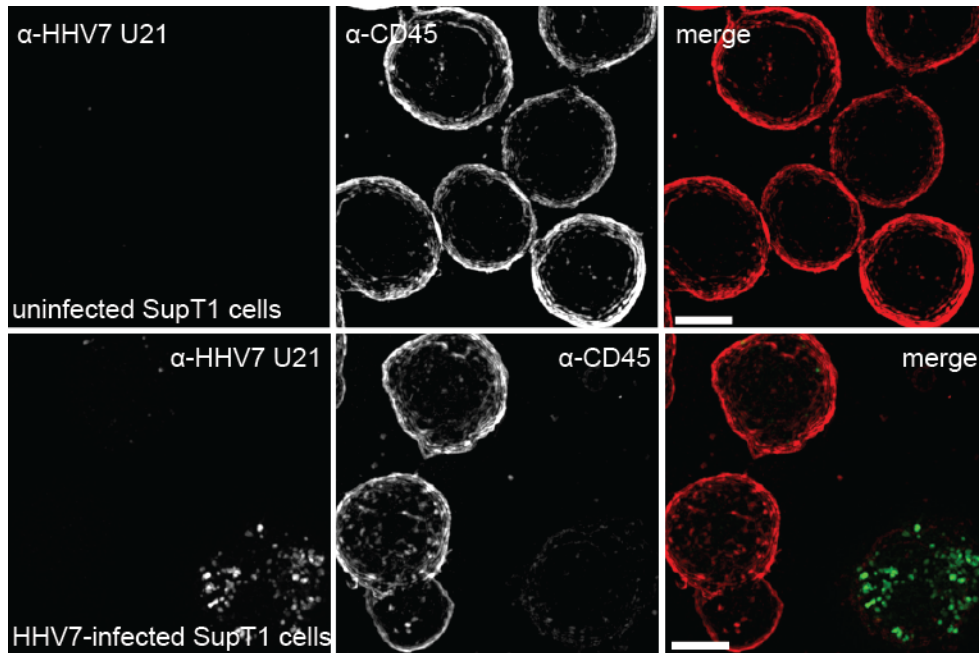


Figure 3. CD45 is downregulated in HHV7-infected T cells. Structured Illumination 3-dimensional microscopy images showing uninfected control T cells (top panels) and HHV7-infected T cell (bottom panels). Fixed and permeabilized cells were labeled with an antibody directed against the U21 open reading frame (ORF) from HHV7 (green) and an antibody directed against human CD45 (red). Images were taken at 100X and are shown as maximum intensity projections. Scale bar = 5 μ m.

208 *CD45 protein stability in HHV6A-infected T cells*

209 Viruses often employ the strategy of degrading host proteins involved in the host
210 response to virus infection. For example, the murine cytomegalovirus m42 gene product
211 induces internalization and subsequent proteasomal degradation of CD45 (60). To
212 further investigate the mechanism by which CD45 is downregulated in HHV6A-infected
213 JJhan cells, we examined the stability of CD45 in the presence of proteasomal and
214 lysosomal protease inhibitors.

215 Lysosomal protease function is compromised at a more neutral pH, thus stabilization of
216 a protein in the presence of the weak base ammonium chloride (NH_4Cl) would suggest
217 the involvement of lysosomal proteases in its turnover. Likewise, stabilization of a
218 protein in the presence of the peptide-aldehyde MG-132, which selectively inhibits

219 proteolytic activity of the 26S
220 proteasome, would suggest
221 involvement of proteasomal
222 proteases in the turnover of that
223 protein. We separately inhibited
224 lysosomal and proteasomal
225 protein degradation, treating
226 cells with NH_4Cl or MG-132, and
227 assessed steady-state CD45
228 protein levels by immunoblot
229 analysis. In cells treated with a
230 DMSO vehicle control, we
231 observed a 95% reduction in
232 CD45 abundance in HHV6A-
233 infected cells (Fig 4a), which is
234 similar to what we observed in untreated cells (Fig. 2c), suggesting the dimethyl
235 sulfoxide (DMSO) vehicle does not affect degradation of CD45. We observed a similar
236 reduction in CD45 in cells treated with MG-132 or NH_4Cl (Fig. 4a).
237 We also examined localization of CD45 in HHV6A-infected cells treated with MG-132 or
238 NH_4Cl by immunofluorescence microscopy. As expected, we observed surface-
239 localization of CD45 in untreated, uninfected JJhan cells (Fig. S2, panel a). We also
240 observed surface-localization of CD45 in uninfected JJhan cells treated with a DMSO
241 vehicle, MG-132, or NH_4Cl (Fig. S2, panels b, c, and d, respectively), suggesting CD45

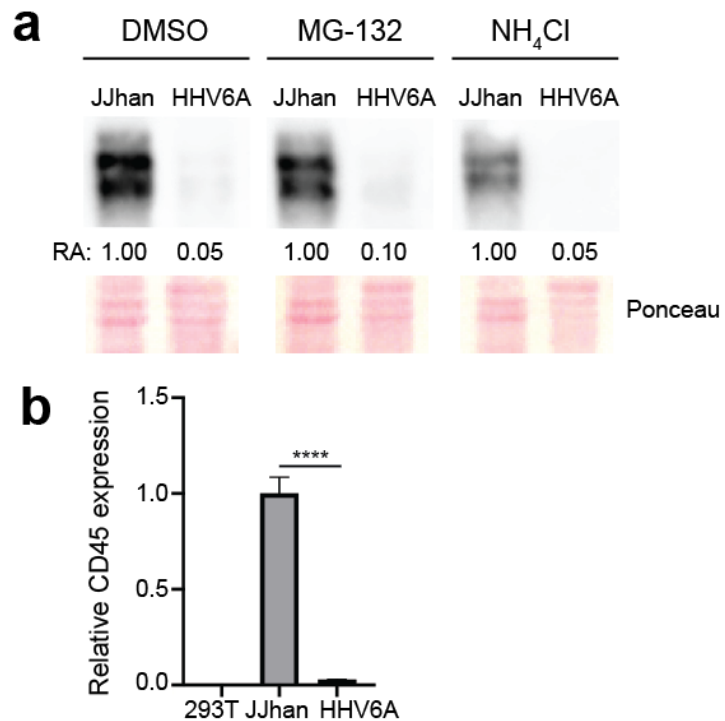


Figure 4. CD45 is downregulated at the RNA level in HHV6A-infected T cells. (a) Immunoblot analysis showing steady-state abundance of CD45 protein in uninfected JJhan cells, or HHV6A-infected cells under different conditions (DMSO vehicle control, MG-132, or NH_4Cl treatment for 19 hours). Equal protein was loaded in each lane, and CD45 abundance was calculated relative to uninfected JJhan cells for each treatment. RA = relative abundance. Ponceau-stained lanes visually illustrate equal protein loading. (b) CD45 RNA abundance in 293T cells (negative control), uninfected JJhan cells, or HHV6A-infected cells. CD45 RNA abundance is calculated relative to a cellular control gene and shown here relative to uninfected JJhan cells. Data shown is from a single representative biological replicate, performed in technical triplicate for each sample, mean \pm SD. **** = $p < 0.0001$.

242 localization is not affected by treatment with MG-132 or NH₄Cl outside the context of an
243 HHV6A infection. Consistent with the immunoblot data, there was little to no CD45
244 labeling present in untreated HHV6A-infected JJhan cells (Fig. S2, panel e). Similarly,
245 there was little to no CD45 labeling in HHV6A-infected treated with a DMSO vehicle,
246 MG-132, or NH₄Cl (Fig. S2, panels f, g, & h, respectively). Taken together, these results
247 suggest that the downregulation of CD45 in HHV6A-infected JJhan cells occurs by
248 some method other than protein degradation.

249 *CD45 gene expression in HHV6A-infected T cells*

250 Because CD45 was not stabilized in the presence of proteasomal or lysosomal
251 inhibitors, we hypothesized that CD45 was downregulated at the transcript level in
252 HHV6A-infected T cells. To test this hypothesis, we quantified CD45 mRNA in
253 uninfected and HHV6A-infected JJhan cells by quantitative reverse transcriptase-
254 polymerase chain reaction (qRT-PCR). CD45 mRNA levels were greatly reduced in
255 JJhan cells infected with HHV6A as compared to uninfected JJhan cells (Fig. 4b),
256 suggesting that CD45 transcripts are downregulated in HHV6A-infected JJhan cells.

257 **Discussion**

258 Herpesviruses, as life-long pathogens, are especially masterful at reprogramming host
259 cells to create a more hospitable environment. Perhaps the greatest challenge to a virus
260 is the detection and killing of its host cell by immune cells. As discussed, the
261 herpesviruses encode multiple gene products that alter host cell biology to prevent the
262 identification of infected cells. These measures are not entirely sufficient, however, and
263 viruses also strategize to target immune cells to inhibit their functional capacity. One

264 way viruses can inhibit these processes is by targeting the proteins that are important
265 for immune cell function, such as the protein tyrosine phosphatase CD45.

266 CD45 is expressed on the surface of all nucleated cells of hematopoietic origin, where
267 its activity is critical for the proper function of immune cells (reviewed in (39-42)). In T
268 cells, CD45's primary substrates are Src family kinases (61-63). CD45
269 dephosphorylates the inhibitory phosphotyrosine residue on the Src kinase Lck, leaving
270 the kinase in a 'primed' state, so that it can be activated through T cell receptor (TCR)
271 signaling (62). The phosphatase activity of CD45 is required for successful signaling
272 through the TCR (43-45), and as such, the downregulation of CD45 could be an
273 attractive strategy for viruses to inhibit T cell signaling.

274 Here we describe the downregulation of CD45 by two roseoloviruses, HHV6A and HHV-
275 7. Expression of CD45 is markedly reduced in HHV6A-infected JJhan cells and HHV7-
276 infected SupT1 cells. While we do not yet fully understand the functional consequences
277 of CD45 downregulation in roseolovirus-infected cells, we can gather clues from three
278 other viruses also known to target CD45: human cytomegalovirus (HCMV), human
279 adenovirus, and murine cytomegalovirus (MCMV).

280 CD45 is targeted by HCMV pUL11 (64). The extracellular domain of pUL11, which is
281 expressed on the surface of HCMV-infected epithelial cells or fibroblasts, interacts with
282 CD45 on nearby uninfected T cells. The interaction between pUL11 and CD45 inhibits
283 the phosphatase activity of CD45, which in turn impairs TCR signaling and ultimately, T
284 cell proliferation (64, 65). Inhibition of CD45 by pUL11 also results in an increase in
285 production of the anti-inflammatory cytokine IL-10 (65). In HHV6A infection, the

286 concentration of secreted IL-10 protein was shown to be increased during HHV6A
287 infection at timepoints up to 72 hours post-infection (66, 67). Since the inhibition of
288 CD45 during HCMV infection results in an increase in IL-10 production (65), it is
289 tempting to speculate that the downregulation of CD45 we observe in HHV6A-infected T
290 cells may be involved in the increase in IL-10 levels shown to occur during HHV6A
291 infection.

292 Another virus that devotes an open reading frame to the downregulation of CD45 is
293 Adenovirus19a (Ad19a). Ad19a E3/49K protein is cleaved to a secreted form (sec49K)
294 that interacts with CD45 on nearby uninfected NK cells and T cells. Sec49K-mediated
295 inhibition of CD45 suppresses T cell activation and signaling, resulting in diminished
296 production of the anti-viral cytokine IFN- γ , and inhibition of NK cell activation (68). After
297 HHV6A infection of stimulated peripheral blood mononuclear cells, the production of
298 IFN- γ was also reported to be reduced (66). Since inhibition of CD45 function during
299 adenovirus infection results in a decrease in IFN- γ production (68), it is possible that the
300 decrease reported in IFN- γ production during HHV6A infection may occur as a result of
301 CD45 downregulation in infected T cells.

302 Unlike HCMV pUL11 and Ad19a sec49K, which act extracellularly to inhibit CD45,
303 MCMV encodes a protein, m42, that induces the internalization and degradation of
304 CD45 within MCMV-infected macrophages (60). MCMV m42 acts as an adaptor or
305 activator of HECT3 E3 ubiquitin ligases, and through ubiquitination, m42 marks CD45
306 for lysosomal degradation (60). The functional outcome of m42-mediated
307 downregulation of CD45 in MCMV-infected macrophages is unclear. HHV6A-mediated
308 downregulation of CD45 is similar to MCMV-mediated downregulation of CD45, in that

309 these viruses downregulate CD45 from within infected cells, as opposed to acting on
310 CD45 *in trans* on the surface of nearby uninfected cells. As yet, the functional outcome
311 of downregulating CD45 within either of these infected immune cells remains elusive.

312 The roseolovirus genomes lack positional or functional homologs of MCMV m42 or
313 HCMV pUL11, and unlike HCMV, MCMV, and Ad19a, HHV6A infection results in the
314 dramatic transcriptional downregulation of CD45. HHV6A therefore downregulates
315 CD45 by a novel mechanism. The separate evolution of four unique mechanisms to
316 target a single host protein strongly suggests that CD45 is an important viral target,
317 though its impact is unclear: how might the roseoloviruses benefit from the
318 downregulation of CD45 in infected T cells? As discussed above, while the influence of
319 CD45 downregulation on cytokine production would certainly benefit HHV6A, it is
320 important to note that HHV6A preferentially infects T cells. Since it takes days to mount
321 a virus-specific T cell response, during a primary infection, the T cells that HHV6A
322 infects are not likely to be HHV6A-specific. Therefore, downregulation of CD45 in
323 infected T cells would not directly impair activation of T cells responding to HHV6A
324 infection. Instead, downregulation of CD45 may be a means to inhibit activation of the
325 HHV6A-infected T cell, possibly preventing activation-induced cell death, and creating a
326 host cell environment conducive to harboring latent virus. Future work is focused on
327 identification of the HHV6A gene products involved in the transcriptional regulation of
328 CD45 and exploring the functional consequences of CD45 downregulation in
329 roseolovirus-infected T cells.

330

331 **Materials & Methods**

332 *Cell lines and viruses*

333 JJhan, ND10^{depl} JJhan, and J.CD45 T cells were cultured in RPMI-1640 medium
334 (ThermoFisher Scientific, Waltham, MA) supplemented with 5% fetal bovine serum
335 (FBS) and 2 mM L-glutamine. JJhan cells depleted of ND10 (ND10^{depl} JJhan cells) were
336 the kind gift of Dr. Benedikt Kaufer (Freie Universität, Berlin, Germany) (69). J.CD45
337 cells (CD45 -negative Jurkat cells) were kindly provided by Dr. Arthur Weiss (UCSF,
338 San Francisco, CA) (70). 293T cells were cultured in Dulbecco's modified Eagle
339 medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 5% FBS, 5% newborn
340 calf serum, and 2 mM L-glutamine. The HHV6A virus used in these studies is a
341 recombinant HHV6A virus (strain U1102) generated from a bacterial artificial
342 chromosome (BAC) containing the HHV6A genome with GFP inserted between the U53
343 and U54 ORFs (71). Infectious HHV6A virus was generated by electroporating HHV6A-
344 GFP BAC DNA into JJhan T cells. HHV6A virus was propagated by mixing uninfected
345 ND10-depleted Jhan cells with infected JJhan cells once HHV6A-infected JJhan cells
346 were >80% GFP+. HHV6A-infected and uninfected JJhan cells as well as J.CD45 cells
347 were stimulated with 3.75 ug/ml Phytohemagglutinin (PHA-P) and 9 ug/ml
348 hydrocortisone. HHV6A-infected cells used for experiments in this study were $\geq 70\%$
349 GFP+ at the time of harvest. SupT1 cells were cultured in RPMI-1640 medium
350 supplemented with 2.5% FBS and 2 mM L-Glutamine. HHV-7 infection (strain SB) was
351 performed by co-culture of HHV7-infected SupT1 cells with uninfected SupT1 cells.

352

353 *Antibodies & reagents*

354 The monoclonal anti-human CD45 antibody (clone S5-Z6, Santa Cruz Biotechnology,
355 Dallas, TX) was used for flow cytometry (1 ug/ 1 x10⁶ cells), immunofluorescence
356 microscopy (1:50), and immunoblotting (1:50). The monoclonal anti-CD45 antibody
357 (clone MEM-28, Millipore-Sigma, St. Louis, MO) was used for immunofluorescence
358 microscopy (1:200). The polyclonal antibody MCW62 (U21-N) was raised against the N-
359 terminus of HHV-7 U21 (1:400) (72). AlexaFluor-405, -488, -594, and -647-conjugated
360 goat-anti mouse and rabbit secondary antibodies were used at dilutions recommended
361 by the manufacturer (ThermoFisher Scientific). Chemicals were purchased from Sigma
362 Millipore unless otherwise noted.

363 *Identification of cell surface N-glycoproteins*

364 Cell Surface Capture (CSC) was applied to control JJhan and HHV6A-infected JJhan T
365 cells (10 million cells per replicate, three biological replicates per condition). Briefly,
366 extracellular glycans on intact cells were oxidized using sodium meta-periodate, and the
367 resulting aldehydes were labelled with biocytin hydrazide to form a 'handle' for
368 enrichment. Cells were then lysed, proteins enzymatically digested, and biotinylated
369 glycopeptides were enriched using streptavidin beads. N-glycopeptides were then
370 selectively released by Peptide-N-Glycosidase F (PNGase:F) and analysed by mass
371 spectrometry. Here, CSC was performed as previously described in detail (47-50), with
372 the exception that glycopeptide enrichment and bead washing was performed using an
373 epMotion 5073m (Eppendorf, Hamburg, Germany). For each sample, 750 µg of total
374 peptide was diluted in binding buffer (80 mM sodium phosphate, 2 M NaCl, 0.2% Tween

375 20, pH 7.8) and incubated with 100 μ L of GenScript Streptavidin MagBeads (GenScript,
376 Piscataway, NJ) for 1 h with mixing. Beads were then washed sequentially with: (1) 2%
377 sodium dodecyl sulfate in ultrapure water, (2) 80 mM sodium phosphate, 2 M NaCl,
378 0.2% Tween 20, pH 7.8, (3) 100 mM sodium carbonate, (4) 80% isopropyl alcohol in
379 ultrapure water, (5) ultrapure water, and (6) 50 mM ammonium bicarbonate. Peptides
380 were released by digestion with PNGase F (Promega, Madison, WI) overnight at 37 °C
381 with vortexing. Deglycosylated peptides were cleaned and desalted using the SP2
382 procedure (73). Peptide Retention Time Calibration (PRTC) Mixture (ThermoFisher
383 Scientific) was added to each sample at a final concentration of 2 nM to enable
384 retention time calibration and assessment of instrument performance throughout the
385 acquisition. A “pooled QC” mixture was generated by combining equal portions of each
386 sample. Individual samples were queued in a randomized order within a technical
387 injection series with two technical replicates each. Pooled QC samples were analyzed
388 at the beginning and end of each technical injection series. Samples were analyzed by
389 liquid chromatography tandem mass spectrometry (LC-MS/MS) using a Dionex UltiMate
390 3000 RSLCnano system in-line with an Orbitrap Fusion Lumos (ThermoFisher
391 Scientific), and data were analysed with Proteome Discoverer 2.3 and Skyline (38).
392 Normalized quantitation ratios were determined by comparisons of each sample type to
393 the pooled QC.

394 *Immunofluorescence microscopy*

395 T cells were adhered onto poly-L-lysine-coated glass coverslips as described in (74)
396 and permeabilized in 0.5% saponin in PBS + 3% BSA + 880 μ M Ca²⁺ + 490 μ M Mg²⁺.
397 Permeabilized cells were incubated with primary antibodies, washed, and then

398 incubated with secondary antibodies conjugated to a fluorophore. 4',6-diamidino-2-
399 phenylindole (DAPI) was added to the final PBS wash at 1 ug/ml to stain DNA.
400 Superresolution microscopy was performed on a Nikon Structured-Illumination
401 Microscope (N-SIM; Nikon) and NIS-Elements AR imaging and 3D reconstruction
402 software (v. 5.11) (Nikon Instruments Inc, Melville, NY). Images were taken using a
403 Nikon 100X Oil-immersion lens (CFI Apo SR TIRF 1.49 NA) and an Andor iXon+897
404 EMCCD camera. Confocal microscopy was performed on a Nikon Eclipse Ti2
405 microscope equipped with a W1 Spinning Disc, Orca Flash CMOS camera, and 100X
406 oil-immersion objective (CFI Plan Apo λ 1.49 NA), and NIS-Elements AR imaging and
407 3D reconstruction software (v. 6.0).

408 *Flow cytometry*

409 Cells were incubated with primary antibodies in 1% bovine serum albumin (BSA) in
410 DMEM -phenol red for 30 min on ice, washed, and incubated with secondary antibodies.
411 Flow cytometry was performed using an LSRII flow cytometer (BD Biosciences, San
412 Jose, CA). Data was analyzed using FlowJo analysis software (v. 10.7, BD
413 Biosciences). Infected cells were selectively analyzed by GFP+ gating. Non-viable cells
414 were excluded from all flow cytometric analyses.

415 *Immunoblotting*

416 Cell lysates were prepared using 1% Triton Tx-100 lysis buffer supplemented with 62.5
417 U/ml Benzonase and 174 μ g/ml phenylmethylsulfonyl fluoride (PMSF) followed by the
418 addition of an equal volume of 2% sodium dodecyl sulfate (SDS) and 100 mM Tris-HCl
419 (pH 7.4). Lysates were normalized to total protein concentration as determined by

420 bicinchronic acid (BCA) assay (Pierce, Rockford, IL). Lysates were resolved by SDS-
421 PAGE and transferred to BA-85 nitrocellulose membrane (Cytivia, Marlborough, MA).
422 Membranes were incubated with primary antibodies, followed by HRP-conjugated
423 secondary antibody (BioRad Laboratories, Hercules, CA). Bands were visualized using
424 SuperSignal West Pico reagent (ThermoFisher Scientific) imaged with an Azure c600
425 gel documentation system and quantified using AzureSpot software (v2.2.167) (Azure
426 Biosystems, Dublin, CA).

427 *Inhibition of protein degradation*

428 HHV6A-infected or uninfected JJhan cells were incubated in RPMI in 50 mM NH₄Cl, 100
429 nM MG-132, or a DMSO control for 19 hours. Cells were then divided for use in
430 immunofluorescence microscopy or immunoblot analysis.

431 *qRT-PCR*

432 Cells were lysed in TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's
433 instructions. Approximately 2 ug of RNA was treated with an AccuRT Genomic DNA
434 Removal Kit (Applied Biological Materials, Richmond, BC, Canada) prior to cDNA
435 synthesis using the 5X All-In-One Master mix (Applied Biological Materials), both
436 according to the manufacturer's instructions. qPCR was performed using a BioRad
437 CFX96 Real-Time System (BioRad Laboratories, Hercules, CA) and data analyzed
438 using BioRad CFX Maestro (v.4.1.2433.1219). CD45 RNA levels were normalized to a
439 cellular control for each sample (28S rRNA). Primer sets are listed in [Table 1](#). All qPCR
440 reactions were run in technical triplicate with corresponding no template and -RT
441 controls, which did not exceed background levels. The delta-delta C_t method was used

442 to calculate the relative abundance of CD45 cDNA from HHV6A-infected JJhan cells
443 and 293T cells relative to the uninfected JJhan cell control.

444 Table 1. Primers used in this study.

CD45 qRT4 FWD	AAAAGTGCTCCTCCAAGCCA
CD45 qRT4 REV	TGGGAGGCCTACACTTGACA
28S rRNA 3783F	GTGACGCGCATGAATGGA
28S rRNA 3846R	TGTGGTTTCGCTGGATAGTAGGT

445 *Statistical analysis*

446 Statistical analysis was done using GraphPad 8 (v.8.4.3). One-way ANOVA analysis
447 with Tukey's multiple comparisons test was used to compare the abundance of CD45
448 mRNA 293T cells, uninfected JJhan cells, and HHV6A-infected T cells. **** < 0.0001

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