Dissection of the Interaction of the Human Cytomegalovirus-derived US2 Protein with Major Histocompatibility Complex Class I Molecules

**PROMINENT ROLE OF A SINGLE ARGinine RESIDUE IN HUMAN LEUKOCYTE ANTIGEN-A2**

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Human cytomegalovirus encodes several proteins that interfere with expression of major histocompatibility complex (MHC) class I molecules on the surface of infected cells. The unique short protein 2 (US2) binds to many MHC class I allomorphs in the endoplasmic reticulum, preventing cell surface expression of the class I molecule in question. The molecular interactions underlying US2 binding to MHC class I molecules and its allele specificity have not been fully clarified. In the present study, we first compared the sequences and the structures of US2 retained versus non-retained human leukocyte antigen (HLA) class I allomorphs to identify MHC residues of potential importance for US2 binding. On the basis of this analysis, 18 individual HLA-A2 mutants were generated and the ability of full-length US2 to bind wild-type and mutated HLA-A2 complexes was assessed. We demonstrate that Arg181 plays a critical role in US2-mediated inhibition of HLA-A2 cell surface expression. The structural comparison of all known crystal structures of HLA-A2 either alone, or in complex with T cell receptor or the CD8 co-receptor, indicates that binding of US2 to HLA-A2 results in a unique, large conformational change of the side chain of Arg181. However, although the presence of Arg181 seems to be a prerequisite for US2 binding to HLA-A2, it is not sufficient for binding to all MHC class I alleles.

Cytomegalovirus (CMV) belongs to the β-herpes virus subfamily and infects human populations at a high frequency worldwide. Primary infection with CMV, usually asymptomatic, is followed by lifelong latency, low level persistence, and occasional episodes of reactivation. Yet for individuals with a defective, immature, or compromised immune system, CMV is a serious threat. Congenital CMV infection is enable its persistence with the ultimate goal of transmission to a new host. One highly sophisticated immune evasion mechanism employed by CMV entails the interference with MHC class I presentation of viral peptides to CD8 T cells to prevent the lysis of infected cells (2). At least five viral unique short (US) proteins (US2, US3, US6, US10, and US11) are involved in this complex intervention. These proteins are expressed during different stages of infection, interfere with various steps of the cellular MHC class I mediated antigen presentation pathway, and act in a partly synergistic manner. US3, an immediate-early protein, retains MHC class I complexes in the endoplasmic reticulum (3). US2 and US11, expressed at early and late stages during infection, bind to certain MHC class I heavy chains, leading to their export from the endoplasmic reticulum to the cytosol for proteasome-mediated degradation (4–6). US6, expressed during late stages of the replication cycle, prevents the access of cytosolic peptides to MHC class I molecules by inhibiting the transporter associated with antigen processing (TAP) (7). Finally, US10 delays trafficking of MHC class I molecules (8).

US2 distinguishes between HLA class I allomorphs, leading to the destruction of some, e.g. HLA-A2 and HLA-Aw68 (9, 10), but not others, e.g. HLA-B7, HLA-Cw3, HLA-Cw4 and HLA-E (10, 11). For some allomorphs the results are less clear. In contrast to soluble HLA-A2 (9), soluble HLA-B27 does not bind US2, whereas full-length HLA-B27 does (10). HLA-C alleles generally do not seem to be retained by US2 (10), but HLA-Cw7 expression levels are reduced upon co-expression of US2 (11). Finally, HLA-G has been described as both susceptible (10) and resistant (12) to US2 binding. Varying experimental set ups, relatively poor MHC expression levels, but also the use of shortened soluble versions of US2 may explain some of the current discrepancies in the literature concerning the HLA class I specificity of US2.

The crystal structure of US2 in complex with HLA-A2 has been solved (13). Although the physicochemical properties of the US2-HLA-A2 complex suggest a 1:1 stoichiometry (9), examination of the structure reveals the presence of two distinct binding sites for US2 in HLA-A2. Site 1 is located at the junction of the peptide-binding region and the α3 domain, whereas site 2 is confined to a smaller area of the HLA-A2 α2-domain. Based on the effects of mutations in three residues site 1 was proposed as the sole physiological binding region (13).

However, the crystal structure of the US2-HLA-A2 complex was determined using a shortened soluble version of US2 (residues 15–140). Furthermore, the final model of US2 in the crystal structure comprises less than half of the US2 protein, residues 43–137 of the full-length 199-residue protein. Studies with truncated versions of US2 have revealed the importance of residues 28–40 and 140–160 for binding to...
MHC class I and II molecules (14). Thus, although the structure of the US2-HLA-A2 complex provides important insights about the interaction between US2 and class I molecules, it does not provide an absolute molecular basis for the retention of specific alleles by US2.

In the present study we examined the relative importance of specific residues in the two putative HLA-A2 contact sites with US2. Site-directed mutagenesis, combined with structural comparisons, strongly suggests that HLA-A2 residue Arg^{181} plays a predominant role in HLA-A2/US2 interaction. The binding of US2 to HLA-A2 results in a large conformational change of the side chain of Arg^{181}, which is used as an anchor position by the CMV-derived protein. Finally, our findings indicate that US2 interacts with other class I allomorphs in a different manner from its interaction with HLA-A2.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—J26 cells (murine Ltk− fibroblasts expressing human β2-microglobulin (β2m) on an H-2k background; American Type Culture Collection, Manassas, VA) (15) were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen), supplemented with 10% fetal calf serum (Integro, Zaandam, the Netherlands), 100 units/ml penicillin, and 100 μg/ml streptomycin. This cell culture medium is referred to as “normal cell culture medium.”

**Antibodies (Ab)**—The following anti-MHC class I monoclonal Ab were used for flow cytometry: FITC-conjugated W6/32 (general HLA class I, conformation-specific; eBioscience, San Diego, CA) (16) and FITC-labeled anti-HLA-A2 (BB7.2; BD Biosciences Pharmingen).

**Plasmids**—The HLA-A*0201 cDNA in pcDNA3, generously provided by Prof. V. Cerundolo (University of Oxford, Oxford, UK), was fully sequenced. All mutations (Table 1) were introduced using the QuikChange site-directed mutagenesis kit (Stratagene) and confirmed by sequencing.

**Transfection**—J26 cells were transfected with wild-type (WT) or mutant HLA-A2 using the Effectene Transfection Reagent (Qiagen, Hilden, Germany). Transfections were conducted as described in the manufacturer’s protocol. Stable transfectants were selected 24 h later in normal tissue culture medium containing 0.3 mg/ml G418 (Geneticin, Invitrogen). After 1–3 weeks, G418-resistant cells were sorted by flow cytometry for HLA-A2 expression using anti-HLA-A2 Ab. Cells were analyzed for HLA class I expression by flow cytometry using a FACSVantage DIVA (BD, San Jose, CA) for surface expression of HLA-A2 using anti-HLA-A2 Ab. Cells were analyzed for HLA class I expression by flow cytometry using a FACSCount (BD) (18). Cells were then treated with 0.2M citric acid/Na2HPO4 buffer at pH 3.0 for 2 min on ice to eliminate MHC class I molecules on the cell surface, as described previously (19). Flow cytometry was performed after an additional 4-h incubation in normal cell culture medium at 37 °C. To test the effect of US2 on HLA-B alleles, non-transfected J26 cells were co-infected with VVUS2 or VVPN as a control and a recombinant vaccinia virus expressing either HLA-B8 (VVB8) or HLA-B27 (VVB27). VVB8 and VVB27 were created following protocols described previously (18, 20). Cells were harvested for analysis of HLA-B expression by flow cytometry after a 6-h incubation in normal cell culture medium. The percentage of infected cells was assessed by monitoring the shape of the cells by microscopy and indirectly, by infecting J26 cells with VV-expressing green fluorescent protein at the same multiplicity of infection, using flow-cytometry as a read out.

**Flow Cytometry**—J26 cells were sorted using a FACSVantage DIVA (BD, San Jose, CA) for surface expression of HLA-A2 using anti-HLA-A2 Ab. Cells were analyzed for HLA class I expression by flow cytometry using a FACSCount (BD). Cells (0.5 × 10^6) were stained with W6/32 Ab for 30 min at 4 °C in FACS buffer (cold phosphate-buffered saline containing 1% fetal calf serum). Cells were subsequently washed twice in FACS buffer and a total of 15,000 events gated on live cells were collected. Non-transfected cells infected with VVNP or VVUS2 were used as negative controls in each test of the HLA-A2 mutations. Non-transfected, non-infected cells were used as a negative control in co-infection tests. Analyses were performed using CellQuest software (BD Biosciences) and FlowJo software (Tree Star). Single-step staining with FITC-conjugated primary Ab was performed to promote linearity when comparing the effects of US2 on these samples.

**RESULTS**

**Identification and Substitution of Residues Important for US2 Binding to MHC Class I Allomorphs**—To test the functional importance of individual residues localized at each of the two defined contact sites between HLA-A2 and US2 (Fig. 2A) we used site-directed mutagenesis to substitute on an individual basis each of the HLA-A2 residues predicted to form hydrogen bonds with US2 (Figs. 1 and 2, B and C, and Table 1). Because regions adjacent to the contact sites could be important for binding to full-length US2 we also mutated residues that differed between HLA-A2 and HLA allomorphs not binding US2. Based on our

FIGURE 1. Sequential comparison of tentative US2 binding sites in MHC class I molecules. Sequence alignment is depicted for MHC class I allomorphs that are retained (top part) and that are not retained (bottom part) by US2. The alignment was created using the program Multalin (64). Regions involved in US2 binding are framed in black (site 1) and green (site 2). Arrows indicate residues in HLA-A2 that were mutated in this study. The sequence residues with high or low consensus are colored red and blue, respectively, neutral residues are colored black.
analyses of available HLA amino acid sequences as well as published crystal structures, HLA-A2 residues were mutated to alanine and/or to the residue present in HLA-B and -C allomorphs not retained by US2. None of the mutations were conservative. Finally, we also created combinations of mutations in locations where we deemed it unlikely that a single substitution would significantly alter US2 interaction with HLA-A2 (Figs. 1 and 2 and Table 1).

**Table 1: Mutations introduced in HLA-A2, within or near US2 binding sites 1 and 2**

| No. | Changed residue | US2 site 1 | Vicinity US2 site 1 | US2 site 2 | Vicinity US2 site 2 |
|-----|----------------|------------|---------------------|------------|---------------------|
| 1   | W107A          | x          |                     |            |                     |
| 2   | Q180A          | x          |                     |            |                     |
| 3   | Q180E          | x          |                     |            |                     |
| 4   | R181E          | x          |                     |            |                     |
| 5   | T182A          | x          |                     |            |                     |
| 6   | A184P          | x          |                     |            |                     |
| 7   | T182A/A184P    | x          |                     |            |                     |
| 8   | A184H          | x          |                     |            |                     |
| 9   | S207G          | x          |                     |            |                     |
| 10  | K144Q          | x          |                     |            |                     |
| 11  | H145R          | x          |                     |            |                     |
| 12  | H151R          | x          |                     |            |                     |
| 13  | K144Q/H145R    | x          |                     |            |                     |
| 14  | K144Q/H145R/H151R | x |                 |            |                     |
| 15  | K127N          | x          |                     |            |                     |
| 16  | M138T          | x          |                     |            |                     |
| 17  | T142I          | x          |                     |            |                     |
| 18  | M138T/T142I    | x          |                     |            |                     |

**DISCUSSION**

Human CMV encodes several US proteins, which have the ability to down-regulate MHC class I expression (42). Herein we focused our analysis on the molecular basis underlying US2 interaction with MHC class I ligands by assessing the expression of MHC class I molecules directly on the cell surface.

Our data demonstrate that the molecular interaction between US2 and HLA-A2 in site 1 is dependent on binding of US2 to the class I heavy chain residue Arg^{181}, in concordance with previous observations by Gewurz and collaborators (13). The observation that the mutation of Arg^{181} alone blocks US2-mediated degradation of HLA-A2 is consistent with previous reports.
with a single site interaction model. Moreover, none of the nine substitutions introduced in or around the so-called site 2 affected US2 activity against HLA-A2 (Figs. 2C and 3), further strengthening the view that site 1 is the only physiological site of interaction between HLA-A2 and US2.

The present site-directed mutagenesis study demonstrates that the interaction of US2 with HLA-A2 is critically dependent on residue Arg181. A hot-spot mutation is a perturbation that destabilizes the bound state ensemble relative to the unbound one (43). Protein-protein binding depends crucially on such energetic hot-spots, as they provide most of the binding energy (44, 45). Thus Arg181 is a hot-spot residue at the interface of the CMV-derived US2 protein and its target HLA-A2. Interestingly, the structural comparison of all known structures of HLA-A2 reveals that the conformation of the side chain of Arg181 is profoundly affected by the binding of US2, whereas the conformation of all other HLA-A2 residues interacting with US2 is conserved. The side chain of Arg181 assumes a diametrically opposed conformation when compared with its most common position, protruding toward and interacting with US2 residues.

Notably, Arg181 is present in all allomorphs retained by US2. Although necessary for US2 interaction, Arg181 is not sufficient, because the MHC alleles HLA-B7 and HLA-B8 are not retained by US2, although they possess Arg181 (10, 11) (Fig. 1). Such a discrepancy is also present among HLA-C alleles. Although all HLA-C alleles have Arg181 (except HLA-Cw0209), HLA-Cw3 and -Cw4 escape retention by US2 (10), whereas HLA-Cw7 is retained (11).

The most obvious explanation for the escape of Arg181-containing MHC allomorphs from US2 activity is interference from surrounding residues in site 1. Presumably these residues impair the US2 interaction by direct effects on surface charges or less directly by...
inducing conformational alterations in surrounding side chains. To better understand these influences, the C backbone atoms of heavy chain residues 1–182 in the US2/HLA-A2 co-complex were superimposed on corresponding residues in retained versus escaping MHC allomorphs (PDB codes 2HLA (46), 1HSA (47), 1FO2 (48, 49), 1M05 (50), 1EFX (51), 1QKD (52), and 1IM9 (53)) (Fig. 6). Clearly, the region surrounding Arg181 in retained HLA-A2 and escaping HLA-B8 and HLA-Cw4 revealed a broad similarity, except a few differences that were tested for their impact on US2 interaction through our substitutions. The most likely candidate to influence the interaction between US2 and the positively charged Arg181 of HLA-B alleles is the negatively charged glutamate residue at position 180, which is present in the escape alleles HLA-B7 and HLA-B8 but not in the retained HLA-B27. However, mutation of the glutamine residue Gln180 in HLA-A2 to either an alanine or a glutamate (Q180A and Q180E) did not impair US2 retention. Furthermore, modification of Ala184 to either a proline or a histidine, both of which occur at this position in escaping alleles (Figs. 1, 2B, 3D, and 6C), did not reduce US2-binding (Fig. 3D); neither did the combined T182A/A184P mutation. Finally, the mutation in HLA-A2 of residue Trp107 to an alanine did not impair the destruction of HLA-A2 by US2. The present study suggests that only the substitution of Arg181 prevents the destructive effects of US2 on HLA-A2.

Structural analysis of the interaction site 1 between HLA-A2 and US2 reveals that the side chain of the HLA-A2 residue Arg181 points into a pocket formed by US2 residues Phe74, Asp75, Pro76, Lys77, Asn86, Leu87, and Lys88. The side chain of Arg181 fits snugly within the narrow US2 pocket, acting as an optimal anchor position. Using molecular modeling, we have tested the potential effect of a rather conservative mutation, such as a change from arginine to lysine, and our analysis suggests that such a conservative mutation would result in sterical hindrance with the US2 residue Leu87 (data not included). Five hydrogen bonds are formed between the side chain of Arg181 and the carbonyl atoms of residues Pro76 (one hydrogen bond interaction), Leu87 (two), and the side chain of residue Asp88 (two) (Fig. 2B). A salt bridge is also formed between the side chain of Arg181 and

![FIGURE 3. The R181E mutation allows HLA-A2 to escape US2 retention.](image-url)

Murine J26 cells transfected with plasmids encoding WT or mutated HLA-A2 molecules were infected with recombinant VVUS2 or the control VVNP. The HLA-A2 cell surface expression levels were analyzed by flow cytometry using FITC-conjugated W6/32 (pan-anti-MHC class I). A, background staining of non-transfected cells is depicted as a dotted gray line, HLA-A2 expression levels on VVNP-infected cells as a solid gray line, and HLA-A2 expression levels on VVUS2-infected cells as a solid black line. Histograms depict representative graphs for the retention capacity of US2 on WT HLA-A2 (A), HLA-A2 (Q180A) (B), and HLA-A2 (R181E) (C). The histograms of all other mutations were similar to that of HLA-A2 (Q180A) (data not included). D, the bar chart summarizes the effect of US2 on all altered HLA-A2 molecules listed in Table 1. Data were collected from three independent experiments. The percentage of MHC molecules that escaped US2 binding was determined for every substituted HLA-A2 as well as the WT by using the equation (escape fraction = (MFI(HLA + US2) − MFI(background))/(MFI(HLA + NP) − MFI(background)) × 100%). The escape fraction obtained for HLA-A2 WT was subtracted from the escape fraction of each mutant in the same experiment. This difference in US2 escape compared with the WT is shown on the y axis, error bars represent ± S.D.

![FIGURE 4. The side chain of residue Arg181 undergoes a large conformational change upon binding of US2 to HLA-A2.](image-url)

We have superimposed the backbone atoms of the peptide binding cleft residues 1–182 in the HLA-A2/US2 crystal structure and all known crystal structures of HLA-A2, either alone or in complex with different ligands such as T cell receptors, CD8 co-receptors, and leukocyte immunoglobulin-like receptor 1. The conformation of the side chain of Arg181 shifts around 180° upon binding of US2 to HLA-A2. For simplicity, only 4 representative structures out of a total of 30 are displayed (1IM3, 1HHK, 1AO7, 1QSF).
FIGURE 5. Cell surface expression of HLA-B8 is not affected by US2. J26 cells were simultaneously infected with either VVUS2 or VVNP (control) and a second recombinant vaccinia virus introducing HLA-A2 (A), HLA-B27 (B), or HLA-B8 (C). MHC class I surface levels were measured 6 h postinfection by flow cytometry using FITC-conjugated W6/32 (pan-anti-MHC class I). Representative histograms indicate HLA surface expression after VVUS2 infection (solid black line) and after control VV infection (solid gray line). Background staining is represented by a dotted line in each plot.

FIGURE 6. The US2 binding site in HLA class I allomorphs. A, overall view of HLA-A2 depicting the US2 binding site 1 within the highlighted area. The three main extracellular domains of the heavy chain (α1, α2, and α3) are indicated. The peptide is red, the β2m subunit is dark gray, and the heavy chain is light gray. B, transparent surface representation of the US2 binding site 1. Negatively and positively charged regions are red and blue, respectively. The side chains of residues Gln180, Arg181, and Ala184 are indicated. C, comparison of US2 binding site 1 in retained (left column) and non-retained (right column) MHC class I complexes. The crystal structures of HLA-A2 (PDB code 1M3 [13]), HLA-B8 (1M05 [50]), and HLA-Cw4 (1M9 [53]), and models of HLA-A2 with substitutions R181E, Q180E, or A184H (based on the crystal structure of HLA-A2/US2 [13]) were used to create the figures. Arrows indicate the position of corresponding residues in altered HLA-A2 molecules and escaping HLA-B or -C allomorphs.
the carbonyl atom of Phe74. The size and the form of the side chain of Arg181 combined with the large amount of hydrogen bonds and salt bridges that are formed with US2 residues provide an explanation to the dominant role of an arginine residue in the specific interaction between US2 and HLA-A2 (when compared with other amino acids). A complemented pocket is a concave region on the interface part of a protein (e.g. US2) that is filled by one or more residues from another protein (e.g. Arg181 in HLA-A2) upon association. These pockets, which represent binding regions on interfaces that have non-trivial geometric shapes and a tight fit, are thus present when two interacting proteins are separated but disappear following association. Complemented pockets and their corresponding protruding residue(s) are among the most important geometric features in protein-protein interactions (45). Structurally conserved residues and energetic hot-spots, such as Arg181, are strongly favored to be located in complemented pockets. Thus we propose that, besides disrupting all the hydrogen bonds formed between this central residue and US2, the R181E mutation would repel the US2 side chains and decrease the binding affinity of US2 to HLA-A2. This specific substitution could also impair a local conformational fit of US2 following binding to Arg181 with potential implications for the consecutive destruction of the targeted MHC allomorph.

Still, the remaining question is that if Arg181 is essential for binding to US2, why do other MHC class I alleles that contain Arg181 escape US2-binding? As we see it, there are two non-excluding potential explanations for this observation. The first explanation could be that parts of US2, which are not visualized in the crystal structure of the US2-HLA-A2 complex, bind to other HLA-A2 regions. This second binding region would increase the overall affinity of US2 to the MHC alleles over HLA-A2 complex, bind to other HLA-A2 regions. This second binding region would increase the overall affinity of US2 to the MHC alleles over a specific threshold. Indeed, studies with different shortened versions of US2 have found that a major part of the US2 luminal domain seems to be necessary to establish a tight interaction with MHC class I molecules (14). However, this explanation does not fit fully with biochemical studies that demonstrate that a shortened US2 binds to HLA-A2 (9). Furthermore, we have scrutinized the alignment of the sequences of the α3 domains in MHC alleles that are retained (or not) by US2, but we were unable to find any hint about the potential localization of such a region (data not included). It should also be noted that the amino acid sequences of the α3 domains of most MHC alleles are rather conserved.

The formation of a disulfide bond in the α3 domain of class I molecules is essential for US2-mediated degradation (54), indicating that either 1) as we stated above, the C-terminal region of US2 (that is lacking in the structural study) binds to a site on α3 or 2) that the refolding of α3 is essential for the presentation of a specific MHC residue (e.g. Arg181). The α3 domain is likely to fold before the α1α2 region, independently of the β2m species (55, 56). The subsequent binding of β2m would then facilitate the folding of the α1α2 domain and thereby increase the likelihood of peptide binding (57). However, the role of β2m in the binding of US2 to MHC complexes is subject to controversy. Previous studies suggested that US2-mediated dislocation of MHC class I heavy chains require assembly with β2m (58). However and although US2 prefers properly folded and assembled heavy chains as targets, unassembled heavy chains are also targeted for degradation by US2 in the absence of β2m (59). All of these studies regarding the role of α3 and β2m suggest that these two regions play an important role in binding of MHC alleles by US2. The structural comparison performed within this study shows that the side chain of Arg181 undergoes a profound conformational change upon binding to US2 (Fig. 4). We propose that the direct environment surrounding the arginine residue in non-retained MHC class I alleles prevents the side chain of Arg181 to undergo such a conformational change, hindering US2 from using this precise residue as a primary anchor position. However, we are unable at the present time to pinpoint a specific residue or MHC complex subunit (such as the α3 domain or the β2m subunit). Only further crystal structure studies of US2 in complex with other MHC class I alleles, or of a longer US2 variant in complex with HLA-A2, for example, will permit us to fully assess whether this theory is valid.

The present study confirmed a previous report that US2 affects the surface expression of HLA-B27 (10). Yet when the binding of HLA-B27 to US2 was previously studied using shorter soluble versions of US2 and HLA-B27, no binding was observed (9), despite the fact that the interacting site 1 is present in both the short and full-length versions of HLA-B27. Thus the puzzling lack of binding by soluble HLA-B27 to US2 observed when using soluble shortened versions of US2 (9) could be explained by either one or a combination of the following possibilities: (i) bacterially produced US2 lacks post-translational modifications necessary for interaction with some allomorphs (14); (ii) full-length US2 is required because HLA-B27 residues in the vicinity of site 1 are involved in binding; (iii) residue Arg181 in HLA-B27 is not important for interaction with US2, and the latter retains this MHC allomorph in a different manner from HLA-A2; (iv) soluble HLA-B27 does not allow a necessary conformational change of the side chain of Arg181, whereas full-length HLA-B27 allows it. The observed interaction between soluble US2 and HLA-A2 (9) may indicate that the slightly different amino acid composition of the defined US2 binding site 1 in HLA-A2 versus that in HLA-B27 accounts for a stronger interaction with US2. Although we have demonstrated here that HLA-A2 residue Arg181 is essential for US2 recognition, the contribution of other regions in the retention/evasion of specific HLA-B and -C allomorphs remains to be assessed, because some MHC allomorphs, although displaying Arg181, still escape US2 retention (Figs. 1 and 6C).

US2 binding to the α-chains of HLA-DR and HLA-DQ was previously demonstrated as a means of triggering proteasome-mediated degradation of MHC class II molecules (14, 60, 61), allowing the virus to avoid CD4+ T cell responses (14). In addition, the same viral protein interferes with metabolic functions; US2 binds to the hereditary hemochromatosis protein HFE, related to MHC class I molecules, and is involved in intracellular iron homeostasis (62). A structural comparison of either MHC class II molecules or HFE with retained MHC class I molecules did not provide an explanation for these interactions (63). Thus US2 seems to be capable of binding to different distinct binding motifs. It is possible that this interaction could occur at site 2. In this scenario, the crystal structure would have revealed a tenuous interaction between US2 and HLA-A2 that is much stronger with other US2 ligands.

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