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Comparison of the HIV-1 VPU interaction with CD4 and CD74

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Review article

Functional roles of HIV-1 Vpu and CD74: Details and implications of the Vpu–CD74 interaction

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ABSTRACT

HIV-1 Vpu has a variety of functions, including CD4 degradation and the downregulation of MHCII. Downregulation of the MHCII occurs through Vpu binding to the cytoplasmic domain of CD74, the chaperone for antigen presentation. The CD74 cytoplasmic domain also plays a vital role in cell signaling through the activation of an NF-κB signal cascade for the maturation, proliferation and survival of B cells as well as by binding the macrophage inhibitory factor. In view of these functions, it follows that the Vpu–CD74 interaction has multiple downstream consequences for the immune system as it not only impairs foreign antigen presentation but may also have an effect on signal transduction cascades. It is thought that Vpu specifically targets intracellular CD74 while other HIV-1 proteins cannot. Therefore, this protein–protein interaction would be a potential drug target in order to reduce viral persistence. We review the functional importance and specific binding site of Vpu and CD74.

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1. Introduction

HIV-1 has several accessory genes, namely *vif*, *vpr*, *nef* and *vpu*. The accessory proteins encoded by these genes play important roles in viral replication [1]. Vif targets the host restriction factor APOBEC3G for proteasomal degradation to prevent the inhibition of viral DNA synthesis [2–4]. Vpr has many functions including activation of cell death and proviral transcription [4,5]. Nef is a functionally diverse protein that is involved in the alteration of cell signaling pathways, disruption of antigen presentation by MHCI and MHCII and alteration of gene and receptor surface expression [4,6]. Lastly, Vpu also has multiple functions within the host cell, some of which are similar to Nef, with the most well-described functions being the degradation of the HIV-1 receptor CD4 and virion release [4,7,8].

Of particular interest, Vpu is only encoded by the genome of HIV-1 and a few simian immunodeficiency virus (SIV) isolates such as SIVcpz (chimpanzee), SIVmon (mona monkey), SIVgsn (greater spot-nosed monkey), SIVmus (mustached monkey) and SIVden (Dent's mona monkey), but is not found within HIV-2 [9]. Related isolates that do not express a functional Vpu protein have far less severity in terms of disease outcome [10] indicating the importance of this viral protein. HIV-1 infections tend to result in chronic immune activation, while HIV-2 infections yield lower levels of immune activation [11] and SIVs of sooty mangabeys and African green monkeys, which do not encode *vpu*, generally also do not cause high levels of immune activation [12]. Furthermore, the functionality of the Vpu protein in the HIV-1 strains of M, N and O has been suggested to be necessary for the spread of the pandemic M strain as this strain expresses a Vpu protein that is not only able to target CD4 for degradation, but also effectively antagonizes tetherin [4,9]. The non-pandemic N and O strains express a Vpu protein that is lacking in one of the primary functions. Although HIV-2 targets tetherin using the Env protein, this has been found to be less effective than that of HIV-1 Vpu [9]. HIV-1 Vpu appears to target the immune system in many different but related ways that are highly effective at sabotaging the immune

Abbreviations: AGTR1, angiotensin II type 1 receptor; β-TrCP, β-transducin repeat-containing protein; CD74-ICD, CD74 intracellular domain; CLIP, class-II-associated Ii-chain peptide; ER, endoplasmic reticulum; ERK-1/2, p44/p42 extracellular-signal-regulated kinase family; HLA-DR/DM, human leukocyte antigen-DR/DM; I-CLiPs, intramembrane cleaving proteases; MCP-1, monocyte chemoattractant protein-1; MIF, macrophage inhibitory factor; NMR, nuclear magnetic resonance; RIP, regulated intramembrane proteolysis; SIV, simian immunodeficiency virus; $TAF_{II}105$, TBP associated factor $_{II}105$; TBP, TATA box binding protein; TFIID, transcription factor IID.

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response and that work in conjunction with other HIV-1 proteins to more effectively enhance viral replication. As each component of the immune system that is targeted by Vpu is important, the impairment of Vpu function would likely increase the ability of the immune system to respond to HIV-1 infection.

One of the more recently described functions of Vpu is the downregulation of the MHCII, specifically through the interaction with the MHCII-associated invariant chain, or CD74 [13]. As CD74 plays a role in many important cellular functions, including the immune response, it is feasible that the binding of Vpu to this host protein has multiple downstream consequences for the immune response apart from the downregulation of the MHCII. Therefore downregulation of this host protein is beneficial for viral persistence. In this review, we take a look at the multiple functions of both Vpu and CD74 within the infected cell and examine the specific interaction and binding site between these two proteins and consider the possible ramifications of this interaction. Finally, we contemplate on the suitability of this protein–protein interaction as a possible novel therapeutic target for drug intervention.

2. HIV-1 Vpu structure

Vpu is an 81-mer type I integral intracellular membrane protein that is expressed in the later stages of viral infection. Vpu has an N-terminal transmembrane hydrophobic helix (residues 1–27) and two amphipathic helices (residues 35–50 and 58–70) that form part of the cytoplasmic domain and are separated by a linker region (residues 47–58) [14–16]. The linker region has two highly conserved serine residues in the cytoplasmic domain, namely Ser52 and Ser56 [16] that are required for the binding of β-transducin repeat-containing protein (β-TrCP) – a necessity for some functions of Vpu. The transmembrane domain tilts at an angle of approximately 6–15 degrees [17,18] and the linker region between the two cytoplasmic helices is largely flexible, allowing for the second cytoplasmic helix to move and possibly lie parallel to the first cytoplasmic helix [16]. The first cytoplasmic helix of Vpu is considered to lie parallel to and be partly buried in the membrane of the endoplasmic reticulum (ER) to shield the hydrophobic residues, leaving the charged residues exposed. However, the exact conformation of Vpu may be dependent on oligomerization or the interaction with other host proteins [17,19,20]. Using molecular simulation studies, it was shown that there are slight structural differences in the cytoplasmic domain between the phosphorylated and unphosphorylated forms of Vpu, with the phosphorylated protein adopting a more compact structure in comparison to the unphosphorylated protein [19]. Nuclear overhauser effect spectra have suggested that the two cytoplasmic helices lie in an antiparallel formation and that there is likely to be spatial proximity between the C-terminal region and the linker region containing the phosphorylated serine residues [20]. The side chains of the serine residues are thought to be exposed to the buffer or solvent, making these residues accessible to casein kinase II for phosphorylation [20].

Vpu is mostly found to be in the ER and Golgi apparatus but may also be found to some extent at the plasma membrane [14,21]; however Vpu is not found in culture fluid or supernatant, suggesting that the protein is not associated with virions [22]. It is thought that Vpu contains two trafficking signals in the hinge region that lies between the transmembrane domain and the cytoplasmic domain as well as within the second or C-terminal α-helix [9]. It has been suggested that there are deviations in the primary sequences of these trafficking signals between HIV-1 subtypes which would explain why Vpu expressed from different subtypes is not localized within the same cellular compartments. This difference is demonstrated between subtype B and subtype C, where subtype B Vpu localizes mainly within intracellular membranes such as the ER, Golgi and endosomes, while subtype C Vpu is reportedly found mostly at the plasma membrane [9].

3. HIV-1 Vpu functions

The two primary functions of Vpu are the degradation of CD4, the primary receptor protein for HIV-1 [23–25] and the release of new virus particles from infected cells either by inhibition of the host restriction factor, tetherin [26–29] or through the viroporin activity of Vpu in which the transmembrane domain is able to form an ion channel in a separate mechanism to that of the antagonism of tetherin [30–32]. Vpu also seems to undermine or impair the immune system through a variety of methods including the induction of apoptosis in infected cells [33] and the downregulation of MHCII by binding CD74 [13] to name a few.

Possibly the best known function of HIV-1 Vpu is the targeting of CD4 for degradation [15,23–25]. CD4 is integral for the functioning of the immune system as it is involved in multiple functions including the production of cytokines, the enlistment of neutrophils and basophils, assisting with the production of antibodies along with B cells and protection against intracellular and extracellular pathogens, bacteria and parasites [34–38]. After infection with HIV-1, CD4–Env complexes are formed in the ER, trapping CD4. Vpu and CD4 interact *via* their cytoplasmic domains and β-TrCP is then recruited to the Vpu–CD4 complex. The ternary complex consisting of Vpu–CD4–β-TrCP recruits Skp1p and ultimately the E3 ligase complex, leading to the ubiquitination and degradation of CD4. CD4 is unable to bind to β-TrCP in the absence of Vpu, indicating that Vpu is a linker protein between CD4 and β-TrCP [39]. By targeting this protein for degradation, Vpu has a deleterious effect on the immune system.

Yet another protein that is inhibited or antagonized by Vpu is the host restriction factor tetherin. Tetherin is a membrane protein that is induced by interferon-α, is expressed on the cell surface of differentiated B cells and bone marrow stromal cells and functions to harness newly assembled virions to each other as well as to the cell membrane [26,27]. Vpu has been found to co-localize with tetherin [26] and these two proteins interact specifically through the binding of their transmembrane domains [28,29]. Not all cell types require Vpu for virion release. However, those cell types that are dependent on Vpu for this function, but that are deficient in Vpu, show the accumulation of mature and assembled virions at the surface which later are internalized to the endosomes [26]. The ability of Vpu to inhibit tetherin at the surface of the cell relies on both the transmembrane domain of Vpu as well as the conserved serine residues in the cytoplasmic linker region. Previous studies reported the possibility that Vpu downregulates tetherin by trapping it within the Golgi network [40] and degrades tetherin by ER-associated protein degradation [41]. Additionally, it was shown that Vpu rather targeted cell surface tetherin and removed it directly from the plasma membrane [28] thus greatly enhancing virion release. It has further been hypothesized that the antagonism and downregulation of tetherin by Vpu functions to disable anti-HIV antibodies from recognizing infected cells which ultimately abrogates antibody-mediated clearance of the infected cells [42].

Vpu is also able to mediate virus particle release through its transmembrane domain ion channel activity and this mode of virus particle release is distinct from tetherin antagonism [30–32]. The Vpu ion channel, or viroporin, is selective for cations such as sodium and potassium [30] and has been predicted to exist in a pentameric state [43,44]. Predictive molecular simulation studies have generated a model of the Vpu ion channel and suggested this channel would be able to exist in either an open or a closed

formation, depending on the positioning of two Vpu residues – Ser23 and Trp22 [44,45]. The Vpu ion channel is blocked by amiloride derivatives, 5-(*N*,*N*-hexamethylene)amiloride and 5-(*N*, *N*-dimethyl)amiloride and in certain cell types results in inhibition of budding of virus-like particles from the plasma membrane [46]. Furthermore a candidate drug, BIT225, has been shown to have anti-HIV activity and specifically acts on Vpu to block the ion channel activity with the ability to significantly abrogate both viral integration as well as viral release [47].

Another way in which Vpu undermines the immune system is in the induction of apoptosis in T cells [33]. Vpu competitively binds β-TrCP thereby inhibiting the degradation of IκB [33]. This, in turn, reduces the activation of NF-κB which ultimately leads to a decrease in the expression of antiapoptotic proteins [33]. Simultaneously, caspase-3 levels are increased causing apoptosis within HIV-1 infected T cells [33]. However, it is important to note that many other HIV-1 proteins are also involved in the induction of apoptosis as well as the modulation of survival genes. Nef has been reported to induce apoptosis in bystander CD4+ T helper cells [48] as well as decreasing the expression of the antiapoptotic protein Bcl-2 [49]. Vpr is able to prevent the continuation of the cell cycle at the G2/M phase as well as activate caspase-9, leading to activation of caspase-3 and ultimately induces apoptosis in primary human cells [50]. Some non-accessory HIV-1 proteins, such as Tat, have also been shown to play a role in inducing the apoptosis of host cells [51]. Taking this into account Vpu does not work alone in terms of inducing apoptosis but rather in conjunction with these other proteins, in particular the Nef protein, to facilitate the impairment of the immune system and target host proteins in cellular compartments that may not be available to the other viral proteins. For example, using fluorescence activated cell sorting-based Försters resonance energy transfer, it was shown that Nef and Vpu were found in their expected locations within the cell, i.e. Nef at the plasma membrane and Vpu at the ER, Golgi and plasma membrane [52]. The binding of Nef and Vpu to CD4 within their respective cellular compartments was also corroborated using this approach [52].

It is possible that Vpu has yet another function by which it contributes to undermining the immune system. By binding to the intracellular cytoplasmic domain (ICD) fragment of CD74 (CD74-ICD) in the ER, Vpu may prevent the translocation of CD74-ICD to the nucleus. Consequently, the NF-κB signal cascade that affects B cell maturation, proliferation and survival cannot be initiated (Fig. 1A). This may add to the arsenal of host proteins that Vpu targets in order to assist in avoiding detection of the viral infection. The ability of the immune system to respond to infections is reliant on the presentation of foreign antigens or peptides to the T cells by the MHC molecules [53]. Vpu has been shown to downregulate MHCI molecules [54] which display particles derived from intracellular microorganisms within the cytoplasm, particularly viruses, to the CD8+ cytotoxic T lymphocyte cells which are known to directly lyse infected cells [55,56]. However the effect of Vpu-mediated downregulation of MHCI may be overshadowed by Nef-mediated downregulation of MHCI [57] indicating that this specific function of Vpu may not be particularly significant. More recently it was shown that Vpu prevents the trafficking of MHCII to the endosome and ultimately inhibits the maturation of the MHCII complex at the cell surface (Fig. 1B) [13]. MHCII molecules present foreign particles derived from extracellular microorganisms within the endosomal–lysosomal system including viruses, bacteria, protozoan parasites and cell surface proteins to CD4+ helper T lymphocytes which activate B lymphocytes as well as macrophages, leading to the production of antibodies and the destruction of bacteria, respectively [55,56,58]. The binding of Vpu to CD74-ICD therefore prevents the presentation of foreign antigens and thus the already weakened immune system is not able to either recognize or respond to these foreign particles. Decreased antigen presentation may only be one reason why MHCII complexes are downregulated in infected cells. It has been found that the assembly and budding of HIV-1 may occur either at the plasma membrane or in late endosomes/multivesicular bodies, depending on the cell type. For example, in T lymphocytes and some human cell lines HIV-1 assembles at the plasma membrane, while in macrophages, HIV-1 assembles in the late endosomes/multivesicular bodies. Interestingly it was shown that the cytoplasmic domains of human leukocyte antigen-DR (HLA-DR), which is an important component of the MHCII molecule, affects the assembly and budding of HIV-1 in such a way that it is directed away from the plasma membrane and rather to the late endosomes/multivesicular bodies [59]. HLA-DR specifically acts on the relocation of the HIV-1 Gag protein, which is needed for the assembly of virus particles. Although the virions in these intracellular compartments are still infectious, this finding suggests that the MHCII molecules may be able to reduce the release of new viruses from the cell surface [59]. Vpu may also influence a set of survival genes by interacting with CD74 in the ER. Although it has been shown that Nef also modulates the functions of CD74, Nef has only been reported to interact with cell surface CD74 [4,6,60]. This indicates the importance of Vpu as it is able to target CD74 within the ER and in so doing, Vpu works in conjunction with Nef to modulate CD74.

4. Human CD74 structure

CD74 consists of a 30-mer N-terminal cytoplasmic domain, a 26-mer transmembrane domain and a 160 amino acid sequence that is either extracellular or projects into the lumen, depending on the subcellular location of the protein [13]. There are several isoforms of CD74, namely p33, p35, p41 and p43 [61]. Ii-p33 and Ii-p35 are 33 kDa and 35 kDa, respectively and are involved in MHCII antigen presentation with the Ii-p33 isoform being the prominent of the two. These isoforms originate in two ways, i.e. by alternative splicing as well as through the use of two different translation initiation sites [61]. The only known difference between these isoforms of CD74 is that Ii-p35 has 16 extra residues at the N-terminus that form part of the cytoplasmic domain. These additional residues differentiate between the routes by which the immature MHCII molecules reach the endocytic compartment for antigen loading. MHCII molecules that are partnered with the Ii-p33 isoform only reach the endosome *via* the cell membrane, while molecules that are partnered with the Ii-p35 isoform are first trafficked to the Golgi network before moving to the endosome [61]. CD74 has been shown to be a regulated intramembrane proteolysis (RIP)-processed protein [62], in which dormant membrane-bound regulatory proteins and transcription factors are activated by proteolytic cleavage along the plane of the membrane allowing the cytosolic fragment to migrate to the nucleus in order to modulate transcription (Fig. 1A). This occurs within the endosome as proteolysis is catalyzed by intramembrane cleaving proteases (I-CLiPs), which are found within this compartment [62]. The cytoplasmic tail of CD74 has a dileucine motif which acts as a trafficking signal to move CD74-associated MHCII molecules out of the ER for further processing [63]. These trafficking signals are DQRDLI and EQLPML in the membrane distal and membrane proximal regions of the cytoplasmic domain respectively, with the first motif lying within a helix and the second motif lying within a turn [64,65]. The DQRDLI motif, in particular, is required for sorting the CD74-bound MHCII molecules from the Golgi network to the endocytic compartment, although both sorting motifs in combination have a greater effect than one motif alone [65]. I-CLiPs hydrolyze the peptide bond of CD74 in the plane of the cell membrane in a two step event in the endosome [62], leaving behind the

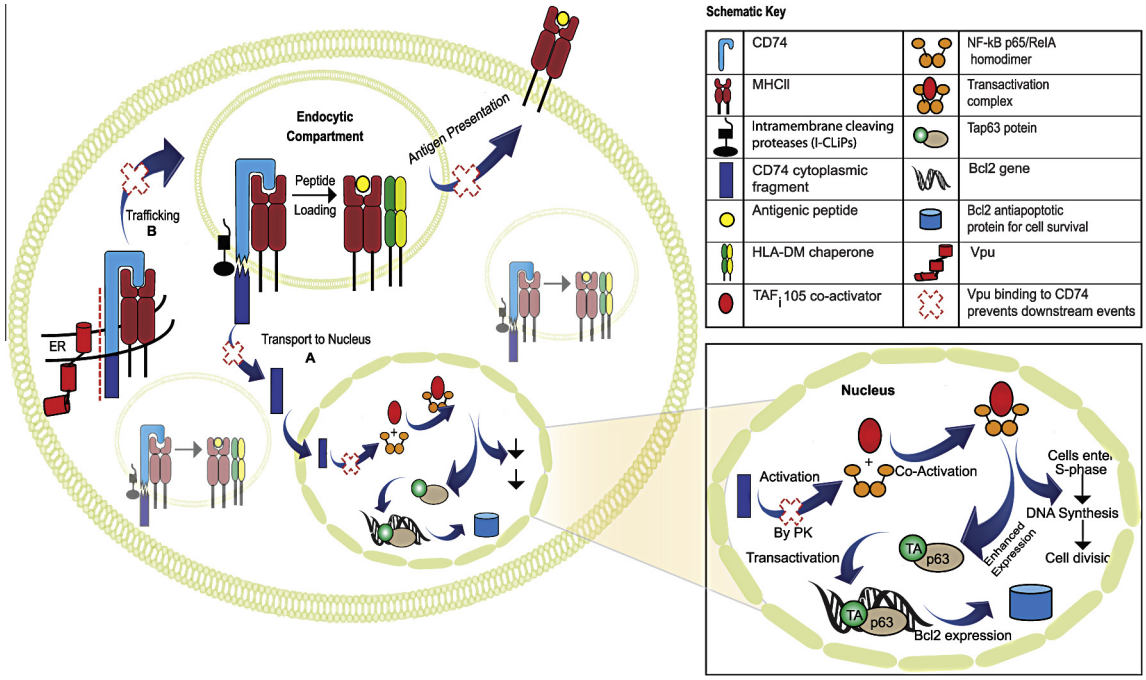


Fig. 1. Schematic of the signal cascade induced by the CD74-ICD for cell survival. CD74 forms part of the MHCII in the endoplasmic reticulum. Two dileucine-based target motifs in the cytoplasmic domain of CD74 direct the complex to the endosome, where intramembrane cleaving proteases hydrolyze the peptide bond along the plane of the cell membrane. The cytoplasmic fragment then moves to the cell nucleus, where a signal cascade is initiated through the activation of protein kinases leading to the activation of the NF-κB p65/RelA homodimer and its coactivator, TAF$_{II}$105. This leads to entry of the cell into the S-phase and increased DNA synthesis, which ultimately results in cell division and proliferation. Simultaneously, the activation of the NF-κB p65/RelA homodimer leads to the upregulation of TAp63 protein expression. TAp63 then binds to the Bcl-2 promoter thereby transactivating the *Bcl-2* gene leading to the expression of the Bcl-2 antiapoptotic protein. This results in enhanced cell survival. Cells that have been infected with HIV-1 express Vpu which then binds to the cytoplasmic domain of CD74 within the endoplasmic reticulum, thus preventing the trafficking of the MHCII to the endosome. This not only prevents antigen presentation on the cell surface, but also prevents the translocation of the CD74 cytoplasmic fragment to the nucleus. Consequently, the signal cascade affecting B cell proliferation and survival cannot be initiated. This adds to the various ways by which HIV-1 Vpu undermines the immune system.

class-II-associated Ii-chain peptide (CLIP) fragment to block the binding cleft in order to prevent the loading of self-peptides. The HLA-DM chaperone then facilitates the exchange of the CLIP fragment in the peptide binding site for a foreign peptide [56]. This allows for the formation of the mature MHCII complex that then moves to the cell surface in order to present foreign antigens to CD4+ T helper cells (Fig. 1B).

Using nuclear magnetic resonance (NMR) and aqueous solution at physiological pH, it was determined that there is an α-helix in the cytoplasmic domain of CD74 from residues glutamine to leucine (Gln4–Leu14) as well as a type I β turn from leucine to leucine (Leu14–Leu17) and two type II β turns from arginine to alanine and from alanine to serine (Arg20–Arg23 and Arg23–Ser26), when examining the Ii-p33 isoform of CD74 [64]. However the type I β turn can possibly be incorporated into the α-helix without disturbing other residues within the helix. As this helix would then contain proline (Pro15), the resulting helix is a kinked proline α-helix [64]. Crystal structures of the CLIP fragment (4AEN; 4AH2), the trimeric ectodomain (1IIE) as well as the p41 fragment (1ICF) have been obtained [66–68]; however no crystal structure of the N-terminal cytoplasmic domain has been obtained as yet. The N-terminal cytoplasmic tail of the Ii-p35 isoform also contains an arginine-based signal motif that behaves as a retention signal to keep the complex in the ER [61,69]. However, association of CD74 with MHCII molecules is thought to mask this retention signal to allow the exit of the complex from the ER. Phosphorylation of Ser6 and Ser8 residues in close proximity to the arginine-based retention motif by protein kinase C may also aid in the trafficking of molecules out of the ER. If this phosphorylation is in any way inhibited, such as by the binding of Vpu, MHCII-associated CD74 is unable to undergo processing within the endosomes, thereby repressing antigen presentation [70].

5. Human CD74 functions

One of the most well described functions of CD74 is its role in antigen presentation through association with the MHCII complex [53,55,56]. However, the expression of CD74 is separate to that of the MHCII molecules and this allows CD74 to have numerous other functions within the cells. During cleavage of CD74 within the endosome, a portion of the CD74-ICD translocates to the cell nucleus (Fig. 1A) and thereby initiates a signal cascade by activating the phosphatidylinositol 3′-kinase, Syk tyrosine kinase and Akt serine/threonine kinase, leading to the activation of the NF-κB p65/RelA homodimer and its coactivator, TATA box binding protein (TBP)-associated factor $_{II}$105 (TAF$_{II}$105) which is a subunit of the general transcription factor IID (TFIID) [71,72]. This signal cascade results in entry of the cell into the S phase and increased DNA synthesis, yielding an increase in cell division and proliferation [72]. Simultaneously TAp63 protein expression is upregulated by CD74-ICD as well as the NF-κB p65/RelA homodimer. By binding to the Bcl-2 promoter TAp63 transactivates the *Bcl-2* gene thereby leading to the expression of the Bcl-2 antiapoptotic protein. This results in enhanced survival of mature B cells due to the decrease of apoptosis [73]. Through this signal cascade the N-terminal CD74-ICD has also been shown to be involved in the differentiation of B cells from immature to mature cells within the spleen [71,74]. However, this signal cascade is eliminated by the degradation of the active CD74 protein [73,75].

Using a similar pathway, CD74-ICD also influences the proinflammatory responses by altering the expression of the monocyte chemoattractant protein-1 (MCP-1), which is involved in the early stages of atherosclerosis. In this case it was found that CD74 colocalizes with NF-κB suggesting that these two proteins may interact within atherosclerotic plaques in order to increase the expression of MCP-1, which is regulated by NF-κB [76]. Overall, an increase in CD74 leads to an increase in inflammation during atherosclerosis [76]. Another way in which CD74 plays a role in inflammation is in the overexpression of CD74 on the surface of gastric epithelial cells due to *Helicobacter pylori* infection. CD74 functions as the receptor for this bacteria and the binding of the bacteria to CD74 not only results in the upregulation of cell surface levels of CD74, but also induces NF-κB pathways that result in the production of proinflammatory cytokines [77,78]. CD74 has not only been associated with inflammatory disorders but is also implicated in gastrointestinal cancers [79].

CD74 is also involved in the mediation of signaling on the cell surface in conjunction with the macrophage inhibitory factor (MIF) thus indicating that CD74 is involved in multiple other functions within the cell [80]. MIF is a cytokine that mediates many important pathways within the cell such as the production of other cytokines and nitric oxide. MIF has also been shown to bring about the phosphorylation and therefore, activation of the p44/p42 extracellular-signal-regulated kinase family (ERK-1/2). The downstream consequence of this is the activation of effector proteins and transcription factors that are involved in inflammation, such as NF-κB. MIF interacts with the CD74 extracellular domain at the cell surface which allows MIF to bind to the cell and induce the ERK-1/2 cascade. Studies have reported that a complex comprising MIF, CD74 and CD44 may exist [81], suggesting that the signal cascade generated by CD74 requires CD44 as a second messenger protein [80]. Therefore, CD74 functions as a binding protein for MIF at the cell surface and in conjunction with CD44 it regulates MIF's functions in the cell [80].

CD74 has been implicated as a negative regulator of angiotensin II type 1 receptor (AGTR1) [63], which plays a fundamental role in regulating blood pressure and is therefore involved in hypertension and atherosclerosis [82]. AGTR1 also regulates the hormone angiotensin II and in so doing, it influences many other signal pathways that include the activation of G proteins, protein kinase C, tyrosine kinases and mitogen-activated protein kinases [82,83]. All of these functions are reliant on the C-terminal tail of AGTR1 which is expressed in the ER and was found to bind to CD74 within this compartment. This interaction causes AGTR1 aggregation in the ER and ultimately leads to the proteasomal degradation of AGTR1 [63]. This indicates that CD74 also plays a role in the regulation of these important signal cascades as well as cardiovascular disorders.

6. Interaction between Vpu and CD74

Vpu and CD74 were found to interact via their cytoplasmic domains within the ER [13], similar to that of the Vpu–CD4 interaction [7]. Specific sequences in the cytoplasmic domain of Vpu were identified to be necessary to induce the degradation of CD4 [24,84]. The CD4 binding site on Vpu overlaps with the immunodominant domain, as CD4 prevents the binding of antibodies directed against the Vpu cytoplasmic domain [7,85]. In a similar study, it was also shown that an antibody directed against a Vpu cytoplasmic peptide with residues 73–81 (HAPWDVDDL) was masked through the binding of CD4 to Vpu [86], suggesting that this sequence is part of the binding region for CD4. Further to this, mutational studies indicated that residues 28–47 (IEYRKILRQRKIDRLIDRIR) and 76–81 (WDVDDL) in the cytoplasmic domain of Vpu are needed in order to bind CD4 [87]. Hussain and coworkers first discovered the interaction between Vpu and CD74 and confirmed the binding site between these two proteins lies within both cytoplasmic domains through an *in vitro* assay. This was done by assessing the binding of Vpu to a synthetic peptide corresponding to the CD74 cytoplasmic domain [13]. It is interesting to note that the binding site on Vpu for CD74 may also overlap with the immunodominant region (Fig. 2), as it was found within our laboratory that the binding of the CD74 cytoplasmic domain to Vpu was able to prevent the binding of an antibody directed against the cytoplasmic domain of Vpu (unpublished data). Further to this, the use of overlapping peptide sets suggested that the binding site between Vpu and CD74 lies between the Vpu sequence of RIRERAEDSGNESEG and the CD74 overlapping sequences of SRSCREDQKP and EDQKPVMDDQ, both of which contain the residues EDQKP (unpublished data).

The exact primary sequences involved in the interaction may not be solely responsible for binding; the presence of secondary structures within the proteins may be equally responsible for the binding site. It has been shown that the presence of a membrane proximal α-helix within the CD4 cytoplasmic domain is needed in order to bind to Vpu as it may provide an interface for this protein interaction, and the disruption of this α-helix abrogates the degradation of CD4 through interaction with Vpu [86]. Furthermore CD4 mutants that have an altered primary sequence, but that are still able to form an α-helix, are also still capable of binding Vpu [86] indicating that structural determinants in combination with the primary sequence may be of a greater significance for binding than the primary sequence alone. The presence of α-helical structures within the cytoplasmic domain of CD74 suggests that the binding between Vpu and CD74 may also occur largely due to the α-helical content (Fig. 2).

Many of the abovementioned functions of CD74 are likely to be influenced by its interaction with Vpu, as it has already been shown that the interaction between Vpu and CD74 leads to a downregulation in MHCII surface expression [13], which is naturally followed by reduced antigen expression and ultimately decreases the activation of T cells. It is not unreasonable to assume that this binding interaction will also impede the role of CD74 in signal transduction and may have even more widespread downstream consequences than this, as CD74 is a ubiquitous protein and it is involved in many important cellular pathways. While many of the functions of CD74 may not have any influence over viral persistence, the downregulation of MHCII expression and ultimate decreased antigen presentation, is highly advantageous for the proliferation and spread of the virus.

7. Targeting of the Vpu/CD74 interaction

Vpu is able to connect viral and host cellular proteins to cellular pathways, as well as regulate these pathways through protein–protein interactions in order to promote viral replication [23], making this protein actively involved in the establishment of infective persistence within the host. As previously mentioned, HIV or SIV isolates that do not express a functional Vpu protein have less severity in terms of disease outcome [10]. Therefore targeting the interactions of this particular accessory protein of HIV-1 within the host would greatly aid in modulation of the disease. There is evidence to indicate that Vpu is a suitable drug target. A single amino acid substitution in the transmembrane domain of Vpu was found to alter the ion channel of this protein as to make it sensitive to the drug rimantadine – an ion channel blocker of the influenza A virus M2 viroporin [88]. More recently the BIT225 candidate drug was found to not only block the ion channel activity of Vpu specifically but also have anti-HIV-1 activity within macro-

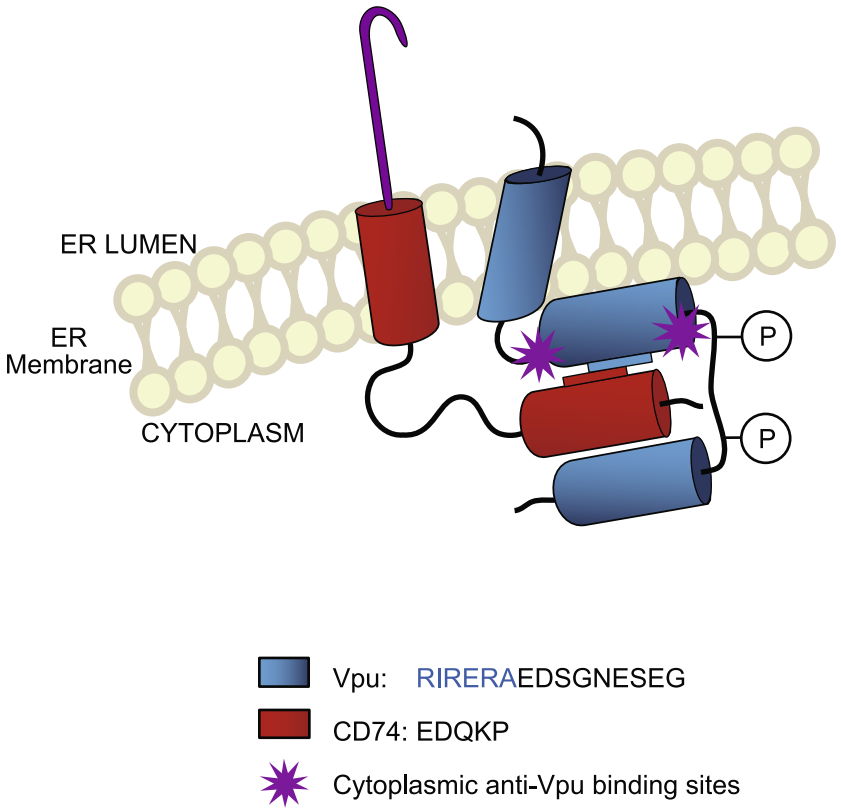


Fig. 2. Schematic indicating the prevention of the binding of anti-Vpu to the Vpu protein in the presence of CD74. HIV-1 Vpu and host CD74 interact via sequences in the cytoplasmic domains of each protein in the endoplasmic reticulum. The suggested binding site is RIRERAEDSGNESEG on Vpu and EDQKP on CD74. Both of these sequences lie within α-helices and the CD74-binding site on Vpu overlaps with that of the immunodominant domain, thus preventing the binding of the anti-Vpu antibody.

phages, with very low cell toxicity [47]. The BIT225 drug was discovered due to exploration of the amiloride analogues that were capable of inhibiting the flow of ions through the Vpu ion channel [46]. The activity of the BIT225 drug was shown to be lacking in HIV-2 indicating that the drug target is specific to HIV-1 Vpu [47] and further investigation of the BIT225 drug has shown that this compound only affects the viroporin activity of Vpu and has no effect on the Vpu–tetherin interaction [32].

As Vpu is considered to be an attractive drug target, we further propose that this protein may be targeted so as to prevent the interaction between Vpu and CD74. The binding between Vpu and CD74 has already been studied *in vitro* [13], therefore this interaction is assayable and it can be considered that the screening and evaluation of potential inhibitors of this interaction may be carried out in a similar way with few adjustments. As previously mentioned, a putative binding site between these two proteins has been found (unpublished data) which will contribute to the design of drug molecules for this interaction. However the three dimensional structure of the CD74 cytoplasmic domain is not readily available despite NMR determination of the alpha-helical content in this region [64]. Knowledge of the three dimensional structure would aid greatly in the *in silico* design of inhibitors for this protein–protein interaction. Furthermore, an animal model is required in order to investigate the efficacy of possible drug compounds to determine the modulation, if any, of the disease. The importance of the multiple functions of both Vpu and CD74 has already been highlighted in preceding sections. Therefore the inhibition of this interaction in an infected individual would not only allow for increased antigen presentation [13], but would also result in a continuation of the cell signaling functionality of CD74, most likely contributing to the increased proliferation and differentiation of B cells [71–75] for better immune functioning. Together this would decrease viral persistence as the immune system would be better equipped to deal with the infection as infected cells may be more easily targeted for lysis, ultimately leading to a better disease outcome.

8. Conclusions

As both HIV-1 Vpu and the host protein CD74 have a wide variety of functions, inhibition of the interaction between these two proteins may be highly disadvantageous for viral persistence. While this interaction is easily assayable and monitored *in vitro*, the lack of a three dimensional structure for CD74 may delay the rational design of novel inhibitors for the specific binding site between CD74 and Vpu. Despite this the Vpu–CD74 interaction is still a viable drug target as Vpu targets intracellular CD74, as opposed to cell surface CD74, and possible inhibitors would prevent the impairment of the immune system by allowing for the continuation of CD74 functionality. In this way, antigen presentation would be increased and the proliferation of B cells would continue during infection.

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Author contributions

D.L. – conceptual idea as well as writing of the review.
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