# The Role of HIV-1 Vpr in Inducing Tat and Gp120 toxicity, Decrease antigen presentation, causes neuronal apoptosis, axonal injury and in Progression of infection and diseases, as potential target for vaccine development (Part Fifth)

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**Abstract**

Vpr could cause more than five-fold up-regulation of cyclic AMP response element (CRE)-directed transcription via a mechanism that did not require Vpr-induced G2/M arrest.

Vpr may act by stabilizing interactions with CREB and its transcriptional cofactor CREB binding protein (CBP).

Vpr as a modulator of the microtubule-dependent endocytic trafficking in HIV-1–infected macrophages, leading to strong alterations in phagolysosome biogenesis; Deletion of the vpr gene reduces the virulence of HIV-1 dramatically, indicating the importance of this protein for the virus.

Thus, HIV-1 Vpr caused neuronal death through convergent pathogenic mechanisms with ensuing in vivo neurodegeneration, yielding new insights into the mechanisms by which HIV-1 injures the nervous system.

Due to the critical role of Vpr in HIV-1 pathogenicity, study of the interactions between Vpr and cellular proteins and function of vpr represents a novel mechanism in the complex strategy evolved by HIV to influence the turnover of T lymphocytes leading to either viral persistence or virus release and Spreading may help us to understand the mechanism(s) of HIV-1 pathogenicity.

In this article, I discuss the Biology of Vpr, Vpr Functions and Amino Acid Residues, Vpr's structure and nuclear function, The Vpr important role to macrophage permissiveness and HIV-1 reservoirs, Immune dysfunction by Vpr, The role of Vpr Protein in Infection, Molecular Mechanisms, and Affected by Vpr Interaction with Cellular Factors and The role of Vpr in pharmaco-therapeutic

**Key Words: HIV-1 Vpr, Transcriptional Regulation, Neuronal apoptosis, Axonal injury DNA Repair and Vaccine**

## The Table Contents

## 1. Introduction

Viral protein R (Vpr) is a 96 amino acid, 14 kDa protein that was originally isolated almost two decades ago (1),(2). Vpr molecular functions include nuclear import of viral preintegration complex (PIC), induction of G2 cell cycle arrest, modulation of T-cell apoptosis, transcriptional co-activation of viral and host genes, and regulation of nuclear factor kappa B (NF-B) activity. Macrophages play crucial functions at the interface between innate and adaptive immunity and also represent niches for intracellular pathogens. They are professional phagocytes that take up pathogens and debris through various opsonic and nonopsonic receptors (e.g., Fc receptors [FcRs] for the Fc portion of immunoglobulins; (3),(4).The molecular machineries required for fusion and fission are thought to be the same as for endosome maturation (5),(6). Human immunodeficiency virus type 1 (HIV-1) infects and kills T cells, which profoundly damages the host-specific immune response but also integrates into memory T cells and long-lived macrophages, establishing a chronic infection (7),(8). Nuclear factor of activated T cells, NFAT, is an important transcription factor in regulation of gene expression in T cells. Together with activator protein-1 (AP-1) it promotes transcription of several cellular genes involved in T cell activation, such as interleukin-2 (IL-2) (9),(10), Increased production of IL-2 is a critical step in T cell activation and also serves to stimulate the surrounding resting T cells in an autocrine fashion. Since the activation of T cells strongly correlates with the ability of HIV-1 to infect and replicate in these cells (11),(12). NFAT has a central role in regulating HIV-1 infection.(13). Vpr is another accessory protein of HIV-1. This small (14–15 kDa) viral protein is packaged into mature virions (14), and localizes mainly in the nuclei of infected cells (15). Vpr increases viral replication and is known to have several functions during different stages of HIV replication cycle (16),(17),(18),(19). Vpr has been reported to enhance the transport of the viral genome into the host cell nucleus (20), by promoting the interaction of the viral preintegration complex with the cellular nuclear import machinery (21),(22),(23). Apoptosis is a regulated mechanism of cell suicide that is essential for normal development and homeostasis in multicellular organisms and provides a defense against virus invasion and oncogenesis (24). Recent evidence suggests that most eukaryotic cells respond to viral disruption of cellular homeostasis by undergoing apoptosis (25). Significant amounts of Vpr protein and anti-Vpr antibodies can be detected in the serum of HIV-1-infected patients (26),(27). Serum Vpr has been linked to the activation of HIV-1 replication in vivo and also with the control of latency (27). Notwithstanding the significant progress achieved, available antiretroviral strategies are not capable of eradicating HIV in treated patients due to viral reservoirs within cells and tissues, emergence of resistant viruses and adverse effects associated with each antiviral drug class (28). The numerous functions of Vpr in the viral life cycle suggest that Vpr would be an attractive target for therapeutic intervention.

## 2. Biology of Vpr

The accessory protein Vpr (viral protein R) is a 14-kDa gene product that confers rapid growth advantage to viruses expressing the protein (1),(29). Vpr-positive strains grow faster and produce moderately higher levels of virus than their Vpr-negative counterparts (30). Vpr is

unique among the accessory proteins in that it is assembled in the virion most likely through interaction with the Gag polyprotein precursor p55 (31),(32). Absence or truncation of p6 (a protein, like Vpr, unique to lentiviruses, located at the distal end of the Gag precursor) prevents Vpr incorporation into the virion (33). The presence or absence of envelope and genomic RNA does not appear to affect Vpr incorporation (32),(33). Recently, Lu et al. demonstrated that at the cellular level HIV-1 Vpr is localized predominantly in the nucleus (34). The virion association of Vpr is highly indicative of its participation in early events during viral replication. Virion associated nonstructural proteins in many viral systems play pivotal enzymatic functions in early replication steps, because cellular homologs either are unavailable or are sequestered, for example, in the nucleus. It is possible that Vpr is one such protein capable of augmenting early virus-specific functions such as reverse transcription, stabilization of RNA-DNA or DNA-DNA structures, migration of the proviral DNA complex to the nucleus, or integration (31). A role for Vpr at the preintegration level is supported by a recent report that identifies Vpr as one of the redundant viral nucleophilic determinants (the other being the matrix protein p17) that ensures efficient nuclear import of the preintegration complexes in nondividing cells such as macrophages (20). In addition, experimental evidence also suggests that Vpr may function at the level of gene expression early in the infection. Vpr is localized primarily in the nucleus and demonstrates moderate trans-activating ability from both HIV long terminal repeat and heterologous promoters in in vitro assays (1). Vpr may alter cellular gene expression to foster a milieu that can both initiate and sustain efficient viral replication such as regulation of cellular activation and differentiation. It is interesting in this regard that Vpr induces differentiation of a rhabdomyosarcoma cell line (35). Such transcriptional activity, most likely mediated through cellular factors may promote basal long terminal repeat activity immediately following integration, a period during which the Tat protein is unavailable. Some evidence indicates an important role for Vpr in vivo: rhesus monkeys infected with Vpr mutants of simian immunodeficiency virus (SIV) reverted to functional ORFs in vivo (36).

## 3. Vpr Functions and Amino Acid Residues

The comparison of amino acid sequence between Vpr proteins from different HIV-1 subtypes reveals a high degree of conservation (37). Several approaches have been undertaken to determine the relationships between sequence amino acid positions and functionality of Vpr. Analysis of Vpr from cultured and natural HIV-1 variants together with site-directed mutagenesis studies have suggested specific domains and residues in the protein sequence that are associated with virus cytopathogenicity and with disease progression. The N-terminal 42 amino acids of Vpr constitute the oligomerization domain of the protein (38). This domain includes helix 1 and several residues including Q3,W18, L22, L23, K27, and F34, which have been associatedwith cytopathicity functions of Vpr (39),(40),(41), (42),(43),(44). The C-terminalmoiety (Vpr binds to ANT and can induce apoptosis (45). Two highly conserved leucine-rich domains are located within helix 1 and helix 3. The first domain is likely involved in the interactions of multimerization and, as a result, virion incorporation, while the second domain binds heterologous proteins such as DCAF1 and GR, which then become co-activated (46),(47),(48),(49). Non-conservative mutations of L64 enhance the pro-apoptotic activity of Vpr, but in a subtype-dependent manner (50). Helix 3 contains several hydrophobic amino acids including I63, L67, I70, and I74 that enable nuclear localization, cell cycle arrest, and oligomerization of the viral protein (51). The protein folds around a hydrophobic core defined by leucine, isoleucine, valine, and aromatic residues located in helix 1, 2, and 3 (52). The

mitochondrial membrane permeabilization-inducing activity of Vpr (MMP) resides within a 12-amino acid moiety (45). This moiety contains two H(F/S)RIG motifs (at 71–75 and 78–82); the conservation of these two motifs correlates with HIV pathogenicity (53). Located between the two motifs is a very well conserved cysteine residue (C76), which is critical for oligomerization and incorporation into HIV-1 virions, while the H(F/S)RIG motifs are necessary for G2 arrest and/or cell death (39),(40),(41),(42),(53),(54),(33),(55). Additionally, arginine residues R73, R77, R80, and R90 are strongly conserved, and theirmutation reduces virus replication and Vpr-induced activities such as apoptosis, LTR activation, IL-12 suppression, and cell cycle (56),(57),(58),(59),(60). The phosphorylation of S79, but not of S94, and S96, is crucial for the cell cycle arrest, although all three serines can be phosphorylated (44). Mutations at positions 3, 36, 37, 41, 55, 63, 68, 72, and 77 have been associated with variations in the disease progression or the degree of neurocognitive deficit in patients. The simultaneous presence of A55 and T63 in patient-derived Vpr sequences has been associated with lower plasma viral load and higher CD4 count compared with those that express either single or none of these residues (61). The impact on the neurocognitive function of patients has been associated with the presence of G41N and A55 (detrimental effect) or I37 and S41 (beneficial effect) (62). Mutations that have been associated with long-term non-progressor (LTNP) patients are Q3R, Q65R, F72L, and R77Q (43), (51),(56),(57),(63). The R77Q mutation is less frequent among patients with progressive disease (36%) than in LTNP patients (about 80%) and shows poor replication (56),(57),(64). Nevertheless, the use of this mutation as a marker of slow disease progression is in disagreement with the lack of correlation of this mutation with the course of disease in progressor patients that were receiving therapy (65),(66). Conversely, rapid progression of disease has been associated with R36W, L68M, and R85Y (56),(67). This moiety contains two H(F/S)RIG motifs (at 71–75 and 78–82); the conservation of these two motifs correlates with HIV pathogenicity (42). Located between the two motifs is a very well conserved cysteine residue (C76), which is critical for oligomerization and incorporation into HIV-1 virions, while the H(F/S)RIG motifs are necessary for G2 arrest and/or cell death (39),(40),(41),(42),(53),(54),(55) Additionally, marginine residues R73, R77, R80, and R90 are strongly conserved, and their mutation reduces virus replication and Vpr-induced activities such as apoptosis, LTR activation, IL-12 suppression, and cell cycle arrest (39),(40),(56),(57),(58),(59),(60). The phosphorylation of S79, but not of S94, and S96, is crucial for the cell cycle arrest, although all three serines can be phosphorylated (41). Mutations at positions 3, 36, 37, 41, 55, 63, 68, 72, and 77 have been associated with variations in the disease progression or the degree of neurocognitive deficit in patients. The simultaneous presence of A55 and T63 in patientderived Vpr sequences has been associated with lower plasma viral load and higher CD4 count compared with those that express either single or none of these residues (61). The impact on the neurocognitive function of patients has been associated with the presence of G41N and A55 (detrimental effect) or I37 and S41 (beneficial effect) (62). Mutations that have been associated with long-term non-progressor (LTNP) patients are Q3R, Q65R, F72L, and R77Q (43),(51),(57),(58), (63). The R77Q mutation is less frequent among patients with progressive disease (36%) than in LTNP patients (about 80%) and shows poor replication (56),(57),(64). These observations would support the need for caution when choosing the HIV-1 clone as a reference sequence in drug discovery studies, in order to ensure potential effectiveness of any Vpr-targeted inhibitor in developing countries (56).Thus, it is possible that several residues in the Vpr sequence and even Vpr itself, as well as other HIV-1 proteins, make a concomitant contribution to the disease progression.

## 4. Vpr's structure and nuclear localization function

Structural studies have been invaluable to understanding HIV-1 viral interaction with host cells, including nondividing macrophages. Relatively recent structural studies have identified three alpha helical domains, a-H1, a-H2, and a-H3 as well as other structural features capable of mediating diverse biological functions (52). Indeed, Vpr's structure allows for direct binding to many cellular proteins, which likely enables Vpr to mediate functions such as nuclear import and G2 arrest. All three alpha helices have been implicated in Vpr mediated nuclear localization (34),(68),(69),(70),(71),(49). while the G2 arrest property has been attributed mainly to the C-terminal region of Vpr (69), Vpr mediates nuclear localization by binding to importin- a via residues located within the alpha helices. While some studies initially reported a low affinity of Vpr for importin-a, others have found that Vpr binds to importin-a using other techniques (23),(21),(72). Vpr/ importin-a binding was shown to be non-competitive with that of the classical the NLS found on MA (22). Kamata and others demonstrated that regions 17-34 (aH1) and 46-74 (aH2+aH3) can both independently localize to the nucleus, albeit to a lower extent than an identified bona fide Vpr NLS consisting of residues 17-74 (73). Mutations in aH1, aLA (L20,22,23,26A), as well as in aH2+aH3, I60P and L69P, completely ablated the ability of the individual peptides to localize to the nucleus. Later, Kamata and others found that Vpr aH1 and aH3 both bind importin-a, that the IBB domain of importin-a primarily determines this interaction, and that the C-terminal domain of importin-a, 393-462, is necessary for nuclear localization of Vpr (74). Although, an importin-a lacking an IBB still facilitated import of Vpr, a mutation in Vpr's first alpha helix, aLA, impaired importin-a binding and nuclear localization but still showed perinuclear accumulation. In contrast, a mutation in the third alpha helix, L67P, failed to localize to both the nuclear and perinuclear areas, but still permitted binding to importin-a. Previous findings from other investigators also showed that the use of IBB peptides failed to inhibit Vpr mediated nuclear localization. Hitahara-Kasahara and others showed that importin-a1, a3, and a5 isoforms are all able to induce Vpr mediated nuclear import (75). Importin-a was shown to be essential for HIV-1 replication in macrophages, suggesting that importin-a nuclear import is a vital process in the infection of these cells. Furthermore, a recent study found that Vpr does not bind to importin-a2 or importin-a2/b1 heterodimers, suggesting that cell-line specific expression of importins may regulate Vpr's karyophilic properties (55).
In addition to the reported binding interaction with importin-a, Vpr has been demonstrated to bind directly to nuclear pore proteins (76),(77),(23),(21),(78). Vpr mutants F34I and H71R have been found to lose the ability to localize to perinuclear areas, suggesting that these residues are involved in nuclear pore interaction (23). Vpr is less than 40kDa. The F34I mutant showed lower binding to importin-a and Nsp1p, a member of the nuclear pore complex. WT Vpr colocalizes with importin-b and nuclear pores in perinuclear regions and binds both Pom 121 and very weakly to Nsp1p (76). An A30P mutant lacked these abilities. FXFG regions on nucleoporins, a form of phenylalanine- glycine (FG) repeat, have been reported to interact with cytoplasmic proteins involved in nuclear import (79),(80),(81). Vpr was reported to bind to FXFG containing proteins p54 and p58 as well as to the FXFG region of Nup1(21). Further, addition of Vpr was shown to stabilize the binding of importin-a/b to Nup1 FXFG.
In a later study, it was found that four Vpr mutants L23F, K27M, A30L, and F34I, which all occur on one face of the first alpha helix, have impaired hCG1 binding and fail to show nuclear

localization (77). Thus, it seems that Vpr is able to bind to importin-a as well as nucleoporin using the same residues on the first helix. In both cases, there is evidence that Vpr binding to nucleoporin components occurs in a way that is distinct from the classical NLS pathway.
The role of importin-b in the nuclear transport of Vpr is an aspect of the mechanism of Vpr's karyophilic properties that remains to be fully understood. Early studies showed that Vpr fails to bind importin-b (22), or that it binds at a low affinity (82). Oddly, the latter study found greater affinity of Vpr to importin-b than to -a. Subsequent studies argued that Vpr's localization is importina, but not -b, dependent. Addition of importin-b to digitonin permeabilized cells, which was required for the classical SV40-NLS localization, was unnecessary for Vpr N17C74, a construct containing the minimal region for nuclear localization (75),(73). Previous studies demonstrating that the use of IBB peptides failed to inhibit Vpr localization also lend some support to these findings (83). Further, importin-b siRNA failed to prevent N17C74 localization to the nucleus (75). Vpr has also been shown to physiologically behave in ways similar to importin-b, leading some authors to suggest that Vpr replaces the role of importin-b, which, like Vpr, also binds to both importin-a and nuclear pores, in the nuclear translocation process (23). Based on these findings Papov and others proposed that Vpr stabilizes the MA and IN NLS complex with importin-a/b to promote nuclear entry. A dominant negative form of importin-b, residues 71-876 (84), has also been shown to inhibit Vpr localization, further suggesting that importin- b plays a role in Vpr mediated nuclear targeting (83). Recent studies have clearly shown binding of Vpr to importin-b3, but not to importin-b1 or to importina2/ b1 complexes (55). The respective roles of the alpha helices and the Cterminal region in nuclear localization and G2 arrest remain controversial. Mahalingam and others put forth a hypothesis that the nuclear localization function resides primarily in the alpha helices while the G2 arrest property is determined by the carboxyl-terminus (69). Previous studies lend support to this assertion as the alpha helices, but not Nterminal or C-terminal regions were involved in nucleoporin binding by Vpr (78). Other reports found that N17C74 Vpr, which lacks the C and N terminal regions and other Vpr constructs lacking the C-terminus are unimpaired in nuclear localization (73). Although the C-terminal region closely resembles a classical NLS, this region does not have NLS function and Vpr functions independently of NLS binding (85),(86). Conversely, many other studies found that the C-terminal is necessary or sufficient for nuclear entry of Vpr (34),(83),(76),(49),(87). These studies may suggest that karyophilic and cell cycle arrest properties rely on multiple domains that may be separable to some degree.

## 5. HIV-1 long terminal repeat and Vpr functions

While Vpr promotes infection of HIV-1 into nondividing cells, the ability of Vpr to activate both viral and endogenous promoter activity likely contributes to increased viral replication and pathogenesis. Initially, it was observed that Vpr can reactivate cells latently infected with HIV-1 (27),(88). Later studies demonstrated more specifically that Vpr transactivates the HIV-1 long terminal repeat (LTR) as well as other promoters (89),(31),(90). The U3 region of the HIV-1 LTR has several activating elements, which include NF-AT, glucocorticoid response elements (GRE), NRF, NF-B, Sp1, a Tat responsive RNA element (TAR), and a TATA box (91),(91), (92),(93),(94).

Studies employing HIV-1 LTR indicator constructs demonstrated that Vpr acts via Sp1 sites (90). Vpr binds to the Sp1/promoter complex and it has been proposed that Vpr exerts its effects by stabilizing promoter complexes containing multiple bound Sp1 proteins. Other studies, however, support the notion that Vpr transactivates primarily the -278 to -176 region of the LTR, which contains the GREs, while the NF-B and Sp1 are utilized by Tat mediated transactivation (95). Vpr appears to act as a coactivator in the presence of other activating elements but not on a bare promoter alone. Vpr was shown to bind transcription factor IIB (TFIIB), suggesting that the effect of Vpr is indeed due to coactivation rather than direct transcription factor function (89), Vpr has also been demonstrated to potentiate the activation of the HIV-1 LTR by p300 (96). and was shown to form a complex with p300 and TFIIH to cooperatively induce GRE activation in a manner independent of G2 cell cycle arrest (97). Several Vpr mutants including R73S, C76S, and Q21P have also been reported to lose HIV-1 LTR transactivation abilities (58). Vpr has also been reported to act cooperatively with Tat, another LTR coactivator. Their cooperative effect was disrupted by the Vpr R73S mutation (98). Therefore, in the presence of Vpr, viral production is likely amplified via coactivation of the HIV-1 LTR by a mechanism that appears to be dependent on multiple binding sites within the viral LTR (99). Vpr was shown to induce R-interacting protein 1 (Rip-1) nuclear translocation in a GR dependent manner and along with Rip-1 form a complex with GR. A later study showed that Vpr transactivates promoters containing GREs (100). The authors also reported that Vpr L64A, a mutant for a signature GR binding motif LXXLL, was found to be defective for binding to GR and in GRE transactivation, but like WT Vpr, Vpr L64A retained the ability to bind TFIIB. A later publication confirmed many of these observations for LXXLL Vpr mutants in the first and third alpha-helices, 22-26 and 64-68 respectively (48),(101), later research has solidified the notion that GR and Vpr function synergistically. Human Vpr interacting protein (hVIP/ Mov34), which binds to both Vpr and GR, translocates to the nucleus following either dexamethasone or Vpr treatment, further suggesting that Vpr and GR form an functional complex within cells (102). Vpr and GR also have a gain of function in inhibiting poly (ADP-ribose) polymerase 1 (PARP-1) nuclear translocation, which is a necessary event in NF-B transcription (103). Tat is known to induce the HIV-1 LTR synergistically with NF-B [98], highlighting the importance of the NF-B pathway for HIV-1 replication. In summary, these studies suggest that Vpr and GR function in a cooperative manner through a mechanism that involves direct binding, and this interaction is at least partly responsible for the transctivation of the HIV-1 LTR by Vpr. The interaction of Vpr with GR and elements of the LTR transcription complex, including p300 (104),(105),(106),(107),(108),(109),(110),(111),(112). Interestingly, a recent study found that extracellular Vpr was capable of increasing IL-6 production in an NF-B and C/EBP-b dependent manner by stimulating Toll-like receptor 4 (TLR4) signaling in macrophages (113). Glucocorticoids and TNF-a have also been shown to increase HIV-1 virus production (114). Therefore, the effect of glucocorticoids on the HIV-1 promoter may be influenced by the presence or absence of pro-inflammatory signals. Increased levels of glucocorticoids have been associated with HIV-1 progression, although some reports suggest that these effects are due to immune system modulation rather than a direct effect on viral

replication (115),(116, 117). GR and progesterone receptor (PR) inhibitor, can reduce HIV-1 LTR activation by Vpr and attenuate virus production in X4 infected PBMCs as well as R5 infected macrophages (118). In contrast, glucocorticoids can increase the permissiveness to infection of unstimulated PBMCs by HIV-1 (119). These studies demonstrated that the viral life-cycle was blocked at a stage of infection before proviral integration. Interestingly, a similar block in HIV-1 replication was also shown to be abrogated by Vpr, further suggesting GR/Vpr cooperativity (120). In summary, Vpr may have varying effects on the HIV-1 LTR depending on the context of proinflamatory and anti-inflammatory signals, in addition to GR pathways.

## 6. The Vpr important role to macrophage permissiveness and HIV-1 reservoirs

Numerous studies have focused on the role of Vpr in macrophage infection and permissiveness to HIV-1. Recent findings in the field, however, suggest the likelihood that both G2 arrest and another, yet unknown, cellular process use similar machinery and that the factors involved in these Vpr functions may have significant overlap. Findings from mutational studies have suggested overlap in G2 arrest and localization of the HIV PIC to the nucleus (77),(121), (23),(69). HIV-1 transcripts in Vpr defective viruses lose the ability to be detected at some time between the reverse transcription and pro-viral DNA replication phases (120), suggesting that in the absence of Vpr the viral life cycle may be inhibited at the nuclear entry phase. The ability of IN to compensate for Vpr loss also suggests that nuclear localization plays a predominant role (85),(122).Therefore, there is ample evidence to support the notion that Vpr can induce nuclear localization independent of G2 arrest. As nuclear localization and G2 arrest seem to be related in some structural studies, it is not surprising that both properties of Vpr have been linked to productive infection of macrophages (123). Upon infecting macrophages with HIV-1 viruses that were Vpr WT, ATG-Vpr (Vpr negative), Vpr R62P (impaired in nuclear localization), and Vpr R80A (impaired in G2 arrest), the authors observed that unlike the Vpr R62P mutant, which only inhibited viral growth at low MOI, the Vpr R80A and ATG-Vpr viruses were the most impaired at higher MOI. However, R80A mutant, as expected, showed no differences as compared to the other mutants in the number of G2 stage cells in terminally differentiated macrophages, as these cells are already arrested. These results suggest that the so called G2 arrest property of Vpr is important in different ways than nuclear localization for productive viral infection in myeloid cells. It is very important to note that the G2 arrest property of Vpr has been recently attributed binding to damaged DNA binding protein 1 and Cullin 4a-associated factor- 1 (DCAF-1) (124),(125),(126),(127),(128),(129),(130), (originally identified as a binding partner called VprBP (46), and is a result of subsequent induction of ataxia telangiectasia-mutated and Rad3 related (ATR) kinase (131),(132). Macrophages are non-dividing cells and are therefore not subject to the cell-cycle arrest function of Vpr and even lack the prerequisite ATR induction in the presence of Vpr (133). The findings that demonstrate the importance of Vpr residues involved in G2 arrest in promoting HIV-1 replication likely suggest that the recruitment of native cellular factors to DCAF-1 promotes both properties. Many studies have shown that Vpr's ability to cause G2 arrest and increase viral production are linked (133),(88),(96),(134),(135). While G2 cell cycle arrest may make HIV-1 infected T-cells and oddly

macrophages, which are not dividing, more permissive to active infection, many studies have shown that Vpr constructs deficient in G2 arrest maintain the ability to function as a coactivator (69),(95),(100),(48),(136). While G2 arrest and transactivation properties of Vpr both impart positive effects on viral replication, whether these effects represent independent functions is a matter of debate. As mentioned previously, Vpr is believed to allow for permissive infection of HIV-1 in many cell types, but is considered particularly important for the infection of non-dividing cells such as macrophages and resting Tcells. As such, Vpr is likely important in generating a long lived reservoir for virus infection. Indeed, it has been suggested based on results in non-human primate studies, that macrophages are likely the main producers of virus in late stage simian/human immunodeficiency virus (SHIV) at a time when CD4+ T-cells have been depleted (137). Vpr and Vpx have discrete functions in HIV-2/SIVSM viruses causing G2 arrest and nuclear localization respectively, whereas Vpr has both properties in HIV-1(138). Recently, it was shown that SIV/HIV-2 Vpx overcomes a block to reverse transcription in macrophages, further suggesting that HIV-1 Vpr may increase viral permissiveness in myeloid cells as well (139),(140). This likely suggests that Vpx acts on cellular targets that may be only partially in common to those of Vpr. Interestingly, Vpx binds DCAF-1 in a way similar to Vpr (127), and such interaction is necessary for the permissive effects described above (131). Therefore, it is likely that the particular macrophage restriction factor antagonized by Vpx is not a target of Vpr. In agreement with this notion, previous studies have attributed Vpr to lifting a post-reverse transcriptional block, whereas Vpx seems to affect an earlier block in viral replication (120). However, Vpr may use the same system to recruit other factors that promote permissive infection of HIV-1 into macrophages. Considering that Vpr has small effects on macrophage permissiveness to HIV-1 during single a round of infection (141), but causes profound changes after long-term culture (30),(116). it is likely Vpr mediated macrophage permissiveness has not been detected as compared to Vpx simply due to the a smaller magnitude of it's effect or due to shortterm culture conditions. HIV-1 virus is known to have anti-apoptotic properties in chronically infected macrophages and microglia (142), and causes a reduction of pro-apoptotic Bax expression in mitochondria of persistently infected cells (143). While Vpr promotes apoptosis (144),(145). it also exhibits anti-apoptotic properties (146). Intriguingly, Vpr was observed to inhibit apoptosis in a lymphoblastoid cell line by inducing Bcl-2, with concomitant downregulation of Bax in a manner seemingly contingent on Vpr expression level (146). Further, Vpr mediates resistance to cell death from Fas ligand and TNF-a in these cells. The G2 arrest function of Vpr in these cells, however, is most likely defective since these clones exhibited cell cycle characteristics similar to those of control transfected cells. If Vpr promotes cell survival, it is conceivable that the pro-survival effects of HIV-1 may involve the action of Vpr, especially in macrophages, possibly in combination with additional host-viral interaction. In combination with the aforementioned abilities of Vpr to increase viral replication by inducing G2 arrest and activating the HIV-1 LTR, the potential of Vpr to promote infection of and survival of macrophages could be a highly significant factor in the development and/or maintenance of macrophage viral reservoirs. The differential mechanism of pro-apoptotic/anti-

apoptotic Vpr activity warrants further investigation and may provide an avenue of therapy as an additive to combination antiretroviral therapy (cART).

## 7. Immune dysfunction by Vpr

Vpr has profound inhibitory effects on many members of the immune system involved in adaptive response. Consequently, Vpr reduces the efficacy of DNA and SIV-Nef vaccination in vivo, suggesting that Vpr may aid in evasion of immune response during HIV-1 (147),(148). The mechanism of immune dysfunction caused by Vpr appears to involve the induction of apoptosis and cell cycle arrest in bystander T-cells, contributing to the depletion of immune cells. While Vpr is seemingly anti-apoptotic in HIV-1 infected cell lines, in vitro studies suggest that bystander T-cells may be induced to undergo apoptosis in response to extracellular or secreted Vpr (146),(149),(150). However, in vivo, Vpr alone has been shown to be contribute to HIV-1 mediated immune dysfunction by promoting depletion of thymic cells (151). Activation induced cell death by apoptosis has been proposed as a mechanism of HIV-1 infected CD4+ lymphocyte depletion, although multiple mechanisms distinct from Vpr likely contribute to this process (152),(153). Vpr can increase Fas dependent caspase- 8 dependent cleavage in T-cells to induce apoptosis, providing a potential mechanism for increased cell death. CD4 promoter-Vpr transgenic mice do show Tcell depletion in a Bcl-x, Bax, and caspase-1 dependent and Fas-Fas ligand independent manner (154). G2 arrest precedes the induction of apoptosis by Vpr and has been reported to be necessary for progression to apoptosis (155). however, the latter findings remain controversial (156). Recently, it was demonstrated that this property depends on Vpr activated phosphorylation of Chk1, an event that begins during the S phase of the cell cycle (157). Apoptosis occurs via caspase-9 and seems to cause apoptosis in cancer cell lines with mutated p53, suggesting that this effect is independent of p53 function (158),(159),(160). Vpr has also been postulated to increase the expression of TNF-a in dendritic cells (DC)s and in this way may indirectly promote apoptosis in CD8+ T-cells (161). The Vpr mediated depletion of uninfected T-cell populations likely contributes, in part, to the immune dysfunction observed in AIDS. Recent studies have identified additional mechanismsVpr mediated T-Cell depletion. Vpr has been shown to up regulate natural killer group 2, member D (NKG2D) ligands in CD4+ lymphocytes, which resulted in natural killer (NK) mediated toxicity to these cells (162),(163). Vpr could induce bystander T-cell killing due to NK mediated toxicity. It should also be mentioned, however, that Vpr has been reported to inhibit NK function (164),(165), which would be predicted to oppose NK mediated toxicity. If Evidence from many studies suggests that Vpr's effect on the immune system seems to be mediated by interaction with the NF-B pathway by a mechanism involving. Glucocorticoids have been shown to have immunosuppressive effects due to NF-B inhibition and induction of I kappa B alpha (IBa), which prevents NF-Btranslocation into the nucleus thereby preventing and immune activation (166),(167). Vpr was first shown to induce T-cell apoptosis in a TCR dependent mechanism by inducing IB and reducing NF-Bactivity (168). Vpr downregulates NF-B inducible cytokines,

including IL-2, IL-12, TNF-a, and IL-4, and chemokines,MIP-1a, MIP-1b, and RANTES (168),(169),(170).

Indeed, Vpr and GR cooperate infected T-cells are depleted due to NK function, this may suggest that the infection of these targets is outweighed by the advantage conveyed by immune suppression. Interestingly, the upregulationNKG2 ligands by Vpr is also related to DCAF-1 binding in an ATR related mechanism, which suggests that these ligands may not be readily upregulated in macrophages that are reported to lack ATR response to Vpr expression (133),(162),(163). In summary, Vpr has been reported to cause apoptosis of bystander T cells by multiple mechanisms, which may contribute to decreased immune function and possibly impaired viral clearance in the host.

Vpr may suppress cellular immunity by modulating antigen mediated activation and cytotoxic killing of surviving T-cells. In vivo, Vpr promotes a shift toward a Th2 response, likely by suppressing IFN-g, a Th1 inducing cytokine (147). Other studies have also confirmed that Vpr promotes Th2 cytokine IL-10 while suppressing the expression of Th1 cytokine IL-12 (171),(172). Recombinant Vpr has been shown to lower activation of macrophages and maturation of DCs by inhibiting the expression of key co-stimulatory molecules including CD40, CD80, CD83, and CD86 (171),(173). This suggests that Vpr may dampen antigen presentation by downregulation of partner molecules on both presenter and effector cells. Vpr has also been shown to suppress immune activation to superantigens in vivo (174). More recently, Vpr has also been shown to modulate NK cell function, causing areduction in cytolytic killing and differential regulation of IL-12 and TGF-b by Smad3 activation (165). Therefore, Vpr may significantly contribute to the immune deficiency seen in AIDS by altering both adaptive and innate immune cellular function to suppress NF-B mediated transcription (96). The cooperativity of Vpr with GR has been proposed as a cause of the hypersensitivity to glucocorticoids seen in HIV infected patients thus amplifying the GR induced immune-suppressive effect (169). Vpr's effects on the immune system seem to be carried out by several and possibly independent mechanisms. Therapeutic strategies targeting Vpr, therefore, may impair virus replication directly and at the same time serve promote functional antiviral immune responses.

## 8. The role of Vpr Protein in Infection

One of the outstanding activities performed by Vpr after HIV-1 infection of dividing cells is the blockade of the cell cycle at G2 (175),(176),(177),(178), a phase where the viral long terminal repeat (LTR) promoter is more active (135). Equally relevant is its activity in non-dividing cells, where its contribution to the nuclear import of the viral preintegration complex (PIC) is critical for virus replication in these cells (21). Other detected activities for Vpr include the regulation of apoptosis and the transcriptional modulation of immune function (179),(168),(180),(181),(38). Vpr is able to form dimers and even multimers that may determine its functions (38). While only oligomerizable Vpr incorporates into virus particles and has nuclear transport ability, non-oligomerizable variants retain some activities of the protein, such as inhibition of cellular proliferation and also bystander cell death (38),(51),(182). The multiple localization of Vpr

protein in infected cells, including inside the nucleus, in mitochondria and dispersed in the cytoplasm, could account for its diverse functions (121),(183),(184). Moreover, differences in the level of its expression might explain the timing of Vpr functionality along the viral replication cycle (185). The variety of molecular events leading to innate recognition of HIV in different target cells, low permissiveness to infection of primary cells, and defects of cell lines in the release of cytokines to the extracellular milieu are key factors to bear in mind when attempting in vitro assessment of the Vpr modulation of antiviral immune response (186).

### 8.1. The role of Vpr on Disease Progression

Vpr protein likely contributes to disease progression in HIV-1-infected patients in several ways: (1) by inhibiting the proliferation of T cells and inducing cellular differentiation (187),(188),(35); (2) by enabling productive infection of primary macrophages and reactivating virus production from latency, which contributes to virus production in the absence of CD4+ T cells and to the establishment of drug resistant reservoirs in patients early in infection (27),(189),(190),(88), (137); (3) by contributing to the bystander cell depletion in lymphoid tissues, peripheral blood, and the CNS (191),(179),(192). In this respect, soluble Vpr can activate cells in an autocrine or paracrine manner, and this activation could contribute to immune deficiency in patients (27). It has been proposed that Vpr intervention enables HIV to circumvent the innate immune sensing of viral infection and to prevent the triggering of an innate immune response (1),(193). Additionally, infected brain microvascular endothelial cells and brain resident cells might also release soluble Vpr in the CNS. Once there, extracellular Vpr might directly contribute to the HIV-associated CNS dysfunction or through bystander effects mediated by factors involved in cellular death pathways (183),(194). In macrophages, HIV-1 productive infection is low and infected-cells survive and become viral reservoirs (177),(142). It is proposed that Vpr induces anti-apoptotic pathways in infected macrophages that facilitate viral replication and long-term cell survival. Furthermore, Vpr impairs the phagocytic function of macrophages, which in turn could contribute to the establishment of opportunistic infections in HIV-infected patients (195),(196).

## 9. Molecular Mechanisms, Affected by Vpr Interaction with Cellular Factors

Several studies revealed Vpr-induced changes to protein mediators and modulators of signaling pathways related to glycolysis and other energy processes, mitochondrial activity, redox homeostasis, cell cycle, cell death, and DNA repair (197),(198),(199),(200). It is also likely that the mitochondrial dysfunction provoked by Vpr affects proteasomal activity (197),(198),(199),(200). which would further impact the regulation of transcription initiation (201). In some cases there appear to be causal links between the effects of Vpr in host cells, undoubtedly because of the variety of signaling pathways affected by viral protein. In addition to transcription and translation, the turn-over of cellular proteins regulates cellular processes. This section is intended to contextualize the significant changes in the amount/activity of some cellular proteins by Vpr.

### 9.1. Energy Pathways, Redox Homeostasis, and Cell Cycle

Molecular biological analyses in a wide variety of virus families suggest that virus production requires glycolysis during later steps in replication (202). HIV-1 infection of T cells increases glycolysis, whereas infection of macrophages suppresses glycolysis (203). This cell type-dependent adaptation of glucose metabolism agrees well with the known differences in virus

production and cell survival in both cell types. In macrophages, vpr transduction enhances the expression of glucose-6-phosphate dehydrogenase (G6PDH), a pentose phosphate pathway (PPP) enzyme that functions as a sentinel for oxidative stress, while it reduces the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a key glycolytic enzyme (198). Decreased GAPDH activity by extracellular Vpr is also observed in astrocytes. Besides GAPDH, several key mitochondrial enzymes involved in glutamate metabolism are significantly downregulated by Vpr in macrophages, among them glutamate dehydrogenase 2 (GLUD2), which may contribute to neuronal pathogenesis (198). Early studies demonstrated that drugs that replenish intracellular glutathione GSH also counteracted oxidative stress and inhibited HIV replication in models of acute and latent infection (204). Specific assays using extracellular Vpr protein demonstrated impaired levels of intracellular ATP and GSH in astrocytes (205). Furthermore, extracellular addition of ATP or GSH and its precursors was sufficient to counter growth arrest by endogenously-produced Vpr in yeasts (206). Whereas physiological concentrations of endogenous ATP downregulates proteasome activity, it is rapidly upregulated by reduced ATP (207). Thus, a Vpr-induced reduction in intracellular ATP might explain the observed decrease of endogenous cellular proteins. HIV-1 infection decreases the abundance of mitochondrial ion channels (VDAC1, VDAC2) and glutathione reductase (GSR), which respectively facilitates the survival of infected cells and protects late stages of virus production (208),(209). It should be noted that the functional interplay of peroxisomes with other subcellular organelles, such as mitochondria, is necessary for the regulation of cellular redox metabolism (210). Hypoxia inducible factor-1 alpha (HIF-1) has been proposed as the major transcription factor participating in the Vpr-mediated activation of the HIV-1 promoter (211). The activation of this transcription factor would occur once Vpr activates the oxidative stress pathway (212). Supporting this model is the finding that the switch from HIV-1 latency to reactivation in infected macrophages is promoted by a marginal increase in glutathione redox potential (EGSH) of about 25 mV (213). A moderate oxidative shift in EGSH, a consequence of GSH oxidation to glutathione disulfide (GSSG), is detected at early stages of viral replication, but as viral replication increases, higher oxidation and also depletion of GSH leads to a robust oxidative shift in EGSH (177). The mechanism by which Vpr protein activates this DNA repair response is, however, not clear since ATR responds to a broad spectrum of DNA damage (214). Alternative models to explain Vpr-mediated G2 arrest rely on the proteasome-mediated downregulation of several cellular factors. Among them are the structure-specific endonuclease regulator SLX4, histone deacetylases (HDAC), the DNA replication factor minichromosome maintenance 10 (MCM10), and also unknown factors (193),(215), (125),(126),(127).

### 9.2. Proteasomal Activity and Cell Death

The discovery of the interaction between Vpr and DCAF1 gave rise to alternative models to explain the potential depletion of cellular factors that could be required for cell cycle progression (125),(126), (127),(130),(124). DCAF1 is an element of the E3 ubiquitin ligase complex. The DCAF1-DDB1-Cul4 E3 ubiquitin ligase complex is involved in the facilitation of macrophage infection and Vpr-mediated protein degradation. Thus, the hijacking of host DCAF1-CUL4 E3 ubiquitin ligase by Vpr enables targeting of the endonuclease complex MUS81 structure specific endonuclease subunit/essential meiotic structure-specific endonuclease 1 (MUS81/EME1) for degradation via the proteasome and also the activation of SLX4 endonuclease complex that promotes G2/M arrest and escape from innate immune sensing (193),(216). In addition, Vpr-induced acceleration of DCAF1 turnover protects viral envelope

(Env) protein from lysosomal degradation and enhances virion production in macrophages (47). Indeed, Vpr and DCAF1 were found to be necessary for efficient cell-to-cell spread of HIV-1 from macrophages to CD4+ T lymphocytes (217). The Vpr-induced mitochondrial dysfunction might affect proteasomal activity and vice versa (207). Ubiquitin-proteasome system and mitochondria are involved in the cellular response to oxidative stress and intracellular variation of ATP levels. Several authors have proposed that Vpr has a dual pro-apoptotic or anti-apoptotic role on programmed cell death that is dependent on its intracellular level, the stage of the infection, and also the cell type (195),(185),(217),(218). Vpr permeabilizes mitochondrial membranes through a specific interaction with the PTPC via interaction with the adenine nucleotide translocator (ANT) that is located in the inner mitochondrial membrane (219). The subsequent swelling of the mitochondrial matrix might result in impairment of the outer mitochondrial membrane (45),(220). Vpr induces a mitochondria-dependent apoptotic pathway in T cells and primary mononuclear Cells (221). In neurons, an alternative mechanism has been proposed where the uptake of extracellular Vpr permeabilizes the plasma membrane by downregulating the plasma membrane Ca2+ ATPase (PMCA) (222). As a consequence, Vpr triggers an increase of intracellular Ca2+ levels, leading to ROS production and impairing signaling in neurons (146). In this manner, and at early stages of infection, low levels of endogenous Vpr may protect T lymphocytes from death, contributing to the virus dispersion. In addition, the alternative anti-apoptotic role that ATR plays in the mitochondria might contribute to the regulation of apoptosis by Vpr (223)

### 9.3. Transcriptional Regulation

Extracellular Vpr was capable of reactivating HIV-1 virus from latency (27),(88). Direct interaction of Vpr with the glucocorticoid receptor (GR) and/or other components of the glucocorticoid-induced transcription initiation complex would signal the transactivation of HIV LTR (100),(95). Further investigation showed that this was due to its ability to transactivate several promoters, among them the viral LTR (1),(90),(89),(224). In T cells Vpr increases the basal ubiquitination of HDAC1 and HDAC3 by 2.2- and 3.4-fold, respectively (215). In infected macrophages, Vpr induced depletion of HDAC1 on specific chromatin regions was associated with hyperacetylation of histones and consequently the activation of the viral promoter (225). Hence, Vpr may enable the virus to overcome latent infection in primary macrophages. In resting CD4+ cells, virion encapsidated Vpr activates NFAT through Ca2+ influx and the nuclear import of this transcription factor (39). Modification of the regulation of several transcription factors, such as NF-_B, AP-1, and C/EBP-delta by Vpr could be the cause of the observed Vpr–induced impairment of cytokines and GR signaling in a broad range of cell types (226),(227),(169).

### 9.4. DNA Repair Mechanisms

Curiously, the HIV-1 Vpr directs two repair enzymes, helicase-like transcription factor (HLTF) and uracil DNA glycosylase (UNG2), for proteasome-dependent degradation, while the HIV-2 Vpr targets the dNTPase SAM and HD domain containing deoxynucleoside triphosphate triphosphohydrolase 1 (SAMHD1) (228). Thus, both Vpr proteins reprogram CRL4 (DCAF1) E3 ligase to remove key enzymes involved in three DNA repair pathways, although each protein uses a different strategy to achieve this (228). HIV-1 Vpr interacts with UNG2, a nuclear DNA repair enzyme that excises uracil from DNA containing miss-incorporated deoxyuridine

triphosphate (dUTP), leading to its degradation (229),(230),(231). Concerning the HIV-1 Vpr-mediated downregulation of the DNA translocase, HLTF, a recent study demonstrated that this occurs independently of cell cycle stage (200). As far as is known, the depletion of this DNA translocase occurs in a DCAF1-dependent manner in T cells and macrophages.

## 10. Targeting Vpr's effects as an adjuvant therapy

The actions of Vpr in the virus life cycle and its role in the pathogenesis of HIV induced immune dysfunction and end-stage organ disease suggest the potential importance of Vpr as a therapeutic target for the treatment of HIV infection. Vpr/Vpx defective SIV virus has been shown to have a greatly attenuated course with no progression to AIDS in rhesus monkeys (232). In HIV- 1, Vpx is absent and Vpr is thought to carry out Vpx functions, suggesting that in humans a Vpr deletion would have similar effects. Infection of Vpr defective HIV-1 into tonsilar histocultures showed a fifty percent reduction in HIV-1 production, even though macrophages represented a small portion of total infectable cells (233). Vpr has also been shown to reduce the efficacy of DNA and SIV-Nef vaccination in vivo, suggesting that in the absence of Vpr a more effective immune response to HIV would be possible (147),(148). Interestingly, all of these mutations involved a decrease in Vpr's apoptotic effects, suggesting that the cytotoxic properties of Vpr are of key clinical importance. One of the major clinical consequences of Vpr in HIV-1 infected patients is the existence of viral reservoirs in macrophages. Nucleoside reverse transcriptase inhibitors (NRTIs) are more effective in macrophages than in CD4+ T-cells for early viral inhibition; non-NRTIs are equally effective in macrophages and in CD4+ T-cells for early infection (for review see (234). Protease inhibitors, however, require a much higher dose to effectively control HIV- 1 infection in macrophages than in CD4+ T-cells, and it is unknown if they achieve the concentrations needed to inhibit macrophage mediated HIV-1 production in compartments such as CNS or testes. While NRTIs, non-NRTIs and protease inhibitors prevent the cell to cell spread of HIV-1 infection, it is unknown how efficiently these drugs address virus produced from infected macrophages in vivo. Nanotechnology-based drug delivery systems have been proposed as one method for delivering drugs more effectively to macrophages, especially those in relatively inaccessible body compartments (235),(236). A therapeutic approach to target HIV-1 infected mononuclear cells would be to employ specific cytokines or cellular kinase inhibitors. One candidate, TNF-related apoptosis-inducing ligand (TRAIL), has been shown to cause HIV-1 infected macrophages to undergo cell death (237). Imatinib, a tyrosine kinase inhibitor that has some cross reactivity to colony stimulating factor-1 receptor (CSF- 1R), the receptor for M-CSF, restores the effect of TRAIL on infected MDM cells (237). TRAIL has been shown to act through the PI3/Akt pathway (238), and consequently other PI3/Akt inhibitors have similar effects on infected MDM cells (239). Additionally, morphine in combination with gp160 has been shown to cause apoptosis in mononuclear cells (240). In combination with cART therapy, a clinical approach to target the anti-apoptotic pathways in HIV-1 infected macrophages may yield more effective therapies. Heat shock proteins have been proposed as cellular targets of Vpr and a mechanism of antiviral response (241). HSP 27 inhibits Vpr dependent G2 arrest and cell death in T-lymphocytes when expressed exogenously, but does not seem to inhibit viral replication in macrophages (242). Another heat shock protein, HSP 70, can inhibit HIV-1 replication in a Vpr dependent manner as well as reduce G2 arrest in proliferating cells (243). As heat shock response is protective, increasing heat shock pathways could promote the survival of chronically infected cells. The anti-apoptotic effects of Vpr in HIV-1 infected cells may contribute to the persistence of viral reservoirs in vivo (244). Several

pharmacological approaches have already been suggested to target Vpr pathways. As many Vpr mediated effects depend on GR activity, RU486 has been proposed as a therapy for HIV-1 and has been shown to suppress HIV-1 replication in infected mononuclear cells and to suppress Vpr mediated downregulation of IL-12 and other cytokines (118),(245). Vpr is necessary for viral PIC entry into the nucleus of nondividing cells and therefore this property of Vpr has also been investigated as a potential avenue of therapy (246). More recently, a study has demonstrated that hematoxylin is a specific inhibitor of the Vpr/importina interaction and consequently prevented the nuclear import of the HIV PIC complex (247). In summary, many studies have proposed targeting the cellular effects of Vpr as a way of treating the consequences of Vpr function in HIV-1 infection. In combination with established cART regiments, these approaches may lower viral loads, increase immune response, and even contribute to the depletion of viral reservoirs thus improving the clinical outcome in HIV patients.

## 11. The role of Vpr in pharmacotherapeutic

Vpr is a multifunctional protein that is able to efficiently facilitate many HIV-1 functions. Importantly, Vpr can traffic into cells (88), and is incorporated into HIV particles (191),(33). Further, the Vpr peptide region from R14-88 has been used to introduce other protein products into HIV-1 particles (248). As a result, Vpr has been explored as a vector system for drug delivery by conjugation to apolipoprotein B mRNA editing enzyme, catalytic peptide 3G (Vpr14-88-Apobec3G) (249). Apobec3G has strong antiviral effects in Vif deficient viruses, but in the presence of Vif loses the ability to incorporate into virons and therefore its therapeutic efficacy (250),(251). The fusion of Vpr 14-88 to Apobec3G facilitates packaging into the HIV-1 particles and restores the ability of Apobec3G to inhibit viral replication. The discoveries of other properties of Vpr, including induction of G2 cell cycle arrest and apoptosis, have led the argument that Vpr has efficacy as an anti-cancer agent (252). Further, Vpr induction of apoptosis seems to be independent of p53 function, suggesting that mutations in p53 commonly seen in various tumor types will not prevent the potential therapeutic efficacy of Vpr (158). However, Vpr, like other chemotherapeutic agents, also possesses the ability to transform cells as double stranded breaks and aneuploidy have been reported in cell lines (253).

## 12. Conclusion

Vpr is that this small polypeptide interacts with variety of proteins and directs them toward different pathways. Vpr promotes infection of dividing as well as non-dividing cells through a variety of effects including, nuclear localization, cell cycle arrest, apoptosis, and other effects due to DCAF-1 binding, as well as transactivation of host and viral genes. Vpr mediated pathogenesis is one avenue of investigation that holds promise when combined with other therapeutic approaches. Vpr to exert so many effects through direct protein–protein interactions The development of novel targets to direct new antiretroviral drugs and broad neutralizing antibodies are necessary to combat residual problems of resistance, toxicity, and persistence of latent virus reservoirs. The development of therapeutic broad neutralizing antibodies and drugs directed towards the multifaceted target Vpr might contribute to reduce these problems. Recent advances in the characterization of its interaction with host cells points to Vpr as having a changeable behavior depending on its multimerization status, protein concentration, and cell type infected. The protection from energetic deficit and antioxidant production nullifying Vpr-induced pathogenesis represents a promising strategy in broad neutralizing antibodies and

drug discovery of Vpr inhibitors. Several cellular models and transgenic animals are available for screening this Vpr-induced inhibition. Hence, a structure-based approach for drug design and therapeutic broad neutralizing antibodies will also be very useful in the development of Vpr as a novel target for antiretroviral therapy.

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