

Review

Human immunodeficiency virus (HIV)-1 proteins and cytoskeleton: partners in viral life and host cell death

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Abstract

Cytoskeletal components play a major role in the human immunodeficiency virus-1 (HIV-1) infection. A wide variety of molecules belonging to the microfilament system, including actin filaments and actin binding proteins, as well as microtubules have a key role in regulating both cell life and death. Cell shape maintenance, cell polarity and cell movements as well as cytoplasmic trafficking of molecules determining cell fate, including apoptosis, are in fact instructed by the cytoskeleton components. HIV infection and viral particle production seem to be controlled by cytoskeleton as well. Furthermore, HIV-associated apoptosis failure can also be regulated by the actin network function. In fact, HIV protein gp120 is able to induce cytoskeleton-driven polarization, thus sensitizing T cells to CD95/Fas-mediated apoptosis. The microfilament system seems thus to be a sort of cytoplasmic supervisor of the viral particle, the host cell and the bystander cell's very fate.

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Keywords: HIV-1; cytoskeleton; apoptosis; microfilaments; microtubules**Abbreviations:** AIDS, acquired immunodeficiency syndrome; ABPs, actin-binding proteins; Cyt, cytochalasins; HIV-1, Human immunodeficiency virus 1; BDM, butanedione monoxime; ERM, ezrin, radixin, moesin; Latr, latrunculin; IP, immunoprecipitation; IL-2, interleukin 2; LTR, long terminal repeats; MTs, microtubules; MycB, mycalolide B; NAC, *N*-acetyl cysteine; PHA, phytohemagglutinin; Nocod, nocodazole; PAF, platelet-activating factor; PAK, p21-activated kinase; VEGF, vascular endothelial growth factor; Ubp, Vpu-binding protein; HIVE, HIV encephalitis

Introduction

Several lines of evidence indicate that the host cell microfilament cytoskeleton plays a key role in the different

steps of the subcellular pathway involved in human immunodeficiency virus-1 (HIV-1) infection. The microfilament system encompasses a number of molecules spread throughout the cell cytoplasm of any eucaryotic cell type, including the main targets of HIV particles, that is, CD4 lymphocytes (about 10% of total cell proteins). A milestone of this network is represented by the actin filaments together with the actin-binding proteins (ABPs) whose activity is controlled by small GTPases of the Rho family.¹ At least three main subfamilies of ABP are devoted to the actin polymerization and depolymerization state, filament cross linking, bundle formation and actin–plasma membrane interaction. In turn, the Rho family small GTPases, encompassing the superfamilies Rho, Rac and Cdc42, are active in their GTP-bound form and regulate microfilament network dynamics. Several membrane-associated processes, including the expression of certain receptors at the cell surface or the structural changes occurring in cell life and death, are also under control of the Rho proteins. For instance, stress fiber formation is associated with cell adhesion and is regulated by Rho GTPase, while surface ruffling, of great importance in macropinocytotic phenomena as well as in cell spreading and motility is instructed by the Rac-1 molecule.² Hence, as a general rule, intracellular trafficking of molecules, cell movements, cell–cell interaction pathways as well as cell–substrate adhesion pattern and cell polarization, which are crucial in infectious processes, need the continuous assembly and disassembly, reorganization and remodeling of the actin network. In a word, the ‘plasticity’ of a cell is essentially due to the microfilament system integrity and function. Also, microtubules (MTs) contribute to cellular plasticity. In fact, MTs are stiff enough to resist forces that may compress or bend the fiber. This property enables them to provide mechanical support much as steel girders support a tall office building. The distribution of cytoplasmic MTs in a cell helps to determine the shape of that cell. Furthermore, MTs and MTs-associated proteins contribute to the molecular machinery that moves material and organelles from one part of a cell to another.

Cytoskeletal components are of great importance in different steps of HIV infection, governing cell polarity, migration and death. All these processes can be profoundly modified by viral infection, leading to a scenario strictly related to the pathogenesis of the disease. For example, some microorganisms trigger their uptake into eucaryotic cells by delivering virulence proteins capable of leading to a remodeling, such as polarization, of the actin cytoskeleton. Similarly, HIV particles appear capable of directly or indirectly hijacking cytoskeletal cell component organization to their own advantage often modifying cell behavior and cell fate.

Actin Cytoskeleton and HIV Infection

Actin filaments and several ABPs have been associated with the interaction of HIV particle with the host cell. Viral entry, viral production and viral release have been studied and some of these findings will be schematically illustrated below.

HIV/host cell interaction: crucial role of the microfilament system

It has been suggested that receptor clustering via actin microfilament redistribution could facilitate virion fusion to the host cell.^{3,4} Live-cell microscopy shows how HIV-1 virion trafficking first occurs in the cortical regions of the host cell cytoplasm. These movements are independent of MTs and likely are due to an interaction of viral particles with the actin cytoskeleton of the host cell.^{5,6} In addition, the dependence on intact cytoskeleton contraction also has implications for host cell selection, beyond selective coreceptor usage. Faced with a multitude of inappropriate potential host cells, the ability to enter only cells that are viable and activated would be a major selective advantage. The idea is that cytoskeletal contraction leading to coreceptor clustering as well as an intact cell membrane could serve as an indicator of host cell fitness, which the virus probes interactively prior to entry. In fact, statins that alter microfilament system dynamic downregulating Rho activation also inhibit HIV-1 envelope fusion with target cell membranes, thus reducing cell infection.⁷ In addition, HIV mutants that are independent of cytoskeletal activation will not be pathogenic or replication competent *in vivo* unless they are also capable of activating quiescent cells upon binding.⁸

HIV-1 penetration of cortical actin meshwork: the role of Nef accessory protein

With respect to HIV/cell interaction, major findings refer to HIV accessory proteins, for example, to the role of Nef accessory protein. This is a small, myristoylated protein with different subcellular activities.⁹ The deregulation of cellular signaling pathways,¹⁰ the regulation of cell surface expression of some proteins, such as CD4,¹¹ and the modulation of the infectivity of HIV-1 virions^{12–14} have been studied in detail. It was hypothesized that Nef viral protein colocalizes with actin in T cells, forming noncovalent high-molecular-weight complexes, thus participating in viral interaction with the actin cytoskeleton in early phases of infection.¹⁵ Nef has also been reported to interact with a number of proteins and molecules associated with actin microfilament network organization. For example, Nef activates P21-activated kinase (PAK) proteins, members of the PAK family known to mediate actin cytoskeletal organization and rearrangement.^{16,17} It also activates the Rho GTPase exchange factor Vav in both T cells¹⁸ and dendritic cells.¹⁹ This is similar to the observations made on larger intracellular pathogens that utilize various mechanisms to breach the cortical actin barrier. For instance, *Salmonella* injects bacterial proteins such as SopE into host cells at the site of invasion. SopE acts as a highly active Rho GTPase exchange factor, and this exchange factor activity could facilitate the entry of the bacterium via a local

remodeling of the host cell actin cytoskeleton.²⁰ In the same vein, Nef binds and activates the cellular Rho GTPase exchange factor Vav determining a modification and a circumvention of the actin cytoskeleton barrier to infection. Based on this, it was hypothesized that Nef could allow the HIV genome to penetrate the cortical actin network, that is, the microfilament meshwork of ABPs directly below the plasma membrane. This meshwork, in fact, represents a barrier for a number of intracellular parasitic organisms including HIV. Nef seems to act by inducing local actin remodeling to facilitate the movement of the viral core. In the absence of Nef-mediated actin reorganization, the HIV core could remain trapped in the cortical actin meshwork and its cytoplasmic trafficking would be blocked, also impairing reverse transcription.^{21,22} Importantly, a specific region has also been identified within the SH3-binding domain as the region required for Nef-mediated infectivity.²³

Cytoskeleton contributes to viral assembly and production

An interaction between HIV-1 Gag precursor molecules and actin has been reported in HIV-1-infected T cells and macrophages.²⁴ In particular, actin and HIV-1 Gag proteins appeared to be colocalized in the pseudopod structures, where virus budding is concentrated.^{25,26} In fact, cytochalasin D, which inhibits actin polymerization,²⁷ modifies the intracellular distribution of HIV-1 Gag²⁴ and partially inhibits HIV-1 production.²⁸ In particular, the functional significance of the interaction between HIV-1, the nucleocapsid core protein domain and actin filaments could allow the intracellular transport of HIV-1 Gag molecules as well as virus assembly and budding. It was further hypothesized that actin molecule may serve as a structural component of HIV-1 particles. Actin has been detected in purified HIV-1 virions at approximately 10% of the molar concentration of Gag.²⁹ The interaction of the nucleocapsid domain with the actin molecule could influence 'packing density' during viral assembly.^{30,31} Furthermore, the interaction between HIV-1 Gag and actin in infected cells enhances cell motility and this ultimately promotes the dissemination of viral particles into the brain and other tissues.³² Finally, other authors also found that ezrin and moesin, together with the ABP cofilin, can be detected in pure preparations of HIV-1 virions.²⁹ It was hypothesized that the microfilament system does not merely play a passive role during viral infection but contributes to viral intracytoplasmic motility and budding.³³

The efficient exit of HIV-1 particles from cells, once correctly assembled, also requires the action of the viral-encoded protein Vpu. Vpu-binding protein (Ubp) is a cellular protein present both in nucleus and cytoplasm that interacts either with Vpu or with the major structural component of the viral Gag. In the cytoplasm, Ubp appears to be associated with MTs. Interestingly, expression of Vpu in cells resulted in redistribution of both Ubp and Gag to a location near the periphery of the cell. The effect of Vpu on both Ubp and Gag protein has implications for Vpu-mediated particle exit from cells and seems to require MTs participation.³⁴

The cytoskeleton integrity is mandatory for viral infection

Several studies have been carried out by using microfilament system or MT-perturbing agents, including latrunculins, cytochalasins, mycalolide B or, for MTs, nocodazole (Table 1). These treatments were, in different ways, able to hinder viral particle entry or trafficking in the cell cytoplasm. For example, *in vivo* fluorescence microscopy demonstrated that GFP-Vpr-labeled subviral HIV particles colocalize with MTs, move through the cytoplasm, and accumulate around the MT-organizing center. MTs are the cytoskeletal components responsible for long distance transport, whereas actin filaments are implicated in short distance motility.^{35–38} HIV motility is therefore blocked in the presence of the MT-depolymerizing drug nocodazole³⁷ (Nocod) as well as in the presence of latrunculin (Latr), causing disassembly of the actin filaments, but resumed if the drug is washed out.^{38,39} Further data obtained with wortmannin, an inhibitor of myosin light chain kinase, also support the importance of the host microfilament system for HIV nascent protein transport. In fact, the interaction of myosin II with actin filaments seems to play an essential role in the final process of HIV production, in particular in the budding process.³⁹ Finally, it was suggested that inhibition of host cell actin polymerization by cytochalasins (Cyt) as well as complete disorganization of F-actin by mycalolide B (MycB), blocks viral envelope formation, with capsid proteins appearing as aggregates in the host cell cytoplasm.³⁹

Viral spread and cell to cell transfer

Once viral particles are formed, viruses can disseminate within an infected host by two mechanisms: (i) release of cell-free virions and (ii) direct passage between infected and uninfected cell. In general, direct cell–cell transfer is more rapid and efficient than cell-free spread because it obviates

rate-limiting early steps in the virus life cycle.⁴⁰ Several studies have been performed to evaluate cell–cell spread of HIV-1 between various cell types: (i) between infected and uninfected CD4+ cells;⁴¹ (ii) between CD4+ T cells and epithelial cells;⁴² (iii) between infected macrophages and epithelial cells or CD4+ T cells⁴³ and (iv) between virus-pulsed dendritic cells and CD4+ T cells.^{44,45} Cells permissive for HIV-1 infection express CD4 and chemokine receptors (coreceptors). HIV-1 virions or HIV-1-infected cells bind these receptors via the viral surface envelope glycoprotein (Env) subunit gp120. The interaction between gp120 and chemokine receptor activates gp41, the Env transmembrane glycoprotein that fuses the Env-containing membrane with target cell membrane. Subsequently, the viral core enters the target cell cytoplasm and initiates the intracellular phase of the virus life cycle. In this context, several studies have been carried out demonstrating that the conjugate formation between effector and target cell is a prerequisite for HIV-1 transfer and that the interaction between CD4 (or CXCR4) and the envelope protein Env induces a clustering of Gag at the interface and transfer into the target cells. This transfer is regulated by microfilament function. In fact, jasplakinolide (Jasp), an actin-stabilizing drug, abolishes either Gag polarization or transfer indicating that actin is also crucial for Gag recruitment in effector cells.⁴⁶

The HIV-1-mediated fusion is considered a two-step process that involves recruitment of actin cytoskeleton elements within the target cells as well as in infected cells.³ In fact, the polarization and concentration of viral particles and virus proteins (such as Env and Gag) were detected in infected cells.⁴⁷ Polarization of viral receptors and coreceptors, as well as of actin, was also found in target cells, where it leads to the formation of a functional immunological synapse.⁴⁶ This strong molecule concentration in the cell–cell contact area is an actin-driven process.⁴⁸ Importantly, the rearrangement of actin filaments induced in target cells by the interaction with effector cells is an Env-dependent process. In

Table 1 Cytoskeleton modulating agents and HIV infection

Cytoskeleton perturbing agent	Activity	Effects on HIV infection	Ref
Jasplakinolide	Polymerizes and stabilizes actin	Block of CD4, CXCR4 and Env copolarization Block of the fusion Block of Gag transfer	Jolly <i>et al.</i> ⁴⁶
Latrunculins	Depolymerize actin	Block of conjugate formation Block HIV motility	Spector <i>et al.</i> ³⁸
Cytochalasins	Depolymerize actin	Reduction of conjugate formation, CD4-Env and actin polarization, Gag redistribution and HIV production Inhibition of Tat-induced JNK activation on endothelial cells	Rey <i>et al.</i> , ²⁴ Sasaki <i>et al.</i> ²⁸
Mycalolide B	Inhibits actin polymerization and actin-activated myosin Mg ²⁺ -ATPase activity	Block of the virus production via inhibition of viral protein transport process	Sasaki <i>et al.</i> ³⁹
Wortmannin BDM	Inhibits myosin light chain kinase activity Inhibits myosin ATPase	Reduction of CD4 and Env polarization Reduction of CD4 and actin polarization Block of CXCR4 recruitment	Sasaki <i>et al.</i> ³⁹ Mitchison and Cram ⁵⁰
Statins Nocodazole	Downregulate activation of Rho GTPase Depolymerizes microtubule network	Reduction of envelope fusion with target cells Block HIV motility into cell cytoplasm Sensitization to Tat-induced apoptosis	del Real <i>et al.</i> ⁷ Bukrinskaya <i>et al.</i> ³⁷ Chen <i>et al.</i> ⁶⁴
Taxol	Stabilizes microtubules	Sensitization to Tat-induced apoptosis	Chen <i>et al.</i> ⁶⁴

particular, Env-dependent recruitment of CD4 and coreceptors to the interface in target cells is actin dependent and probably involves myosin motor proteins.⁴⁶ The key role played by actin/myosin cytoskeleton was confirmed by using different actin-perturbing agents. Treatment of target cell with Cyt or Latr, which depolymerize actin³⁸ significantly reduces formation of immunological synapses and CD4-Env polarization. In contrast, Jasp, which polymerizes and stabilizes actin⁴⁹ has no effect on conjugate formation but completely blocks CD4, CXCR4 and Env copolarization to the interface. Thus, the inhibition of actin remodeling is sufficient to prevent receptor recruitment. The direct involvement of myosin in actin filament movements and in receptor polarization has also been demonstrated by using butanedione monoxime (BDM), an inhibitor of myosin ATPase.⁵⁰ In the same vein, wortmannin (Wort), an inhibitor of myosin light chain kinase activity,⁵¹ blocks myosin motor function by significantly reducing CD4 and Env polarization. This is a compelling argument that myosin is also essential for immunological synapse formation.

As described above, it has been shown that HIV-1 associates with actin during its intracellular maturation and assembly, thus favoring virion budding to the sites of cell-to-cell contact. In this condition, an aberrant polarization of virion-producing cells leading to a cytoskeletal-driven unidirectional budding was observed.^{24,28} A direct consequence of the interaction between the cytoskeleton and HIV-1 virions is that some adhesion molecules, such as CD54, and HIV-1 colocalize during cell-to-cell contact. This may result in the formation of the so-called viral 'synapse'⁵² with consequent viral entry in target cells, but also in profound cytopathic events, such as cell-to-cell fusion.⁵³ The generation of

syncytia, leading to the well-known cytopathic effect of HIV-1, can result in immune cell death.⁵⁴ Together, these findings point to the cortical boundary line of actin-associated cytoskeleton as regulator of cell polarity in terms of cell infection, viral particle spreading and, as further described below, in cell death. Main cytoskeletal components involved in HIV-1 infection process are shown in Figure 1.

Cytoskeleton in AIDS-associated disease

Tat, a small *trans*-acting regulatory protein essential for viral replication with the primary role in regulating transcription from the HIV-1 long terminal repeats (LTR), has been shown to impinge upon many cellular functions. Importantly, some of these are consistent with the fact that Tat can be secreted by HIV-1-infected cells and can act upon the uninfected cells through its protein transduction domain.⁵⁵ Tat protein may interact with endothelial cells by vascular endothelial growth factor (VEGF). Some integrins, for example, $\alpha 5 \beta 1$ and $\alpha V \beta 3$, or chemokine receptors CCR2 and CCR3 may act as additional Tat receptors. In this regard, Tat protein represents a good candidate as vasculopathic factor. It seems in fact to play a role in the vascular endothelium disorders associated with HIV-1 infections, which contribute to the spectrum of AIDS-associated disease. In particular, chaotic endothelial cell morphology was observed in the most of the HIV-1-infected patients⁵⁶ and the alterations observed in coronary intima speak for a disorganized angiogenic behavior.⁵⁷ Several studies also indicate that Tat molecule has a relevant role in the pathogenesis of Kaposi's sarcoma, a hemoangiosarcoma associated with HIV-1 infection. The mechanisms underlying these effects seem to involve Tat activity on

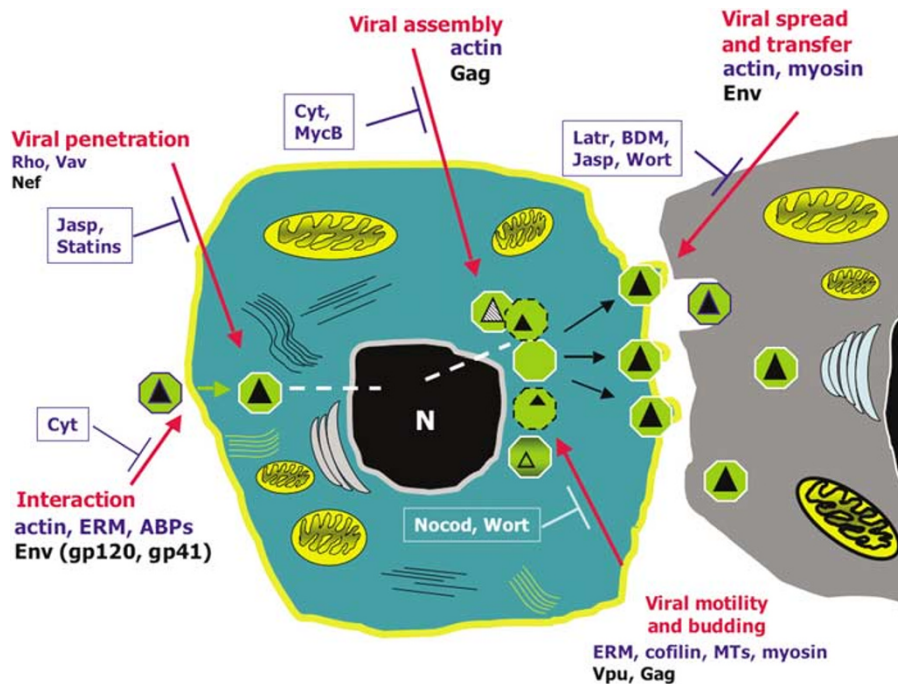


Figure 1 Schematic draw summarizing the principal steps of virus/cell interaction: viral entry, penetration, assembly and particle release. In cyan, the main cytoskeleton components participating to host cell/virus interaction; in white, cytoskeleton-perturbing agents able to interfere with viral infection, and, in green, the main viral proteins involved in HIV–host cell interaction

endothelial cell actin microfilament dynamics. Tat is able to induce a dramatic actin cytoskeleton rearrangement and remodeling leading to membrane ruffling. This results in endothelial cell migration and matrix invasion *in vitro* and angiogenesis *in vivo*.^{58,59} In particular, *in vitro* studies have demonstrated that Tat molecule did not induce apoptosis in endothelial cells⁶⁰ but, conversely, it increased cell motility by stimulating platelet-activating factor (PAF) synthesis. PAF acts by inducing alterations of actin filament organization that also lead, in turn, to a modification in the expression of cell surface molecules of importance in cell/extracellular matrix interaction, that is, integrins.⁶¹ Tat seems also to exert its effects on microfilament cytoskeleton through JNK, PAK1 and downstream activation of the NADPH oxidase.⁶² The upstream JNK activation seems to be mediated by intracellular oxidant products since it is significantly prevented by oxidant scavengers such as *N*-acetyl cysteine (NAC). Importantly, JNK activation by Tat is also suppressed by microfilament destabilizer cytochalasin B. This strongly suggests that an intact microfilament system is required for Tat-initiated signaling in endothelial cells.

The neurodegenerative process in HIV encephalitis (HIVE) is associated with extensive damage to the dendritic and synaptic structure that often leads to cognitive impairment. Several mechanisms might be at play, including release of neurotoxins, oxidative stress and decreased activity of neurotrophic factors. Furthermore, downregulated genes included neuronal molecules involved in synaptic plasticity and transmission (e.g. ion channels, synapsin II), signaling molecules (e.g. PI3K), transcription factors and cytoskeletal components (e.g. tubulin, adducin-2) may contribute to neurodegeneration. In fact, HIV-1 products released from infected cells can act on uninfected cells, such as neurons, regulating cytoskeletal molecules such as microfilaments and their associated proteins important in maintaining synaptodendritic functioning and integrity.⁶³

HIV, Cytoskeleton and Cell Death

Infection with HIV-1 is associated with a progressive decrease in CD4⁺ T-cell number and a consequent impairment in host immune defense. Since the virus predominantly infects CD4⁺ lymphocytes, it was suggested that HIV directly kills the infected cells, and/or that the anti-HIV immune response destroys them. However, apoptosis of bystander cells was also detected and different mechanisms have been taken into account.

Tat targets MTs and induces apoptosis

Extracellular Tat, secreted by the HIV-infected cells, has been shown to induce apoptosis in primary CD4⁺ cells and in T lymphoblastoid cells, contributing in part to the progressive loss of T cells that is associated with AIDS. Tat induces apoptosis by perturbing MT dynamics. In fact, the tubulin-binding domain, a four-amino-acid segment, seems to be required for its activity. Like other MT-damaging drugs, such as the stabilizing drug taxol, Tat may exert its proapoptotic effects by stabilizing MTs.⁶⁴ Furthermore, the glutamine-rich

region of Tat was hypothesized to be involved in the Tat-mediated apoptosis of T cells.⁶⁵ The death signal initiated by MT perturbation is transduced by Bim, a proapoptotic Bcl-2 relative, leading to apoptosis through the mitochondrial pathway. In particular, Tat induces release of the long form of Bim, normally sequestered to MTs, which binds and neutralizes antiapoptotic activity of Bcl-2.⁶⁴

Microfilament system and apoptosis: role of cortical microfilament meshwork in T-cell death

The actin cytoskeleton seems to be required for viral infection process and viral particle production, but little detail has been learned about the role of microfilament system in apoptosis of both infected and bystander cells or in cell pathology associated with HIV-induced cytoskeleton damage. A substantial loss of actin filaments arranged into stress fibers and vimentin intermediate filament clumping and collapsing around the nucleus followed by rounding up and detachment from the substratum were previously described in infection of adhering cell types, for example, fibroblasts and epithelial cells.^{66–68} Although microfilament cytoskeleton appears poorly developed in T cells, recent studies also focused on certain aspects of the changes occurring in actin cytoskeletal components in T-cell injury and death.

After activation, T lymphocytes become motile cells, switching from a spherical to a polarized shape and rapidly orienting their components towards one pole of the cell. The ability to polarize is under control of cytoskeletal elements, including the membrane/cytoskeleton interactions. In particular, a major role in this process is played by a microfilament system remodeling that results in a polarized T-cell.^{69,70} Thanks to these events, a reorganized cytoplasmic protrusion is formed: the uropod (Figure 2). This sort of structural 'device', an 'antenna-like' protrusion or keraiosome⁷⁰ could allow T cells to 'favor' a series of subcellular events including those leading to cell death. The formation of this multifaceted structure seems to be due to a rearrangement of microfilament system meshwork underlying the plasma membrane, that is, to the activity of a subset of ABP, the ERM proteins (ezrin, radixin, moesin), which exert their function in the cortical cytoplasmic region. Ezrin, radixin and moesin belong to a family of proteins involved in the linking of transmembrane proteins to the actin cytoskeleton^{71,72} and have been detected in virions.²⁹ ERM proteins expose their binding sites to both actin and membrane proteins when in an opened/activated form, that is, through their phosphorylation.^{73,74} The state of activation/differentiation and/or polarization of human lymphocytes is a crucial factor in the CD95-induced apoptosis.^{75,76}

Gp120-induced cytoskeleton-mediated T-cell polarization

Similarly to Tat, gp120, the glycoprotein forming the viral envelope known to be involved in the viral binding to the CD4 receptor, has been found in the peripheral blood serum of HIV-infected patients. Gp120 has also been shown to be involved in the increased sensitivity of human lymphocytes to

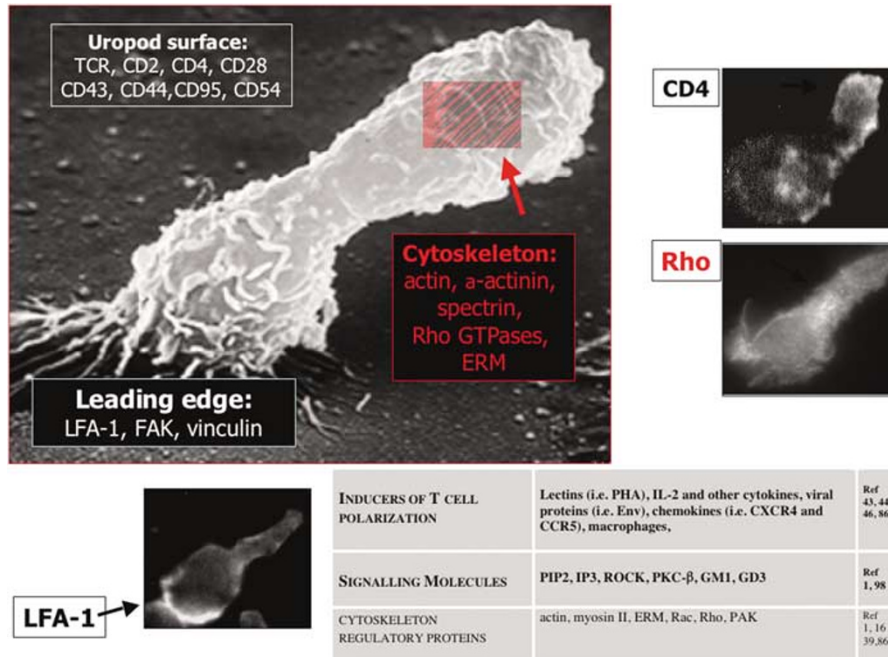


Figure 2 T-cell polarization events. A scanning electron microscopy micrograph of a T cell forming a leading edge and a well evident uropod. The inset indicates the actin cytoskeleton beneath the cell uropod. Immunofluorescence microscopy shows the polarization of CD4 antigen on the surface of the uropod, the arrangement of Rho molecule in the uropod cytoplasm and LFA-1 integrin expression at the leading edge. Methods are reported in Parlato *et al.*⁸⁶ Yellow box indicates agents able to induce T-cell polarization, molecules involved in uropod-associated cell signaling and the cytoskeletal elements known to modulate uropod formation

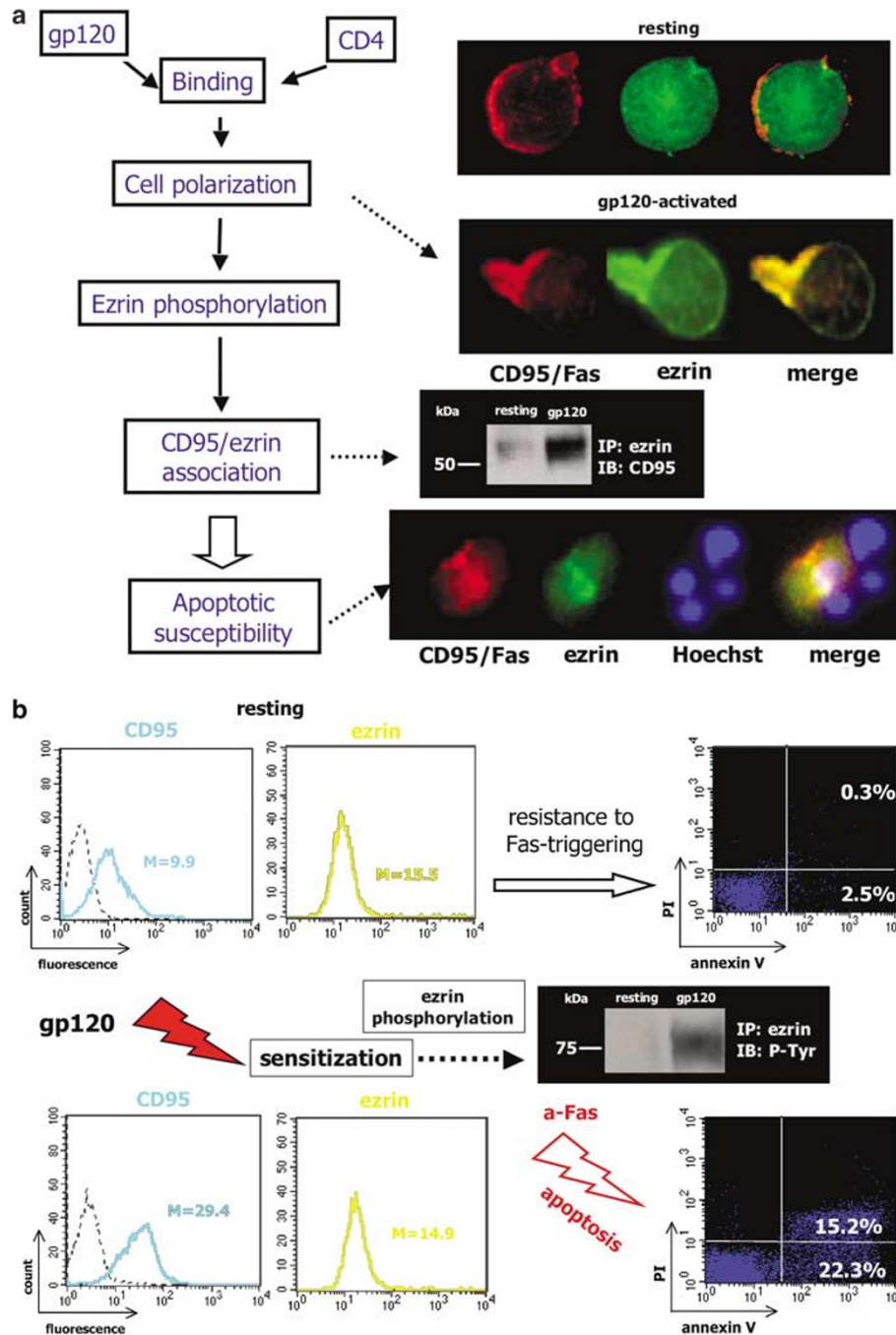
CD95-mediated apoptosis.^{77–79} CD95 (APO-1/Fas) is a member of the tumor-necrosis factor receptor family involved in both the physiological control of cell proliferation and in the pathogenesis of several human pathologies including viral diseases.^{80–85} Connection to actin is of crucial importance in predisposing T lymphocytes to CD95-mediated apoptosis^{86,87} as well as in allowing the early steps of CD95 signaling.⁸⁸ Specifically, CD95 polarization at the uropod mediated by ezrin has a role in rendering human CD4⁺ T lymphocytes susceptible to Fas-mediated apoptosis.⁸⁸ Following CD4 crosslinking in human T lymphocytes, a rapid ezrin tyrosine phosphorylation^{89–91} does occur, and cells become susceptible to CD95-mediated apoptosis. Importantly, *in vitro* treatment with HIV-1 gp120 can induce: (i) lymphocyte polarization with uropod formation; (ii) CD95/ezrin colocalization on lymphocyte uropods and (iii) a stable ezrin phosphorylation, with the presence of an 80 kDa tyrosine-phosphorylated protein corresponding to ezrin in CD95 immunoprecipitates (Figure 3a).⁹² All these events determine a high susceptibility to CD95-mediated apoptosis (Figure 3b). Thus, gp120/gp41 complexes, shed from both infected cells and HIV-1 virions, may interact with receptor molecules expressed also on uninfected cells and contribute in this way to the depletion of both infected and bystander CD4⁺ cells in acquired immunodeficiency syndrome (AIDA).⁹³

Further important changes occurring in the cortical regions of T-cell cytoplasm are related to lipid raft-associated glycosphingolipids. These are carbohydrate-bearing lipid components of biological membranes that have been implicated as mediators of cell adhesion as well as modulators of signal transduction.⁹⁴ In human lymphocytes, monosialoganglioside

GM3 is the major ganglioside constituent⁹⁵ and disialoganglioside GD3 is also well expressed, although to a lesser degree.⁹⁶ A significant increase in GD3 content in HIV-infected lymphocytes can be detected.⁹⁷ A GD3 overexpression strictly associated with the early events leading to apoptosis was also observed in activated lymphocytes.⁹⁸ This increase was accompanied by an intracellular redistribution of GD3 molecules in the uropods⁹⁸ and its association with ezrin molecule.⁹⁷ Altogether these results seem to depict a scenario in which modulation of T-cell apoptosis in HIV infection can also occur via gp120-mediated resting T-cell changes.⁹⁹ These bolster Fas-mediated T-cell apoptosis via actin meshwork-associated changes involving the ezrin molecule as well as gangliosides.

Vpr acts on actin microfilament system

The HIV accessory protein vpr is a small basic protein of approximately 96 amino acids that is highly conserved in HIV-1, HIV-2 and simian immunodeficiency virus (SIV). Vpr is the only accessory protein packaged into viral particles in significant amounts, through an interaction with the p6 domain of the p55 Gag protein.^{100–102} The incorporation of vpr into viral progeny is highly suggestive of its participation in early events of viral infection. In this regard, functions ascribed to vpr include transport of the viral core into the nucleus of nondividing cells and upregulation of viral gene expression was reported to determine host cell fate. It has been shown that vpr induces alterations of cellular morphogenesis in fission yeast that can be attributed to a change of cell growth polarity and disruption of cytoskeletal structures.¹⁰³



Moreover, it has been shown that vpr can regulate cell cycle progression on human T cells by inducing cell cycle arrest in G2 and apoptosis as a function of its expression level.^{104–107} Some results clearly associate the subcellular effects of vpr with actin cytoskeleton integrity and function. For instance, vpr expression in Jurkat cells results in remarkable modification of cell-to-cell and cell-to-substrate^{108,109} interactions by specifically modifying the actin cytoskeleton, including cortical meshwork, and the expression of certain adhesion molecules (i.e. cadherin and integrins $\alpha 5$ and $\alpha 6$) at the cell surface.¹⁰⁹ In turn, integrins mediate cell migration and cell adhesion, and regulate cell proliferation and apoptosis. Hence, vpr protein could act, via cytoskeleton, as a viral modulator of cell behavior and cell fate.

In conclusion, it has become apparent that numerous viruses interact with cytoskeletal components at various stages throughout their life cycle deranging or remodeling cytoskeletal networks to their own advantage. Although insufficient literature data regarding the role of cytoskeletal components in HIV–host cell interaction, it seems to be conceivable that viral manipulations of the actin cytoskeleton could represent a clearcut feature of the HIV-infective process. However, the microfilament system plays no mere passive role. In fact, microfilament-driven cell polarization could represent host cell fitness prerequisite with a key role in cell infection and HIV particle production and release. In turn, the occurrence of actin filaments and actin meshwork changes leading to cell polarity seem to be a requisite for bolstering apoptosis of HIV-infected and uninfected cells. Actin cytoskeleton may therefore act as supervisor of both viral and cellular survival by instructing and hijacking their fate.

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