Cell Host & Microbe
Review

Immune Evasion and Counteraction of Restriction Factors by HIV-1 and Other Primate Lentiviruses

Frank Kirchhoff[1,*]
[1]Institute of Molecular Virology, University Hospital Ulm, Meyerhofstrasse 1, 89081 Ulm, Germany
*Correspondence: frank.kirchhoff@uni-ulm.de
DOI 10.1016/j.chom.2010.06.004

Retroviruses have evolved effective strategies to evade the host immune response, such as high variability and latent infection. In addition, primate lentiviruses, such as HIV-1, have acquired several "accessory" genes that antagonize antiviral host restriction factors and facilitate viral immune evasion, thereby allowing continuous and efficient viral replication despite apparently strong innate and acquired immune responses. Here, I summarize some of our current knowledge on the acquisition and function of the viral *vif*, *vpr*, *vpu*, and *nef* genes, with a particular focus on the evolution and specific properties of pandemic HIV-1 strains that may contribute to their efficient spread and high virulence.

Introduction

Since their introduction into the human population by a single transmission event from a chimpanzee infected with a simian immunodefiency virus (SIVcpz) to a human early in the last century (Korber et al., 2000; Worobey et al., 2008), pandemic HIV-1 group M (major) strains have infected about 60 million people. Despite this rapid spread, the efficiency of sexual virus transmission that accounts for more than 80% of all HIV-1 infections is usually surprisingly poor (~1 per 1000 sexual contacts) and most often results from a single "founder" virus (Keele et al., 2008). It is not well understood why most genital exposures to HIV-1 do not lead to a spreading infection. Possible explanations are protective effects of the mucosal layer, limited target cell availability, or elimination of HIV-1 by innate immunity factors (reviewed in Haase, 2010). Once an infection is established, however, HIV-1 spreads very rapidly and eliminates most memory CCR5+CD4+ helper T cells in lymphoid tissues within a few weeks (reviewed in Brenchley and Douek, 2008). Although apparently vigorous innate and virus-specific adaptive immune responses are induced, HIV-1 is capable of replicating continuously and efficiently in the infected host. One reason for this lack of immunological control is that the virus has evolved multiple properties to evade or counteract the host defense mechanisms (Table 1). Some features helping HIV-1 to evade the immune system are (1) Hiding/latency: the retroviral genome is integrated into that of its host cell. Many HIV-1-infected cells are minimally activated or dormant and cannot be recognized and eliminated by the immune system as long as they do not express viral antigens. Some latently infected cells survive for many years, thus precluding virus eradication. (2) Variation/escape: the reverse transcriptase makes about one error per 10,000 nucleotides, and HIV-1 is highly recombinogenic. Because billions of viruses are produced each day and the viral generation time is short, the potential for variation is enormous. Thus, HIV-1 evolves in fast motion, resulting in the rapid selection of viral quasi-species that have a growth advantage because they are not recognized by the antibodies or cytotoxic T lymphocytes (CTLs) in the respective host. (3) Camouflage: most antibodies react very poorly with the native oligomeric envelope proteins present on HIV-1 particles. Reasons for this are that about half of the mass of the Env glycoprotein consists of variable carbohydrates and that conserved functional domains are masked by variable loops and only transiently exposed during viral entry (reviewed in Johnson and Desrosiers, 2002). Thus, broadly neutralizing antibodies are extremely rare. (4) Cell tropism: helper CD4+ T cells sense viral infections by T cell receptor (TCR)-mediated interactions with foreign peptides on MHC and release cytokines and chemokines to promote antibody and CTL responses. However, activated CD4+ T cells are also the ideal viral targets. Usually, the virus infects and eliminates most of them during acute infection and leaves the infected host with little CD4 help. Only a small minority of virally infected individuals maintain strong HIV-specific CD4 proliferative capacity and can achieve long-term immunological control of viral replication. (5) Cell-to-cell spread: HIV-1 can spread directly from infected to uninfected cells via virological synapses. This may protect the virus sterically and kinetically from immune effector mechanisms (reviewed in Sattentau, 2008).

Another reason for the lack of immunological control of HIV-1 is that the adaptive immune response takes time to develop and can only become effective in response to an infection that is already ongoing. Thus, by the time CTLs emerge, the virus has already spread efficiently in the host, damaged the immune system, and established latent reservoirs. The innate immune response occurs more rapidly but is also largely dependent on the response to ongoing viral replication, e.g., virus-triggered signaling, to induce the interferon (IFN) response. However, in addition to conventional innate and acquired immune responses, humans and other mammals have also evolved specific antiviral factors. These "intrinsic immunity" or "host restriction" factors are constitutively expressed in some cell types and are induced by interferons as part of the innate immune response in others. They have the advantage that they do not have to "learn" to combat viruses but are already active at the first virus-cell interaction (reviewed in Malim and Emerman, 2008; Neil and Bieniasz, 2009). Three major classes of retroviral restriction factors have been identified. APOBEC3G (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G) was the first host gene identified as an inhibitor of HIV-1 infection (Sheehy et al., 2002). APOBEC3G is a cytidine deaminase that suppresses reverse

Table 1. Host Defenses and Mechanisms of Primate Lentiviral Evasion or Antagonism

Immune Response	Host Defense	Antiviral Effect	Viral Evasion or Antagonistic Mechanism	Viral Factor(s) or Properties
Innate	NK cells	lysis of infected cells	selective downmodulation of HLA-A and -B, but not HLA-C and -E	Nef
Intrinsic	ABOBEC 3G	lethal hypermutations	polyubiquitination and degradation	Vif
	TRIM5α	untimely uncoating	variation in capsid	high variability
	tetherin	blocks virion release	sequestration from the site of virion budding	Vpu, Nef, Env
Acquired	cytotoxic CD8+ T cells	lysis of infected cells, inhibitory cytokines	MHC-I downmodulation, escape mutations, latent infection	Nef, high variability
	CD4+ helper T cells	helper function to promote antibody and CTL responses	destruction by infection or bystander apoptosis; downmodulation of CD4, CD3, CD28, and CXCR4	Nef, Vpu, viral cytopathicity
	B cells, antibodies	neutralization	antigenic variation, glycosylation, shielding of functional epitopes, inhibition of IgG2, and IgA switching	high variability, N-linked glycosylation sites, Env structure, Nef
	antigen-presenting cells	viral antigen presentation, helper T cell activation	upmodulation of Ii surface expression	Nef

transcription and introduces G-to-A substitutions in the HIV-1 genome, which are detrimental to viral replication. TRIM5α (tripartite motif 5-α) proteins were originally discovered as important determinants of the resistance of monkey cells to HIV-1 infection (Stremlau et al., 2004). TRIM5α binds incoming viral capsids in the cytoplasm and seems to mediate rapid uncoating. The most recently identified restriction factor, tetherin (BST-2, CD317, or HM1.24), inhibits viral release by "tethering" mature virions to the cell surface (Neil et al., 2008; Van Damme et al., 2008). However, it is obvious from the high levels of viremia during acute infection that this front line of antiviral defense fails to protect humans against the spread of HIV-1. The reason for this is that HIV-1 and other primate lentiviruses have evolved specific "tools" to antagonize these host restriction factors. These viral factors were named "accessory" because they are not absolutely required for viral replication in cell lines. However, they allow HIV-1 to replicate continuously at high levels in the presence of strong innate, intrinsic, and adaptive virus-specific immune responses and thus play important roles for viral persistence, pathogenesis, and transmission in vivo. The mechanisms underlying the function of the HIV-1 accessory Vif, Vpr, Vpu, and Nef proteins have been the topic of several recent in-depth reviews (Ariën and Verhasselt, 2008; Chiu and Greene, 2009; Kirchhoff et al., 2008; Malim and Emerman, 2008; Malim, 2009; Neil and Bieniasz, 2009; Planelles and Benichou, 2009). Here, I describe the acquisition of these factors, summarize (some of) their functions, highlight specific features of pandemic HIV-1 M strains, and mention future questions and challenges. The main objective is to give an impression of the multitudes of strategies evolved by primate lentiviruses to evade or counteract the host defenses.

Acquisition of Accessory Genes by HIV-1

HIV-1 belongs to the genus of lentiviruses. These viruses are characterized by their ability to infect nondividing cells and to cause diseases with long incubation periods. Lentiviruses have been divided into five groups, each restricted to a single mammalian family, i.e., ovines-caprines, bovines, felines, equines, and primates. Until recently, only exogenous lentiviruses were known, and their evolutionary analysis suggested that they emerged relatively recently. However, though the extremely high rates of evolution of infectious primate lentiviruses facilitate the reconstruction of recent evolutionary events, such as the emergence of HIV-1, they preclude the reliable analysis of their distant evolutionary history and origin. The recent discovery and analysis of distinct endogenous lentiviruses has provided important information on the origin and evolution of current lentiviruses and indicates a considerably more ancient origin than previously anticipated (Gifford et al., 2008; Katzourakis et al., 2007; Keckesova et al., 2009). For example, the presence of an endogenous lentivirus in a lemur from Madagascar (named gray mouse lemur prosimian immunodeficiency virus, pSIVgml) suggests that lentiviruses have been infecting primates for more than 10 million years (Gifford et al., 2008).

In addition to the *gag*, *pol*, and *env* genes, which encode structural and enzymatic proteins and are present in the genomes of all retroviruses, the most primitive known lentivirus (i.e., rabbit endogenous lentivirus type K, RELIK) contains just the *tat* and *rev* genes encoding essential regulatory proteins (Figure 1). The discovery of a RELIK ortholog in the genome of European hares revealed that these lagomorph lentiviruses are at least 12 million years old (Keckesova et al., 2009). In comparison, the genomes of present day primate lentiviruses, found in about 40 African nonhuman primate species (reviewed in Hahn et al., 2000; Pandrea et al., 2008), contain at least three additional genes (*vif*, *vpr*, and *nef*). Vif is also present in the genomes of the ovine-caprine, bovine, and feline (but not in the equine) groups of lentiviruses and was thus acquired early during lentiviral evolution. In contrast, *vpr* and *nef* genes are characteristic for

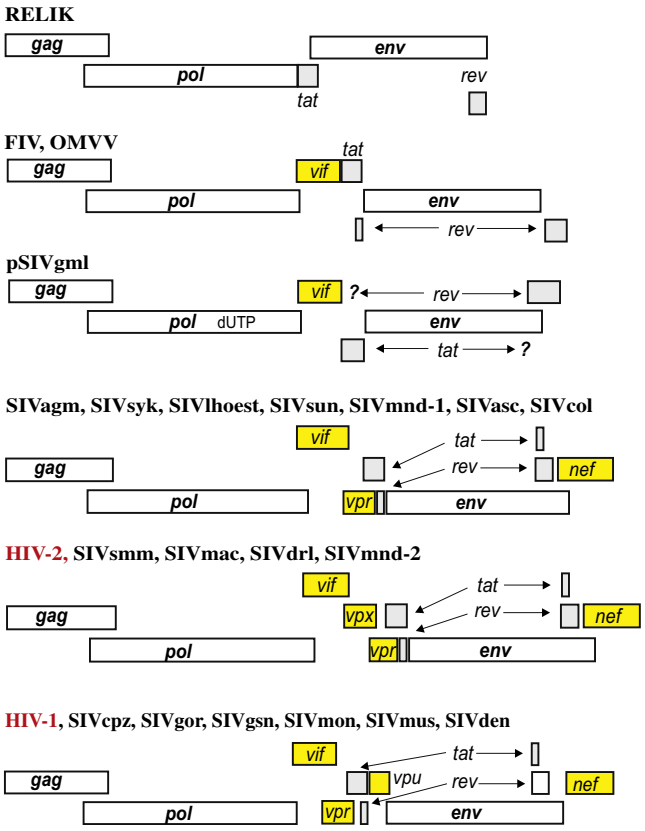

Figure 1. Genomic Organization of Lentiviruses

The organization of the consensus endogenous RELIK and pSIVgml proviral genomes is shown in comparison to that of other infectious lentiviruses. RELIK, rabbit endogenous lentivirus type K; FIV, feline immunodeficiency virus; OMVV, ovine maedi-visna virus; pSIVgml, gray mouse lemur prosimian immunodeficiency virus. Question marks indicate the possible presence of short, spliced Rev and Tat exons.

primate lentiviruses. Notably, the "prosimian" lentivirus pSIVgml that evaded the host genome several million years ago contains a dUTPase, which is otherwise only found in nonprimate lentiviruses (Gifford et al., 2008). In addition to *vif*, *vpr*, and *nef*, SIVs infecting the *Papionini* tribe of monkeys (mangabeys, drills, and mandrills) and HIV-2 contain a *vpx* gene. *Vpx* may have arisen from a duplication of the *vpr* open reading frame (Tristem et al., 1990) or by the acquisition of a heterologous *vpr* gene (Sharp et al., 1996). Finally, another gene (*vpu*) was most likely acquired by a common ancestor of SIVs nowadays found in *Cercopithecus* monkeys (Bailes et al., 2003). To our current knowledge, this *vpu* containing SIV was transmitted to chimpanzees and formed a hybrid with the ancestor of an SIV found in red-capped mangabeys (*Cercocebus torquatus*) (Bailes et al., 2003). Chimpanzees prey on these monkeys, and their ranges in west and central Africa overlap. Most likely, one chimpanzee became coinfected by both simian viruses. They recombined, and the *vpu*-containing hybrid virus (SIVcpz) then spread in chimpanzees and was later transmitted to humans and gorillas to become HIV-1 and SIVgor. Thus, Vpu distinguishes HIV-1 and its SIV counterparts in chimpanzees, gorillas, and some *Cercopithecus* monkeys from most other primate lentiviruses that do not encode a homolog to Vpu.

Accessory HIV-1 Proteins Antagonizing Host Restriction Factors

Compared to the most primitive retroviruses, HIV-1 is equipped with six additional genes. Two of them encode essential regulatory proteins (Tat and Rev) and four small accessory factors (Vif, Vpr, Vpu, and Nef) that are dispensable for viral replication in some cell types. Accumulating evidence suggests that the main function of three of the latter, i.e., Vif, Vpu, and (most likely) Vpr, is to counteract intracellular proteins that humans and other mammals have evolved primarily or exclusively as a defense against viral pathogens (reviewed in Malim and Emerman, 2008). The necessity of such antiviral factors and the enormity of encounters with invading retroviruses over millions of years are evident from the fact that about 8% of our genome consists of the defective remnants of once infectious retroviruses (reviewed in Bannert and Kurth, 2004). This is a larger proportion of our genetic material than that encoding proteins. Thus, retroviruses have not only left their imprints, but have also driven our evolution and shaped our genetic repertoire. Similarly to highly active antiretroviral therapy (HAART), antiviral "host restriction" factors interfere with different stages of the viral life cycle: APOBEC3G induces lethal hypermutations of the retroviral genome; TRIM5α proteins restrict the incoming retroviral capsid; and tetherin inhibits the release of viral particles (reviewed in Huthoff and Towers, 2008; Malim and Emerman, 2008; Neil and Bieniasz, 2009) (Figure 2). Its high variability allows HIV-1 to evade the adaptive immune response. In comparison, it is more difficult for the virus to avoid factors targeting viral components in a less-specific manner because it cannot easily become resistant by escape mutations. TRIM5α proteins inhibit viruses with limited homology and thus seem to target viral capsids with a relatively relaxed specificity. However, escape by capsid mutations is clearly possible, as retroviruses from a given species are not restricted by the TRIM5α variants found in the same species (reviewed in Song, 2009). Of interest, binding of the HIV-1 capsid protein to cyclophilin A increases its susceptibility to simian TRIM5α and may thus reduce its potential for cross-species transmission (Keckesova et al., 2006). Incorporation of APOBEC3G proteins into budding virions and inhibition of virus release by tetherin are both relatively unspecific processes (reviewed in Bieniasz, 2004; Malim and Emerman, 2008). Thus, HIV-1 is unable to avoid them by escape mutations but has taken the highly demanding step of acquiring new tools to antagonize these intrinsic immunity factors. Of note, the functional characterization of the viral antagonists was frequently instrumental in identifying the host restriction factors. As outlined in a recent review (Malim and Emerman, 2008), Vif, Vpu, and Vpr all seem to target antiviral factors for ubiquitin-dependent proteasomal degradation to render the intracellular environment more conducive to viral replication. In comparison, the multifunctional HIV-1 Nef protein interacts with numerous cellular factors and evolved to facilitate viral immune evasion from adaptive immune responses and to directly promote viral spread.

Virion infectivity factor (Vif) is a basic protein of 23 kDa that is essential for viral replication in primary T cells and in vivo (reviewed in Malim, 2009; Chiu and Greene, 2009). Its cellular target, APOBEC3G, was initially discovered by the Malim laboratory by comparing the mRNA expression profiles of cells that do or do not support efficient replication of *vif*-defective HIV-1

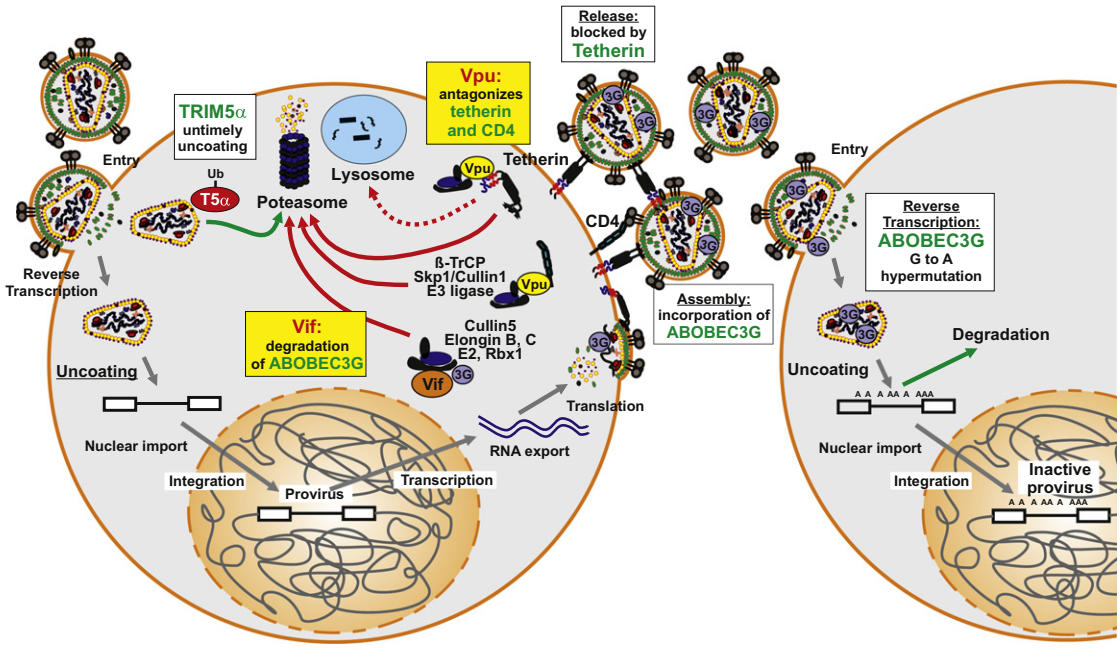

Figure 2. Host Restriction Factors and Their Viral Antagonists

As schematically indicated, TRIM5α (T5α) interacts with incoming HIV-1 capsids and may induce accelerated uncoating by proteasomal degradation. Vif binds to a cullin 5-based ubiquitin ligase complex and to APOBEC3G (3G) to induce the degradation of the restriction factor in proteasomes. Without Vif, APOBEC3G is incorporated into budding virions and causes lethal G-to-A hypermutations of the retroviral genome in the next round of infection. Tetherin inhibits the release of mature viral particles from the cell surface and is antagonized by the Vpu protein. The exact mechanism remains to be defined but most likely involves direct interaction and β-TrCP2-dependent degradation of tetherin, leading to its sequestration from budding virions.

(Sheehy et al., 2002). In the absence of Vif, APOBEC3G is incorporated into HIV-1 virions (Sheehy et al., 2003; Stopak et al., 2003; Mariani et al., 2003), inhibits viral DNA synthesis during reverse transcription (Holmes et al., 2007; Miyagi et al., 2007; Bishop et al., 2008), and catalyzes deamination of cytidine to uridine during negative-strand DNA synthesis (Conticello et al., 2005). These changes lead to the degradation of the viral DNA and/or become fixed as guanosin-to-adenosin transitions in the proviral sequences (known as G-to-A hypermutations). APOBEC3G is particularly effective in inactivating the virus because it preferentially targets GG dinucleotides, thereby changing TGG (W) to TAA (stop) codons. Such mutations seem to be responsible for the inactivation of some ancient retroviral sequences (Esnault et al., 2005; Jern et al., 2007). Vif serves as an adaptor molecule to link a cullin 5-based E3 ubiquitin ligase complex to APOBEC3G and induces its polyubiquitination and subsequent proteasomal degradation, thereby preventing its packaging into budding virions (Figure 2). It has been reported that ABOBEC3G exists in an enzymatically active low-molecular mass form that restricts HIV-1 in quiescent T cells but is recruited into high-molecular mass RNA-protein complexes upon T cell activation (Chiu et al., 2005). Of interest, the latter also contain Alu/hY retroelements and may reduce their transposition (reviewed in Chiu and Greene, 2009). Some recent studies, however, did not confirm the role of low-molecular mass ABOBEC3G complexes in the HIV-1-restrictive phenotype of quiescent T cells (Kamata et al., 2009; Santoni de Sio and Trono, 2009). Thus, further investigation is required to fully understand the regulation and antiviral properties of APOBEC3G. Of note, APOBEC3G is only one of seven related cytidine deaminases found in humans. Vif also mediates the degradation of APOBEC3F, which is another potent inhibitor of HIV-1 (Mehle et al., 2004; Yu et al., 2003). Furthermore, the expression of truncated or misfolded viral proteins due to APOBEC3G editing enhances the recognition of HIV-1-infected T cells by CTLs and thus links the innate and adaptive immune response (Casartelli et al., 2010a). Low levels of APOBEC3G-mediated mutations, however, may actually be beneficial for the virus because they may help HIV-1 to diversify rapidly and to escape from adaptive immunity (Simon et al., 2005; Sadler et al., 2010).

HIV-1 viral protein R (Vpr) is a virion-associated factor of about 14 kDa. Multiple activities of Vpr, including activation of proviral transcription, cell-cycle arrest in the G2 phase, induction of cell death, and enhancement of the fidelity of reverse transcription, have been reported (reviewed in Malim and Emerman, 2008; Planelles and Benichou, 2009). Of note, an intact *vpr* gene is not required for efficient viral replication and progression to AIDS in the SIVmac/macaque model (Gibbs et al., 1995), although its conservation between all primate lentiviruses suggests a relevant role in natural SIV and recent HIV infections. The ability of Vpr to cause a G2 cell-cycle arrest is preserved between HIV and SIV (Fletcher et al., 1996). Several recent studies suggest that it involves the interaction of Vpr with the cullin 4A-DDB1 complex via DCAF-1 (initially named VprBP) (reviewed in Malim and Emerman, 2008; Planelles and Benichou, 2009). It is currently unclear whether Vpr increases the activity of the cullin 4A-DDB1-DCAF-1 complex for its normal substrates or allows it to recruit a novel one for ubiquitination and degradation. In either case, the characterization of this substrate will be highly important for our understanding of Vpr function. It may also help

to clarify whether G2 arrest is beneficial for the virus because it increases HIV-1 transcription (Goh et al., 1998) or whether it is just a side effect of the destruction of a factor playing a dual role in cell-cycle progression and host-mediated viral restriction. Of note, HIV-1 Vpr also facilitates infection of macrophages (Balliet et al., 1994; Connor et al., 1995). In the HIV-2/SIVsmm lineage that also contains a *vpx* gene, the two major functions of Vpr are segregated: Vpr induces cell-cycle arrest, and Vpx facilitates infection of macrophages (albeit with much higher efficiency than HIV-1 Vpr) (Goujon et al., 2007; Sharova et al., 2008). Importantly, Vpx also binds DCAF-1 to interact with the cullin 4A-DDB1 complex, and this interaction seems critical for its ability to promote macrophage infection (Le Rouzic et al., 2007; Srivastava et al., 2008). Thus, it is tempting to speculate that Vpx (and Vpr) may target an as yet unknown restriction factor expressed in macrophages for polyubiquitination and degradation.

HIV-1 viral protein U (Vpu) is a 16 kDa integral membrane protein produced together with Env during the late stage of the viral life cycle. Vpu has two main functions. First, it interacts with newly synthesized CD4 in the endoplasmatic reticulum and recruits a ubiquitin ligase complex to its cytoplasmic tail to mediate polyubiquitinylation and proteasomal degradation (Bour et al., 1995; Willey et al., 1992). CD4 is the primary receptor of all primate lentiviruses. Thus, its degradation may facilitate virus release, avert superinfection, and enhance the incorporation of functional Env proteins into progeny virions by preventing the formation of gp120/CD4 complexes in virally infected cells. Second, Vpu is required for efficient viral particle release in some cell types but only after type I interferon treatment in others (Strebel et al., 1989; Göttlinger et al., 1993). Microarray screening for membrane-associated proteins constitutively expressed in cells in which Vpu is required for efficient virion release and induced by IFN-α treatment in those in which it is dispensable allowed the identification of BST-2 as the antiviral factor antagonized by Vpu (Neil et al., 2008). An independent study (Van Damme et al., 2008) also identified this long-sought "tethering" factor by following up on a previous report showing that BST-2 is downmodulated from the plasma membrane by the Kaposi's sarcoma associated herpes virus (KSHV) protein K5, a viral ubiquitin ligase, and by Vpu (Bartee et al., 2006). BST-2, or "tetherin" as it is now commonly called, is a type II single-pass transmembrane protein with a cytoplasmic N-terminal region, followed by a transmembrane (TM) domain, a coiled-coil extracellular domain, and a C-terminal glycophosphatidylinositol (GPI) anchor (Kupzig et al., 2003). It also contains three cysteine residues mediating homodimerization. Since its discovery by the Bieniasz and Guatelli labs, there has been a flurry of papers on tetherin and its viral antagonists, and this has been the topic of several recent reviews (Malim and Emerman, 2008; Neil and Bieniasz, 2009; Ruiz et al., 2010; Sauter et al., 2010). In brief, tetherin dimers seem to directly tether nascent virions to the surface of the producer cells with one membrane anchor sticking in the virion and the other in the cell membrane (Figure 2). In agreement with this straightforward mechanism, the cytoplasmic tail and the GPI anchor are both critical for its antiviral activity (Neil et al., 2008). Furthermore, an artificial "tetherin" composed of entirely different sequences but with comparable topology inhibits virus release (Perez-Caballero et al., 2009). Recent data suggest that its conformational flexibility may help tetherin to maintain its anchoring in both the cellular and viral membranes during virion budding (Hinz et al., 2010). Usually, tetherin is not expressed at high levels in primary CD4+ T cells. However, its expression is strongly induced by type I interferons (Neil et al., 2007). Vpu interacts with the TM domain of tetherin in a highly specific manner and targets the restriction factor to the *trans*-Golgi network or to early endosomes for proteasomal and/or lysosomal degradation by a β-TrCP-dependent mechanism (Douglas et al., 2009; Goffinet et al., 2009; Gupta et al., 2009; Mangeat et al., 2009; McNatt et al., 2009). Vpu reduces the levels of tetherin expression at the cell surface (Van Damme et al., 2008; Mitchell et al., 2009; Douglas et al., 2009). However, downmodulation and/or degradation of tetherin may not always be required for the capability of Vpu to promote virion release (Dubé et al., 2010; Goffinet et al., 2010; Miyagi et al., 2009; Neil et al., 2008). Thus, though it is clear that Vpu keeps tetherin away from the sites of virion budding, the exact mechanism of tetherin antagonism needs further study.

The finding that HIV-1 developed an effective tetherin antagonist clearly supports a relevant role of this restriction factor in vivo. However, HIV-1 O strains can cause AIDS, although their Vpu and Nef proteins are poor tetherin antagonists (Sauter et al., 2009). Early data suggested that Vpu may not be required for effective cell-to-cell spread (Gummuluru et al., 2000). Thus, ineffective tetherin antagonism could just shift the spread of HIV-1 to cell-to-cell transmission. However, a recent study suggests that tetherin also restricts cell-to-cell spread of the virus, albeit with limited efficiency (Casartelli et al., 2010b). Furthermore, HIV-1 replication is hypersensitive to IFN-α (which induces tetherin) in the absence of Vpu (Neil et al., 2007), and intact *vpu* genes are required for effective replication in ex vivo-infected human lymphoid tissues (Schindler et al., 2010). Thus, the importance of tetherin antagonism for viral replication both in vitro and in vivo remains to be clarified.

All-Rounder Nef: Manipulation and Evasion of Adaptive Immunity

The negative factor (Nef) of HIV-1 is a myristoylated protein of about 27 kDa associated with cytoplasmic membranes and abundantly expressed early during the viral life cycle. Its name is misleading because intact *nef* genes are critical for the maintenance of high virus loads and accelerate disease progression in HIV-1-infected human individuals and in SIVmac-infected rhesus macaques (Kestler et al., 1991; Deacon et al., 1995; Kirchhoff et al., 1995). Nef performs a striking number of activities and induces complex changes in cellular trafficking, gene and receptor surface expression, antigen presentation, and signal transduction (reviewed in Ariën and Verhasselt, 2008; Kirchhoff et al., 2008). Furthermore, Nef may also affect the survival and function of bystander cells (Lenassi et al., 2010) and deregulate the communication between T cells and antigen-presenting cells (Arhel et al., 2009; Thoulouze et al., 2006). Thus, most likely, multiple Nef activities cooperate to delay the elimination of HIV-1-infected cells by the immune system to turn them into more effective producers of fully infectious virions and to render the cellular environment more conducive for viral spread.

In HIV-1-infected T cells, Nef downmodulates CD4, MHC-I, and (less efficiently) CD28 and CXCR4 (CXCL12) from the

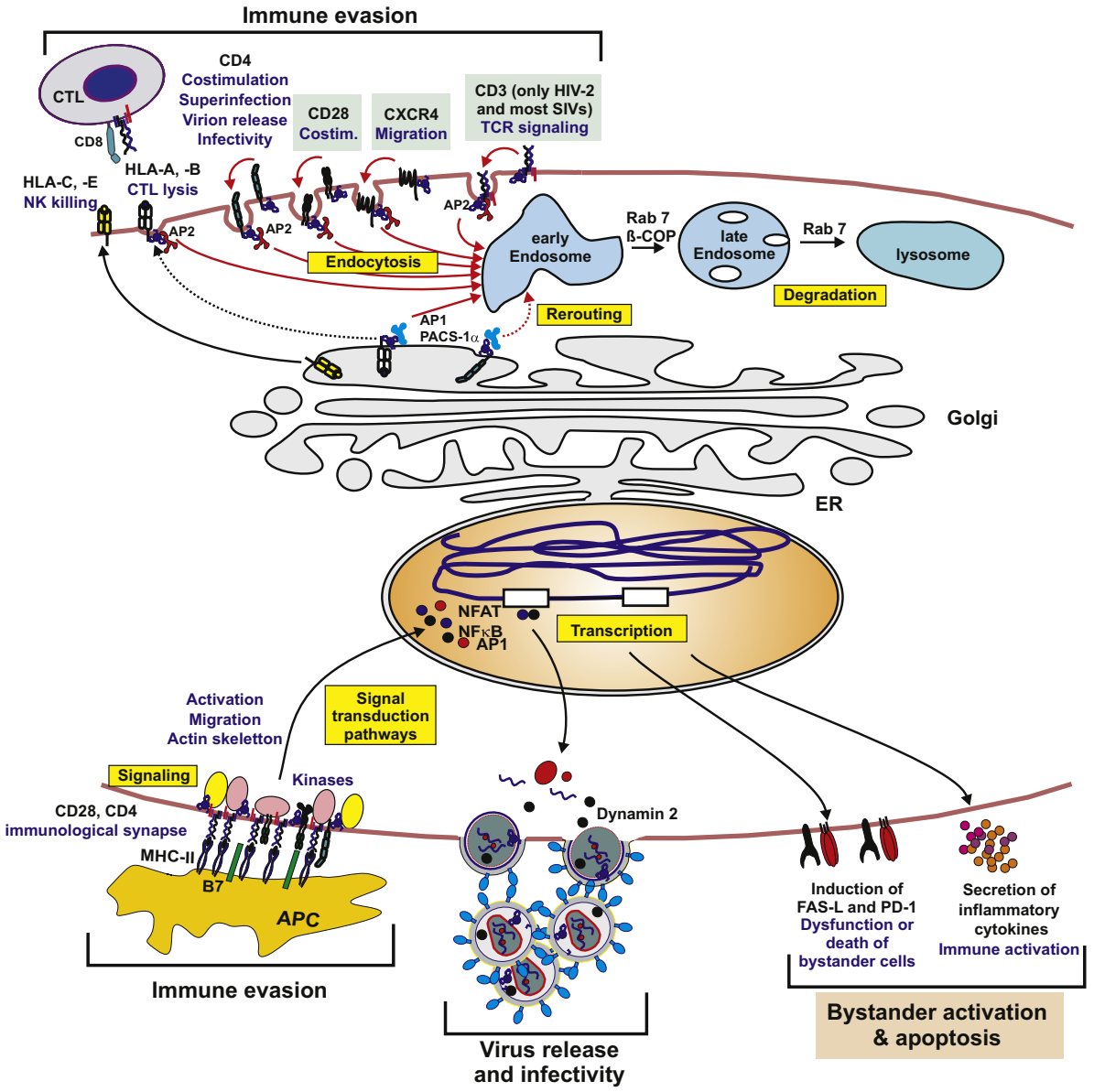

Figure 3. Schematic Presentation of Selected Nef Functions

Nef downmodulates HLA molecules, CD4, CD28, CXCR4, and CD3 from the surface of infected CD4+ T cells by recruiting them to the endocytic machinery via interactions with adaptor protein 2 (AP2) complexes or by rerouting them to endosomes. These Nef functions reduce CTL lysis, suppress cell migration, facilitate virus release, and modulate signal transduction by the immunological synapse. Furthermore, Nef interacts with cellular kinases and induces downstream signaling events to modulate T cell activation; to induce rearrangements of the actin skeleton; to activate NF-AT, NF-κB, and AP-1; and to induce the efficient transcription of the viral LTR promoter, as well as of cellular genes, including those encoding inflammatory cytokines, activation markers, and death receptors. Nef also directly enhances the infectivity of progeny virions. As indicated by green boxes, SIVagm and SIVsmm Nef alleles are more effective than those of HIV-1 in modulating CD28 and CXCR4 and, in addition, also downregulate CD3. Thus, in contrast to HIV-1 Nefs, they prevent the formation of the immune synapse and not just deregulate it. As indicated by the orange box, cells expressing HIV-1 Nefs express higher levels of death receptors and inflammatory cytokines than those expressing SIVsmm or SIVagm Nef proteins. Modified from Arhel and Kirchhoff, 2009.

surface by recruiting these receptors to the endocytic machinery or by rerouting them to lysosomes for degradation (reviewed in Roeth and Collins, 2006; Arhel and Kirchhoff, 2009). These Nef functions protect virally infected T cells against CTL lysis, reduce their migration in response to the chemokine SDF-1 (CXCL12), prevent superinfection, and may facilitate the release of fully infectious virions (Figure 3). Of note, Nef selectively downmodulates HLA-A and –B, but not HLA-C or –E, alleles from the cell surface (Cohen et al., 1999) to balance escape from CTL lysis with protection from attack by natural killer cells. Furthermore, Nef interacts with various cellular kinases and modulates signal transduction pathways to manipulate the responsiveness of virally infected T cells to TCR-mediated stimulation. As a consequence, the HIV-1 Nef protein promotes the induction of cellular transcription factors, such as NF-AT, NF-κB, and AP-1, that elevate the transcription of the viral LTR promoter and thus viral

replication (Fenard et al., 2005; Fortin et al., 2004; Manninen et al., 2001). Nef also affects MHC-II antigen presentation by efficient upmodulation of the Invariant chain (Ii or CD74) at the cell surface (Stumptner-Cuvelette et al., 2001). Ii usually caps the MHC-II peptide-binding site during its transport to endosomal compartments to prevent premature peptide loading. Upmodulation of immature MHC-II-Ii complexes at the cell surface perturbs MHC-II-restricted antigen presentation (Roche et al., 1992). Thus, this Nef function may contribute to the impaired helper T cell responses observed in AIDS patients. Although Nef is commonly considered an early viral gene product, it also acts during the late stage of the virus life cycle. For example, Nef enhances the infectivity of progeny virions by a poorly defined mechanism that involves the interaction of Nef with the GTPase Dynamin-2, an essential regulator of clathrin-mediated endocytosis (Pizzato et al., 2007). Furthermore, as discussed below, some SIVs use their Nef proteins to antagonize tetherin (Jia et al., 2009; Sauter et al., 2009; Zhang et al., 2009).

Accumulating evidence suggests that Nef may not only manipulate HIV-1-infected host cells, but also cause significant changes in its cellular environment. For example, Nef may induce the secretion of factors from HIV-1-infected macrophages that attract T cells and render them more susceptible to HIV-1 infection (Swingler et al., 1999, 2003). Furthermore, it has been proposed that the HIV-1 Nef protein itself is secreted and may trigger apoptosis in bystander CD4+ T cells (James et al., 2004; Lenassi et al., 2010). Finally, it has been reported that Nef induces the formation of long-range actin-propelled conduits in infected macrophages to mediate its own transfer to neighboring B cells (Xu et al., 2009). B cells containing Nef showed impaired IgG2 and IgA class switching, suggesting that HIV-1 may exploit Nef to manipulate the antibody response and to evade humoral immunity (Qiao et al., 2006).

The relevance of most Nef activities for viral replication and pathogenesis in vivo is far from clear and has been discussed in greater depth in recent reviews (Foster and Garcia, 2008; Kirchhoff et al., 2008). It has become clear that different Nef functions require distinct elements and are often genetically separable. In some cases, this allowed the specific elimination of individual Nef activities. For example, the analysis of highly selective SIVmac239 mutants containing changes in the C-terminal domain of Nef demonstrated that MHC-I downmodulation is associated with a strong selective advantage and reduces $CD8^+$ T cell responses in infected rhesus macaques (Münch et al., 2001; Swigut et al., 2004). More often, however, mutations in Nef have pleiotropic effects, and this is a major obstacle for conclusive studies on the relevance of specific Nef functions in vivo. Altogether, the results obtained in the SIV/macaque model show that mutations in Nef that disrupt some but not all of its activities usually result in a phenotype intermediate between wild-type and *nef*-deleted SIVmac infection (reviewed in Kirchhoff et al., 2008). This implies that both the Nef functions that are maintained, as well as those that are disrupted, play some role for viral replication in vivo. The emerging picture suggests that HIV-1 and SIV evolved Nef as a multifunctional tool to manipulate the key cell types of the acquired immune system (helper CD4+ T cells, CTLs, B cells, and APCs) and to interfere with the various mechanisms (such as antigen presentation, cellular migration, signal transduction, apoptosis, and cytokine secretion) critical for the immunological control of the virus.

Adaptations Preceding the Emergence of Pandemic HIV-1 Strains

Genes encoding host restriction factors, such as TRIM5α, APOBEC3G, and tetherin, evolve unusually fast (Sawyer et al., 2004, 2007; McNatt et al., 2009). This positive selection for diversification is most likely driven by the need to antagonize new emerging pathogens or to escape viral antagonists over millions of years of virus-host coevolution. As a consequence, TRIM5α, APOBEC3G, and tetherin all show a high degree of sequence divergence and constitute barriers to zoonotic viral transmissions because the viral antagonists often act in a species-specific manner. Nonetheless, primate lentiviruses have crossed species barriers on many occasions, and HIV-1 is the product of successive zoonotic transmission and recombination events (Figure 4). As mentioned above, SIVcpz most likely arose from a recombination between ancestors of SIVs presently infecting red-capped mangabeys and *Cercopithecus* monkeys (Bailes et al., 2003). Subsequently, SIVcpz was transmitted from chimpanzees to gorillas and to humans to give rise to SIVgor and HIV-1. Recent studies provide the first insights on how these primate lentiviruses managed to jump from one species to another (Gaddis et al., 2004; Kratovac et al., 2008; Sauter et al., 2009; Schindler et al., 2006).

TRIM5α has been first identified as a major barrier to HIV-1 replication in rhesus macaque cells (Stremlau et al., 2004). Human and chimpanzee TRIM5α proteins, however, seem to be unable to restrict different primate lentiviruses, including SIVgsn and SIVcpz (Kratovac et al., 2008). The inability of chimpanzee TRIM5α to restrict SIVs found in small monkeys most likely facilitated coinfection by different lentiviruses and thus the generation of the chimeric virus that adapted to chimpanzees. Species-specific differences in the susceptibility of ABOBEC proteins may also play a role in cross-species viral transmission. Indeed, the anti-APOBEC activity of various SIV Vif proteins in transfected H9 cells seems to correlate with the capability of these viruses to infect humans (Gaddis et al., 2004). However, the capability of these SIVs to antagonize ABOBEC did not correlate with their replicative capacity in human cells, and some SIVs were partly resistant to human APOBEC proteins irrespectively of Vif function. Thus, the role of ABOBEC in primate lentiviral replication is complex, and it remains elusive whether ABOBEC constituted a barrier for the transmission of SIVs from small monkeys to chimpanzees. This was clearly not the case for the later transmission of the virus from chimpanzees to humans because the SIVcpz Vif is fully capable of antagonizing human ABOBEC proteins (Gaddis et al., 2004).

Tetherin most likely posed a significant hurdle to the cross-species transmissions that preceded the emergence of HIV-1. The chimeric virus that gave rise to SIVcpz contained the *vpu* gene of the precursor of SIVgsn/mus/mon and the *nef* gene of the progenitor of SIVrcm (Schindler et al., 2006). Most likely, the ancestor of SIVrcm used Nef (because it does not encode Vpu) and that of the SIVgsn/mus/mon lineage used Vpu (because all descendants do) to antagonize tetherin. Thus, the hybrid virus was equipped with two potential tetherin antagonists. However,

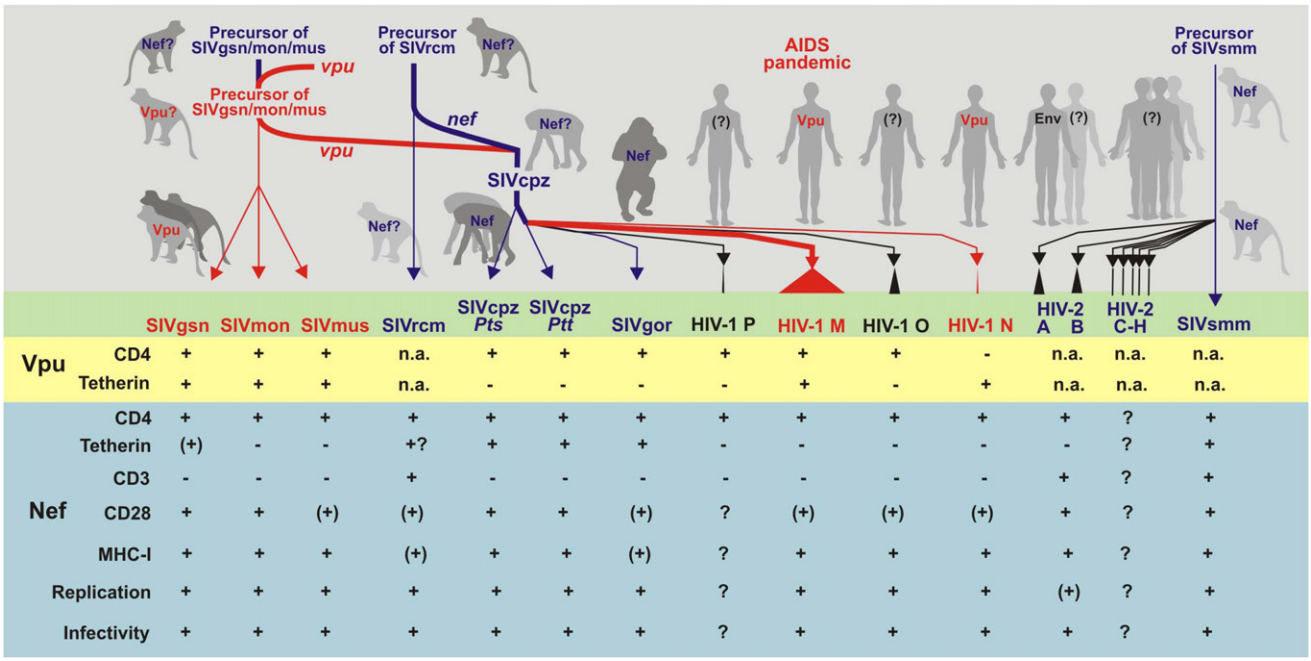

Figure 4. Tetherin-Driven Evolution of Vpu and Nef Function and the Emergence of HIV-1

SIVcpz represents a recombinant of the precursors of viruses nowadays found in red-capped mangabeys and *Cercopithecus* monkeys and was transmitted to humans and gorillas. *Vpu* was first acquired by a common precursor of SIVgsn/mus/mon and then transferred from monkeys to apes and to humans by zoonotic primate lentiviral transmissions. The events that led to the emergence of pandemic HIV-1 group M strains are indicated by thick lines. Nef-mediated tetherin antagonism is indicated by blue and Vpu-mediated tetherin antagonism by red lines, respectively. +, active; (+), poorly active; −, inactive; ?, unknown; n.a., not applicable because these viruses do not have a *vpu* gene. Adapted from Sauter et al., 2009.

both were presumably poorly active immediately after cross-species transmission because Vpus and/or Nef proteins of SIVs infecting smaller monkeys are poor antagonists of chimpanzee tetherin (Sauter et al., 2009). Subsequently, Nef, and not Vpu, evolved to become an effective tetherin antagonist in SIVcpz-infected chimpanzees, most likely because the cytoplasmic domain targeted by Nef is somewhat less divergent between the chimpanzee and monkey tetherin sequences than the transmembrane domain targeted by Vpu (reviewed in Sauter et al., 2010). In contrast, Vpu lost its anti-tetherin activity during adaptation to chimpanzees but maintained its capability to degrade the CD4 receptor. After transmission of SIVcpz from chimpanzees to gorillas, it was easy for the virus to adapt to this new host because the CPZ and GOR tetherin sequences differ only by two amino acid changes in the cytoplasmic domain targeted by Nef (Sauter et al., 2009). This was different after the species jump of SIVs from chimpanzees and gorillas to humans. The human tetherin variant contains a deletion in the cytoplasmic region, which most likely evolved to escape an ancient viral antagonist and renders human tetherin resistant to Nef (Jia et al., 2009; Sauter et al., 2009; Zhang et al., 2009). Pandemic HIV-1 M strains mastered this hurdle perfectly by switching from Nef to Vpu to regain efficient anti-tetherin activity in the new human host (Sauter et al., 2009). In contrast, the Vpu proteins of nonpandemic HIV-1 O strains remained poor tetherin antagonists, and those of the rare HIV-1 group N strains gained some anti-tetherin activity but lost their capability to degrade CD4. Thus, this deletion poses a significant, but not insurmountable, barrier for viral transmissions from chimpanzees to humans. The result that only pandemic HIV-1 M strains evolved fully functional Vpu proteins suggests a possible role in sexual transmission. Thus, it will be interesting to determine whether Vpu-mediated tetherin antagonism and/or CD4 degradation may affect the shedding of infectious virions into the genital fluids.

The direct simian precursor of HIV-2, SIVsmm from sooty mangabeys, does not contain a *vpu* gene and counteracts tetherin by Nef (Jia et al., 2009; Zhang et al., 2009). Recently, it has been shown that HIV-2 instead uses its Env protein to antagonize human tetherin (Le Tortorec and Neil, 2009). Thus, the deletion in human tetherin obviously forced both human immunodeficiency viruses to switch from Nef to a different tetherin antagonist, i.e., Vpu or Env. This illustrates the enormous plasticity by which lentiviruses can adapt to new hosts. Of note, only two (groups A and B) of at least seven cross-species transmissions of SIVsmm from sooty mangabeys to humans resulted in significant spread in the human population (Butler et al., 2007). Thus far, anti-tetherin activity has only been demonstrated for the Env protein of the group A HIV-2 ROD strain, but not for the remaining groups of HIV-2 (Le Tortorec and Neil, 2009). To assess a possible role of tetherin in the spread of HIV-2, it will be interesting to determine whether also the HIV-2 group B Env proteins, but not those of group C-H HIV-2 strains, show anti-tetherin activity.

Possible Effects of Vpu on Nef Function

As outlined above, the functions of Vpu and Nef overlap because both reduce the levels of CD4 at the cell surface and

may antagonize tetherin. Furthermore, the acquisition of *vpu* may have facilitated evolutionary changes in Nef function. The great majority of primate lentiviral Nef proteins remove TCR-CD3 from the cell surface, whereas *vpu*-containing viruses, such as HIV-1, fail to perform this function (Schindler et al., 2006). Furthermore, HIV-2 and most SIV Nefs also downmodulate CD28 much more efficiently than those of HIV-1. As a consequence, the great majority of primate lentiviral Nefs block the responsiveness of virally infected T cells to stimulation (Figure 4). In contrast, the effect of HIV-1 Nef on T cell activation is somewhat controversial. Some studies showed that HIV-1-infected CD4+ T cells are hyperresponsive to stimulation (Fortin et al., 2004; Schindler et al., 2006), whereas others reported inhibitory effects (Thoulouze et al., 2006). In either case, it is evident that Nef proteins derived from nonpathogenic SIVs that downmodulate CD3 interfere with T cell activation and the formation and function of the immunological synapse between T cells and APCs much more severely than HIV-1 Nefs (Arhel et al., 2009). It remains elusive why the acquisition of a *vpu* gene reduced the selective pressure for the suppression of T cell activation. I have proposed that viruses expressing Vpu could perhaps afford to lose the ability to block T cell activation and thus to cause higher levels of immune activation because they are better equipped to counteract the host restriction factors induced by high levels of inflammatory IFN-α than viruses lacking a *vpu* gene (Kirchhoff, 2009). This still seems plausible, although recent results show that this issue is more complex than anticipated because many SIVs lacking Vpu use their Nef proteins to antagonize tetherin (Jia et al., 2009; Sauter et al., 2009; Zhang et al., 2009). Furthermore, SIVcpz and SIVgor Nefs did not regain the "lost" CD3 downmodulation function, although their Vpu proteins are poor tetherin antagonists (Sauter et al., 2009). However, it may be much easier for the virus to lose than to regain specific activities. Furthermore, HIV-1 and SIVgsn/mus/mon Vpus may be more effective tetherin antagonists than SIVsmm and SIVagm Nef proteins (Jia et al., 2009; Lim and Emerman, 2009; Sauter et al., 2009; Zhang et al., 2009). It seems logical that SIVagm and SIVsmm need less-effective tetherin antagonists than HIV-1 because they only transiently induce interferon production in vivo (Bosinger et al., 2009; Jacquelin et al., 2009). Thus, the expression of tetherin during chronic infection should be lower than in pathogenic HIV-1 infection. Furthermore, it seems reasonable that a more specialized tetherin antagonist like Vpu may evolve to become more effective than an "all-rounder" protein like Nef. However, many results on Vpu and Nef function come with the caveat that both accessory proteins were expressed in *trans* by expression constructs. Different expression levels and the time frame that they are produced in the viral life cycle may have an important impact on the anti-tetherin activity of Vpu and Nef. Thus, more studies in virally infected primary cells are needed to draw definitive conclusions about the efficiency by which different primate lentiviruses antagonize tetherin.

Whatever the reason is for why *vpu*-containing primate lentiviruses lost their capability to block T cell activation, it may have relevant implications for their pathogenicity. It is well known that some SIVs, such as SIVagm and SIVsmm, do not cause disease in their natural simian hosts despite high levels of viral replication (Paiardini et al., 2009; Sodora et al., 2009). For some reason, these natural hosts of SIV are able to avoid the chronic, generalized immune system activation that seems to drive disease progression in HIV-infected individuals. It has been proposed that an inborn defect in the ability of plasmacytoid dendritic cells to produce IFN-α in response to viral infection is responsible for the lack of aberrant chronic immune activation in natural SIV infection (Mandl et al., 2008). Subsequent studies have shown, however, that SIV infection triggers rapid and strong IFN-α responses in both African green monkeys and sooty mangabeys (Bosinger et al., 2009; Jacquelin et al., 2009). However, in contrast to pathogenic HIV-1 and SIVmac infections, this response was transient. Thus, the lack of a sustained type I IFN response during chronic natural SIV infection is not due to a genetic inability to produce IFN-α but, rather, occurs because this response can rapidly be controlled. It is evident that both viral and host factors contribute to the development of the different levels of immune activation after the acute phase of pathogenic and nonpathogenic infection (Paiardini et al., 2009; Sodora et al., 2009). It is conceivable that a virus that blocks T cell activation may make it easier for the infected host to develop lower levels of immune activation than a virus that renders infected T cells hyperresponsive to stimulation. These viral properties may not only affect the fate of virally infected cells, but also the survival and function of uninfected bystander cells because hyperactivated HIV-1-infected T cells express death receptors and secrete inflammatory cytokines. Downmodulation of CD3 and (to a lesser extent) CD28 would be expected to impair the function of helper CD4+ T cells and thus mainly the initiation and strength of the acquired immune response, but not the induction of IFN by HIV-1 during acute infection. This may potentially explain why different levels of immune activation in pathogenic and nonpathogenic infection develop after acute infection with the rise of the acquired immune response. In support of a protective role in vivo, inefficient downmodulation of TCR-CD3 by Nef correlates with loss of CD4+ T cells in natural SIVsmm (Schindler et al., 2008) and increased levels of immune activation in HIV-2-infected individuals (Feldmann et al., 2009). However, Nef-mediated downmodulation of TCR-CD3 to suppress T cell activation and programmed death is only one of several mechanisms contributing to nonpathogenic infection, and host factors also play an important role (reviewed in Pandrea et al., 2008; Paiardini et al., 2009; Sodora et al., 2009). This is most evident from the fact that efficient CD3 downmodulation is insufficient to prevent progression to AIDS in SIV-infected macaques and in HIV-2-infected individuals (Kestler et al., 1991; Feldmann et al., 2009). Nonetheless, accumulating evidence suggests that the acquisition of a *vpu* gene may have allowed the viral lineage that gave rise to HIV-1 to evolve toward greater pathogenicity by removing the selective pressure for a protective Nef function that prevents damaging levels of immune activation (reviewed in Kirchhoff, 2009). The findings that HIV-1 is more pathogenic than HIV-2 and that SIVcpz causes AIDS in its natural chimpanzee host (Keele et al., 2009) are in agreement with this hypothesis. Obviously, AIDS is a consequence of imperfect virus-host adaptation because a perfectly adapted virus does not "commit suicide" and reduces its chance for transmission by killing its hosts. However, it is conceivable that a virus that causes higher levels of cellular activation may outgrow a less virulent form, and the

fact that pandemic HIV-1 strains infected about 60 million people within less than a hundred years after zoonotic transmission is daunting evidence that the time frame from virus infection to death is sufficient for effective viral spread.

Conclusions and Perspectives

Current data show that the ongoing ''arms race'' between lentiviruses and their hosts started long ago, is inextricably intertwined, and involves a considerably more sophisticated arsenal of ''weapons'' than previously anticipated. Basically, humans and other mammals had already developed a kind of combination therapy long before HAART because they evolved specific antiviral factors that interfere with different steps of the viral life cycle, such as reverse transcription, uncoating, and virion release. In some aspects, these cellular antiviral factors are even superior to antiretroviral drugs because they have broad antiviral activity and often HIV-1 cannot just avoid them by escape mutations. Instead, HIV-1 and other primate lentiviruses have acquired specific tools to antagonize these ancient antiviral defense mechanisms. Furthermore, they evade adaptive immunity by their high variability and by a striking combination of Nef activities. As a consequence, primate lentiviruses are capable of replicating efficiently and continuously in the presence of apparently strong antiviral immune responses. In fact, recent data show that the expression of IFN-stimulated genes encoding intrinsic antiretroviral defense factors in HIV-1-infected individuals correlates with increasing viral loads (Rotger et al., 2010), suggesting that they have become indicators rather than suppressors of HIV-1 replication. Of note, the high viral loads damage the immune system and cause AIDS mainly in poorly adapted recent or experimental hosts, such as humans or macaques. Most primate lentiviruses seem to coexist in a relatively benign relationship with their natural primate hosts because they can avoid damaging high levels of immune activation during chronic infection. It is possible that the acquisition of Vpu facilitated the emergence of primate lentiviruses, such as HIV-1, that cause higher levels of immune activation and damage because they are unable to block T cell activation. Furthermore, the evolution of a fully functional Vpu protein may have facilitated the spread of pandemic HIV-1 group M strains.

Although exciting progress has been made, we are only just beginning to understand the complex interactions between lentiviruses and their hosts. For example, it is currently largely unclear whether known host restriction factors contribute to the control of HIV-1 in vivo and how many as-yet-unknown antiviral factors remain to be identified. A better understanding of the virus-host interactions seems important for many reasons. For example, comparative studies of pathogenic and nonpathogenic primate lentiviral infections may teach us how the high levels of damaging immune activation can be avoided. This seems particularly relevant because immune activation seems to be a problem even under HAART and may cause a premature aging of the immune system. Studying the viral immune evasion mechanisms may teach us how better immunological control of HIV-1 can be achieved because it is evident that viruses evolve to manipulate exactly those immune functions that would be otherwise most relevant for their control. It will also be interesting to assess whether the inhibition of the viral antagonists or the specific induction of the natural cellular defense mechanisms to overpower them represent useful strategies to improve antiretroviral therapy. It may even be possible to develop improved artificial restriction factors with broad-based antiviral activity that are resistant to the viral antagonists (Perez-Caballero et al., 2009). Such studies are challenging but may be very rewarding because it will be difficult for HIV-1 to become resistant against host restriction factors. Furthermore, host restriction factors do not only inhibit HIV-1 and may be effective against a variety of viral pathogens.

ACKNOWLEDGMENTS

We thank Beatrice H. Hahn and Guido Silvestri for helpful discussions and Jan Münch, Dré van der Merwe, and Daniel Sauter for critical reading of the manuscript. I apologize to the authors of many interesting studies that could not be cited due to space limitations. The author is supported by grants from the Deutsche Forschungsgemeinschaft.

REFERENCES

Arhel, N.J., and Kirchhoff, F. (2009). Implications of Nef: host cell interactions in viral persistence and progression to AIDS. Curr. Top. Microbiol. Immunol. *339*, 147–175.

Arhel, N., Lehmann, M., Clauss, K., Nienhaus, G.U., Piguet, V., and Kirchhoff, F. (2009). The inability to disrupt the immunological synapse between infected human T cells and APCs distinguishes HIV-1 from most other primate lentiviruses. J. Clin. Invest. *119*, 2965–2975.

Ariën, K.K., and Verhasselt, B. (2008). HIV Nef: role in pathogenesis and viral fitness. Curr. HIV Res. *6*, 200–208.

Bailes, E., Gao, F., Bibollet-Ruche, F., Courgnaud, V., Peeters, M., Marx, P.A., Hahn, B.H., and Sharp, P.M. (2003). Hybrid origin of SIV in chimpanzees. Science *300*, 1713.

Balliet, J.W., Kolson, D.L., Eiger, G., Kim, F.M., McGann, K.A., Srinivasan, A., and Collman, R. (1994). Distinct effects in primary macrophages and lymphocytes of the human immunodeficiency virus type 1 accessory genes vpr, vpu, and nef: mutational analysis of a primary HIV-1 isolate. Virology *200*, 623–631.

Bannert, N., and Kurth, R. (2004). Retroelements and the human genome: new perspectives on an old relation. Proc. Natl. Acad. Sci. USA *101* (*Suppl 2*), 14572–14579.

Bartee, E., McCormack, A., and Früh, K. (2006). Quantitative membrane proteomics reveals new cellular targets of viral immune modulators. PLoS Pathog. *2*, e107.

Bieniasz, P.D. (2004). Intrinsic immunity: a front-line defense against viral attack. Nat. Immunol. *5*, 1109–1115.

Bishop, K.N., Verma, M., Kim, E.Y., Wolinsky, S.M., and Malim, M.H. (2008). APOBEC3G inhibits elongation of HIV-1 reverse transcripts. PLoS Pathog. *4*, e1000231.

Bosinger, S.E., Li, Q., Gordon, S.N., Klatt, N.R., Duan, L., Xu, L., Francella, N., Sidahmed, A., Smith, A.J., Cramer, E.M., et al. (2009). Global genomic analysis reveals rapid control of a robust innate response in SIV-infected sooty mangabeys. J. Clin. Invest. *119*, 3556–3572.

Bour, S., Schubert, U., and Strebel, K. (1995). The human immunodeficiency virus type 1 Vpu protein specifically binds to the cytoplasmic domain of CD4: implications for the mechanism of degradation. J. Virol. *69*, 1510–1520.

Butler, I.F., Pandrea, I., Marx, P.A., and Apetrei, C. (2007). HIV genetic diversity: biological and public health consequences. Curr. HIV Res. *5*, 23–45.

Brenchley, J.M., and Douek, D.C. (2008). HIV infection and the gastrointestinal immune system. Mucosal Immunol. *1*, 23–30.

Casartelli, N., Guivel-Benhassine, F., Bouziat, R., Brandler, S., Schwartz, O., and Moris, A. (2010a). The antiviral factor APOBEC3G improves CTL recognition of cultured HIV-infected T cells. J. Exp. Med. *207*, 39–49.

Casartelli, N., Sourisseau, M., Feldmann, J., Guivel-Benhassine, F., Mallet, A., Marcelin, A.G., Guatelli, J., and Schwartz, S. (2010b). Tetherin restricts productive HIV-1 cell-to-cell transmission. PLOS Pathog. *6*, e1000955.

Chiu, Y.L., and Greene, W.C. (2009). APOBEC3G: an intracellular centurion. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *364*, 689–703.

Chiu, Y.L., Soros, V.B., Kreisberg, J.F., Stopak, K., Yonemoto, W., and Greene, W.C. (2005). Cellular APOBEC3G restricts HIV-1 infection in resting CD4+ T cells. Nature *435*, 108–114.

Cohen, G.B., Gandhi, R.T., Davis, D.M., Mandelboim, O., Chen, B.K., Strominger, J.L., and Baltimore, D. (1999). The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. Immunity *10*, 661–671.

Connor, R.I., Chen, B.K., Choe, S., and Landau, N.R. (1995). Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. Virology *206*, 935–944.

Conticello, S.G., Thomas, C.J., Petersen-Mahrt, S.K., and Neuberger, M.S. (2005). Evolution of the AID/APOBEC family of polynucleotide (deoxy)cytidine deaminases. Mol. Biol. Evol. *22*, 367–377.

Deacon, N.J., Tsykin, A., Solomon, A., Smith, K., Ludford-Menting, M., Hooker, D.J., McPhee, D.A., Greenway, A.L., Ellett, A., Chatfield, C., et al. (1995). Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. Science *270*, 988–991.

Douglas, J.L., Viswanathan, K., McCarroll, M.N., Gustin, J.K., Früh, K., and Moses, A.V. (2009). Vpu directs the degradation of the human immunodeficiency virus restriction factor BST-2/Tetherin via a betaTrCP-dependent mechanism. J. Virol. *83*, 7931–7947.

Dubé, M., Roy, B.B., Guiot-Guillain, P., Binette, J., Mercier, J., Chiasson, A., and Cohen, E.A. (2010). Antagonism of tetherin restriction of HIV-1 release by Vpu involves binding and sequestration of the restriction factor in a perinuclear compartment. PLoS Pathog. *6*, e1000856.

Esnault, C., Heidmann, O., Delebecque, F., Dewannieux, M., Ribet, D., Hance, A.J., Heidmann, T., and Schwartz, O. (2005). APOBEC3G cytidine deaminase inhibits retrotransposition of endogenous retroviruses. Nature *433*, 430–433.

Feldmann, J., Leligdowicz, A., Jaye, A., Dong, T., Whittle, H., and Rowland-Jones, S.L. (2009). Downregulation of the T-cell receptor by human immunodeficiency virus type 2 Nef does not protect against disease progression. J. Virol. *83*, 12968–12972.

Fenard, D., Yonemoto, W., de Noronha, C., Cavrois, M., Williams, S.A., and Greene, W.C. (2005). Nef is physically recruited into the immunological synapse and potentiates T cell activation early after TCR engagement. J. Immunol. *175*, 6050–6057.

Fletcher, T.M., III, Brichacek, B., Sharova, N., Newman, M.A., Stivahtis, G., Sharp, P.M., Emerman, M., Hahn, B.H., and Stevenson, M. (1996). Nuclear import and cell cycle arrest functions of the HIV-1 Vpr protein are encoded by two separate genes in HIV-2/SIV(SM). EMBO J. *15*, 6155–6165.

Fortin, J.F., Barat, C., Beausejour, Y., Barbeau, B., and Tremblay, M.J. (2004). Hyper-responsiveness to stimulation of human immunodeficiency virus-infected CD4+ T cells requires Nef and Tat virus gene products and results from higher NFAT, NF-kappaB, and AP-1 induction. J. Biol. Chem. *279*, 39520–39531.

Foster, J.L., and Garcia, J.V. (2008). HIV-1 Nef: at the crossroads. Retrovirology *5*, 84.

Gaddis, N.C., Sheehy, A.M., Ahmad, K.M., Swanson, C.M., Bishop, K.N., Beer, B.E., Marx, P.A., Gao, F., Bibollet-Ruche, F., Hahn, B.H., and Malim, M.H. (2004). Further investigation of simian immunodeficiency virus Vif function in human cells. J. Virol. *78*, 12041–12046.

Gibbs, J.S., Lackner, A.A., Lang, S.M., Simon, M.A., Sehgal, P.K., Daniel, M.D., and Desrosiers, R.C. (1995). Progression to AIDS in the absence of a gene for vpr or vpx. J. Virol. *69*, 2378–2383.

Gifford, R.J., Katzourakis, A., Tristem, M., Pybus, O.G., Winters, M., and Shafer, R.W. (2008). A transitional endogenous lentivirus from the genome of a basal primate and implications for lentivirus evolution. Proc. Natl. Acad. Sci. USA *105*, 20362–20367.

Goffinet, C., Allespach, I., Homann, S., Tervo, H.M., Habermann, A., Rupp, D., Oberbremer, L., Kern, C., Tibroni, N., Welsch, S., et al. (2009). HIV-1 antagonism of CD317 is species specific and involves Vpu-mediated proteasomal degradation of the restriction factor. Cell Host Microbe *5*, 285–297.

Goffinet, C., Homann, S., Ambiel, I., Tibroni, N., Rupp, D., Keppler, O.T., and Fackler, O.T. (2010). Antagonism of CD317 restriction of human immunodeficiency virus type 1 (HIV-1) particle release and depletion of CD317 are separable activities of HIV-1 Vpu. J. Virol. *84*, 4089–4094.

Göttlinger, H.G., Dorfman, T., Cohen, E.A., and Haseltine, W.A. (1993). Vpu protein of human immunodeficiency virus type 1 enhances the release of capsids produced by gag gene constructs of widely divergent retroviruses. Proc. Natl. Acad. Sci. USA *90*, 7381–7385.

Goujon, C., Rivière, L., Jarrosson-Wuilleme, L., Bernaud, J., Rigal, D., Darlix, J.L., and Cimarelli, A. (2007). SIVSM/HIV-2 Vpx proteins promote retroviral escape from a proteasome-dependent restriction pathway present in human dendritic cells. Retrovirology *4*, 2.

Goh, W.C., Rogel, M.E., Kinsey, C.M., Michael, S.F., Fultz, P.N., Nowak, M.A., Hahn, B.H., and Emerman, M. (1998). HIV-1 Vpr increases viral expression by manipulation of the cell cycle: a mechanism for selection of Vpr in vivo. Nat. Med. *4*, 65–71.

Gummuluru, S., Kinsey, C.M., and Emerman, M. (2000). An in vitro rapid-turnover assay for human immunodeficiency virus type 1 replication selects for cell-to-cell spread of virus. J. Virol. *74*, 10882–10891.

Gupta, R.K., Hué, S., Schaller, T., Verschoor, E., Pillay, D., and Towers, G.J. (2009). Mutation of a single residue renders human tetherin resistant to HIV-1 Vpu-mediated depletion. PLoS Pathog. *5*, e1000443.

Haase, A.T. (2010). Targeting early infection to prevent HIV-1 mucosal transmission. Nature *464*, 217–223.

Hahn, B.H., Shaw, G.M., De Cock, K.M., and Sharp, P.M. (2000). AIDS as a zoonosis: scientific and public health implications. Science *287*, 607–614.

Hinz, A., Miguet, N., Natrajan, G., Usami, Y., Yamanaka, H., Renesto, P., Hartlieb, B., McCarthy, A.A., Simorre, J.P., Göttlinger, H., and Weissenhorn, W. (2010). Structural basis of HIV-1 tethering to membranes by the BST-2/tetherin ectodomain. Cell Host Microbe *7*, 314–323.

Holmes, R.K., Koning, F.A., Bishop, K.N., and Malim, M.H. (2007). APOBEC3F can inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation. Comparisons with APOBEC3G. J. Biol. Chem. *282*, 2587–2595.

Huthoff, H., and Towers, G.J. (2008). Restriction of retroviral replication by APOBEC3G/F and TRIM5alpha. Trends Microbiol. *16*, 612–619.

Jacquelin, B., Mayau, V., Targat, B., Liovat, A.S., Kunkel, D., Petitjean, G., Dillies, M.A., Roques, P., Butor, C., Silvestri, G., et al. (2009). Nonpathogenic SIV infection of African green monkeys induces a strong but rapidly controlled type I IFN response. J. Clin. Invest. *119*, 3544–3555.

James, C.O., Huang, M.B., Khan, M., Garcia-Barrio, M., Powell, M.D., and Bond, V.C. (2004). Extracellular Nef protein targets CD4+ T cells for apoptosis by interacting with CXCR4 surface receptors. J. Virol. *78*, 3099–3109.

Jern, P., Stoye, J.P., and Coffin, J.M. (2007). Role of APOBEC3 in genetic diversity among endogenous murine leukemia viruses. PLoS Genet. *3*, 2014–2022.

Jia, B., Serra-Moreno, R., Neidermyer, W., Rahmberg, A., Mackey, J., Fofana, I.B., Johnson, W.E., Westmoreland, S., and Evans, D.T. (2009). Species-specific activity of SIV Nef and HIV-1 Vpu in overcoming restriction by tetherin/BST2. PLoS Pathog. *5*, e1000429.

Johnson, W.E., and Desrosiers, R.C. (2002). Viral persistence: HIV's strategies of immune system evasion. Annu. Rev. Med. *53*, 499–518.

Kamata, M., Nagaoka, Y., and Chen, I.S. (2009). Reassessing the role of APOBEC3G in human immunodeficiency virus type 1 infection of quiescent CD4+ T-cells. PLoS Pathog. *5*, e1000342.

Katzourakis, A., Tristem, M., Pybus, O.G., and Gifford, R.J. (2007). Discovery and analysis of the first endogenous lentivirus. Proc. Natl. Acad. Sci. USA *104*, 6261–6265.

Keele, B.F., Giorgi, E.E., Salazar-Gonzalez, J.F., Decker, J.M., Pham, K.T., Salazar, M.G., Sun, C., Grayson, T., Wang, S., Li, H., et al. (2008). Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. Proc. Natl. Acad. Sci. USA *105*, 7552–7557.

Keele, B.F., Jones, J.H., Terio, K.A., Estes, J.D., Rudicell, R.S., Wilson, M.L., Li, Y., Learn, G.H., Beasley, T.M., Schumacher-Stankey, J., et al. (2009). Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz. Nature *460*, 515–519.

Keckesova, Z., Ylinen, L.M., and Towers, G.J. (2006). Cyclophilin A renders human immunodeficiency virus type 1 sensitive to Old World monkey but not human TRIM5 alpha antiviral activity. J. Virol. *80*, 4683–4690.

Keckesova, Z., Ylinen, L.M., Towers, G.J., Gifford, R.J., and Katzourakis, A. (2009). Identification of a RELIK orthologue in the European hare (Lepus europaeus) reveals a minimum age of 12 million years for the lagomorph lentiviruses. Virology *384*, 7–11.

Kestler, H.W., III, Ringler, D.J., Mori, K., Panicali, D.L., Sehgal, P.K., Daniel, M.D., and Desrosiers, R.C. (1991). Importance of the nef gene for maintenance of high virus loads and for development of AIDS. Cell *65*, 651–662.

Kirchhoff, F. (2009). Is the high virulence of HIV-1 an unfortunate coincidence of primate lentiviral evolution? Nat. Rev. Microbiol. *7*, 467–476.

Kirchhoff, F., Greenough, T.C., Brettler, D.B., Sullivan, J.L., and Desrosiers, R.C. (1995). Brief report: Absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. N. Engl. J. Med. *332*, 228–232.

Kirchhoff, F., Schindler, M., Specht, A., Arhel, N., and Münch, J. (2008). Role of Nef in primate lentiviral immunopathogenesis. Cell. Mol. Life Sci. *65*, 2621–2636.

Korber, B., Muldoon, M., Theiler, J., Gao, F., Gupta, R., Lapedes, A., Hahn, B.H., Wolinsky, S., and Bhattacharya, T. (2000). Timing the ancestor of the HIV-1 pandemic strains. Science *288*, 1789–1796.

Kratovac, Z., Virgen, C.A., Bibollet-Ruche, F., Hahn, B.H., Bieniasz, P.D., and Hatziioannou, T. (2008). Primate lentivirus capsid sensitivity to TRIM5 proteins. J. Virol. *82*, 6772–6777.

Kupzig, S., Korolchuk, V., Rollason, R., Sugden, A., Wilde, A., and Banting, G. (2003). Bst-2/HM1.24 is a raft-associated apical membrane protein with an unusual topology. Traffic *4*, 694–709.

Lenassi, M., Cagney, G., Liao, M., Vaupotic, T., Bartholomeeusen, K., Cheng, Y., Krogan, N.J., Plemenitas, A., and Peterlin, B.M. (2010). HIV Nef is secreted in exosomes and triggers apoptosis in bystander CD4+ T cells. Traffic *11*, 110–122.

Le Rouzic, E., Belaïdouni, N., Estrabaud, E., Morel, M., Rain, J.C., Transy, C., and Margottin-Goguet, F. (2007). HIV1 Vpr arrests the cell cycle by recruiting DCAF1/VprBP, a receptor of the Cul4-DDB1 ubiquitin ligase. Cell Cycle *6*, 182–188.

Le Tortorec, A., and Neil, S.J. (2009). Antagonism to and intracellular sequestration of human tetherin by the human immunodeficiency virus type 2 envelope glycoprotein. J. Virol. *83*, 11966–11978.

Lim, E.S., and Emerman, M. (2009). Simian immunodeficiency virus SIVagm from African green monkeys does not antagonize endogenous levels of African green monkey tetherin/BST-2. J. Virol. *83*, 11673–11681.

Malim, M.H. (2009). APOBEC proteins and intrinsic resistance to HIV-1 infection. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *364*, 675–687.

Malim, M.H., and Emerman, M. (2008). HIV-1 accessory proteins—ensuring viral survival in a hostile environment. Cell Host Microbe *3*, 388–398.

Mandl, J.N., Barry, A.P., Vanderford, T.H., Kozyr, N., Chavan, R., Klucking, S., Barrat, F.J., Coffman, R.L., Staprans, S.I., and Feinberg, M.B. (2008). Divergent TLR7 and TLR9 signaling and type I interferon production distinguish pathogenic and nonpathogenic AIDS virus infections. Nat. Med. *14*, 1077–1087.

Mangeat, B., Gers-Huber, G., Lehmann, M., Zufferey, M., Luban, J., and Piguet, V. (2009). HIV-1 Vpu neutralizes the antiviral factor Tetherin/BST-2 by binding it and directing its beta-TrCP2-dependent degradation. PLoS Pathog. *5*, e1000574.

Manninen, A., Huotari, P., Hiipakka, M., Renkema, G.H., and Saksela, K. (2001). Activation of NFAT-dependent gene expression by Nef: conservation among divergent Nef alleles, dependence on SH3 binding and membrane association, and cooperation with protein kinase C-theta. J. Virol. *75*, 3034–3037.

Mariani, R., Chen, D., Schröfelbauer, B., Navarro, F., König, R., Bollman, B., Münk, C., Nymark-McMahon, H., and Landau, N.R. (2003). Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell *114*, 21–31.

McNatt, M.W., Zang, T., Hatziioannou, T., Bartlett, M., Fofana, I.B., Johnson, W.E., Neil, S.J., and Bieniasz, P.D. (2009). Species-specific activity of HIV-1 Vpu and positive selection of tetherin transmembrane domain variants. PLoS Pathog. *5*, e1000300.

Mehle, A., Goncalves, J., Santa-Marta, M., McPike, M., and Gabuzda, D. (2004). Phosphorylation of a novel SOCS-box regulates assembly of the HIV-1 Vif-Cul5 complex that promotes APOBEC3G degradation. Genes Dev. *18*, 2861–2866.

Mitchell, R.S., Katsura, C., Skasko, M.A., Fitzpatrick, K., Lau, D., Ruiz, A., Stephens, E.B., Margottin-Goguet, F., Benarous, R., and Guatelli, J.C. (2009). Vpu antagonizes BST-2-mediated restriction of HIV-1 release via beta-TrCP and endo-lysosomal trafficking. PLoS Pathog. *5*, e1000450.

Miyagi, E., Opi, S., Takeuchi, H., Khan, M., Goila-Gaur, R., Kao, S., and Strebel, K. (2007). Enzymatically active APOBEC3G is required for efficient inhibition of human immunodeficiency virus type 1. J. Virol. *81*, 13346–13353.

Miyagi, E., Andrew, A.J., Kao, S., and Strebel, K. (2009). Vpu enhances HIV-1 virus release in the absence of Bst-2 cell surface down-modulation and intracellular depletion. Proc. Natl. Acad. Sci. USA *106*, 2868–2873.

Münch, J., Stolte, N., Fuchs, D., Stahl-Hennig, C., and Kirchhoff, F. (2001). Efficient class I major histocompatibility complex down-regulation by simian immunodeficiency virus Nef is associated with a strong selective advantage in infected rhesus macaques. J. Virol. *75*, 10532–10536.

Neil, S., and Bieniasz, P. (2009). Human immunodeficiency virus, restriction factors, and interferon. J. Interferon Cytokine Res. *29*, 569–580.

Neil, S.J., Sandrin, V., Sundquist, W.I., and Bieniasz, P.D. (2007). An interferon-alpha-induced tethering mechanism inhibits HIV-1 and Ebola virus particle release but is counteracted by the HIV-1 Vpu protein. Cell Host Microbe *2*, 193–203.

Neil, S.J., Zang, T., and Bieniasz, P.D. (2008). Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature *451*, 425–430.

Pandrea, I., Sodora, D.L., Silvestri, G., and Apetrei, C. (2008). Into the wild: simian immunodeficiency virus (SIV) infection in natural hosts. Trends Immunol. *29*, 419–428.

Paiardini, M., Pandrea, I., Apetrei, C., and Silvestri, G. (2009). Lessons learned from the natural hosts of HIV-related viruses. Annu. Rev. Med. *60*, 485–495.

Perez-Caballero, D., Zang, T., Ebrahimi, A., McNatt, M.W., Gregory, D.A., Johnson, M.C., and Bieniasz, P.D. (2009). Tetherin inhibits HIV-1 release by directly tethering virions to cells. Cell *139*, 499–511.

Pizzato, M., Helander, A., Popova, E., Calistri, A., Zamborlini, A., Palù, G., and Göttlinger, H.G. (2007). Dynamin 2 is required for the enhancement of HIV-1 infectivity by Nef. Proc. Natl. Acad. Sci. USA *104*, 6812–6817.

Planelles, V., and Benichou, S. (2009). Vpr and its interactions with cellular proteins. Curr. Top. Microbiol. Immunol. *339*, 177–200.

Qiao, X., He, B., Chiu, A., Knowles, D.M., Chadburn, A., and Cerutti, A. (2006). Human immunodeficiency virus 1 Nef suppresses CD40-dependent immunoglobulin class switching in bystander B cells. Nat. Immunol. *7*, 302–310.

Roche, P.A., Teletski, C.L., Karp, D.R., Pinet, V., Bakke, O., and Long, E.O. (1992). Stable surface expression of invariant chain prevents peptide presentation by HLA-DR. EMBO J. *11*, 2841–2847.

Roeth, J.F., and Collins, K.L. (2006). Human immunodeficiency virus type 1 Nef: adapting to intracellular trafficking pathways. Microbiol. Mol. Biol. Rev. *70*, 548–563.

Rotger, M., Dang, K.K., Fellay, J., Heinzen, E.L., Feng, S., Descombes, P., Shianna, K.V., Ge, D., Gunthard, H.F., Goldstein, D.B., and Telenti, A.; Swiss HIV Cohort Study; Center for HIV/AIDS Vaccine Immunology. (2010). Genome-wide mRNA expression correlates of viral control in CD4+ T-cells from HIV-1-infected individuals. PLoS Pathog. *6*, e1000781.

Ruiz, A., Guatelli, J.C., and Stephens, E.B. (2010). The Vpu protein: new concepts in virus release and CD4 down-modulation. Curr. HIV Res. *8*, 240–252.

Sadler, H.A., Stenglein, M.D., Harris, R.S., and Mansky, L.M. (2010). APOBEC3G contributes to HIV-1 variation through sublethal mutagenesis. J. Virol. *84*, 7396–7404.

Santoni de Sio, F.R., and Trono, D. (2009). APOBEC3G-depleted resting CD4+ T cells remain refractory to HIV1 infection. PLoS ONE *4*, e6571.

Sauter, D., Schindler, M., Specht, A., Landford, W.N., Münch, J., Kim, K.-A., Votteler, J., Schubert, U., Bibollet-Ruche, F., Keele, B.F., et al. (2009). Tetherin-driven adaptation of Vpu and Nef function and the evolution of pandemic and nonpandemic HIV-1 strains. Cell Host Microbe *6*, 409–421.

Sauter, D., Specht, A., and Kirchhoff, F. (2010). Tetherin: holding on and letting go. Cell *141*, 392–398.

Sattentau, Q. (2008). Avoiding the void: cell-to-cell spread of human viruses. Nat. Rev. Microbiol. *6*, 815–826.

Sawyer, S.L., Emerman, M., and Malik, H.S. (2004). Ancient adaptive evolution of the primate antiviral DNA-editing enzyme APOBEC3G. PLoS Biol. *2*, E275.

Sawyer, S.L., Emerman, M., and Malik, H.S. (2007). Discordant evolution of the adjacent antiretroviral genes TRIM22 and TRIM5 in mammals. PLoS Pathog. *3*, e197.

Schindler, M., Münch, J., Kutsch, O., Li, H., Santiago, M.L., Bibollet-Ruche, F., Müller-Trutwin, M.C., Novembre, F.J., Peeters, M., Courgnaud, V., et al. (2006). Nef-mediated suppression of T cell activation was lost in a lentiviral lineage that gave rise to HIV-1. Cell *125*, 1055–1067.

Schindler, M., Schmökel, J., Specht, A., Li, H., Münch, J., Khalid, M., Sodora, D.L., Hahn, B.H., Silvestri, G., and Kirchhoff, F. (2008). Inefficient Nef-mediated downmodulation of CD3 and MHC-I correlates with loss of CD4+ T cells in natural SIV infection. PLoS Pathog. *18*, e1000107.

Schindler, M., Rajan, D., Banning, C., Wimmer, P., Koppensteiner, H., Iwanski, A., Specht, A., Sauter, D., Dobner, T., and Kirchhoff, F. (2010). Vpu serine 52 dependent counteraction of tetherin is required for HIV-1 replication in macrophages, but not in ex vivo human lymphoid tissue. Retrovirology *7*, 1.

Sharova, N., Wu, Y., Zhu, X., Stranska, R., Kaushik, R., Sharkey, M., and Stevenson, M. (2008). Primate lentiviral Vpx commandeers DDB1 to counteract a macrophage restriction. PLoS Pathog. *4*, e1000057.

Sharp, P.M., Bailes, E., Stevenson, M., Emerman, M., and Hahn, B.H. (1996). Gene acquisition in HIV and SIV. Nature *383*, 586–587.

Sheehy, A.M., Gaddis, N.C., Choi, J.D., and Malim, M.H. (2002). Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature *418*, 646–650.

Sheehy, A.M., Gaddis, N.C., and Malim, M.H. (2003). The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. Nat. Med. *9*, 1404–1407.

Simon, V., Zennou, V., Murray, D., Huang, Y., Ho, D.D., and Bieniasz, P.D. (2005). Natural variation in Vif: differential impact on APOBEC3G/3F and a potential role in HIV-1 diversification. PLoS Pathog. *1*, e6.

Sodora, D.L., Allan, J.S., Apetrei, C., Brenchley, J.M., Douek, D.C., Else, J.G., Estes, J.D., Hahn, B.H., Hirsch, V.M., Kaur, A., et al. (2009). Toward an AIDS vaccine: lessons from natural simian immunodeficiency virus infections of African nonhuman primate hosts. Nat. Med. *15*, 861–865.

Song, B. (2009). TRIM5alpha. Curr. Top. Microbiol. Immunol. *339*, 47–66.

Srivastava, S., Swanson, S.K., Manel, N., Florens, L., Washburn, M.P., and Skowronski, J. (2008). Lentiviral Vpx accessory factor targets VprBP/DCAF1 substrate adaptor for cullin 4 E3 ubiquitin ligase to enable macrophage infection. PLoS Pathog. *4*, e1000059.

Stopak, K., de Noronha, C., Yonemoto, W., and Greene, W.C. (2003). HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol. Cell *12*, 591–601.

Strebel, K., Klimkait, T., Maldarelli, F., and Martin, M.A. (1989). Molecular and biochemical analyses of human immunodeficiency virus type 1 vpu protein. J. Virol. *63*, 3784–3791.

Stremlau, M., Owens, C.M., Perron, M.J., Kiessling, M., Autissier, P., and Sodroski, J. (2004). The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. Nature *427*, 848–853.

Stumptner-Cuvelette, P., Morchoisne, S., Dugast, M., Le Gall, S., Raposo, G., Schwartz, O., and Benaroch, P. (2001). HIV-1 Nef impairs MHC class II antigen presentation and surface expression. Proc. Natl. Acad. Sci. USA *98*, 12144–12149.

Swigut, T., Alexander, L., Morgan, J., Lifson, J., Mansfield, K.G., Lang, S., Johnson, R.P., Skowronski, J., and Desrosiers, R.C. (2004). Impact of Nef-mediated downregulation of major histocompatibility complex class I on immune response to simian immunodeficiency virus. J. Virol. *78*, 13335–13344.

Swingler, S., Brichacek, B., Jacque, J.M., Ulich, C., Zhou, J., and Stevenson, M. (2003). HIV-1 Nef intersects the macrophage CD40L signalling pathway to promote resting-cell infection. Nature *424*, 213–219.

Swingler, S., Mann, A., Jacqué, J., Brichacek, B., Sasseville, V.G., Williams, K., Lackner, A.A., Janoff, E.N., Wang, R., Fisher, D., and Stevenson, M. (1999). HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. Nat. Med. *5*, 997–1003.

Thoulouze, M.I., Sol-Foulon, N., Blanchet, F., Dautry-Varsat, A., Schwartz, O., and Alcover, A. (2006). Human immunodeficiency virus type-1 infection impairs the formation of the immunological synapse. Immunity *24*, 547–561.

Tristem, M., Marshall, C., Karpas, A., Petrik, J., and Hill, F. (1990). Origin of vpx in lentiviruses. Nature *347*, 341–342.

Van Damme, N., Goff, D., Katsura, C., Jorgenson, R.L., Mitchell, R., Johnson, M.C., Stephens, E.B., and Guatelli, J. (2008). The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. Cell Host Microbe *3*, 245–252.

Willey, R.L., Maldarelli, F., Martin, M.A., and Strebel, K. (1992). Human immunodeficiency virus type 1 Vpu protein induces rapid degradation of CD4. J. Virol. *66*, 7193–7200.

Worobey, M., Gemmel, M., Teuwen, D.E., Haselkorn, T., Kunstman, K., Bunce, M., Muyembe, J.J., Kabongo, J.M., Kalengayi, R.M., Van Marck, E., et al. (2008). Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. Nature *455*, 661–664.

Xu, W., Santini, P.A., Sullivan, J.S., He, B., Shan, M., Ball, S.C., Dyer, W.B., Ketas, T.J., Chadburn, A., Cohen-Gould, L., et al. (2009). HIV-1 evades virus-specific IgG2 and IgA responses by targeting systemic and intestinal B cells via long-range intercellular conduits. Nat. Immunol. *10*, 1008–1017.

Yu, X., Yu, Y., Liu, B., Luo, K., Kong, W., Mao, P., and Yu, X.F. (2003). Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science *302*, 1056–1060.

Zhang, F., Wilson, S.J., Landford, W.C., Virgen, B., Gregory, D., Johnson, M.C., Munch, J., Kirchhoff, F., Bieniasz, P.D., and Hatziioannou, T. (2009). Nef proteins from simian immunodeficiency viruses are tetherin antagonists. Cell Host Microbe *6*, 54–67.