Manipulation of immunometabolism by HIV – accessories to the crime?

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Abstract

Evolutionary pressure has produced an “arms race” between cellular restriction factors (limiting viral replication) and viral proteins (overcoming host restriction). The host factors SAMHD1 and SLFN1 patrol metabolic bottlenecks required for HIV replication. Conversely, the HIV accessory proteins Vpx, Vpu and Nef manipulate cellular metabolism to enable viral replication. Recent work identifying Vpu-mediated downregulation of the alanine transporter SNAT1 and Nef-mediated downregulation of the serine carriers SERINC3/5 has uncovered the importance of HIV manipulation of the amino acid supply. Interference with CD4^{+} T-cell amino acid metabolism suggests a novel paradigm of viral immunomodulation, and signposts fundamental aspects of lymphocyte biology.

Introduction

Human Immunodeficiency Virus (HIV) infects almost 40 million people worldwide and causes more than 1 million AIDS-related deaths every year (latest data available from www.unaids.org). In addition to the Gag, Pol and Env polyproteins common to all retroviruses, HIV encodes the so-called "accessory proteins" Vif, Vpr, Nef and Vpu/Vpx, dispensable for viral replication *in vitro*, but essential for viral pathogenesis *in vivo* (**Figure 1**; reviewed in [1]). Vif, Vpr and Nef are common to all strains of HIV, but Vpu is found only in HIV-1 (responsible for the global AIDS pandemic), and Vpx is found only in HIV-2 (responsible for a minority of infections, particularly in West Africa). As well as counteracting host-cell restriction and promoting immune evasion (reviewed in [2]), recent results suggest that HIV accessory proteins directly manipulate cellular metabolic pathways to optimise the intracellular environment for productive infection (**Figure 2**). They are therefore accomplices, rather than accessories to the crime.

Whilst all viruses co-opt cellular metabolism to enable their replication, two characteristics of HIV infection *in vivo* present specific challenges. First, like all retroviruses, HIV relies on reverse transcription for replication of its genome. Second, the HIV entry receptor CD4 is expressed by both resting (naïve) and activated helper T-cells, as well as terminally differentiated macrophages, a range of cells with markedly different metabolic programmes. In this review, we describe how HIV accessory proteins enable the virus to overcome these challenges by manipulating the metabolism of nucleotides, glucose, amino acids and lipids. We discuss cellular restriction factors which patrol metabolic pathways required for viral replication, and focus on recent discoveries highlighting HIV-mediated remodelling of the plasma membrane and interference with the T-cell nutrient supply.

Expansion of the nucleotide pool

Like all retroviruses, HIV encodes reverse transcriptase to generate complementary ssDNA from its positive-sense ssRNA genome, utilising cellular deoxynucleotide triphosphates (dNTPs) as substrates for DNA polymerisation. This metabolic bottleneck is exploited therapeutically by nucleoside/nucleotide and non-nucleoside reverse transcriptase inhibitors, which have formed the backbone of anti-retroviral drug therapy since zidovudine (AZT) was approved for the treatment of HIV in 1987. Productive HIV-1 infection in resting primary $CD4^+$ T-cells and myeloid cells is inhibited at the level of reverse transcription by the scarcity of intracellular dNTPs (reviewed in [3]). Low concentrations of dNTPs are maintained by the recently described deoxynucleotide phosphohydrolase SAMHD1, which hydrolyses dNTPs to deoxynucleosides and free triphosphates, and therefore acts as a metabolic restriction factor [4,5]. Conversely, an expanded dNTP pool is characteristic of activated T-cells and

transformed/cancerous cell lines, enabling cellular DNA replication and relieving the block to reverse transcription [3].

Vpx of HIV-2 targets SAMHD1 for ubiquitin-dependent proteasomal degradation and, when incorporated into virions, overcomes the block to HIV reverse transcription in resting T-cells and myeloid cells [4,6,7]. Vpx is paralogous to Vpr, and Vpr variants of most primate lentiviruses lacking Vpx demonstrate species-specific antagonism of SAMHD1 [8]. This activity was surrendered by the cross-species transmission and recombination event that generated SIV_{cpz}, and has not been reacquired by viruses of the SIV_{cpz}/HIV-1 lineage [9]. The absence of SAMHD1 antagonism in HIV-1 may have been partially compensated by the enhanced affinity (low Km) of HIV-1 reverse transcriptase for dNTPs [10], but it is also possible that the avoidance of productive replication in professional antigen presenting cells is itself an adaptive strategy for immune evasion.

Induction of glycolysis

As well as increasing dNTP availability, T-cell activation results in profound upregulation of glucose uptake and glycolytic flux, a process similar to oncogenic transformation (reviewed in [11] and [12]). As with cancer cells, aerobic glycolysis provides ATP, NADPH and the molecular building blocks for cellular biomass [13], and glycolysis is required to support the replication of a range of viruses (reviewed in [14] and [15]). Similarly, HIV infection of primary T-cells enhances glucose uptake and glycolytic flux [16,17], and the cell surface glucose transporter GLUT1 is required for both T-cell activation and efficient HIV replication [18,19]. The mechanism by which HIV enhances glycolysis has not been elucidated. However, it appears to be specific for primary T-cells, because enhanced glycolysis was not observed in HIV-infected Jurkat or CEM-SS (T-cell) or U937/U1 (monocyte/macrophage) models [16,17], and unlike T-cell activation, does not reflect increased expression of GLUT1 at the plasma membrane [17].

Cutting the amino acid supply

Whilst facilitating HIV replication, T-cell activation also triggers activation-induced cell death (AICD), limiting the life-span of infected cells [20]. Likewise, whilst glucose starvation inhibits virus production, it also prolongs survival of infected cells [17]. HIV-1 replication *in vivo* is therefore likely to be favoured by intermediate levels of T-cell activation [21], and Nef modulates signalling from the T-cell receptor (TCR) at several levels [22]. Attention has focussed on downregulation of the TCR-CD3 T-cell receptor complex by Nef variants of non-pathogenic SIVs, which may limit immune activation by reducing AICD and inflammatory

cytokine release from infected cells [20]. Conversely, the ability to downregulate TCR-CD3 is attenuated in Nef variants of HIV-1 and most Vpu-containing primate lentiviruses [20],

We recently used Tandem Mass Tag (TMT)-based plasma membrane proteomics to gain a comprehensive, unbiased overview of global changes in the cell surface landscape of HIV-1-infected T-cells, including expression timecourses of >800 plasma membrane proteins [23]. As well as HIV-mediated downregulation of numerous immunoreceptors with important functions in T-cell activation, we identified downregulation of a range of transmembrane transporters without well-characterised roles in the immune system, particularly amino acid transporters. Amongst these, the System A transporter SNAT1 is specifically targeted for destruction by Vpu, and phylogenetic analysis has shown that the ability to downregulate SNAT1 arose in Vpu variants from the lineage of SIV_{cpz}/HIV-1 viruses which gave rise to pandemic HIV-1. Since a correlation is observed between the presence of Vpu and the rate of disease progression in natural HIV-1/2 and experimental SHIV (SIV/HIV-1) infections, SNAT1 downregulation may therefore be important for the enhanced pathogenicity of HIV-1 [24-27]. Many γ-retroviruses use amino acid transporters as their principal entry receptors [28], and some viruses (including HCMV) induce and rely on glutaminolysis for virus production and/or host cell survival [14]. Nonetheless, to our knowledge, downregulation of SNAT1 by Vpu is the first example of viral antagonism of amino acid metabolism to be defined at the molecular level.

SNAT1 is dramatically upregulated at the surface of activated primary human $CD4^+$ T-cells and, although best known as a neuronal glutamine transporter, in T-cells mediates quantitatively important transport of alanine, rather that glutamine ([23] and unpublished data). Whilst alanine is a non-essential amino acid, uptake of extracellular alanine by System A transport is required to maintain the intracellular pool of free alanine, explaining why a supply of exogenous alanine is required for optimal T-cell mitogenesis [29,30]. Vpu-mediated targeting of SNAT1, amongst all possible amino transporters, implies a specific role in HIV replication and T-cell metabolism. It is plausible that alanine starvation specifically contributes to a “partially activated” T-cell phenotype, favourable for HIV-replication.

By modulating the composition of the tRNA pool of HIV-1-infected cells, the IFN-inducible restriction factor SLFN11 exploits unusual HIV codon bias to specifically inhibit viral (but not cellular) protein synthesis [31]. How SLFN11 differentially regulates specific tRNAs is unknown, and no viral antagonist has been identified. It remains to be determined whether this process (which operates at the tRNA level) intersects with antagonism of SNAT1 or other amino acid transporters (which operates at the amino acid level). However, the

unusual A-rich nucleotide composition of the HIV genome has long been recognised, and is known to result in biased amino acid composition of HIV proteins [32].

Antagonism of SERINC-mediated restriction

As well as downregulation of SNAT1 by Vpu, we also found downregulation of SERINC3 and SERINC5 by the accessory protein Nef [23], and these proteins were independently identified as potent HIV-1 restriction factors using orthogonal approaches [33,34]. Whilst poorly characterised, SERINC proteins enhance incorporation of serine into membrane phospholipids when over-expressed in E. coli and COS cells, and are therefore proposed to carry serine molecules into the hydrophobic milieu of lipid bilayers for synthesis of phosphatidylserine [35]. It is not currently known whether modulation of phosphatidylserine metabolism is required for SERINC-mediated restriction of HIV-1. However, the SERINC-mediated block to virion infectivity appears to impact viral fusion pore formation or expansion [33,34], a bioenergetically costly process sensitive to phospholipid membrane composition [36]. HIV-1 virions are enriched in phosphatidylserine [37-39], exogenous phosphatidylserine or phosphatidylserine incorporated into enveloped virions regulates the infectivity of numerous viruses including HIV-1 [40-43], and the phosphatidylserine-binding protein TIM-1 inhibits release of HIV-1 virions from the cell surface [44]. Together with SAMHD1 and SLFN11, SERINC proteins may therefore represent a third class of metabolic restriction factors.

Interference with cholesterol efflux

In addition to phosphatidylserine, HIV virions are enriched in cholesterol, reflecting viral budding from raft-like lipid microdomains [37-39]. Membrane recruitment and multimerisation of Gag is dependent on protein-lipid interactions, and leads to cholesterol-dependent reorganisation of lipid rafts and tetraspanin-enriched microdomains [45,46]. Treating producer cells with cholesterol-depleting agents impairs Gag assembly and HIV infectious virus production [47,48], and Nef increases cholesterol levels in macrophages by depleting the ABCA1 cholesterol efflux transporter, enhancing lipid raft formation and virion infectivity [49-52]. Like induction of glycolysis, Nef-mediated modulation of lipid metabolism is likely to be cell-type specific, because ABCA1 expression *in vivo* is seen in macrophages [53], but Nef has also been reported to induce expression of genes involved in cholesterol synthesis and uptake in T-cells [54].

Dysregulation of iron metabolism

As well as downregulating SERINC3/5, ABCA1 and its canonical substrates CD4 and MHC-Ia, Nef has also been reported to downregulate the non-classical MHC-Ib protein HFE [55]. Mutations in HFE cause dysregulation of iron homeostasis in hereditary haemochromatosis, and infection of macrophages with HIV led to Nef-dependent accumulation of intracellular iron [55]. HFE downregulation is not conserved across different HIV/SIV Nef variants [56], however, and the transferrin receptor may be downregulated [55,57] or upregulated [56] depending on the HIV/SIV Nef variants and cell type used. We saw downregulation of the transferrin receptor in HIV-infected T-cells, but this effect was Nef independent, and also seen in the presence of reverse transcriptase inhibitors [23]. Interference with iron metabolism by HIV is therefore likely to be pleiotropic and cell type dependent, and the biological significance remains to be determined.

Conclusions

HIV encodes accessory proteins which regulate the supply of intracellular metabolites, enabling reverse transcription and fine-tuning T-cell activation to optimise viral replication *in vivo*. By encoding 2 accessory proteins (Nef and Vpu) which specialise in the regulation of cell surface proteins, the virus is particularly well placed to modulate flux of small molecules across the plasma membrane. Differential regulation of amino acid transport in CD4+ T-cells is increasingly recognised to be crucial in shaping the immune response [58,59]. The study of pathways targeted by HIV therefore offers unique insights into both viral pathogenesis and critical aspects of immunobiology.

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Figure 1. Evolutionary origins of the HIV accessory proteins

Indicative phylogenetic relationships of selected primate lentiviruses (left panel) and genomic structures of HIV-1 and HIV-2 (right panels) are shown. Viruses encoding Vpr (circles), Vpx (squares) and Vpu (triangles) are indicated, and variants able to antagonise SAMHD1 (Vpr or Vpx; red) and SNAT1 (Vpu; green) are highlighted. Almost all HIV infections are caused by HIV-1 group M viruses, which arose from chimpanzee SIV_{cpz} by cross-species transmission in the Congo river basin in the early 1900s [60]. SIV_{cpz} was itself derived from recombination between red capped mangabey SIV_{rcm} and an ancestral Vpu-containing SIV related to the modern guenon monkey SIV_{gsn}, SIV_{mus} and SIV_{mon} viruses (SIV_{guenon}; reviewed in [61]). This recombination endowed SIV_{cpz}/HIV-1 viruses with Vpu, but restructuring of the C-terminus of Vif to allow antagonism of human APOBEC3G resulted in loss of Vpx [9]. Downregulation of SNAT1 is restricted to Vpu variants of HIV-1 group M viruses and some SIV_{cpz}/HIV-1 group N viruses [23]. A minority of HIV infections are caused by HIV-2 viruses, which arose from sooty mangabey monkey SIV_{smm} by multiple cross-species transmissions in West Africa, and therefore encode Vpx but not Vpu [61]. Regions of HIV-1/2 derived from mangabey $SIV_{rcm/smm}$ (blue) and guenon monkey SIV_{guenon} (tan) viruses are highlighted. All primate lentiviruses encode Vif and Nef, and Vif is also found in other non-primate lentiviral lineages. Adapted from published phylogenetic trees [8,9,23,61] and genomic maps for HIV-1 HXB2 and HIV-2 BEN available from www.hiv.lanl.gov. LTR, long terminal repeat.

Figure 2. Metabolic regulation of the HIV replication cycle

Host factors regulating metabolic pathways required for HIV replication (steps 1-7) are shown (blue) with the metabolic restriction factors SAMHD1 and SLFN11 highlighted (red borders). Viral accessory proteins Vpx, Vpu and Nef (beige) antagonise key host factors to overcome restriction and optimise the intracellular environment for viral replication. SAMHD1 inhibits reverse transcription of the viral genome by reducing the availability of dNTPs, and is targeted for degradation by Vpx [4-7]. SLFN11 inhibits codon-usage-dependent viral protein synthesis by regulating the composition of the tRNA pool [31]. Downregulation of SNAT1 by Vpu restricts the supply of exogenous alanine and inhibits T-cell mitogenesis [23], but the specific downstream consequences of alanine restriction (for example, on the charged tRNA pool) remain to be determined. SERINC3 and SERINC5 are recently described restriction factors, antagonised by Nef, that regulate serine incorporation into membrane phospholipids [23,33-35]. Whether this activity is required for restriction of HIV remains to be determined. ssRNA, single-stranded RNA; dsDNA, double-stranded DNA; dNTPs, deoxynucleotide triphosphates; mRNAs, messenger RNAs; gRNA, genomic RNA; tRNAs, transfer RNAs.

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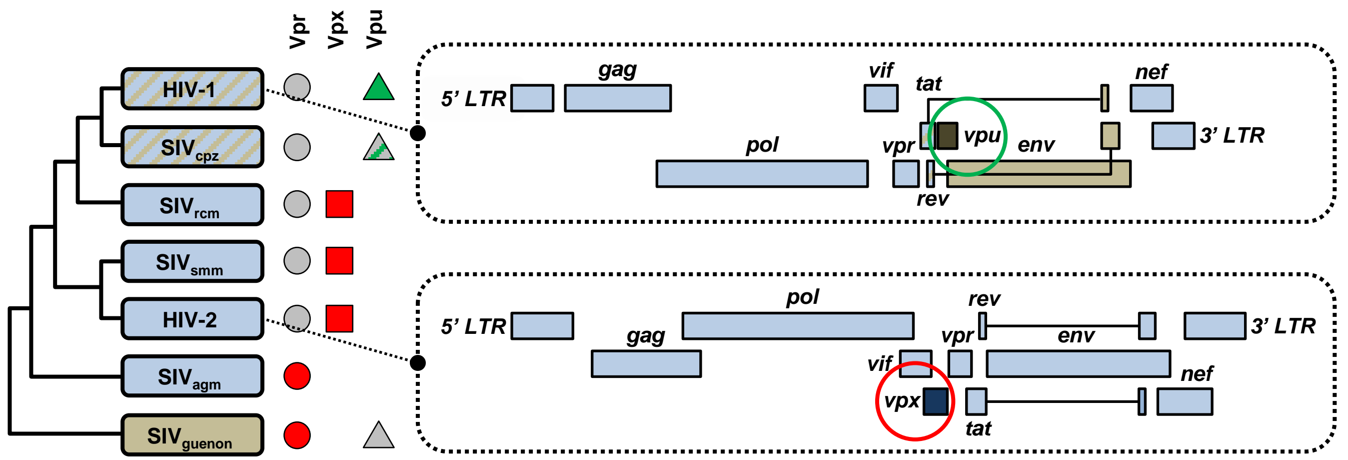
Vpr
Vpx
Vpu
HIV-1
SIV cpz
SIV rcm
SIV smm
HIV-2
SIV agm
SIV guenon
5' LTR
gag
pol
vif
vpr
tat
vpu
rev
env
nef
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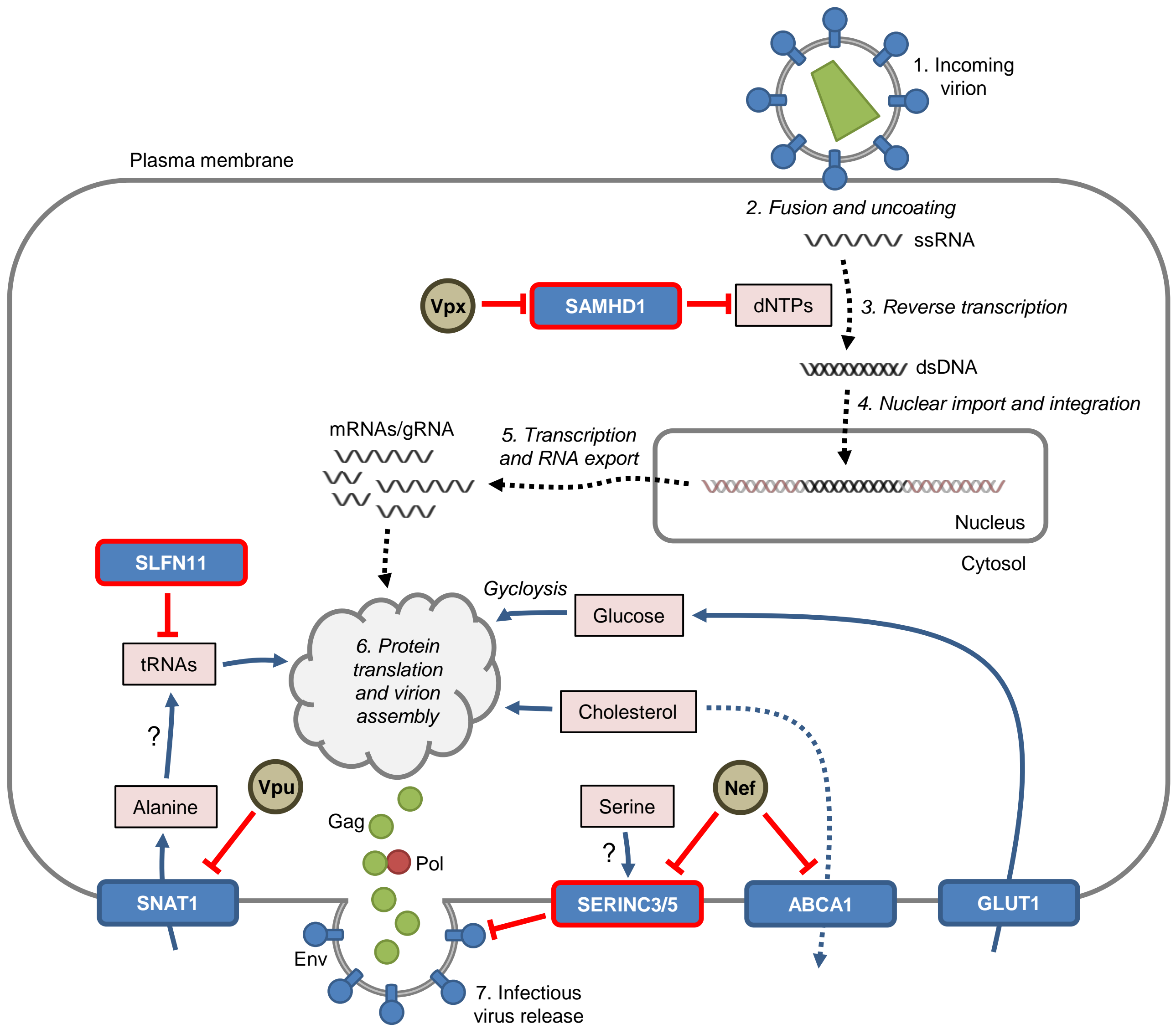
5' LTR
gag
pol
rev
vpr
env
vif
vpx
tat
nef
3' LTR


1. Incoming virion
Plasma membrane
2. Fusion and uncoating
ssRNA
Vpx
SAMHD1
dNTPs
3. Reverse transcription
dsDNA
4. Nuclear import and integration
mRNAs/gRNA
5. Transcription and RNA export
Nucleus
Cytosol
SLFN11
Gycloysis
Glucose
tRNAs
6. Protein translation and virion assembly
Cholesterol
?
Alanine
Vpu
Gag
Serine
Nef
?
Pol
SNAT1
SERINC3/5
ABCA1
GLUT1
Env
7. Infectious virus release

Highlights

- Host restriction factors exploit metabolic dependencies to limit HIV replication
- HIV accessory proteins manipulate cellular metabolism and counteract restriction
- Conflict between SAMHD1 and Vpx regulates the dNTP pool for reverse transcription
- Vpu and Nef downregulate cell surface transporters to control the nutrient supply
- Targeting by HIV identifies critical pathways in T-cell immunometabolism