HIDE, SHIELD AND STRIKE BACK: HOW HIV-INFECTED CELLS AVOID IMMUNE ERADICATION

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Viruses that induce chronic infections can evade immune responses. HIV is a prototype of this class of pathogen. Not only does it mutate rapidly and make its surface components difficult to access by neutralizing antibodies, but it also creates cellular hideouts, establishes proviral latency, removes cell-surface receptors and destroys immune effectors to escape eradication. A better understanding of these strategies might lead to new approaches in the fight against AIDS.

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Despite vigorous innate and adaptive, cellular and humoral immune responses against HIV, this pathogen is almost never eliminated from an infected individual. Similarly, although highly active anti-retroviral therapy (HAART) can control viraemia, high levels of HIV return rapidly after its cessation[1]. Even with HAART, estimates so far indicate that the level of virus decays with kinetics such that it would take more than the lifetime of an individual for the infection to be cleared[2]. With time, HIV also destroys the immune system that is supposed to keep it in check[3]. How does the virus fend off immune attack? 'Forward escape', through mutations that alter recognition of the virus by virus-specific antibodies and cytotoxic T lymphocytes (CTLs), is certainly important. Furthermore, the high degree of glycosylation of the viral envelope and its 'last-second' unfolding at the time of viral entry make HIV difficult to block with neutralizing antibodies. In this review, we concentrate on additional strategies of immune evasion that have been evolved by HIV, namely those that prevent its elimination by the defences of the body — the infection of immunological sanctuaries; the establishment of proviral latency; the perturbation of antigen processing and presentation; and a counter-attack against HIV-specific lymphocytes.

HIV in context

The common cold, influenza and many acute diarrhoeas are rapidly resolving diseases, because their aetiological agents are cytopathic viruses that are cleared quickly by the immune system. In these infections, viruses kill some target cells; but innate immune effectors, such as interferons, erect barriers to virus replication rapidly, antibodies are produced to block the further spread of infectious particles, and cellular immunity eliminates any remaining infected cells. If no complications, such as a bacterial super-infection, occur and if the virus does not have special pathogenic features (two conditions that were not fulfilled during the epidemics of Spanish influenza)[4], infected individuals recover rapidly, and they conserve, at least for a while, immunity that prevents re-infection with the same viral strain.

By contrast, once inside a host, many other viruses are there to stay[5]. Such agents — for example, members of the herpesviridae and retroviridae families — usually have three properties. First, they have mechanisms to ensure the long-term persistence of their genetic information in cells. The genome of herpes simplex virus remains as a stable episome in non-dividing neurons. Epstein–Barr virus, another herpes virus, couples the replication of its genome with the replication of cellular DNA. Retroviruses, amongst them HIV, integrate their genes irreversibly into cellular chromosomes. Second, persistent viruses refrain from killing their host cells too systematically; that is, they modulate their cytopathicity in a time- or target-specific manner. Third, such pathogens evade, to an extent, the immune response that is mounted against them[6]. For these viruses, symptoms or the absence thereof indicate the agent's propensity to express its pathogenic potential or the immune system's ability to block these manifestations, respectively.

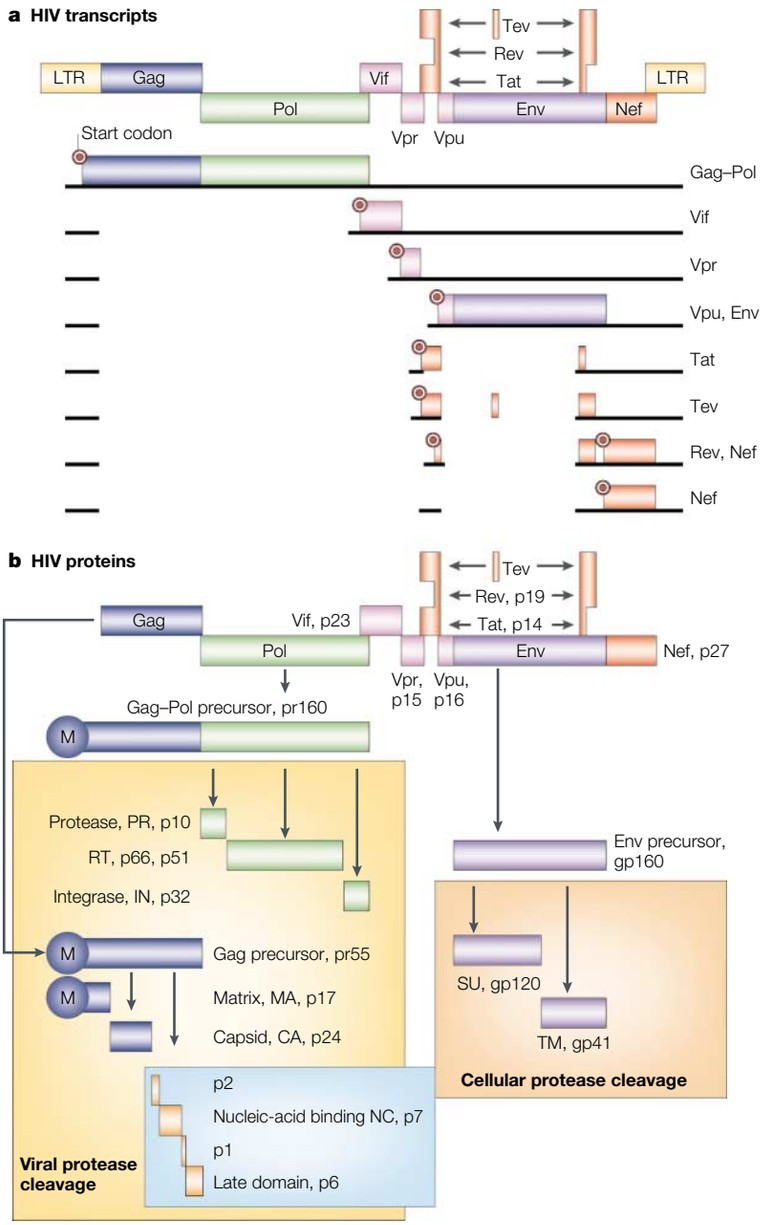

Figure 1 | **The HIV genome, transcripts and proteins. a** | HIV transcripts. Integrated into the host chromosome, the 10-kb viral genome contains open reading frames for 16 proteins that are synthesized from at least ten transcripts. Black lines denote unspliced and spliced transcripts, above which coding sequences are given, with the start codons indicated. Of these transcripts, all singly spliced and unspliced transcripts shown above those encoding the transcriptional transactivator (Tat) require regulator of virion gene expression (Rev) for their export from the nucleus to the cytoplasm. The RNA target for Rev, the Rev response element (RRE), is contained in the gene encoding envelope protein (Env). **b** | HIV proteins. Group-specific antigen (Gag) and Gag–Pol (polymerase) polyprotein precursors are processed by the viral protease into nine subunits: protease (PR), reverse transcriptase (RT), which contains RNAse H, integrase (IN), matrix (MA), capsid (CA), p2, nucleocapsid (NC), p1 and p6 (shown in the yellow box). Env is cleaved by cellular proteases, such as furin, into surface (SU) gp120 and transmembrane (TM) gp41 moieties (shown in the orange box). Tat is the main transcriptional regulator of the long terminal repeat (LTR). Its RNA target, the transactivation response (TAR) element, is present at the 5′ end of all viral transcripts. Rev is the main nuclear-export protein and it regulates the shift between early and late viral gene expression. The viral-infectivity factor (Vif), viral protein r (Vpr), viral protein u (Vpu) and negative effector (Nef) proteins are known as accessory proteins because they are dispensable for viral growth in some cell-culture systems. Nevertheless, they have essential roles in viral replication and progression to AIDS *in vivo*. Arrows below polyprotein precursors point in the direction of their processing to mature proteins. Tev contains Tat, Env and Rev sequences and functions as Tat and Rev.

Although all of the proteins that are encoded by HIV have a role in its replication and transmission — for example, by promoting viral gene expression, by providing the virion with essential structural and enzymatic components, or by altering the intracellular environment to the benefit of the virus — several of these proteins have additional functions that are directed towards immune escape[7]. To contrast the roles of these proteins, we need to understand first how they affect the replicative cycle of HIV.

The basics of HIV replication

HIV (HIV-1) is a human retrovirus that is related most closely to other animal lentiviruses[8]. Its genome is 10 kb in length and encodes 16 distinct proteins[9] (FIG. 1). Those proteins that are derived from the *gag* (group-specific antigen) *pol* (polymerase) and *env* (envelope) genes are classical structural and enzymatic factors that are required by all retroviruses. In addition, HIV encodes two regulatory proteins, the transcriptional transactivator (Tat) and the regulator of virion gene expression (Rev). Finally, the virus contains four genes that encode so-called accessory proteins: the ill-named 'negative effector' (Nef), viral infectivity factor (Vif), and the viral proteins r (Vpr) and u (Vpu). Viral proteins are synthesized from more than 30 messenger RNA species (eight of which are shown in FIG. 1), which are all derived from the same primary transcript. Whereas transcripts encoding the early, mostly regulatory, proteins Tat, Rev and Nef are fully spliced, those that encode the late viral proteins, which are mainly structural and enzymatic components of the virion and factors that fine-tune infectivity, are singly spliced or unspliced. Rev regulates the transition between the early and late phases of viral gene expression by allowing the transport of singly spliced and unspliced viral mRNA species from the nucleus to the cytoplasm. Tat and Nef are not only crucial for high levels of HIV replication, but also have important roles in promoting viral immune evasion (see later).

HIV enters the body through the exchange of bodily fluids, and it infects mainly T helper (T_H) cells, macrophages and, to some extent, MICROGLIAL CELLS and dendritic cells (DCs). This tropism is determined at the level of viral entry by the use of CD4 as a primary receptor and the use of co-receptors that are strain and target specific (FIG. 2). R5 strains of HIV use CC-chemokine receptor 5 (CCR5) as their co-receptor and can, therefore, enter macrophages, DCs and T cells, whereas X4 strains of HIV use CXCR4 as a co-receptor and can infect T cells only[10]. Early in infection, only R5 viruses can be detected in infected individuals. At this stage, it might be that the virus needs to transit through DCs and macrophages, which, in turn, could pass the virus to $CD4^+$ T cells — for example, during the process of antigen presentation. With time, X4 viruses come to predominate, which hastens the demise of T_H cells, the hallmark of AIDS[3]. DCs are likely to have an important role in transporting the virus from its portal of entry to lymphoid organs. These cells can be productively infected by HIV or they can capture the virus through DC-specific ICAM3-grabbing non-integrin (DC-SIGN;

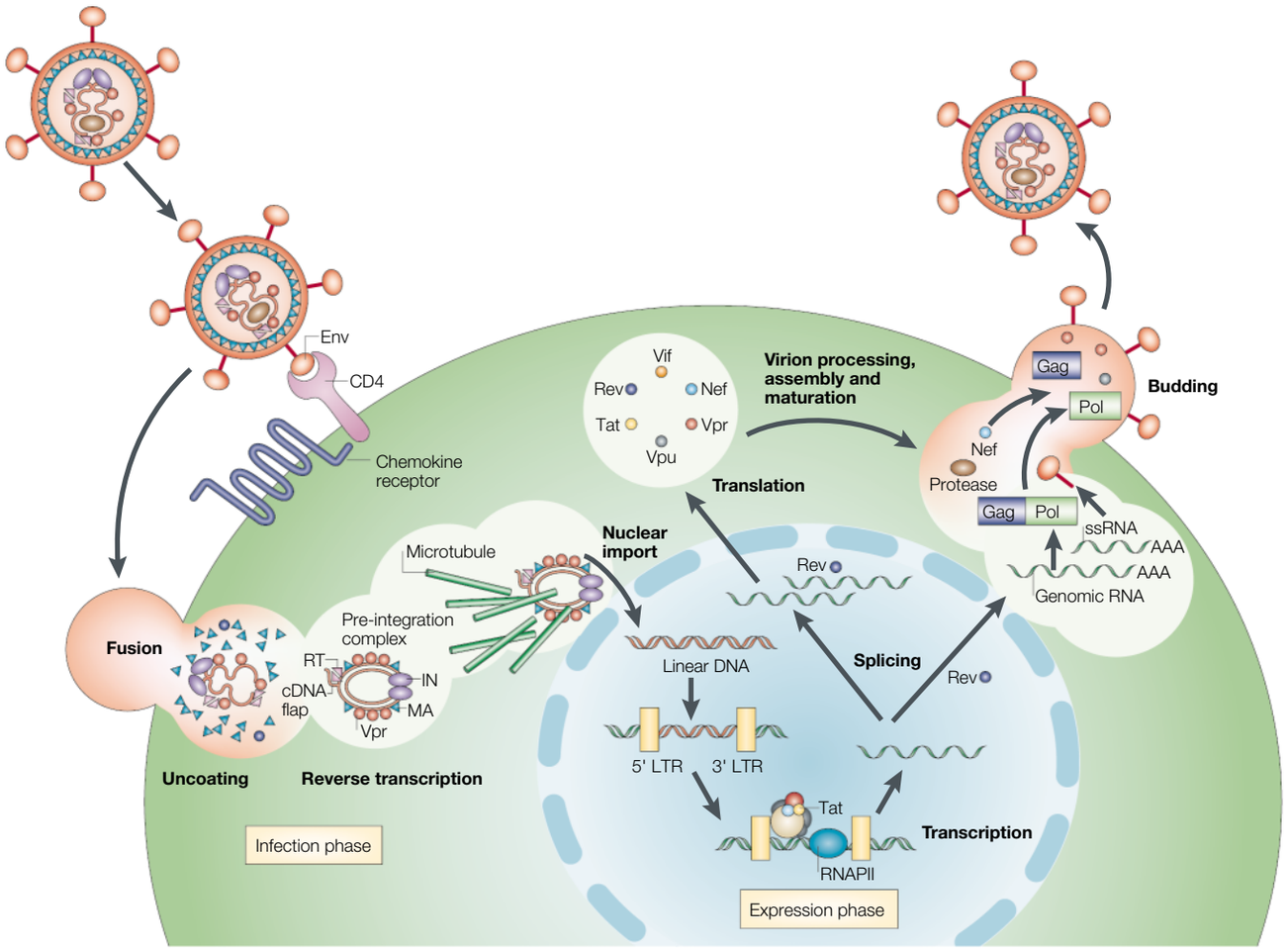

Figure 2 | **The replicative cycle of HIV.** The viral envelope protein (Env) of HIV binds CD4 first, undergoes a conformational change, then binds one of two chemokine receptors — CCR5 (R5 strains) or CXCR4 (X4 strains) — and enters cells by fusion of the viral and cellular membranes. Uncoating of the viral capsid releases the PRE-INTEGRATION COMPLEX, which is recognized by the cellular nuclear-transport machinery and is routed to nucleopores, seemingly along the microtubular network. Reverse transcription begins, yielding double-stranded viral complementary DNA — one and two long terminal repeat (LTR)-containing circles, as well as linear forms — of which only the linear form integrates into the host genome. Because of the large number of nuclear-localization signals on integrase (IN), matrix (MA) and viral protein r (Vpr) proteins, and with the help of the cDNA flap, the pre-integration complex can enter the nucleus without cell division. Integration is mostly into active euchromatin. At this point, the 5′ LTR behaves like any eukaryotic promoter, and the 3′ LTR acts as the polyadenylation and termination site. Cellular activation increases the level of transcription of the provirus, which is augmented greatly by the viral transcriptional transactivator protein (Tat). Regulator of virion gene expression (Rev) transports singly spliced (ss) and unspliced genomic transcripts from the nucleus to the cytoplasm. Viral structural and enzymatic proteins are synthesized and transported to the plasma membrane, where they localize in lipid rafts. Negative effector (Nef) facilitates this viral assembly. The four accessory proteins — Nef, viral infectivity factor (Vif), Vpr and viral protein u (Vpu) — and two regulatory proteins (Rev and Tat) are represented by coloured circles. Late domains in group-specific antigen (Gag) then recruit components of multivesicular bodies to the site of budding, so that progeny virions are released from the infected cell. Arrows point in the direction from infection to the production of new progeny virions. Pol, polymerase; RNAPII, RNA polymerase II; RT, reverse transcriptase.

MICROGLIAL CELLS
Resident brain macrophages. Bone-marrow-derived cells that express CD4 and chemokine receptors

PRE-INTEGRATION COMPLEX
A large complex of viral complementary DNA, integrase (IN) protein, matrix (MA) protein, reverse transcriptase (RT), viral protein r (Vpr) and host proteins that is docked at the nuclear envelope. The viral genome then crosses the nucleopore, together with an as-yet-undefined set of these proteins, before integrating into host chromosomes.

a lectin-like receptor) and store it in an infectious form before 'regurgitating' it to T cells that simultaneously become primed for infection[11,12]. Once internalized, HIV is uncoated, and its RNA genome is reverse transcribed to a double-stranded complementary DNA that is integrated into the chromosome of the target cell, yielding the long terminal repeat (LTR)-flanked provirus. Unlike other retroviruses, HIV does not require disintegration of the nuclear membrane during cell division to enter the nucleus. Instead, several nuclear-localization signals on integrase (IN)[13], matrix (MA)[14] and Vpr[15] proteins ensure that the viral genome passes through the nuclear pores. The linear double-stranded cDNAs have IN and chromatin-remodelling complexes at their termini[16]. They integrate in the genome with a preference for active genes, although other regions, for example heterochromatin-rich centromeric regions, are targeted also[17,18]. However, in resting lymphocytes, there are several barriers that preclude the completion of these early steps — for example, reverse transcription is inefficient and energy levels are probably too low for effective nuclear import — and double-stranded

TATA BOX
A highly conserved DNA sequence (consensus TATAA) that is found in the promoter of many (mainly rapidly transcribed) cellular and viral genes, 25–35 bases upstream of the RNA start site.

POSITIVE TRANSCRIPTION ELONGATION FACTOR B (P-TEFb). This complex consists of the carboxy-terminal domain kinase CDK9 and the C-type cyclin CYCT1, CYCT2a, CYCT2b or CYCK. It is required for the elongation of transcription.

NEGATIVE TRANSCRIPTION ELONGATION FACTOR (N-TEF). This complex consists most probably of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB)-sensitivity-inducing factor (DSIF) and negative elongation factor (NELF); the subunit containing arginine–glutamate repeats (RD) binds TAR.

viral genomes accumulate without integrating[19–24]. Nevertheless, once they have been activated even partially, T cells become fully permissive for HIV infection[25–27]. The nature of the induced factors that are responsible for the acquisition of HIV permissiveness is not known yet.

Once integrated in the host genome, the provirus behaves like any human gene, with transcription being initiated at the 5′ end and terminating at the 3′ end[28]. The LTR contains enhancer and promoter sequences, with binding sites for several transcription factors, and a polyadenylation signal (FIG. 3). Moving upstream from the transcription start site, the initiator (Inr), the TATA BOX and three SP1-binding sites are found[28]. These elements position RNA polymerase II (RNAPII) at the correct site for initiating transcription. Further upstream is the enhancer, which binds nuclear factor-κB (NF-κB) and nuclear factor of activated T cells (NFAT), as well as members of the ETS family of transcription factors[28]. These activators ensure that the virus replicates at a high level in activated T cells and differentiated macrophages. The most unusual feature of the LTR is the presence of a strong regulatory element located 3′ to Inr. This RNA structure, which is found at the 5′ end of all viral transcripts, is known as the transactivation response (TAR) element and it binds Tat[29]. In the absence of Tat, HIV transcription begins, but elongation is inefficient[28]. Tat and its cellular co-factor, POSITIVE TRANSCRIPTION ELONGATION FACTOR B (P-TEFb), cooperate to bind TAR with high affinity, allowing RNAPII to produce full-length viral transcripts[30,31] (FIG. 4). P-TEFb contains two components — cyclin T1 (CYCT1) and cyclin-dependent kinase 9 (CDK9). Once recruited to the nascent HIV RNA, CDK9 phosphorylates the carboxy-terminal domain of RNAPII and NEGATIVE TRANSCRIPTION ELONGATION FACTOR (N-TEF)[32], thereby allowing efficient elongation. The ability of Tat to recruit P-TEFb through an RNA sequence is unique among transcriptional activators, and it renders HIV replication particularly sensitive to inhibition by compounds that target CDK9 (REF. 33). By contrast, Tat is inefficient at recruiting RNAPII, and it requires a strong basal promoter for optimal effects[28]. NF-κB, which also recruits P-TEFb, can substitute partially for Tat[34]. Indeed, in activated peripheral-blood mononuclear cells (PBMCs), mutant viruses that have mutations of TAR still replicate[35]. Nevertheless, Tat leads to higher levels of gene expression and is essential for HIV replication in the host. Furthermore, Tat and P-TEFb affect the ability of HIV to establish latency and affect the transcription of MHC class II genes (see later).

The timely production of viral gene products requires Rev. To function optimally, this protein needs to reach threshold levels[36], the splicing of genomic transcripts must be slow and an active CRM1/RANGTP COMPLEX must be present in the cell[37]. After their translation, viral structural and enzymatic proteins travel to the plasma membrane, where immature virions assemble in cholesterol-rich lipid rafts[38]. The carboxy-terminus of Gag, p6, is ubiquitylated[39], and it recruits components of multivesicular bodies, such as tumour-susceptibility gene 101 (TSG101) and vacuolar protein sorting 4 (VPS4)[40–42], which facilitate the release of progeny virions from the cell. Processing of Gag and Gag–Pol yields mature HIV particles. Some accessory viral proteins (such as Vpr and Nef), as well as cellular components (for example, MHC class I and II molecules, and some CD proteins), are incorporated into virions[43]. The envelope glycoprotein is an essential viral component that allows the infection of new cells. The high cholesterol content of the virion, which is a consequence of its budding through rafts, is crucial for this process as well[44,45].

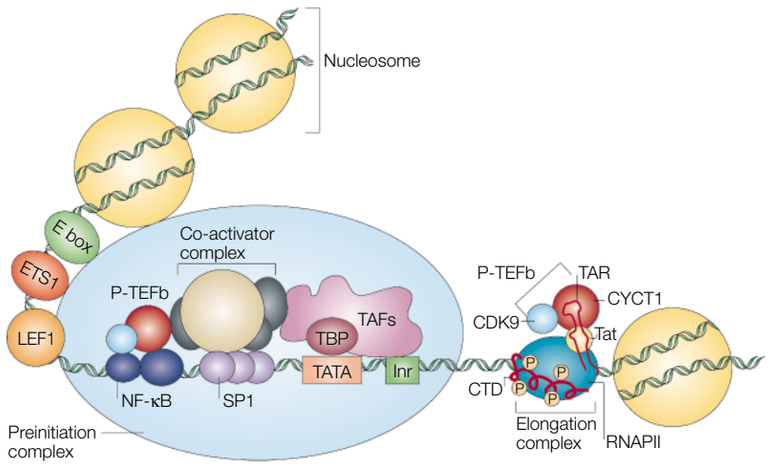

Figure 3 | **The HIV long terminal repeat.** The viral promoter contains proximal (core) and distal (upstream) promoter elements, as well as enhancer sequences and the transactivation response (TAR) element. The core promoter consists of the initiator (Inr) and TATA box (TATA). TATA-binding protein (TBP) and TBP-associated factors (TAFs) bind the core promoter. They are flanked upstream by three SP1-binding sites and downstream by the TAR RNA structure. The co-activator complex binds SP1, and together, they recruit and position RNA polymerase II (RNAPII) in the PRE-INITIATION COMPLEX on the HIV long terminal repeat (LTR). RNAPII then clears the promoter. Positive transcription elongation factor b (P-TEFb), which consists of cyclin T1 (CYCT1) and cyclin-dependent kinase 9 (CDK9), and transactivator (Tat) bind the 5′ bulge and central loop in TAR. After transcription passes TAR, this recruitment results in extensive phosphorylation of the carboxy-terminal domain (CTD; 52 heptapeptide repeats of YSPTSPS) of RNAPII and of negative transcription elongation factor (N-TEF; which consists of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB)-sensitivity-inducing factor, DSIF, and negative elongation factor, NELF) (not shown). This phosphorylation converts an initiating transcription complex to an elongating transcription complex. The enhancer binds members of the nuclear factor-κB (NF-κB), nuclear factor of activated T cells (NFAT) and ETS families. In the absence of Tat, NF-κB also recruits P-TEFb, thereby elongating HIV transcription. So, TAR can be viewed as an RNA enhancer. LEF1, lymphoid enhancer-binding factor 1.

HIV hides

Infection of immune-privileged sanctuaries. If a virus fails to express its genetic information or if it resides in immune-privileged cellular sanctuaries without inducing lethal cytopathic effects, it can establish a persistent infection. Herpesviruses and a few other viruses can be re-activated by stress or intercurrent infections[5,6]. However, they are prevented from uncontrolled spread by the immune system. HIV has evolved similar strategies (as have other lentiviruses), which contribute greatly to the chronicity of the infection. HIV can hide from the immune system in at least two sites: microglial cells of the central nervous system, where cell-mediated responses are reduced normally, and resting T cells[2]. In these latter cells, as well as perhaps in some other targets, HIV can adopt a state of proviral latency.

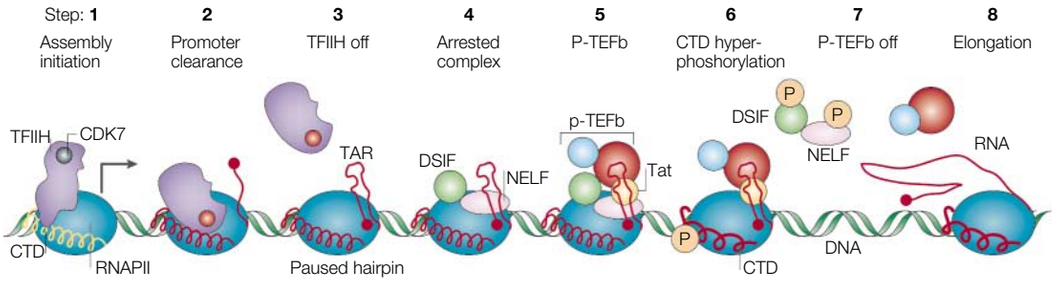

Figure 4 | **Mechanism of Tat transactivation.** Activators that bind the promoter recruit RNA polymerase II (RNAPII) to the long terminal repeat (LTR). In the pre-initiation complex, the unphosphorylated carboxy-terminal domain (CTD) of RNAPII, which is shown as a yellow coil, binds mediators. Together with the general transcription factor TFIIH, which contains DNA-helicase and CTD-kinase activities, RNAPII clears the promoter and starts copying the viral genome. Cyclin-dependent kinase 7 (CDK7) in TFIIH is shown as a grey changing to red ball, indicating its activation as a kinase. The partially phosphorylated RNAPII arrests at or near the transactivation response element (TAR), synthesizing TAR and/or an alternative paused hairpin. 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB)-sensitivity-inducing factor (DSIF) and negative elongation factor (NELF) then ensure that RNAPII does not elongate. RD — so named for its many repeats of arginine and glutamate residues — in NELF contains an RNA-recognition motif that binds the stem in TAR. For formation of the tripartite complex with transcriptional transactivator (Tat) and TAR, positive transcription elongation factor b (P-TEFb), which contains cyclin T1 (CYCT1) and CDK9, must be free of 7SK RNA, and CDK9 must be autophosphorylated. After its recruitment to TAR, P-TEFb phosphorylates suppressor of Ty 5 (SPT5) in DSIF and RD in NELF, and completes the phosphorylation of the CTD of RNAPII, thereby modifying RNAPII for efficient elongation. The phosphorylated CTD now binds elongators, which consist of capping enzymes, splicing aparatus and polyadenylation factors. Efficient elongation of transcription and viral replication ensue. The change in colour of the CTD from yellow to red and its increased thickness indicate increased levels of phosphorylation.

HIV proviral latency. In the early 1990s, it was realized that the long asymptomatic period that follows HIV infection and precedes AIDS is not due to complete viral latency, but is instead accompanied by high levels of viral replication[46,47]. This prompted many researchers to dismiss the concept of proviral latency, and it raised hopes for the discovery of a quick medical cure for the infection. However, much pre-existing evidence called for caution. For example, it was known already that a far greater number of PBMCs harbour HIV proviruses than harbour transcripts[48]. Furthermore, cell lines had been derived that synthesize little or no viral RNA unless stimulated. In two of these models — monocytoid U1 cells and T-lymphoblastoid ACH2 cells — mutations of Tat and TAR accounted for this transcriptionally silent, but inducible, phenotype[49,50]. In one study, multiply spliced viral RNA species predominated in the peripheral blood of asymptomatic infected individuals[51]. In another study, proviral latency could be detected at seroconversion, as indicated by the selective accumulation of non-elongated HIV transcripts, corresponding to TAR, in untreated infected individuals[52]. Furthermore, fully competent virus could be recovered by stimulating these resting PBMCs[52]. The clinical importance of this proviral latency was confirmed by the finding that optimal HAART cannot eradicate HIV, even when viral replication remains undetectable for long periods of time. Indeed, a small proportion of memory T cells harbour intact proviruses that can be induced to produce infectious virus readily after cell activation[2,53–55]. As a large proportion of memory T cells cycle at any given time[56,57] and low levels of viral replication persist[58–60], this proviral reservoir most probably expands, or at least is maintained, despite the progressive loss of a fraction of latently infected cells. Additional targets that harbour latent viruses might include DCs, monocytes, astrocytes, microglia and seminal cells[2,56,57,61,62].

Mechanisms of proviral latency. Both cellular and viral factors can contribute to proviral latency. Amongst the latter, Tat seems to have a pivotal role. First, HIV occasionally integrates into regions of the genome where transcription is barely active or inactive, unless the cell is activated[17]. In these heterochromatin regions, neither initiation nor elongation of transcription is seen. Second, levels of NF-κB and NFAT in resting cells might be too low to recruit P-TEFb to the LTR[63]. So, only the initiation of viral transcription is observed. Third, levels of the Tat co-factor CYCT1 are low in resting T cells, and increase after their activation[63]. Moreover, other cellular factors modulate the activity and/or distribution of P-TEFb. Whereas CDK9 is active when bound only to CYCT1, CYCT2 or CYCK[32], it is inactive when part of a much larger, 500-mDa complex that contains, in addition, the small 7SK RNA and several unidentified proteins[64,65]. Interestingly, stress and ultraviolet light dissociate P-TEFb from this 7SK RNA complex[64,65]. At this point, CDK9 is active, but unless it is autophosphorylated on up to five serine and threonine residues near its carboxyl terminus, it does not form the tripartite complex between P-TEFb, Tat and TAR[66,67]. Moreover Tat–SF1 (splicing factor 1) complexes must engage the carboxyl terminus of CYCT1 and relieve intramolecular interactions between its amino and carboxyl termini that block intermolecular interactions with TAR and RNAPII[67]. Finally, P-TEFb is ubiquitylated, which could strengthen these RNA–protein and protein–protein interactions[68]. All of these events are thought to facilitate the ability of P-TEFb to modify RNAPII and promote the elongation of transcription. Moreover, they counteract the effects of N-TEF, which consists of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB)-sensitivity-inducing factor (DSIF)[69] and negative elongation factor (NELF)[70]. DSIF consists of suppressor of Ty protein 4 (SPT4) and SPT5, of which

PRE-INITIATION COMPLEX
The transcription complex that is recruited to promoters through activators, consisting of RNA polymerase II and mediators that bind its unphosphorylated carboxy-terminal domain.

CRM1/RANGTP COMPLEX
A complex that transports proteins containing a nuclear-export signal from the nucleus to the cytoplasm. The cargo is released in the cytoplasm after the hydrolysis of GTP to GDP.

SPT5 is phosphorylated by P-TEFb[71,72]. The level of expression of SPT5 correlates with lower basal levels of viral gene expression and increased Tat transactivation[73,74]. The smallest subunit of NELF, RD, also binds the RNA stem in TAR and could contribute to maintenance of the arrested transcription complex at or near TAR[75]. Of note, marked decreases in the level of basal transcription from the LTR also affect the quantity and function of Tat negatively, which impacts further on the expression of viral genes. It is not clear whether Rev-based partial latency — in which early, but not late, viral gene products accumulate[76] — contributes to perpetuating the infection by sheltering cells from the immune system. Indeed, in the absence of Rev, although late viral proteins are not produced, their early counterparts — Nef, Rev and Tat — are still present. These early products do elicit immune responses, but CTLs that are specific for them can be lost rapidly[77].

HIV makes the infected cell invisible

Normally, cells that are infected by a virus are recognized and eliminated by the immune system. This is due mainly to the surface presentation of viral peptides by MHC class I molecules, which allows for recognition and killing by virus-specific CTLs. Viruses that establish long-term infections, such as members of the herpes virus family, typically alter MHC class-I-mediated antigen presentation[6]. Not unexpectedly, this effect is mediated usually by proteins that are expressed early in the viral replicative cycle, ensuring that replication can proceed in a host cell that is protected from immune destruction. HIV is no exception, as it decreases the expression of MHC class I molecules on the cell surface through Nef, a viral protein that is expressed immediately after integration[78]. To some extent, HIV-infected cells escape detection by CTLs in this manner, which most probably contributes to the chronicity of the infection[79].

MHC class I molecules form a heterodimeric complex, which contains a highly polymorphic, membrane-anchored heavy chain non-covalently associated with β2-microglobulin (β2-m). The assembly of these two components and the loading of antigenic peptides that are generated by the proteasome occur in the endoplasmic reticulum (ER), and the secretory pathway transports only fully assembled complexes to the cell surface[80]. Viruses can interfere with various stages of this process. For example, the herpes simplex virus protein ICP47 inhibits the function of transporter associated with antigen presentation (TAP), the peptide transporter that translocates antigenic determinants from the cytosol to the ER and mediates their loading onto MHC class I molecules[81]. Similarly, distinct proteins that are encoded by the unique short (US) region of human cytomegalovirus (HCMV) can independently block the function of TAP, dislocate MHC class I heavy chains from the ER to the cytosol or retain MHC class I complexes in the ER[82].

Nef does not affect these early events, but it interferes subsequently with the migration to the cell surface and persistence of MHC class I molecules[78,83] (FIG. 5). Nef is a cytoplasmic protein of approximately 200 amino acids in length that is recruited to the cell membrane through amino-terminal myristoylation. Being mainly a conglomerate of protein–protein interaction domains, Nef does not have any enzymatic activity, and it carries out its functions by establishing connections between its targets and effectors, which are usually part of trafficking or signalling pathways. So, Nef decreases the expression of CD4, the primary receptor for HIV, on the surface of infected cells, thereby ensuring that released virions are fully infectious by making sure that viral gp120 is free to bind CD4 and chemokine receptors on recipient cells[84]. To achieve this, it triggers the sequential accelerated endocytosis and lysosomal degradation of CD4 by connecting the cytoplasmic domain of CD4 with CLATHRIN-COATED PITS at the plasma membrane and with the COPI coatomer, another coat structure, in early endosomes[85–89].

In the presence of Nef, MHC class I molecules are diverted from the cell surface towards endosomes, from where they are retrieved to the trans-Golgi network (TGN)[70,83,90–92]. This effect is blocked by inhibitors of phosphatidylinositol 3-kinase (PI3K)[83], and it involves the binding of Nef to phosphofurin acidic cluster sorting protein 1 (PACS1)[93,94]. Interestingly, PI3K is important also for the incorporation of mannose 6-phosphate receptor (M6PR) into TGN-derived clathrin-coated vesicles[95]. Furthermore, PACS1 controls the endosome-to-Golgi trafficking of furin and M6PR by connecting these molecules with the adaptor-protein complex of endosomal clathrin-coated pits[96]. So, Nef seems to decrease the expression of MHC class I molecules by hijacking the furin–M6PR transport pathway. Recent evidence indicates further that after interacting with PACS1, Nef activates the GTPase ADP ribosylation factor 6 (ARF6) through PI3K and the guanosine-exchange factor ARNO, thereby triggering the clathrin-independent routing of MHC class I molecules from the cell surface to the so-called 'ARF6 endosomal compartment', followed by their retrieval to and trapping in the TGN[97]. By analogy with the removal of CD4, it is probable that Nef functions as a physical bridge between MHC class I molecules and this pathway. However, direct binding between Nef and MHC class I molecules awaits formal demonstration.

Natural killer (NK) cells usually destroy cells that are devoid of surface MHC class I molecules. HIV avoids this trap by decreasing the expression of HLA-A and HLA-B, but not of HLA-C and HLA-E, which bind inhibitory receptors on NK cells[98]. This target specificity resides in amino-acid differences between the cytoplasmic tails of Nef-sensitive and Nef-resistant HLA molecules[99], which provides further evidence in favour of direct binding between Nef and MHC class I molecules.

By acting at the level of transcription, another HIV protein, Tat, can decrease the expression of MHC class I molecules[100], possibly through its ability to bind TATA-box-binding protein (TBP)-associated factor 250 ($TAF_{II}250$)[101]. $TAF_{II}250$ is part of the $TF_{II}D$ complex that interacts with the TATA box and/or Inr, and it has intrinsic kinase and histone acetyl transferase (HAT) activities. However, it is unclear how such an effect of Tat, possibly mediated by blocking the HAT activity of

CLATHRIN-COATED PITS
Subdomains of the plasma or endosomal membranes that are involved in endocytosis.

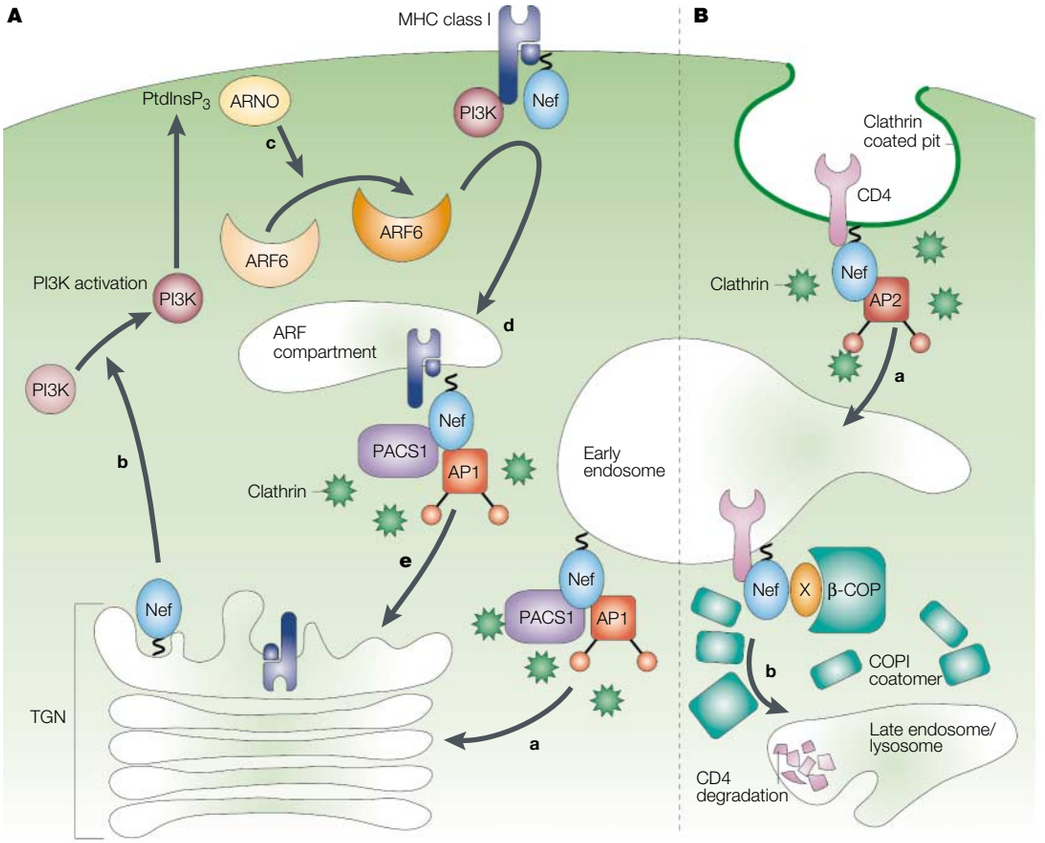

Figure 5 | **Nef-induced downregulation of expression of MHC class I molecules and CD4.** Models are proposed in which the sequential steps governing the downmodulation of expression of MHC class I molecules and CD4 are illustrated. **A** | Negative effector (Nef) accelerates the endocytosis of MHC class I molecules through the phosphofurin acidic cluster sorting protein 1 (PACS1)/phosphatidylinositol 3-kinase (PI3K)-dependent activation of ADP ribosylation factor 6 (ARF6)-mediated endocytosis (**a**). Nef is probably targeted first to the trans-Golgi network (TGN), where it acquires the ability to activate PI3K (**b**). The formation of phosphatidylinositol-3,4,5-triphosphate ($PtdInsP_3$) ensues, which recruits the guanosine exchange factor ARNO to the plasma membrane, where ARF6 becomes activated (**c**). Together with Nef, the latter mediates the internalization of MHC class I molecules from the plasma membrane to an ARF6-specific early endosomal compartment (**d**). From there, MHC class I molecules are retrieved to the TGN, where they remain trapped (**e**). To explain the target specificity of this process, an interaction between Nef and the cytoplasmic tails of HLA-A and HLA-B is probable, although it remains to be shown formally. **B** | The two steps of Nef-induced CD4 downmodulation. At the plasma membrane, Nef connects the cytoplasmic tail of CD4 with clathrin-coated pits through an interaction with adaptor protein 2 (AP2) and the vacuolar ATPase (v-ATPase), triggering rapid endocytosis of the CD4 receptor (**a**). In the early endosome, Nef then interacts with the COPI coatomer, which targets CD4 for lysosomal degradation (**b**). An as-yet-unidentified co-factor (X) potentiates the interaction between Nef and the COPI coatomer. Activation of PI3K and ARF6 is shown as a change in colour from light to dark.

$TAF_{II}250$, could interfere only with the transcription of MHC class I genes. Moreover, a recent report disputes these findings[102].

HIV strikes back against immune effectors

Infection with HIV is the tale of a prey that not only hides out and manages to slip between the claws of its hunter, but that also strikes back. *In vivo* infection of lymphatic tissue with HIV is accompanied by enhanced apoptosis, which affects mainly bystander cells[103,104]. This effect might result, at least in part, from the Nef-induced upregulation of expression of FAS ligand (FASL) on the surface of infected cells, a phenomenon for which the mechanism remains unclear but seems to involve the T-cell receptor (TCR) pathway[105] (FIG. 6). FASL on the surface of HIV-infected cells could interact with FAS molecules displayed on neighbouring cells, including virus-specific CTLs, thereby triggering their apoptosis. Furthermore, recent analyses of HIV-infected patients have shown that HIV-specific CTLs are more prone to FAS-mediated apoptosis than are HCMV-specific CTLs from the same individuals[104]. This preferential killing might explain, in part, the accumulation of incompletely mature HIV-specific CTLs in infected individuals[105,106].

Other FAS-related events contribute to HIV-induced cell death of bystander lymphocytes. For example, crosslinking of CD4 by the HIV envelope in the presence of soluble Tat can induce the expression of FASL and the apoptosis of uninfected cells[107]. Furthermore, interaction of Env with CXCR4 on macrophages leads to the death of bystander $CD8^+$ T cells through the

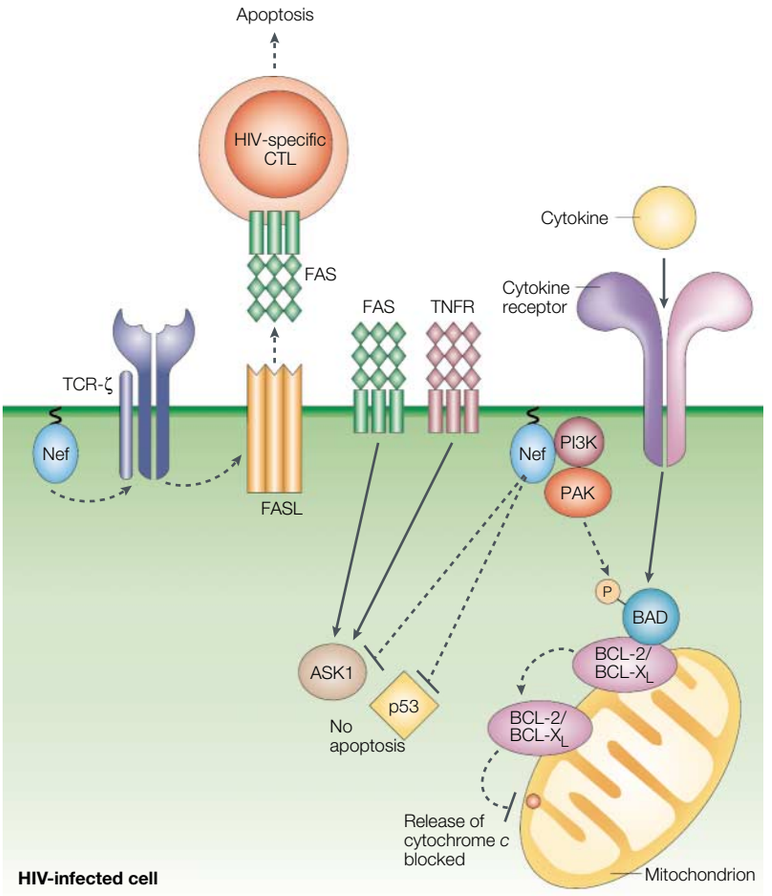

Figure 6 | **HIV and apoptosis.** Through an as yet poorly characterized process that involves interaction with the T-cell receptor ζ-chain (TCR-ζ), negative effector (Nef) stimulates expression of FAS ligand (FASL) on the surface of HIV-infected cells. When virus-specific cytotoxic T lymphocytes (CTLs) come into contact with their targets, they are killed by FAS–FASL-induced apoptosis. By contrast, in infected cells, Nef blocks apoptotic pathways mediated by FAS and tumour-necrosis factor receptor (TNFR) (through inhibition of apoptosis signal-regulating kinase 1, ASK1) and by p53 (through direct binding), and unleashes the anti-apoptotic effects of BCL-2 and BCL-X_L (by inducing the PAK-mediated phosphorylation of BAD, releasing the anti-apoptotic effectors, and thereby mimicking cytokine-induced signals). BAD, BCL-2-antagonist of cell death; PAK, p21/Cdc42/Rac1-activated kinase; PI3K, phosphatidylinositol 3-kinase. Dashed arrows indicate events that are triggered by Nef.

induction of expression of tumour-necrosis factor (TNF) and activation of its cognate receptor[108].

Although HIV uses several weapons to induce the apoptosis of various immune effectors, at the same time, it avoids a similar fate for the infected cell, at least until the cell has produced its load of new virions (FIG. 6). First, apoptosis of HIV-expressing cells through FAS–FASL interactions is prevented by the Nef-dependent inhibition of apoptosis signal-regulating kinase 1 (ASK1), a serine/threonine kinase that is an important intermediate in the FAS and TNF death-inducing signalling pathways[109]. The kinase activity of ASK1 is inhibited by thioredoxin (TRX), a redox regulator protein, and it is possible that Nef might function by inhibiting the stimulus-dependent release of ASK1 from TRX. In addition, Nef inactivates the pro-apoptotic protein BAD (BCL-2 antagonist of cell death), thereby blocking the mitochondria-induced apoptotic pathway[110]. Pro-apoptotic members of this family (BAD, BAX, BAK, BID and others) form heterodimers with, and thereby inactivate, pro-survival members of the same family (BCL-2, BCL-X_L, BCL-W and others). When pro-apoptotic effectors such as BAD are phosphorylated on specific serine residues, BCL-2 is released and can exert its pro-survival activity[111]. Anti-apoptotic signals are induced usually by ligation of cytokine or growth-factor receptors, through the activation of PI3K and then the kinase AKT, which phosphorylates BAD. Nef shortcuts part of this pathway by binding to and activating PI3K, which phosphorylates PAK, another Nef-associated kinase; in turn, PAK phosphorylates BAD. Finally, Nef binds p53 and thereby suppresses its pro-apoptotic activity[112]. The blockade of these last two, 'inside-in', apoptotic pathways probably prevents the premature death of an infected cell by virus-induced cytopathicity, therefore facilitating the completion of the viral replicative cycle.

A strong $CD4^+$ T-cell-specific antiviral proliferative response seems to correlate with the long-term non-progression of infection[113]. However, as activated T cells are ideal targets for the virus, T_H cells that are attracted to and stimulated at the site of infection will be destroyed rapidly. This finding might explain why there is a preferential attrition of HIV-specific T_H cells in infected individuals[114]. Another explanation lies in the ability of HIV to impair the function of antigen-presenting cells (APCs), which are crucial regulators of T_H-cell responses. Viral infection of monocytes and macrophages interferes with antigen processing and presentation through the MHC class II pathway[115], owing to Tat-mediated blockade of MHC class II transcription[116]. Tat competes with the MHC class II master transcriptional regulator CIITA for binding of CYCT1 (REF. 116), and levels of another MHC class II regulator, NF-YA, are decreased in HIV-infected cells[117]. It is expected that early in infection, this impairment of APCs would not inhibit the overall adaptive immune response greatly. Uninfected monocytes/macrophages and DCs could still take up and process free virions and present their peptides to T_H cells. However, later in the disease, when the functions of the thymus and lymph nodes are severely compromised and most mononuclear cells replicate HIV, this block in MHC class II transcription could be more deleterious to the host. In addition, sufficient Tat protein could be circulating and taken up by cells to lead to a functional impairment of antigen processing and presentation by uninfected cells by the same mechanism. Indeed, extracellular Tat has been shown to block the presentation of tetanus toxoid, but not of its peptides, by APCs[118].

Conclusions

The multi-pronged strategy that has been developed by HIV to escape immune eradication explains why therapeutic interventions are unlikely to be curative if based on the sole use of drugs that block viral replication. The costs and side-effects of HAART, as well as the realization that the full control of HIV will require not only the inhibition of viral growth, but also a strong antiviral immune response, have led to investigations into new therapeutic approaches. In a scheme known as structured treatment

interruption (STI), carefully monitored drug 'holidays' are orchestrated. The goal is two-fold: first, to reduce the cost and side-effects of the treatment; second, to stimulate the antiviral immune response by short bursts of viraemia, which are brought under control immediately by re-instating the use of antiviral drugs. Some encouraging results of STI have been obtained in patients who started HAART within weeks of becoming infected, with a significant lengthening of the lags between halting treatment and viral spikes and the apparent boosting of HIV-specific CTLs. However, large-scale studies of STI in chronically infected individuals have not been able to confirm the value of this approach, as if some irremediable step is crossed once an infection is established[119–121]. In another scheme, HAART is coupled with the administration of cytokines, such as interleukin-2, and antibodies specific for the TCR to activate T cells and viral gene expression. The rationale is to boost the immune system while forcing the virus out of its cellular hide-outs, to expose latently infected cells to virus- or immune-mediated killing. So far, however, such attempts to purge the HIV reservoirs have had limited success[122–124].

This cumulated evidence, together with our increasing awareness of the many ways by which HIV disrupts host defences, indicates that effective immune interventions will require a greater degree of sophistication. One could predict that drugs blocking Nef-induced internalization of MHC class I molecules will increase the susceptibility of HIV-infected cells to recognition and destruction by CTLs. Nef-defective simian immunodeficiency virus (SIV) isolates have been used successfully as live-attenuated vaccines in the rhesus macaque model, and animals that were inoculated with these strains subsequently resisted challenge with the wild-type virus[125]. Based on this premise, anti-Nef drugs, if given early during the course of an infection, might promote the emergence of an efficient cellular antiviral immune response that would prevent the establishment of a latent reservoir. Our increasing understanding of the functions and mechanisms of action of Nef, its tertiary structure and its cellular partners, and the availability of several *in vitro* assays to study its activity, should facilitate the discovery of suitable inhibitors. Such inhibitors, combined with agents targeting reverse transcriptase, proteases or other factors that are crucial for viral replication, could constitute a basis for the 'pharmaco-vaccination' of HIV-infected individuals.

Of note, host genetic factors might occasionally favour a more efficient immune response against HIV. There is growing evidence that disease progression is slower in individuals who express certain HLA types, for example HLA-B57 (REF. 126). In some cases, this might be due to the recognition by given HLA molecules of viral epitopes that are harder for the virus to mutate without losing fitness, as was shown for an HLA-B27-specific p24 capsid epitope[127]. The crucial involvement of HLA proteins in shaping variations in HIV proteins has been shown at the population level, supporting a fundamental role for HLA-restricted immune responses in driving and shaping the evolution of HIV-1 *in vivo*[128]. Clinically important polymorphisms could exist also at the level of other parameters, such as the transcriptional regulation of MHC molecules or their susceptibility to the effects of Nef. These putative inter-individual differences could determine vulnerability to strategies that are used by the virus to fend off the immune system. Their identification might help to design therapeutic approaches with a better chance of keeping the virus in check, and could perhaps show the way to better vaccines.

With the realization that antiretroviral therapies are costly, can result in prohibitive side-effects and fail ultimately to eradicate the virus, our interest in the gene therapy of AIDS should be revived also. Virus-resistant $CD4^+$ cells can be genetically engineered by various means[129], including the expression of protein decoys, antisense RNA, ribozymes or, as shown recently, small interfering RNA[130–132]. So far, the delivery of these genetic inhibitors to the appropriate targets has been a formidable challenge. However, the situation has changed with the emergence of new gene-delivery systems that allow for the efficient transduction of haematopoietic stem cells, which are the precursors of all HIV-susceptible cells. The most promising of these vehicles, ironically, are lentiviral vectors derived from HIV itself[133]. The door is now open to attempt the reconstitution of the immune systems of HIV-infected individuals with virus-resistant cells. If successful, this type of approach could not only revolutionize the treatment of AIDS, but would probably also have its place in the management of other viral diseases caused by agents whose ability to escape immune eradication allows them to cause life-long and devastating infections — for example, hepatitis C virus.

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