Host factors in HIV-1 replication: The good, the bad and the ugly

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CHAPTER 10

General Discussion

Host Factors in HIV-1 Replication:

*The Good, the Bad and the Ugly*
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The human immunodeficiency virus type 1 (HIV-1) infects CD4 T-cells, dendritic cells, and macrophages. Its ability to replicate in these cells is influenced by a multitude of host factors that act on different steps of the viral life cycle. The effects of these host factors on the replication cycle can be cell type specific and they can either support or restrict viral replication. However, recent studies have shown that there is a third group of host factors that play a more intricate role. In first instance these factors seem to have a restrictive effect on replication but when the bigger picture is taken into account, these factors actually allow HIV-1 to evade the innate immune response and thus act in a pro-viral manner. Understanding the interplay between these host factors and the virus is crucial in order to develop novel antiviral therapies to eradicate HIV-1 from the human body. Here we discuss how several known and novel host factors affect the viral life cycle and how these factors contribute to disease pathogenesis.

Host factors supporting HIV-1 replication

HIV-1 exploits many host factors to successfully replicate. These so-called HIV-1 dependency factors (HDFs) are recruited during all stages of the viral life cycle. In addition to HDFs that were already implicated in the HIV-1 life cycle, recent genome wide RNAi screens have identified 997 unique HDFs from which the majority was not found to be involved in HIV-1 replication before1-4. Although the overlap in the identified HDFs between the screens was limited, the recovery of host factors that were already implicated in HIV-1 replication was generally good when multiple screens were combined.

Early steps of the replication cycle of HIV-1 are facilitated by host factors like CypA, CPSF6 and TNPO35-8. CypA and CPSF6 interact with the incoming viral capsid. While CypA is required for uncoating of the viral capsid and affects the stability of the capsid, CPSF6 facilitates transport of the capsid particle to the nucleus were it associates with TNPO32;7;9-13. The balance between capsid stability and trafficking to the nucleus is essential for efficient replication of the virus. Mutations in the capsid that block binding of these host factors or alter capsid stability result in abortive infection7;8;14-17.

Reverse transcription of the viral RNA into proviral DNA is initiated by binding of a pre-packaged cellular tRNA^Lys3 to the primer binding site of 5'LTR18;19. This tRNA serves to prime reverse transcriptase-catalyzed synthesis of minus-strand DNA18;19. Reverse transcription is supported by PDE8A that
facilitates proviral DNA synthesis (Chapter 4). PDE8A was identified as a HDF in two genome wide RNAi studies and a single nucleotide polymorphism (SNP) in PDE8A was associated with HIV-1 replication in primary macrophages (Chapter 3), which emphasizes that this factor plays an important role in HIV-1 replication. Import of the proviral DNA into the nucleus is facilitated by several nuclear importins such as TNPO3, RanBP2/NUP358, NUP153 and importin. Furthermore, proteins like HMG I(Y) and LEDGF/p75 determine integration site selection and assist integration of the proviral DNA into the host chromatin.

Transcription of the provirus is facilitated by several host transcription factors like NF-kB, NFAT, C/EBPs and SP1 and the viral protein Tat which recruits the positive transcription elongation factor b (P-TEFb), composed of CDK9 and Cyclin T1. Viral mRNAs are subsequently spliced and translated in to the viral proteins using the cellular machinery. Host factor ADAR1 was implicated to play a role in the regulation of these post-transcriptional events. During HIV-1 infection, ADAR1 is thought to inhibit protein kinase RNA-activated (PKR) and thus the subsequent phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), which is important for the initiation of viral mRNA translation. By using primary T cells from patients carrying ADAR1 mutations and thus lacking functional ADAR1 activity, we showed that ADAR1 supports viral protein synthesis (Chapter 6). However, eIF2α was not involved in this process, suggesting that ADAR1 supports HIV-1 replication via a yet unidentified mechanism.

During the late stages of the replication cycle, new viral particles are assembled and subsequently bud from the cellular membrane. These steps are facilitated by several proteins from the ESCRT family, TSG101 and ALIX, that are required for efficient budding of the virion from the cellular membrane.

**HIV-1 restriction factors**

Several host factors are described that potently restrict HIV-1 replication at several steps in the viral life cycle. These so-called restriction factors have evolved as part of the innate immune system to combat viral infections. In turn, HIV-1 has adapted and has evolved several mechanisms to counteract these restriction factors. Restriction factors, TRIM5α, MX2 and the family of APOBEC3 proteins restrict HIV-1 at early steps in the viral life cycle. TRIM5α interacts with the incoming viral capsid in the cytoplasm resulting in premature destabilization and degradation of the viral capsid. HIV-1 has adapted to TRIM5α by the
introduction of amino acid variations in the viral capsid that render HIV-1 largely insensitive to human TRIM5α\textsuperscript{59,60}. HIV-1 is sensitive to several simian TRIM5α variants indicating that it is indeed a very potent restriction factor, that plays a role in prevention of cross-species transmission of retroviruses\textsuperscript{59,60}. MX2 was recently identified as an interferon-α (IFN-α) induced gene and is thought to inhibit replication at early steps in the viral life cycle like uncoating and transport of the viral capsid to the nucleus\textsuperscript{62-65}. Interestingly, the sensitivity of HIV-1 to MX2 is determined by amino acid residues in capsid, that are also involved in binding of CypA, TRIM5α, and CPSF6 which might explain why MX2 interferes with early steps in the replication cycle\textsuperscript{62-64}.

Members of the APOBEC family of cytosine deaminases are packaged in newly synthesized virions and restrict HIV-1 replication in the next round of infection by deamination of cytidine in uridine in the HIV-1 negative strand DNA during reverse transcription\textsuperscript{66-72}. These mutations either destabilize HIV-1 reverse transcripts, resulting in direct degradation of the uridine containing proviral DNA or render the resulting integrated proviruses replication defective due to the hypermutations\textsuperscript{73,74}. HIV-1 Vif prevents incorporation of APOBEC proteins in new virions by inducing APOBEC ubiquitination and subsequent degradation by the proteasome\textsuperscript{66,67}.

In addition, several restriction factors have been described that potently block virus production after the HIV-1 provirus has been integrated into the host genome. TRIM22 acts on HIV-1 replication by inhibiting transcription of the HIV-1 provirus\textsuperscript{75-77}. The exact mechanism by which TRIM22 inhibits proviral transcription is not known, although it has been shown that it is independent of HIV-1 Tat and the transcription factor NF-kB\textsuperscript{78}. DYRK1A also controls HIV-1 replication at a transcriptional level by limiting the nuclear localization of transcription factor NFAT (Chapter 5). Inhibition of DYRK1A activity by the inhibitor INDY can reactivate latent HIV-1 provirus to a similar extent as broad-spectrum HDAC inhibitors, suggesting that DYRK1A plays a role in the regulation of viral latency.

Furthermore, Schlafen11 has been described as an HIV-1 restriction factor inhibiting HIV-1 protein translation\textsuperscript{79}. Schlafen11 is induced by IFN-β and exploits the unique codon-usage of HIV-1 by limiting tRNAs that are required for viral protein synthesis while expression of tRNAs required for cellular protein synthesis remains unaffected\textsuperscript{79}. Restriction factor Tetherin/BST-2 acts on a late step in the viral replication cycle by preventing the release of de novo produced viral particles\textsuperscript{80-83}. Tetherin is an integral membrane protein and forms parallel disulfide-bond homo-dimers thereby bridging virions and cellular membranes via
its N- and C-terminal membrane anchoring domains thereby physically trapping the virion at the surface of the producer cell\textsuperscript{80-85}. HIV-1 encodes accessory protein Vpu to counteract the antiviral activity of Tetherin\textsuperscript{80;81}. HIV-1 Vpu decreases cell surface expression of Tetherin by interfering with its intracellular trafficking to the plasma membrane\textsuperscript{84;86-88}. 

Although, most restriction factors are constitutively expressed in many cell types, their expression can be potently increased by type I IFNs\textsuperscript{89-93}. Polarization of macrophages with type I IFNs and polarizing cytokines potently inhibits HIV-1 replication in these cells (Chapter 2)\textsuperscript{94-103}. Interestingly, none of the known restriction factors was found to be solely responsible for the inhibition of HIV-1 observed in these cells. This suggests that the inhibitory effects of these interferons and cytokines are mediated via yet unknown effector molecules and undiscovered restrictive mechanisms.

**Host factors involved in evasion of innate recognition of HIV-1**

Recently, several studies showed that HIV-1 exploits several host factors to avoid detection by innate pattern recognition receptors (PRRs) that recognize proteins, lipids and nucleic acids of the invading viruses. Recognition of these so-called pathogen associated molecular patterns (PAMPs) results in the potent induction of type I interferons and pro-inflammatory cytokines. Type I IFNs are potent mediators of antiviral activity and besides creating an antiviral environment they also promote the maturation of antigen presenting cells thereby contributing to potent antiviral T cell responses\textsuperscript{104}. Toll-like receptors (TLRs) located in the endosomes can detect virus derived single-stranded RNA (TLR-7 and TLR-8) and double-stranded DNA (TLR-9)\textsuperscript{105}. Furthermore, cytosolic RIG-I like receptors detect viral RNA whereas viral DNA can be recognized by cyclic GMP-AMP synthase (cGAS), interferon-γ-inducible protein 16 (IFI16) and DNA-dependent activators of interferon regulatory factor (DAI)\textsuperscript{106-109}. To date, many of these sensors have been implicated in sensing of components of the virus\textsuperscript{110-112}. However, HIV-1 has presumably evolved to avoid detection by innate immunity receptors. The HIV-1 RNA genome is concealed by the viral capsid and is not a potent inducer of an interferon signaling\textsuperscript{7;8}. Additionally, it was proposed that the cytosolic sensor RIG-I could be cleaved by HIV-1 protease\textsuperscript{113}. Furthermore, HIV-1 is able to avoid detection by cytosolic DNA sensor cGAS by keeping its viral DNA hidden from PRRs within the viral capsid with help of host factors like CypA and CPSF6\textsuperscript{7;8;107}. The amount of CypA bound to the viral capsid is critical as binding of CypA is required to open up the capsid enough for
efficient RT to take place while excessive CypA binding opens the capsid too much resulting in exposure of viral DNA to PRRs. The HIV-1 capsid has evolved in such a way that it binds enough CypA for efficient uncoating to allow for reverse transcription, while the viral DNA is not sensed by the innate immune system. In addition, binding of CPSF6 to the capsid facilitates efficient transport of the capsid to the nuclear pore thereby minimizing exposure to cytosolic PRRs. This hypothesis is supported by the finding that mutations in the viral capsid that block the interaction with CypA or CPSF6 result in detection of the virus as indicated by an increased type I IFN production.

Furthermore, HIV-1 escape from recognition by PRRs is thought to be the result of TREX1 and SAMHD1 activity that keeps the levels of viral nucleic acids below the detection limit of PRRs. SAMHD1 contains a dNTPase and a RNase domain and potently restricts reverse transcription of the HIV-1 in resting cells such as macrophages, dendritic cells and resting CD4+ T-cells by depletion of the intracellular dNTP pool. However, a recent study suggested that not is dNTPase activity of SAMHD1 but the RNase activity that is responsible for its restriction activity. TREX1 exerts an exonuclease function that degrades both single and double-stranded viral DNA produced during reverse transcription of the virus. Despite the restrictive activity of both SAMHD1 and TREX1, HIV-1 exploits these factors to escape from detection by innate immunity sensors.

Role of host factors in HIV-1 pathogenesis

Candidate gene analyses and unbiased genome-wide association studies have explored the role of host factors in HIV-1 pathogenesis and identified many host genetic variants that partly explain the variation in HIV-1 progression and control of virus set-point. These studies identified associations between HIV-1 acquisition and/or disease progression and genetic polymorphisms in restriction factors such as TRIM5α, TRIM22, DYRK1A and different APOBEC3s, and host factors required for replication such as CCR5, CypA and TSG101. Furthermore, an association was identified between a polymorphism in Trex1 and HIV-1 disease progression, indicating that TREX1 mediated evasion of innate recognition is indeed a very important mechanism in HIV-1 pathogenesis. Besides cellular factors that directly affect replication of the virus, genetic polymorphisms in the several chemokines, cytokines and killer-cell immunoglobulin-like receptors and the MHC region were found to be associated with HIV-1 progression and control of virus set-point. From these, the MHC region was shown to be the major
genetic determinant in HIV-1 pathogenesis\textsuperscript{120-130}. Recently, we observed an association between a genetic polymorphism in the innate sensor IFI16 and CD4+ T cell counts at set-point and delayed disease progression (Chapter 9). IFI16 is able to sense incomplete viral DNA transcripts in resting CD4+ T cells resulting in a proinflammatory response that ultimately leads to the death of the abortively infected resting CD4+ T cell by caspase 1 dependent pyropotosis\textsuperscript{108;155}. Furthermore, an association between a genetic polymorphism in transcription factor PREP1 and HIV-1-associated dementia (HAD) was observed (Chapter 7). Although, this factor had no apparent relation with HIV-1 replication, it was shown to bind to the promotor of MCP-1, a protein thought to be involved in the recruitment of infected monocytes and macrophages to the central nervous system\textsuperscript{156;157}.

**Future perspectives**

The introduction of combined antiretroviral therapy had dramatically increased the survival of HIV-1 infected individuals. However, current lifelong therapies do not restore the immune dysfunction completely and treated individuals still suffer from co-morbidities such as cardiovascular disease and neurological impairments\textsuperscript{158;159}. Therefore, it is of great importance to develop new and curative therapies for which a better understanding of the interactions between the virus and host is crucial.

Novel and effective strategies might result from manipulation of host factors required for viral infection and replication. The identification of many new HIV-1 dependency factors has revealed many novel therapeutic targets. Feasibility of this approach was recently shown by a clinical trial in which the co-receptor CCR5 was safely removed from CD4+ T cells in an ex-vivo gene therapy setting. Subsequent antiretroviral treatment interruption resulted in a slower rebound of their HIV-1 RNA levels while their CD4 T cell levels remained high for weeks\textsuperscript{160}. However, not all HDFs will be suitable targets and it might be difficult to target these HDFs in a way that selectively targets viral replication without interfering with cellular processes essential to the host\textsuperscript{161;162}.

Host factors that restrict viral replication can potentially be used in a therapeutic setting either by using them as a transgene in an ex-vivo gene therapy setting or by blocking the interaction between the viral antagonist and host factor. Feasibility of the latter approach was shown by small-molecule inhibitors of Vif that displayed antiviral activity in cell culture\textsuperscript{163;164}. In addition, factors that regulate transcription of the HIV-1 provirus are interesting.
pharmacological targets for new therapies targeting the latent viral reservoir. The persistence of this reservoir during therapy is the major hurdle in the search for a cure for HIV-1 infection. Reversal of viral latency in an attempt to purge the viral reservoir through killing of infected cells by cytolytic T cells or cytopathic effects of viral replication, using several HDAC inhibitors was successful to some extent\textsuperscript{165-167}. However, HDAC inhibitors were only able to reactivate a small proportion of the proviruses present in the latently infected cells of the viral reservoir\textsuperscript{168;169}. This underscores the need to better understand viral latency and cellular factors involved. The identification of cellular factors such as DYRK1A that regulate viral transcription offers more insights in the complex process of viral transcription and might provide new therapeutic opportunities for drug development targeting the viral reservoir.

A better understanding of how HIV-1 avoids innate recognition may provide new ideas and strategies to increase the innate and possibly the adaptive immune response against the virus. For example, compounds that block the interaction between the capsid and CypA can be used to induce stronger innate responses to the virus, which could be applied in pre-exposure prophylaxis. This is also relevant for the development of a vaccine. Immunogens that provoke a strong innate immune response may also be able to induce a stronger adaptive immune response\textsuperscript{170;171}. On the other hand, preventing signaling of HIV-1 in resting CD4+ T cells via IFI16 could help to prevent the depletion of CD4+ bystander cells.

The results presented in this thesis provide new insights in the complex interplay between HIV-1 and its host and will hopefully contribute to the development of future therapies to stop the ongoing HIV-1 pandemic.
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Gener al Discussion


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