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

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General Introduction
HIV-1 and the AIDS pandemic

The human immunodeficiency virus type 1 (HIV-1) originates from cross-species transmissions of the simian immunodeficiency virus (SIV) from chimpanzees in the beginning of the previous century\(^1\);\(^2\) and was identified as the causative agent of the acquired immune deficiency syndrome (AIDS) in humans. Since its discovery in 1983, it has been estimated that 25 million people died as a consequence of infection with this virus, while another 34 million individuals are currently infected (www.unaids.org).

HIV-1 transmission occurs predominantly through unprotected sexual intercourse, however the virus can also be transmitted through blood-blood contact, or from mother to child during pregnancy, birth and breast-feeding\(^3\). Infection with HIV-1 causes a slow progressive disease, which is characterized by the breakdown of the immune system, which eventually results in severe immunodeficiency and death by opportunistic infections and malignancies. The course of HIV-1 infection is highly variable between individuals but in general individuals develop AIDS within 7-11 years after infection\(^4\);\(^5\).

HIV-1 target cells and pathogenesis

CD4\(^+\) T cells, macrophages and dendritic cells (DCs) are the main target cells for HIV-1 and the virus is able to replicate efficiently in these cells. DCs are important during sexual transmission of the virus as these cells capture the virus with DC-SIGN and facilitate transport to the lymph nodes or other secondary lymphatic organs where HIV-1 is efficiently transmitted to the residing CD4\(^+\) T cells\(^6\);\(^11\). In addition, HIV-1 infects macrophages residing in the mucosa and while these cells do not usually migrate to the lymph node, they efficiently transmit virus to CD4\(^+\) T cells in the mucosal tissue thereby contributing to the establishment of infection at the sites of viral entry\(^12\);\(^18\). During the first 3-6 weeks, infection of the CD4\(^+\) T cells residing in the lymphoid tissues results in a massive depletion of these cells, especially in the gut-associated lymphoid tissue (GALT)\(^19\);\(^20\) (Figure 1). During this process the integrity of the gut mucosa is breached resulting in microbial translocation and continuous immune activation during the subsequent chronic phase of infection\(^21\);\(^25\). The chronic phase of infection is further characterized by a decrease in viral load due to HIV-1 specific adaptive and cellular mediated immune responses such as cytotoxic T lymphocytes CTLs\(^26\);\(^29\). The rapid turnover of virions and infected CD4\(^+\) T cells during this phase contributes to further immune activation, gradual loss of CD4\(^+\)
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T cells and exhaustion of the immune system. Ultimately, the CD4\(^+\) T cell numbers decrease to such an extent that the immune system can no longer protect the body against opportunistic infections and malignancies, which eventually will lead to death of the infected individual (Figure 1).

Macrophages play an important role in HIV-1 infection during different stages of the disease. They are relatively resistant to HIV-1 induced apoptosis and due to the ability of these cells to migrate into the tissue, they disseminate the virus throughout the body including the brain. Infection of macrophages contributes to the viral reservoir and a multitude of tissue-specific pathologies, including AIDS-related lymphomas, cardiovascular diseases, and HIV-1-associated neurocognitive disorders (HAND), of which HIV-1-associated dementia (HAD) is the most severe complication.

*Figure 1:* The clinical course of HIV-1 infection.

**Immune responses to HIV-1**

The immune system recognizes invading viruses with several innate pattern recognition receptors (PRRs). Activation of these PRRs results in the production of type I interferons (IFNs), which initiate direct antiviral responses immediately as well as the adaptive immune response for long-term protection. HIV-1 does not induce a strong type I IFN response, which indicates that HIV-1 is able to evade innate recognition. Recently, restriction factors like TRIM5\(\alpha\),...
APOBEC3G and Tetherin have been identified as part of the innate immune response directed against viral infections\cite{50-58}. These factors have been shown to potently restrict the replication of many viruses at different steps of the viral life cycle. Most restriction factors are constitutively expressed in many cell types and their expression can be potently increased by type I IFNs\cite{59-63}. HIV-1 has evolved to efficiently counteract innate restriction factors by either introducing mutations in its proteins that confer resistance or by expressing accessory proteins that mediated the degradation of these factors\cite{50;64-67}.

Natural killer (NK) cells play an important role in the innate defense to viral infections, as they can recognize infected cells through a variety of activating and inhibitory receptors. Upon recognition of an infected cell, NK cells secrete proteins that lyse the infected cells but also pro-inflammatory cytokines that shape adaptive immune responses. It was suggested that NK cells contribute to the control of HIV-1 infection as it has been shown that combinations of certain killer immunoglobulin-like receptors (KIR) and certain HLA alleles are associated with HIV-1 disease progression\cite{68-71}. Furthermore, it was shown that NK cells positive for the KIR2DL2 inhibitory receptor place immunological pressure on the virus resulting in the selection for viral escape variants that enhance binding of the infected cell to this receptor thereby escaping NK cell mediated killing\cite{72}.

Cytotoxic T cell responses play an important role in the adaptive immune response against viral pathogens. Cytotoxic T lymphocytes express a T-cell receptor that recognize infected cells through epitopes derived from viral proteins presented by HLA molecules and subsequently lyse the infected cells. The CTL response is important for viral control during the chronic phase of HIV-1 infection, which is indicated by the decrease in viral load that coincides with HIV-1 specific CTL activity\cite{26-29}. Furthermore, host polymorphisms in the HLA alleles are the major genetic determinants affecting the outcome of the HIV-1 disease course\cite{73-76}. HLA-B*27 and HLA-B*57 alleles are associated with delayed disease progression, whereas the HLA-B*35 allele and the HLA-Cw*04 allele are associated with a more rapid disease progression\cite{73-76}. The important role of CTLs was further emphasized by the finding that CD8+ T cell depletion in pigtailed macaques during acute HIV-1 infection caused rapid progression towards AIDS whereas the untreated animals exhibited an elite controller phenotype\cite{77}. Although CTLs contribute to the control of HIV-1 replication during the chronic phase of infection, the CTL responses allow for selection of viral variants that contain escape mutations that prevent peptide presentation by
HLA-molecules or recognition by TCR thus allowing the virus to escape CTL mediated killing\textsuperscript{78}.

During the first weeks of infection, HIV-1 also elicits an antibody response against different HIV-1 antigens. Although, some of these antibodies are directed against epitopes in the viral envelop they are not able to neutralize viral infectivity. The first neutralizing antibodies (Nabs) against HIV-1 appear approximately 3 months after infection\textsuperscript{79-82}. These Nabs can potentially inhibit HIV-1 infection by binding to the viral envelop, the sole target for Nabs, thereby preventing the attachment to the host cell receptors or prevent conformational changes necessary for fusion with the host cell membrane. However, Nabs can only neutralize autologous viruses and the presence of these antibodies allow for rapid selection of viral escape variants that are insensitive to these Nabs. The envelop of viruses that escape from these neutralizing antibodies contain longer variable regions, difference in charge and extended glycosylation thereby shielding the envelop from recognition by antibodies\textsuperscript{83-89}. Therefore, the neutralizing antibody response always lags behind the viral escape and does not contribute to the control of viremia or protect against disease progression\textsuperscript{80;87-90}.

**Antiretroviral therapy**

The first therapy for HIV-1 became available in 1987 and consisted of a single nucleoside analog azidothymidine (AZT), which inhibits reverse transcription of the virus. This therapy was unsuccessful due to the rapid emergence of viruses that were resistant to the drug due to mutations in the reverse transcriptase gene\textsuperscript{91}. The development of protease inhibitors and nonnucleoside reverse transcriptase inhibitors, which could inhibit different steps of the HIV-1 replication cycle, allowed for the introduction of combination antiretroviral therapy (cART) in 1996. Combination therapy is very efficient in suppression of viral replication and has significantly reduced transmission rates of the virus and increased life expectancy of infected individuals. Nevertheless, current therapies do not completely restore the immune dysfunction and treated individuals still suffer from co-morbidities such as cardiovascular disease and neurological impairments\textsuperscript{92;93}. Furthermore, lifelong treatment with cART is required as interruption of antiretroviral therapy leads to the rebound of detectable viral replication and progression towards AIDS.

Current antiretroviral therapies are unable to eradicate HIV-1 due to the persistence of a viral reservoir consisting out of resting memory CD4\textsuperscript{+} T cells and macrophages that harbor latent proviruses. Resting memory T cells are
considered to be a major reservoir for HIV-1 because of their long half-life and their ability for self-renewal, which allows for persistence of infected cells even after long-term cART. In addition, infected macrophages are an important reservoir for the virus due to their relative longevity and their ability to reside in multiple tissue compartments, including the CNS. The suboptimal tissue penetration of the drugs combined with the low efficacy of cART in macrophages makes it hard to target viral replication in these cells. Therefore, infected macrophages are thought to remain a source of residual virus replication even during treatment with cART\textsuperscript{37-41,94}.

Figure 2: (A) Schematic representation of the organization of the HIV-1 genome\textsuperscript{127}. (B) Schematic representation of the HIV-1 virion\textsuperscript{128}.
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The HIV-1 virion and replication cycle

HIV-1 is a *Lentivirus* belonging to the family of *Retroviridae*. Retroviruses are enveloped RNA viruses containing two positive single-stranded RNA genomes surrounded by a cone-shaped core. The HIV-1 genome encodes the structural proteins Gag, Env and Pol, common for all retroviruses, and the regulatory and accessory proteins Tat, Rev, Vif, Vpr, Vpu and Nef (Figure 2A). The coding regions in the genome are flanked by two long terminal repeats (LTR) that are important for reverse transcription, integration, provirus transcription, and polyadenylation of mRNA. The structural polyproteins Gag, Pol and Env are further processed into the core proteins (capsid, nucleocapsid and matrix), the viral enzymes (reverse transcriptase, integrase and protease) and the envelope proteins (Gp120 and Gp41) (Figure 2B). Furthermore, regulatory proteins Tat and Rev facilitate transcriptional and post-transcriptional steps of the replication cycle, whereas the function of the accessory proteins Vif, Vpr, Vpu and Nef is dedicated to mechanisms to escape or manipulate innate and adaptive immunity.

Entry of the virion into its target cells is mediated by binding of the envelope spike to the CD4 receptor and CCR5 or CXCR4 co-receptors. The envelope spike consists of a trimeric structure composed of three subunits of the exterior protein Gp120, which are non-covalently bound to three subunits of the transmembrane protein Gp41. Gp120 binds to the CD4 receptor, which induces conformational changes that opens up the trimer thereby exposing the co-receptor binding site. Subsequent binding of the CD4 receptor and co-receptor trigger the insertion of Gp41 into the membrane of the target cell, which ultimately results in fusion of the viral and cellular membrane (Figure 3).

Once the viral capsid containing the RNA genomes is released into the cytoplasm, the uncoating and reverse transcription process can begin. The viral reverse transcriptase copies the single-stranded RNA genomes into linear double-stranded DNA. During the late stages of this process, the pre-integration complex (PIC) is formed which contains the dsDNA genomes and viral integrase. The PIC is transported into the nucleus where the dsDNA is integrated into the host genome by viral integrase.

Transcription of the integrated HIV-1 provirus is driven from the 5’ long terminal repeat (LTR) that is recognized by RNA polymerase II as promotor. Initial binding of RNA polymerase II results in synthesis of short mRNA transcripts from which Tat is translated. Subsequently, Tat binds to the transactivation response (TAR) element and act as a stimulator of transcriptional
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elongation of the viral mRNAs\textsuperscript{103,104}. After transcription, the mRNAs are spliced using host cellular machinery. HIV-1 replication requires multiple spliced species, singly spliced species and unspliced mRNA. To ensure the proper balance between spliced and unspliced mRNA, HIV-1 encoded Rev facilitates the export of single and unspliced RNAs\textsuperscript{105-107}.

Host ribosomes translate the multiply spliced mRNAs into Tat, Rev, and Nef and the singly spliced mRNAs into Vif, Vpr, Env and Vpu. The unspliced mRNA encodes Gag and Gag-Pol but also serves as a viral RNA genome. Assembly of the HIV-1 virion is driven by the Gag polyprotein. The viral genomic RNA interacts with the Nucleocapsid part of the Gag polyprotein thereby promoting Gag multimerisation. Upon multimerisation of Gag, the myristate domain of the Matrix subunit is exposed which results in the Gag-RNA complex being targeted to the plasma membrane\textsuperscript{108}. Further oligomerisation of the Gag polyproteins initiates the budding process. When the newly formed core buds through the membrane, it encapsulates itself with a layer of membrane containing the viral envelop spikes. The virion matures further with help of the HIV-1 protease that digests the Gag polyprotein into the different structural proteins\textsuperscript{109,110}.

\textbf{Figure 3}: Schematic representation of the HIV-1 replication cycle\textsuperscript{129}
Cellular factors involved in HIV-1 infection

Like all viruses, HIV-1 travels light and therefore needs to exploit many host proteins for its replication. In the search for host factors involved in HIV-1 replication several candidate gene and genome wide screens were performed. These screens have identified many host factors from which the majority was not found to be involved in HIV-1 replication before\textsuperscript{111-115}. In addition, several host genetic determinants of HIV-1 infection and pathogenesis were identified by classical candidate-gene approaches and genome-wide association studies (GWAS) performed in multiple cohorts of HIV-1 infected individuals\textsuperscript{75,116-125}. These GWAS primarily confirmed the classical candidate-gene approach studies and found variants in the HLA-region to be the major genetic determinants in HIV-1 infection and pathogenesis. Overall, a very limited overlap in host factors identified in the different screens was observed, which can partially be explained by the use of different cell types, culture conditions, and readout phenotypes. Due to the different approaches taken to identify host factors, these results can also be considered complementary, however it is difficult to exclude false positive/negative hits from these data sets. To confirm the role and mechanism by which these host factors affect HIV-1 replication further experimental validation will be required.
SCOPE OF THIS THESIS

The studies described in this thesis focus on the role of host factors in HIV-1 replication and HIV-1 pathogenesis. In Chapter 2, we studied whether known HIV-1 restriction factors are responsible for the restriction of HIV-1 replication observed in macrophages polarized with different interferons and cytokines. In Chapter 3 and Chapter 4, we describe the identification of host factor PDE8A and we confirm that this cellular protein is an HIV-1 dependency factor that supports HIV-1 replication at the level of reverse transcription. In Chapter 5, we describe that DYRK1A, a host factor identified in a GWAS on HIV-1 replication in macrophages, controls HIV-1 replication at a transcriptional level via nuclear factor of activated T cells (NFAT). In Chapter 6, we investigated the role of ADAR1 in HIV-1 replication and we show that this factor facilitates translation of viral proteins in CD4+ T cells. In Chapter 7, several candidate gene polymorphisms were analyzed for their association with HIV-1 associated dementia in a case-control study. We observed that a polymorphism in Prep1 was associated with HIV-1 associated dementia in this cohort. PREP1 is a transcription factor that binds to the promotor of MCP-1, a protein with a well-established role in the etiology of HAD. In Chapter 8 and Chapter 9, we demonstrate that genetic polymorphisms in host factors TREX1 and IFI16, that were shown to affect innate recognition of HIV-1 in vitro, affect HIV-1 pathogenesis in the Amsterdam Cohort Studies on HIV-1 and AIDS. In Chapter 10, we provide an overview of known and new host factors involved in HIV-1 replication and discuss their relevance in HIV-1 infection and therapy development.
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