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Cellular determinants of HIV-1 persistence

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Abstract

The era of anti-retroviral therapy has made HIV-1 infection a manageable chronic disease for those with access to treatment. Despite treatment, virus persists in tissue reservoirs seeded with long-lived infected cells that are resistant to cell death and immune recognition. Which cells contribute to this reservoir and which factors determine their persistence are central questions that need to be answered to achieve viral eradication. In this chapter, we describe how cell susceptibility to infection, resistance to cell death and immune-mediated killing as well as natural cell life-span and turnover potential are central components that allow persistence of different lymphoid and myeloid cell subsets that were recently identified as key players in harbouring latent and actively replicating virus. The relative contribution of these subsets to persistence of viral reservoir is described and the open questions are highlighted.

Keywords: HIV reservoirs, CD4+ T cell subsets, macrophages, dendritic cells, HIV susceptibility, cell survival, turnover potential

Introduction

Current antiretrovirals (ARVs) have achieved impressive success in preventing progression of HIV-1 infection. However, ARVs, although highly efficient in blocking diverse steps of HIV-1 replication cycle, do not eliminate cells containing proviruses. Thus, despite long-lasting suppressed viremia on ARVs, HIV-1 persists in viral reservoirs (1) that are at the origin of viral rebound if antiretroviral treatment (ART) is discontinued. A better characterisation of the factors governing these reservoirs is fundamental in the search for an HIV cure (2, 3). HIV-1 persistence under ART has been associated to (i) low level viral replication, in particular in deep tissues where concentration of ARVs may not always reach optimal action levels (4-6), and (ii) long half live and self-renewal of latently HIV-1 infected cells (7). Although it is likely that HIV-1 persistence is a consequence of the combination of these two processes, we will focus here on intrinsic cell properties that determine the maintenance of infected cells.

HIV-1 infects cells from the myeloid and T cell lineages but not all cell populations contribute equally to HIV persistence (1). Most of the HIV-1 reservoir is constituted in CD4+ T cells (7, 8), while macrophages and dendritic cells play a critical role as early targets for HIV infection and as vehicles for HIV-1 dissemination throughout the body (9, 10). CD4+ T cells are typically described as cellular subsets that follow a gradient of maturation stages including naïve (TNA), stem cell memory (TSCM), central memory (TCM), transitional memory (TTM), effector memory (TEM), and terminally differentiated (TTD) cells (11). Under ART most persistent HIV-1 DNA is contained within memory CD4+ T cells, and in particular TCM and TTM (7). However the relative contribution of these subpopulations to the HIV reservoir may vary depending on whether the

treatment was initiated in primary or chronic infection, and long periods of treatment appears to enrich the contribution of very long lived cells such as TSCM (12-15).

The establishment and maintenance of HIV-1 reservoirs is a multifaceted process that depends (i) on the relative cell susceptibility to HIV infection, (ii) the capacity of the infected cell to resist HIV induced apoptosis and escape immune surveillance, (iii) the infected cell's life span and turnover potential (**figure 1**). All these processes are determined by each cell type's program and regulated by tissue location, activation and differentiation state of the cells in response to environmental conditions and stress signals. This chapter analyses each of these processes and relates them to the different cell subsets that are thought to more critically contribute to HIV reservoirs.

Being a good or a bad host to the virus

HIV-1 cell tropism is determined by the expression on the cell surface of the main HIV-1 receptor CD4 and at least one additional co-receptor, mainly CCR5 or CXCR4 (16). CD4+ T cells, monocytes/macrophages and dendritic cells are the major targets of HIV-1 (17, 18). The rate of viral replication largely diverges from one cell type to another, and even within a cell type depending on activation and differentiation. Thus, HIV-1 replicates strongly in activated CD4+ T cells and less efficiently in macrophages and immature dendritic cells while resting CD4+ T cells, monocytes and mature dendritic cells are strongly resistant to HIV-1 infection (19-23). These differences are mostly explained by the relative abundance of cell factors that intervene in the virus life cycle. Several studies have identified hundreds of cellular factors potentially required

for HIV-1 to complete each step of its replication cycle (HIV dependency factors, HDF) (24-28). The expression of chemokine receptors varies with T cell differentiation, impacting the susceptibility of the cells to HIV-1 (29, 30). Among CD4+ T helper lineages, Th2 cells are relatively resistant to HIV-1 infection (in particular to R5 viruses due to low CCR5 expression)(31, 32) although they might be targeted by X4 viruses late in infection (33). Th1 are susceptible to both R5 and X4 viruses but to a lower extent than Th1/Th17 or Th17 CCR6 expressing cells (31, 32, 34). The enhanced susceptibility of CCR6+ CD4+ T cells to HIV-1 infection was linked to an enhanced expression of HDF in these cells (28). Beside entry receptors some of the best known HDF include: cyclophilin, which binds to HIV-1 capsid and facilitates decapsidation/reverse transcription through a mechanisms that is still unclear (35); cytoskeleton, which is required for intracellular trafficking of the virus (36, 37); LEDGF/p75, which interacts with integrase and is responsible for the tethering and selective integration of HIV into active transcription units of the chromatin and possibly also in the regulation of HIV Latency (38, 39); P-TEFb (composed of cyclin-dependent kinase 9 (CDK9) and of cyclin T1 or T2 (CycT1/T2)) and NFκb and NAFT transcription factors, which are required for HIV-1 transcription (40); Endosomal Sorting Complex Required for Transport (ESCRT), which participates in HIV-1 budding (41). While relying on numerous cellular factors, HIV-1 needs to overcome restriction factors devised as intrinsic immunity by the cells to counteract infections (42). Although several cellular factors have been suggested to potentially inhibit HIV-1 infection (43), to date only a handful of restriction factors have been clearly validated. SERINC3 and SERINC5 interfere with the delivery of viral particles to target cells (44, 45); APOBEC3G, TRIM5alpha and SAMHD1 impair reverse transcription (46, 47); Mx2 hinders nuclear accumulation and integration of

proviral DNA into the host chromatin (48); BST-2 retains newly produced viral particles at the surface of infected cells (49). The strong expression of HDF present in immune cells suggests that HIV-1 has evolved to replicate in cells performing activities optimally matching the requirements of its replication cycle and has adapted to circumvent the action of restriction factors (24, 28). Thus, the susceptibility of host cells to HIV-1 infection is largely dictated by the availability of HDF rather than the expression of restriction factors, with the exception of SAMHD1.

SAMHD1 possesses dNTPase and nuclease activities and can potentially interfere with HIV-1 reverse transcription by reducing the pool of intracellular dNTP and by degrading incoming viral nucleic acids (50-52), although the relative contribution of each of these activities is still unclear. This may vary as a function of cell type and cell cycle. SAMHD1 efficiently blocks HIV-1 infection in quiescent CD4+ T cells and monocytes, strongly decreases HIV-1 dynamics in differentiated myeloid cells but it is inefficient in cycling CD4+ T cells (51, 53). The differences in the antiviral activity of SAMHD1 between these cell types are not related to its relative expression, but SAMHD1 antiviral activity is ablated by the phosphorylation of its threonine 592 (T592) residue (53, 54). Phosphorylation of SAMHD1 is regulated by cyclin-dependent kinases (CDK) 1/2, which coordinate T cell division and differentiation in response to antigen recognition (55). A recent report shows that tissue resident macrophages susceptible to HIV-1 infection such as microglial cells, which are responsible for HIV-1 persistence and compartmentalisation in the brain (56), are in a G1-like status and express high levels of phosphorylated (inactive) SAMHD1 (57). The influence of the cell cycle on HIV-1 replication is well known (58). P21, a CDK inhibitor which regulates cell cycle arrest and is involved in

monocyte differentiation (59, 60), is a potent inhibitor of HIV-1 infection (61). On one hand, p21 controls the *de novo* synthesis of dNTPs by regulating the expression of the main enzymes involved in this process (62). Through its CDK inhibitor activity, p21 also controls the phosphorylation state of SAMHD1 (63, 64). p21 has also been shown to interfere with HIV-1 replication in hematopoietic stem cells (65) and with CDK9-dependent transcription of HIV-1 in CD4+ T cells (66).

The differentiation state of the cells also influences the relative capacity of HIV-1 to replicate. HIV-1 replicates less well in naïve CD4+ T cells and monocytes than in memory CD4+ T cells and macrophages, and this is not only related to differential expression of HIV-1 co-receptors. Successful HIV infection requires stable integration of viral cDNA into the host cell genome. HIV integration occurs preferentially within transcription units of transcriptionally active genes (67, 68). However several mechanisms including epigenetic gene silencing, transcription gene silencing, and post-transcriptional gene silencing have been described to explain the establishment and maintenance of latency in target cells (reviewed in (69)). Although these mechanisms are described in detail elsewhere in this book, it is interesting to note here that HIV gene expression is heavily dependent on the presence of several transcription factors, such as NFAT and NFκB. These factors are critical regulators of T cell activation and differentiation and their expression is necessary for rapid production of cytokines or effector molecules (70, 71). Accordingly, NFAT and NFκB are expressed at very low levels in naïve and resting T cells and strongly expressed in activated and differentiated T cells. In vitro studies suggest that latency can be established in both resting and activated CD4+ T cells (72). However, it is reasonable to think that HIV-1 latency per integration event may be achieved more frequently

in less differentiated CD4+ T cells subsets than in effector cells, but this remains to be proven. The mammal target of rapamycin (mTOR) is a pivotal regulator of cell differentiation, cell cycle, proliferation and survival (73). mTOR directly regulates many HDF (e.g. cytoskeleton, NFAT) and it has been shown to control HIV-1 latency (74). mTOR also regulates the metabolic activity of the cells (75) and it is worth noting that expression of the glucose transporter Glut1 is required for HIV-1 replication (76). Thus, it is likely that mTOR has an important part in the regulation of HIV-1 infection in different cell subsets.

Much less evidence is available about the establishment of HIV latency in infected macrophages. As in CD4+ T cells, in macrophages HIV preferentially integrates into the transcriptionally active region of the chromatin (18). However, it is not clear whether the mechanisms driving latency on CD4+ T cells act similarly in macrophages. Latency can be established in macrophages in vitro (77) and HIV transcription is regulated in response to external signals and macrophage activation (78). Moreover, the transcription factor CTIP2, which is involved in multiple cellular processes including cell proliferation and survival, has been shown to repress HIV gene transcription in microglial cells by inhibiting the elongation factor P-TEFb and by inducing a compact, transcriptionally inactive, heterochromatic environment at the HIV promoter (79).

Although HIV-1 has evolved mechanisms to avoid the action of restriction factors such as APOBEC3G, it is interesting to notice that this factor is expressed at very high levels in the cells that are more susceptible to HIV-1 infection and this may come at a cost for the virus in terms

replication capacity of viruses produced in more differentiated cell subsets (80). Finally, some cells, such as dendritic cells, are poorly or not susceptible to HIV-1 replication but can internalize free virions in non-cytolytic vesicles and transfer them at high concentrations to CD4 T cells upon interaction (81). Along these lines, follicular DCs have been shown to trap and retain infective HIV-1 particles for extended periods of time (82).

Dodging cell death upon infection

Although apoptosis is a major mechanism of defense against infection, viruses have evolved means to influence the balance of death and survival of the host cell in order to promote efficient virus replication and persistence of infection. Progressive CD4+ T cell loss is a defining characteristic of uncontrolled HIV-1 infection. HIV-1 can provoke direct cytotoxicity on target cells. However, it is now well accepted that decline of CD4+ T cells in vivo is not solely due to direct viral cytotoxicity but to a multifactorial process that also includes apoptosis of "bystander" cells (83-85) and killing of productively infected cells by immune effectors. Yet persistence of HIV-1 infected cells for long periods of time requires avoiding all these forms of cell death. Studies performed in vitro and ex vivo have shown the contribution of many different molecules in apoptosis induction in CD4+ T cells and other HIV-1 susceptible cell subsets but not much information is available in vivo. The exact molecular mechanisms of HIV-1 induced cytotoxic or anti-apoptotic effects on infected cells that lead to the establishment of persistence are still not well understood.

Viral proteins such as tat (86, 87), env (88), vpr (89) and nef (90, 91) have been shown to have an apoptotic effect in vitro. However, the action of these proteins at physiological concentrations and in a complex immunological setting remains unclear. Moreover, the regulation of apoptosis depends on the interaction of viral factors with cell pathways and the equilibrium between pro-apoptotic and anti-apoptotic signals may vary as a function of the stage of viral replication, nature and state of the target/bystander cell and external signals. Thus, nef has been shown to prevent apoptosis in productively infected cells upon Fas and TNFalpha ligation (92) by inhibiting Fas signaling (93). Nef was also shown to inactivate pro-apoptotic Bad protein hence rendering infected T cells more resistant to apoptosis (94). On the other hand, myeloid lineage cells including monocytes and macrophages, appear less sensitive to the cytopathic effect of HIV replication than T cells, suggesting that intrinsic properties of myeloid cells may render them selectively resistant to HIV-induced apoptosis (18, 79). Along these lines, telomerase activity increases in macrophages upon infection, rendering them more resistant to DNA damage or oxidative stress (95). Macrophages were observed to be more apoptosis resistant at least in part due to env (96) and nef (97) dependent alterations in apoptotic pathways.

Under ART the contribution of direct viral cytotoxicity to cell death is likely minimal. Latently infected cells remain largely unnoticed due to lack of expression of viral products, which favors their persistence. However, infected CD4+ T cells have been reported to undergo integration-dependent cell death, linked to the recruitment of DNA-PK (98). It is also unclear that latency is an absolute phenomenon and it is possible that episodes of viral reactivation occur episodically in vivo even in the presence of effective cART (99, 100). On the other hand, recent studies have

shown that reactivation of latent HIV-1 reservoirs in cells from ART-suppressed HIV-1 subjects with HDAC inhibitors or other latency reversal agents was insufficient to promote cell death ex vivo or to decrease the proportion of integrated viral DNA or infectious units in vivo (101-105). Moreover, cells carrying latent replication competent viruses might be particularly resilient to CD8+ T cell-mediated killing even after viral reactivation (106). Therefore, long-term HIV-1 persistence may be caused by cells that are particularly resistant to cell death, either due to their intrinsic properties or because a peculiar anti-apoptotic status was induced by infection. Until recently the characterization of latently HIV-1 infected cells has been severely hampered by the lack of a phenotypic marker allowing the identification of these cells ex vivo (107). However, different studies using in vitro models of HIV-1 latency have described that establishment of latent HIV-1 infection is accompanied by the induction of anti-apoptotic proteins (e.g. BCL-2, cFLIP, Mcl-1) (108-110) or the downregulation of pro-apoptotic proteins (e.g. BAX, FADD) (111, 112). Interestingly, in vivo, highly persistent cells carrying the virus such as TCM (113) and monocytes (114) were also observed to have an increase in anti-apoptotic gene signature in HIV+ patients vs healthy controls.

Death of non-productively infected non-activated T cells that do not express viral antigen, have been reported to result from the detection of viral reverse transcription products by DNA sensor IFI16, which leads to caspase-1 activation thereby triggering pyroptosis (83, 115). However, the contribution of this form of cell death is probably limited once ART is initiated (116). Apoptosis of bystander cells has also been linked to persistent immune activation seen in chronic infection via signaling by TNF family members (TRAIL, FasL and TNFalpha) (117-120). Death signals delivered via Fas ligation (119, 120) were shown to have an important

contribution to bystander cell depletion in HIV patients (121-123). However, these signals were observed to be counteracted, at least in part, by viral proteins expressed by infected cells. For example, Env was observed to induce resistance to TRAIL-induced apoptosis in macrophages (96).

Overall, it seems that the activation status of cells plays a role in the susceptibility to cell death during HIV infection, which is also reflected by differential loss of cell populations from different tissues, where more or less activated cell phenotypes are found. For example, in the lymph nodes of HIV-positive patients, the degree of apoptosis has been correlated with virus and microbial-driven immune activation observed in infection and not the viral load (124). It has been further shown that highly activated effector memory CD4+ T cells are depleted fastest and first from gut mucosal sites (125) and naïve T cells displaying resting phenotype are resistant to depletion in lymphoid tissues (30). Additionally, it was shown that blood-derived CD4+ T cell that display deeper resting state than lymphoid tissue derived cells are more resistant to pyroptosis despite carrying viral genetic material (126). Thus, infected naïve, central memory and stem cell like memory T cells displaying less activated phenotype could be less prone to these mechanisms of induced cell death than highly activated effector memory T cells, which would contribute to shaping the HIV-1 reservoir before treatment initiation. However, how these mechanisms play out during ART is unknown. ART undoubtedly halts loss of CD4+ T cells but some level of abnormal chronic inflammation persists (127) and it is likely that this may contribute to selective elimination during treatment of the most apoptosis-susceptible HIV-carrying cells. The susceptibility of infected cells to cell death may also vary in tissues depending on the cytokine milieu. For example, IL-7 protected resting CD4+ T cells from death

during in vitro HIV infection (128), whereas IL-12 protected while IL-10 augmented Fas-mediated cell death of CD4 T cells from HIV-1 patients (129).

HIV-1 specific cytotoxic CD8+ T cells and NK cells are able to eliminate infected cells and these responses have been linked to protection against HIV transmission or natural control of infection (130-136). However, during progressive infection selection in vivo of viral variants that escape this immune pressure occurs progressively diminishing the capacity of these cells to counteract infection (137-140). HIV-1 reservoirs persist for many years even in the presence of highly efficient CD8+ T cell responses observed in HIV-1 elite controllers (141), suggesting that persistent infected cells are able to avoid immune surveillance. Latently infected resting CD4+ T cells that do not actively express viral epitopes escape immune surveillance although just transient expression of viral antigens may trigger their killing (100). Viral proteins such as Nef (142) and Vpu (143) downregulate MHC class I and may contribute to protect infected CD4+ cells from the CD8+ T cell response (144, 145) (although this could make these targets susceptible to NK mediated killing (142)). Infected macrophages might be more resistant than CD4+ T cells to killing by CD8+ T cells in vitro independently of nef (146, 147) although they may be eliminated by HIV-specific cytotoxic CD4+ T cells (148), which have been found to increase during acute infection (149) and in elite controllers (150). In addition, in macrophages, HIV-1 particles assembly in intracellular virus containing compartments (VCCs) (151, 152) that may provide a protective shelter from immune recognition (153).

In addition to escape mutations and latency, effective immune responses are also curtailed by the physical separation of effector cells from their targets residing in tissue sanctuaries. The

central nervous system (CNS) has long been considered an “immune privileged” site where infected macrophages, astrocytes and microglial cells are relatively inaccessible to anti-viral immune responses and variably accessible to cART (154, 155), thus constituting an important viral reservoir. Cerebrospinal fluid (CSF) of non-infected individuals contains CD4+ T cells with an activated central memory phenotype (156), which are a preferential target of HIV-1. Compared to the blood, the ratio of CD8/CD4 T cells is much lower in the CNS, and it has been suggested that antigen-specific CD8+ T cells found in the CNS do not provide durable immune surveillance in the absence of antigen (157). However, resident memory CD8+ T cells can be found in the CNS in the context of viral infection (158) and it is now recognized that functional HIV-1 specific CD8+ T cells infiltrate CNS during acute (159) and chronic (160) infection. Remarkably, CD8+ T cell responses in the CNS are detected in elite controller patients with undetectable viral load in the CSF and blood (161) and during cART therapy (162, 163) and contribute to the control of infection in the CNS (164). Similarly, several reports have shown accumulation of virus bearing Tfh cells in germinal centers of lymphoid follicles (165, 166) where CD8+ T cells are found in low frequencies as compared to T cell zones (167-170). However, recently identified follicular cytotoxic T cells (Tfc) were shown to enter B cell follicles of HIV+ subjects and to have a cytotoxic potential (171, 172). Cytotoxic CD8+ T cells displaying viral target lysis are also detected in the lamina propria (173) as well as vaginal epithelium and submucosa (174) of SIV infected rhesus macaques. Activated CD8+ T cells with cytotoxic potential were also located in adipose tissue (which carried infected CD4+ T cells and macrophages) of SIV infected monkeys (175). It is, therefore, probable that tissue CD8+ T cell responses contribute to the elimination of infected cell populations in non-lymphoid tissues

and even the CNS, although more studies will be needed to define how they compare in terms of phenotype, function and abundancy per infected target cells when compared to lymphoid tissues.

Endurance and renovation

The number of cells containing HIV DNA decreases sharply during the first months following initiation of ART (176, 177). A steady state appears to be reached by 2 years after treatment, although this may occur later when treatment is initiated during primary infection (177). Modeling the dynamics of viral decrease showed that this decay occurs in several phases that have been attributed to the sequential loss of viral reservoirs of varying half-life (176, 178). In the context of ART efficiently blocking systemic HIV replication, cells with active viral replication are expected to be eliminated within a few days due to cytopathic effects or immune clearance (see above). However, some cells like macrophages can produce infectious viral particles for long periods of time without being killed and resting CD4+ T cells carrying latent provirus persist despite multiple decades of treatment. The maintenance of infected CD4+ T cells under ART is driven by survival of long-lived cells and homeostatic proliferation (7).

The lifespan of quiescent CD4+ T cell progressively decreases with differentiation. Naïve CD4+ T cells are much longer lived than memory cells, and early differentiated memory CD4+ T cells have a longer half-life than terminally differentiated cells. TNA, TSCM and TCM upregulate genes associated with survival and are less prone to undergo apoptosis, at least in vitro (11, 179). It has been estimated that one TNA has a half-life of one to several years, a TCM a few

months or a year while a TEM would last just a few weeks (180, 181). TSCM have the highest survival capacity among memory T cells in the absence of cognate antigen (179, 182). However, these estimations, largely based on the in vivo analysis of incorporation of deuterated glucose or water on CD4+ T cells, are limited by the lack of resolution on cells that migrate to the tissues. The recently described resident memory T cells are programmed to persist locally and not recirculate even in the absence of antigen (183). The role of these cells in the context of HIV-1 infection has not been clarified yet but their potential contribution to the persistence of HIV reservoirs deserves analysis.

The contribution of infected macrophages to the persistent HIV reservoir during ART is debated (184). Surely resting CD4+ T cells constitute the bulk of persisting infected cells, but the potential implication of infected macrophages as source of rebounding virus if treatment is interrupted should not be overlooked (185). Although it is accepted that viral decay in monocytes/macrophages is slower than in activated CD4+ T cells, it is often assumed that the half-life of infected monocytes/macrophages is lower than that of quiescent CD4+ T cells (69, 176, 186). However, the life span of macrophages, as for CD4+ T cells, also varies greatly. Depending on their tissue location, macrophages can live from a few months to several years. Alveolar macrophages, which can be infected by HIV (187), have been found to persist for over 3 years in analyses performed after lung transplant (188, 189), and microglial cells persist for years in the CNS (190). Moreover, macrophages may be best prepared than CD4+ T cells to resist apoptosis under conditions of metabolic stress (191-193).

It was previously assumed that activation of HIV-infected CD4+ T cells driving them to proliferation would reverse viral latency and decrease the half-life of cells carrying productive viruses. However, recent phylogenetic studies using ultra deep whole genome sequencing have shown the presence of proviruses with identical sequences in clonally expanded infected CD4+ T cells (7, 194-198). Moreover, it is now clear that these expanded infected CD4+ T cells do not only carry replication incompetent virus but can also harbor intact proviruses able to spread infection (196, 199). Several reports have shown that proliferation of infected CD4+ T cells could be at least partially driven but the selective integration of HIV-1 into genes that have been associated with cell growth, division and cancer (195, 197). In addition, CD4+ T cells can divide in response to antigenic stimulation or to homeostatic signaling to balance cell numbers although capacity of self-renewal is lost with progressive differentiation of memory CD4+ T cells (11, 200). Antigenic stimulation through the T cell receptor entails the activation of the cell and triggers cell differentiation. However, naïve and early differentiated cells require higher signaling threshold and prolonged contact with antigen presenting cells and they also depend more on co-stimulatory signals than more differentiated cells to respond to antigens. Thus low levels of antigen during treated infection might provide a suboptimal signal allowing some degree of activation of these cells. It is however unknown whether, in vivo, some transiently activated cells might escape cell death despite some degree of viral production to later regain a quiescent state. In contrast in vitro studies have confirmed that infected CD4+ T cells can undergo homeostatic proliferation without significant viral production or cell death (201).

Homeostatic proliferation is governed by members of the common gamma chain family of cytokines in the absence of antigenic stimulation (202-204). In particular IL-7 plays a central role

in CD4+ T cell homeostasis and survival. In the case of naïve CD4+ T cells, IL-7 signaling and contact with self MHC-peptides complexes promotes cell survival without inducing proliferation. In contrast, IL-7 signaling can promote proliferation of memory CD4+ T cells independently of TCR signal. Responsiveness to IL-7 is not equal among all CD4+ T cell memory subsets. TSCM and TCM express high levels of the IL-7 receptor (CD127) and have strong proliferation potential while TEM express lower levels of CD127 and have a limited proliferative potential (11). Other common gamma chain cytokines (such as IL-2 or IL-15) are also going to influence the survival and turnover of the T cells in their inflammatory environment (205). Overall, once established memory CD4+ T cells can persist for decades in the absence of antigens (206).

Macrophages and dendritic cells are terminally differentiated cell populations that cannot be propagated in vitro. However, macrophages subsets that are susceptible to HIV-1 infection are not in a quiescent state and share some characteristics of cycling cells (57, 207, 208). Although infected macrophages did not show evidence of division in vitro, it is now clear that in vivo some macrophages can proliferate locally in tissues in response to inflammatory signals (209-211). Thus the possible persistence of some infected macrophages through cell division cannot be discarded.

Conclusion

The persistence of HIV-1 infected cells under antiretroviral treatment depends on a combination of cell intrinsic characteristics including the susceptibility of the cells to infection

and their capacity to survive and proliferate **(Figure 2)**. However it is also influenced by the responsiveness of these cells to external signals (such as inflammatory cytokines or contact with antigen presenting cells) or their localization and capacity to circulate. For instance, HIV-specific CD4+ T cells have been reported to be preferentially infected by HIV during treatment interruption when compared to other antigen-specific memory CD4+ T cells and this is likely due to the selective localization of activated HIV-specific cells to the sites of viral replication (212). On the other hand, while effector CD4+ T cells are highly susceptible to infection, this cell subset is quickly depleted during infection (30). In contrast, TSCM, rare among memory CD4+ T cell subsets, increase their contribution to the total pool of infected CD4+ T cells with time on treatment (13, 14). Similarly, despite their relative lower frequency, CCR6+ CD4+ T cells (with Th17 or Th1/Th17 polarization) are enriched for HIV-DNA in patients on ART when compared to other Th lineages (32, 213), and this could be the result of enhanced susceptibility to HIV-1 infection (31, 32, 34), resistance to apoptosis, long life-span and proliferation potential (214, 215). HIV-1 can thus reside in multiple cell subsets in multiple tissues while the main mechanisms of persistence may diverge from one to another. It is of the outmost importance to define whether specific cell subsets are more likely to give rise to viral rebound if treatment is discontinued and determine if some of these subsets should be preferentially targeted. In any case, tackling HIV persistence will require the combined neutralization of different mechanisms set for cell survival.

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FIGURE 1

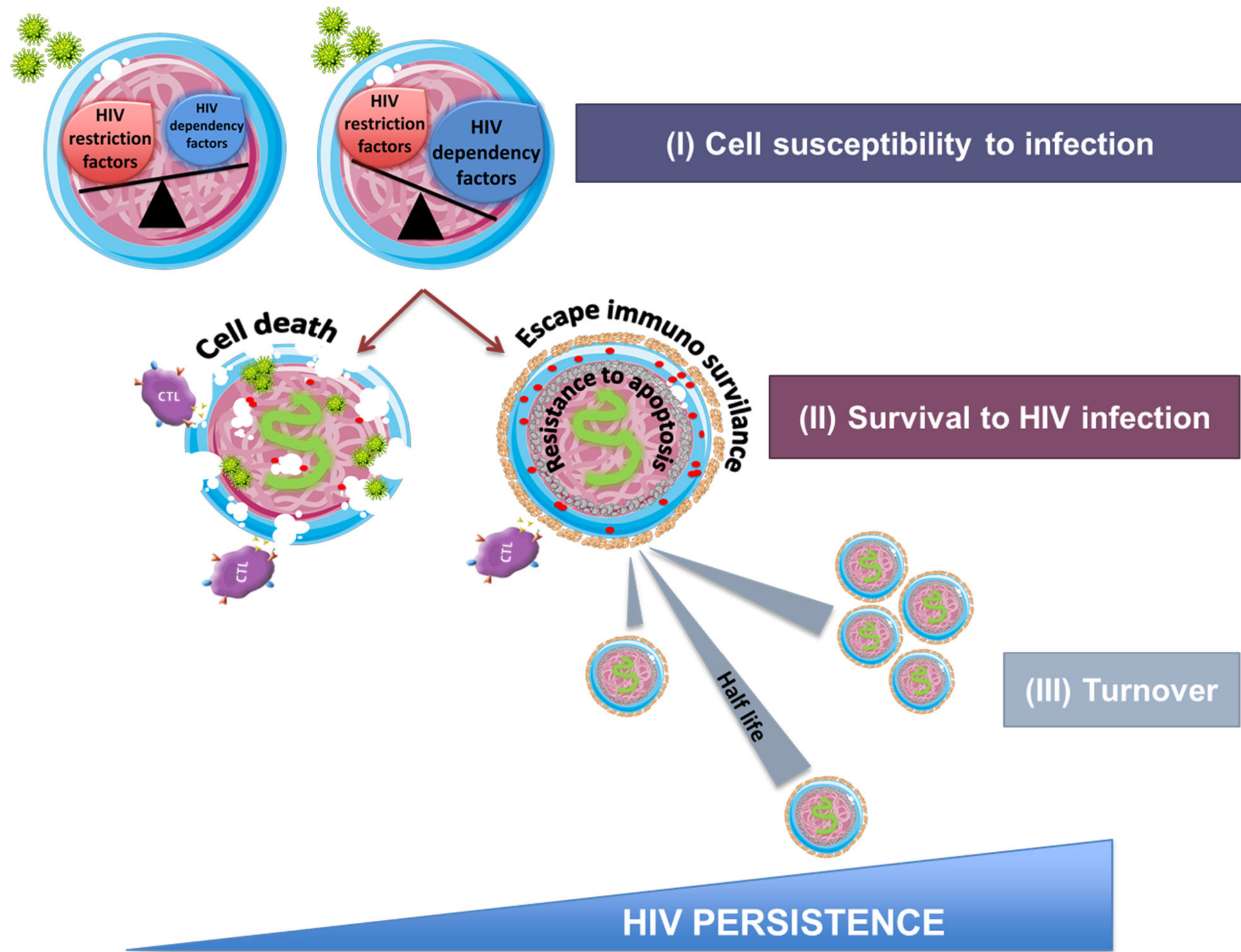

Figure 1. Cellular determinants for the establishment and persistence of HIV reservoirs. The establishment of viral reservoirs is first determined by the susceptibility of cells to infection (i), which is regulated by the balance of HIV host dependency factors and viral restriction factors present in the cells. In order to persist, infected cells need first to resist apoptotic signals induced by viral infection and avoid immune surveillance (ii). These resistant infected cells will persist for variable periods of time depending on their specific life span and capacity to proliferate without enhancing HIV dependent cell death signals (iii).

FIGURE 2

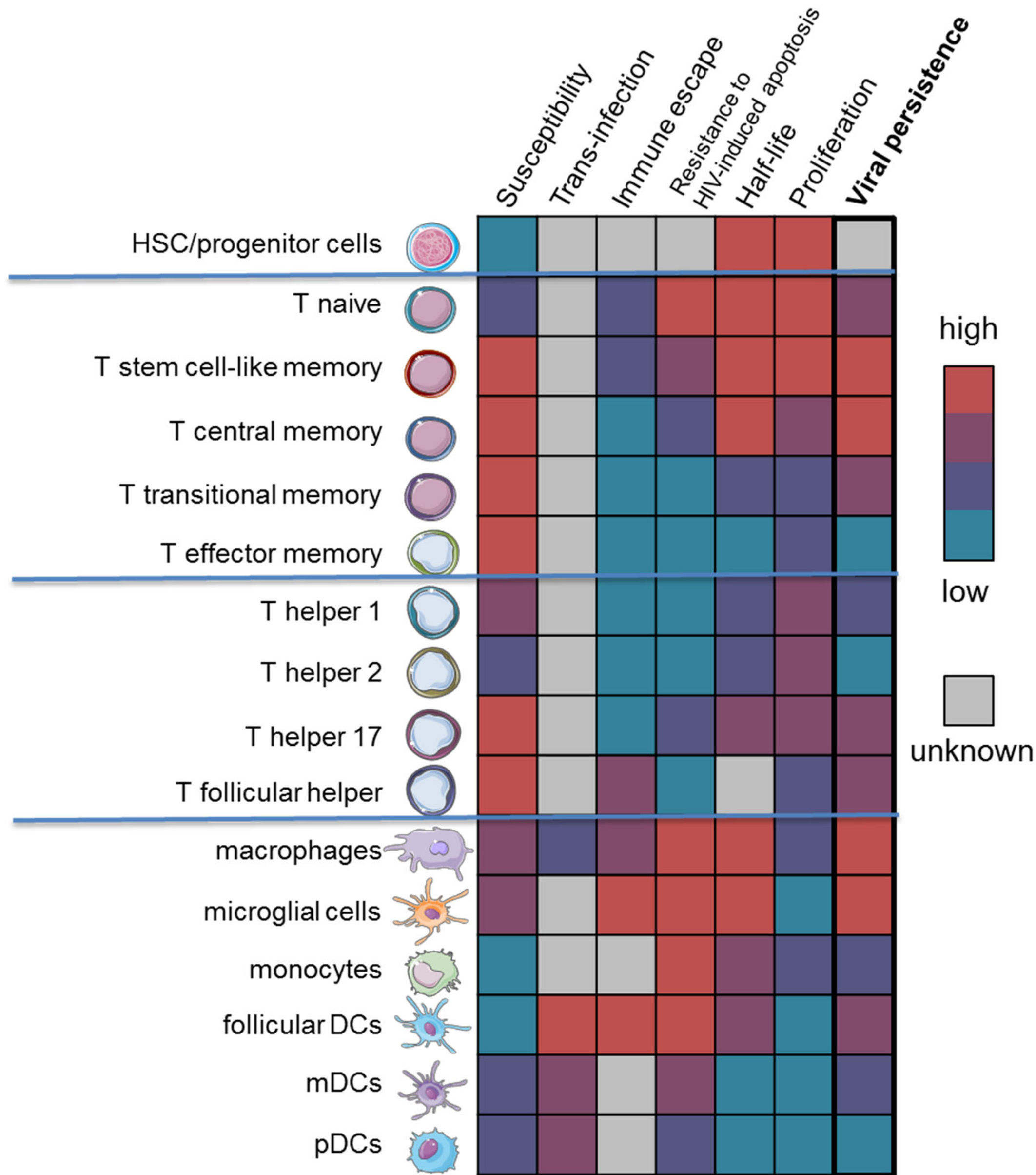

Figure 2. Estimation of potential of different cell subsets to HIV persistence. Among lymphoid cell subsets, naïve, central memory and stem-cell like memory T cells are thought of as major contributors to long-term HIV cellular reservoir, while effector memory and terminally differentiated helper T cell subsets are depleted first during infection and have limited turnover

potential. Myeloid cells, despite their relatively low susceptibility to infection, are now increasingly recognized as important contributors to the reservoir in both lymphoid and non-lymphoid tissues due to their long half-life and resistance to apoptosis and immune-mediated killing. Although not reflected here, tissue localization and activation status influences various parameters of persistence, with actively producing cells being more susceptible to cell death thus reducing their contribution to long term reservoir.