Review

Host Restriction Factors Modulating HIV Latency and Replication in Myeloid Cells

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Abstract: In addition to CD4+ T lymphocytes, myeloid cells, and, particularly, differentiated macrophages, are targets of the human immunodeficiency virus type-1 (HIV-1) infection via interaction of gp120Env with CD4 and CCR5 or CXCR4. Both T cells and macrophages support virus replication although with substantial differences. In contrast to activated CD4+ T lymphocytes, HIV-1 replication in macrophages occurs in nondividing cells and it is characterized by virtual absence of cytopathicity both *in vitro* and *in vivo*. These general features should be considered in evaluating the role of cell-associated restriction factors aiming at preventing of curtailing virus replication in macrophages and T cells particularly in the context of designing strategies to tackle the viral reservoir in infected individuals receiving combination antiretroviral therapy. In this regard, we will here also discuss a model of reversible HIV-1 latency in primary human macrophages and the role of host factor determining restriction or reactivation of virus replication in myeloid cells.

Keywords: HIV, Macrophages, MDM, restriction factors, transcription factors, macrophage polarization

1. Introduction. Macrophages as targets of HIV replication.

The *Lentiviridae* genus of retroviruses are known to privilege mononuclear phagocytes for their replication although they can also infect other cell types [1]. In the case of the human immunodeficiency virus (HIV), the selection of CD4 as primary entry receptor has evolutionarily determined an expansion of its cell tropism for a major subset of T lymphocytes with "helper" function. The consequence of HIV replication in CD4+ T cells is their depletion in association with a profound cytopathic effect, including formation of large syncytia *in vitro*. *In vivo*, HIV infection of CD4+ T cells leads to their progressive depletion leading to a state of profound immunodeficiency known as acquired immunodeficiency syndrome (AIDS) with emergence of opportunistic infections and peculiar types of cancer resulting in the death in >95% of infected individuals if combination antiretroviral therapy (cART) is not administered [2, 3]. Conversely, HIV infection of tissue macrophages neither causes their depletion *in vitro* nor *in vivo*, perhaps reflecting the coevolution between lentiviruses and myeloid cells.

HIV infection of macrophages has been largely studied *in vitro* upon differentiation of human peripheral blood monocytes into differentiated cells (i.e., monocyte-derived macrophages, MDM). Although MDM have been largely interpreted as a surrogate model of the physiological differentiation pathway of myeloid cells, it has been recently demonstrated that it actually represents only an "emergence" pathway of cell recruitment into inflamed tissues. Indeed, most tissue-resident macrophages (TRM) derive from primordial embryonic structures and seed the peripheral tissues before the development of the vessels, blood and bone marrow [4, 5]. TRM play a role in tissues and organs as scavenger cells removing apoptotic bodies and maintain themselves through the release of cytokines inducing their homeostatic

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slow turnover [4, 5]. However, in the case of infection or inflammation, circulating monocytes are recruited in the damaged tissue through in response to chemokines and other chemotactic factors released by the site of infection and rapidly differentiate into MDM, therefore mixing with TRM in the orchestration of the local inflammatory, anti-microbial response.

There is robust evidence that TRM are targets of HIV infection *in vivo*, as highlighted particularly in the central nervous system (CNS) in which microglia, upon infection in the absence of cART, drives the development of a deadly encephalitis associated with a clinical condition known as AIDS-associated dementia [6]. HIV infection of macrophages has been shown also in other tissues and organs, as reviewed in [7-9] and reproduced in relevant animal models such as non-human primates (NHP) experimentally infected with the simian immunodeficiency virus (SIV) [10] and immunodeficient mice reconstituted with human progenitor cells before HIV infection [11].

Thus, with the caveat that *in vitro* infection of MDM might not reflect accurately all features of TRM, some of the main similarities and differences between CD4+ T cell and MDM infection can be summarized as in **Table 1.**

Table 1. Similarities and differences between CD4+ T cell and MDM infection *in vitro* and *in vivo*

	CD4+ T cells	Macrophages	Refs.	Notes
Entry receptors	CD4, CCR5, CXCR4	CD4, CCR5, CXCR4	[2]	Although macrophages express CXCR4 productive infection is usually associated with CCR5 use
Cell proliferation	Yes	No	[12, 13]	
Cytopathic effect, cell depletion *in vitro*	Yes	No	[9]	
Cytopathic effect, cell depletion *in vivo*	Yes	No	[2, 9]	CD4 T cell depletion *in vivo* is likely the result of different processes and not only of direct virus-induced cytopathicity
Main pathogenetic consequence	Profound immunodeficiency, opportunistic infections, cancer	Tissue pathology, brain infection (encephalitis)	[2, 14]	
Virus budding and release	Plasma membrane only	Plasma membrane and virus containing compartments (VCC)	[15, 16]	VCC have been defined as invaginations of the plasma membrane that can be connected or not with the cell surface
Role as viral reservoirs in cART-treated individuals	Well-demonstrated in the case of latently infected "resting memory" cells	Strong evidence in support of TRM	[17-21]	TRM are credited with a longer ½ life than MDM

2. Host cell restriction factors, HIV infection and replication

There is abundant evidence of a complex network of intracellular factors already expressed in many cell types in the absence of an infection thereby providing a state of "intrinsic immunity" [22]. A general feature is their upregulation by interferons (IFNs) that are generated in response to viral infection or vaccination. In the case of HIV, the first clear-cut evidence of "intrinsic immunity" came by studying cell lines with either "permissive" or "nonpermissive"

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phenotypes in terms of virus infection and replication. Permissive cell lines were demonstrated to be devoid of an intracellular gatekeeper belonging to the apolipoprotein B mRNA-editing catalytic polypeptide (APOBEC) family [23], namely **APOBEC3G (A3G),** a cytidine deaminase targeted to proteasomal degradation by the virion-associated accessory protein **Vif**. In the case of viruses lacking Vif expression, A3G present in host cells interferes with the process of reverse transcription of the genomic viral RNA into DNA by converting the cytosines present in the minus strand into uracils thereby resulting in accumulation of G-to-A mutations in the plus strand. This seminal discovery inspired the paradigm that restriction factors such as A3G are often counteracted by specific viral genes consequent to a long coevolution process [24]. After A3G, other members of the APOBEC family have shown similar anti-HIV activity, with particular regard to **A3C, A3D, A3F** and **A3H**, as reviewed in [25]. Like A3G also A3F and A3H (but not other members) are inhibited by Vif [24].

In the case of HIV, and of retroviruses in general, the goal of restriction factors is best achieved when acting before integration of proviral DNA into host cell chromosomes in order to curtail the number of persistently infected cells unaffected by cART that contribute to the establishment of the so-called "viral reservoir", nowadays a major obstacle in HIV eradication. Once proviral integration has occurred, however, certain transcription factors could be also considered operationally as "restriction factors" by favoring latent vs. productive infection, as later discussed. Finally, additional restriction factors have been shown to target late steps in the viral life cycle, therefore affecting virus spreading to target cells, as in the case of BST-2/Tetherin and SERINC5, as later discussed.

An overall list of the main HIV restriction factors relevant to HIV infection of macrophages and their main mechanism of action (when identified) is summarized in **Table 2** and visualized in **Figure 1**.

Table 2. Main Restriction and Transcription Factors Curtailing HIV Infection or Replication in Human Macrophages

Restriction Factor	HIV Life Cycle Step Affected	Mechanism of Action	Counteracting Viral Protein	Key refs	Notes
STING, IRF3	Viral entry	Low levels IFN response	VpR, VpX	[26-28]	
REAF	Early post-entry events	Unclear/unknown	VpR	[29]	
TRIM5α	Early post-entry events	Degradation of incoming viral capsid		[24, 30]	Human TRIM5α prevents animal lentivirus infection, but not HIV
APOBEC-3 members	Reverse transcription	C to A hypermutation	Vif	[24, 31]	
Tet2	Reverse transcription	Cytosine demethylation	VpR	[32]	
SAMHD1	Reverse transcription	Depletion of dNTP pool	VpX (HIV-2)	[9]	SAMHD1 is involved in the Aicardi Goutières Syndrome
MxB	Reverse transcription	Interaction with PIC		[33-35]	PIC: Pre-Integration Complex
TREX-1	Complex with HIV DNA	prevention of IFN induction		[36, 37]	TREX1 is involved in the Aicardi Goutières Syndrome
TRIM22	Integrated provirus	Transcriptional repression		[38]	

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NF-kB1 (p50) homodimers	Integrated provirus	Transcriptional repression		[39, 40]	Active NF-kB is composed of a heterodimer of p50 and p75
p21/Waf1	Integrated provirus	Transcriptional repression		[41, 42]	
CIITA	Integrated provirus	Transcriptional repression		[43, 44]	CIITA also repressed HTLV-1/2 Tax transcriptional activity
BST-2/ Tetherin	Budding and virion release	Prevention of virion release from plasma membrane	VpU (Nef**)	[31, 45, 46]	
IFITM1, 2, 3	Budding and virion release	Incorporation into nascent HIV-1 virions	VpR	[47]	
Mannose Receptor	Budding and virion release	Envelope incorporation into virions	VpR, Nef	[48, 49]	
GBP-5	Budding and virion release		VpU	[50]	
MARCH1, 2 and 8	Budding and virion release	Envelope incorporation into virions		[51, 52]	
SERINC5	Budding and virion release	Prevention of cell fusion	Nef	[53, 54]	
Cholesterol 25-hydroxylase	Budding and virion release	Prevention of cell fusion		[55]	

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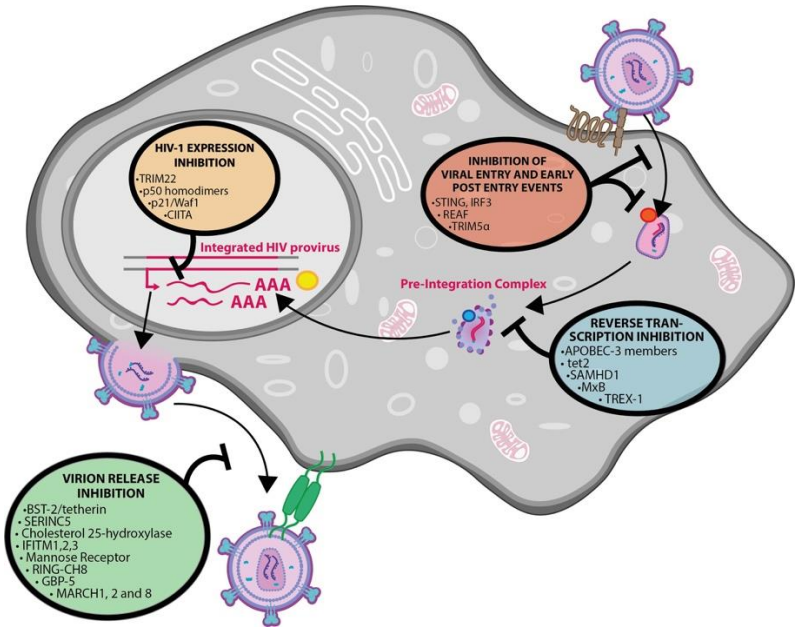

Figure 1. Restriction and transcription factors acting on HIV-1 infection in myeloid cells. See Table 2 for details.

3. Restriction factors and counteracting viral proteins active in myeloid cells

Among other accessory genes encoded by HIV, **VpR**, a virion-associated protein, has been early characterized as a relevant factor to allow efficient virus replication in macrophages [56, 57]. Its mechanism(s) of action has/have been debated for several years and likely does not rely on a single modality. Experimental evidence supports an active role of VpR as transcriptional booster of provirus expression potentially involving its interaction with the intracellular glucocorticoid receptor [58] followed by its translocation from cytoplasm into the cell nucleus and leading to cell cycle arrest in G_2/M phase [59-61]. The importance of VpR in HIV pathogenesis is supported by *in vivo* studies in NHP [62] and in HIV-1+ long-term nonprogressors (LTNP) infected with VpR-defective viruses [63] as well as by a rare case of human infection in a laboratory setting [64], as discussed in detail in [26]. It has been suggested that VpR could cooperate with Vif in the interaction with **A3G** leading to its proteasomal degradation [65].

In addition to A3G, other members of the APOBEC family have shown restrictive effects on HIV-1 infection. Although **A3A** was not initially included among those, we [66] and others [67, 68] have collected evidence of its potential role as restriction factor for HIV-1 infection in monocytes and macrophages, as later discussed. Of interest, an IFN-independent regulation of A3A expression has been observed in association with the downregulation of endogenously released chemokine CCL2/MCP-1 [69]. In the same study, CCL2 downregulation led to NF-KB mediated upregulation of the microRNA miR-155 that modulates the expression of several genes, including chemokines and their receptors [69].

Another target of VpR potentially relevant for HIV infection of macrophages is **Tet2 (ten eleven translocation 2),** a member of the family of DNA dioxygenase that leads to cytosine demethylation [70]. Vpr-dependent degradation of Tet2 has been associated with an increased secretion of interleukin-6 (IL-6) by HIV-infected macrophages enhancing virus replication by acting in an autocrine/paracrine fashion [71]. In addition, Tet2 upregulates the expression of **iFN-induced transmembrane protein 3 (IFITM3)**, restricting virus replication in cooperation with **IFTIM2** [32]. Finally, VpR has been shown to interfere with the transcription of type 1 IFNs [72, 73] as well as with the recently described **RNA-associated Early-Stage Antiviral Factor (REAF)** acting on early steps of the virus life cycle both in cell lines [74] and in primary human MDM [29].

As mentioned above, a peculiar property of macrophages is their long survival in culture in a nonproliferating state [although tissue-resident macrophages undergo a slow homeostatic proliferation driven by cytokines secreted in

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an autocrine fashion in order to persist indefinitely in the host [4, 5]] caused by a physiological arrest of their cell cycle. This aspect of their biology has been recently revisited for its relevance to HIV infection and the role of restriction factors, with particular regard to **SAMHD1** (SAM domain and HD domain-containing protein 1), a hydrolase processing deoxynucleotides triphosphates (dNTPs) physiologically involved in DNA repair mechanisms [13]. SAMHD1 acts by depleting the pool of dNTPs that are the "building blocks" necessary to the reverse transcription process in order to synthesize viral DNA before its integration into the host cell genome. **Vpx** is an accessory protein of the HIV-2, but not of the HIV-1 genome, that targets SAMHD1 for proteasomal degradation thus allowing efficient virus replication [75, 76]. As HIV-1 is devoid of Vpx it has been highly debated whether a similar mechanism of SAMHD1 inactivation would be expressed by other accessory viral proteins. in this regard, Ferreira and colleagues have reported that, although in the absence of cell division [13], macrophages in G_0 phase express **p21/Waf1**, previously shown to represent a negative regulator of virus replication in macrophages [41], together with high levels of SAMHD1 thereby resulting in a highly restricted state in terms of virus replication. Their switch to G_1 has been associated with downregulation of p21/Waf1, increased expression of cyclin dependent kinase 1 (CDK1) and inactivation of SAMHD1 by means of its phosphorylation leading to increased levels of dNTPs and unleashing of virus replication. This pathway was reverted by conditions such as genotoxic stress and response to "danger signals" that favored a back-transition from G_1 to G_0, as reviewed in [13].

The IFN-inducible human myxovirus resistance protein B (**MxB)**, related, but distinct from MxA, has been recently described as restriction factor for HIV-1 infection by acting after the completion of the reverse transcription process but before proviral integration likely trough interaction with capsid-cyclophilin A [77-80].

A well-described feature of HIV-1 replication in macrophages is the lack of induction of a robust type 1 IFN response upon infection, likely explained by the activity of the **TREX1** exonuclease [81, 82], although a modest induction of IFN induced genes (ISG) can be observed upon infection [83, 84]. In the absence of a specific cytosolic sensor, it has been hypothesized that the perturbation of the plasma membrane caused by the interaction with HIV-1 virions would be sufficient to trigger a canonical activation of a STimulator of INterferon Genes (**STING)**-dependent pathway involving the **IFN-responsive factor 3 (IRF-3)** [85, 86]. In addition, a second wave of ISG expression has been reported to occur a few days after infection following proviral integration and synthesis of the viral regulatory protein Tat, as reviewed in [51].

Once integrated as proviral DNA, the expression of the HIV genome falls under the control of both viral and host factors influencing its transcription, RNA splicing and export from the nucleus to the cytoplasm, translation into viral proteins and their assembly with full-length viral RNA at the plasma membrane to generate new progeny virions. The role of negative regulators of proviral transcription (described in Table 2) will be discussed later.

The last steps of the viral life cycle, namely the budding and release of new virions, is a target of modulation by several RF, as earlier described in the case of **Tetherin/BST-2**, an IFN-inducible tetraspanin that keeps the virions stuck at the cell surface and not released; its action is counteracted by the viral accessory protein **Vpu** that promotes its degradation [87, 88]. In addition to IFN, also the presence of the viral accessory protein **Nef** has been associated with increasing levels of Tetherin expression in macrophages [89]. In addition, proteins of the **IFITM family** (in particular, **IFITM1, 2** and **3**) interfere with the release of new virions by inserting themselves into the Env of nascent virions thereby impairing cell fusion in models of cell-to-cell viral spreading [90, 91]. Of note is the fact that their restriction seems particularly effective in macrophages [92]. Furthermore, two recently identified restriction factors **(membrane-associated RING-CH 8 (MARCH8)** and guanylate binding protein-5, **GBP-5** (this latter counteracted by the viral accessory protein **Vpu**) expressed in macrophage target HIV-1 gp120 Env, therefore playing a role in the final phases of budding and release of new progeny virions [50, 52]. Members of the **MARCH family** of E3-ubiquitin ligases are under the control of IFN and were previously shown to downregulate several host cell proteins from the plasma membrane and limiting the levels of HIV-1 Env incorporation into budding virions therefore resulting in their decreased infectivity. In particular, **MARCH8** is endogenously expressed by MDM and dendritic cells whereas its KO results in a

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significant boost of virion infectivity [52]. Similarly, **MARCH1** and **MARCH2** likely interfere with Env incorporation into virions [93], as reviewed in [51].

SERINC3 and **SERINC5** are host molecule that significantly decreases virion infectivity by interfering with the fusogenic properties of HIV-1 Env glycoproteins whereas the accessory viral protein **Nef** prevents their incorporation into nascent virions at the plasma membrane [53, 54]. Their effect has been mostly studied in cell lines, but although they seem to be dispensable for virus replication in primary activated CD4+ T cells, recent studies have described its relevance particularly for primary macrophages, although with significant inter-donor variability [94]. Of interest, SERINC5 expression is upregulated during differentiation of monocytes into MDM [95] and its incorporation into virions has been linked to the upregulated production of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), IL-6 and others, an effect that was also prevented by Nef [94]. SerinC5-induced release of pro-inflammatory cytokines by MDM occurred in synergy with the CCR5 antagonist Maraviroc that blocks virions bound to CD4 on the plasma membrane [96]. In this regard, SERINC5-expressing virions showed a greater susceptibility to inhibition by either Maraviroc or neutralizing antibodies [97]. Thus, in addition to its direct antiviral effect, SERINC5 incorporation into virions may serve as "danger signal" to the infected cells to trigger or enhance a pro-inflammatory response finalized to counteract the infection.

A restriction factor lately emerged as capable of influencing the capacity of HIV to infect cells is the IFN-inducible **cholesterol 25-hydroxylase** that produced **oxysterols,** mediators of several process including inflammation and immune activation [98, 99]. In addition to HIV, this ER-associated enzyme can limit the infectivity of several enveloped viruses, including Ebola, Influenza A viruses and poliovirus, an RNA virus lacking the envelope. Overall, oxysterols are believed to restrict infection at the levels of viral entry by interfering with the fusion of the virion-target cell membranes, a reviewed in [55].

The **mannose receptor (MR)** expressed by macrophages plays a fundamental role as extracellular sensor of bacterial and fungal invasion promoting their phagocytosis. Bacterial countermeasures include the synthesis of lipoarabinomannan by *Mycobacterium tuberculosis* that binds and downregulated the MR leading to the inhibition of a protective inflammatory response, as reviewed in [100]. In addition, MR can serve as entry receptor for Dengue virus and can also interact with HIV-1 virions like other cell surface molecules including DC-SIGN [101] and the integrin α4β7 [102]. Interaction of HIV virions with the MR may facilitate trans-infection of CD4+ T lymphocytes. Interestingly, MR is dowregulated by the HIV-1 accessory proteins **VpR** [48] and **Nef** [49]. As MR destabilizes gp120 Env expression on the MDM cell surface, VpR dual targeting of MR and IFITM3 may ultimately favor the capacity of infected MDM to spread the infection, as discussed in [100].

4. **Macrophage polarization to a pro-inflammatory mode. A model of HIV-1 restriction.**

In addition to a profound revisitation of their ontogenesis macrophage biology has been reinterpreted beyond the classical view of a cell responding to pro-inflammatory signals according to a simple "on-off" modality. A paradigm of an "M1/M2" functional polarization was generated in analogy to the well-defined Th1/Th2 established modality of diversification of CD4+ T helper cell activation. Classically activated M1 macrophages are induced by well-known pro-inflammatory signals, including bacterial endotoxin and cytokines such as IFN-γ and tumor necrosis factor-α (TNF-α) whereas M2 macrophages are considered "alternatively activated" by anti-inflammatory and immunoregulatory signals including IL-4, IL-10 and others. M1 macrophages contribute to the inflammatory process and, in general, exert anti-microbial and anti-cancer effects, whereas M2 macrophage polarization exerts anti-inflammatory effects together with tissue-regenerating activities, including neoangiogenesis, for which it is usually considered as cells favoring cancer growth and exerting variable effects in terms of antimicrobial activities, as reviewed in (ref). Of note is the fact that, unlike Th1/Th2 lymphocytes, macrophage polarization is a transient condition with cells returning to their basal state a

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few days after the polarizing signals have been withdrawn. This dichotomous view has been tempered by considering "M1" and "M2" as the two extremes of a spectrum of functional profiles [103].

We have originally investigated the implications of M1/M2 polarization for HIV-1 infection of primary human MDM). In order to distinguish cell polarization from the direct effect of the polarizing cytokines on HIV-1 infection, differentiated MDM were preincubated for 18 h with IFN-γ and TNF-α or with IL-4, in order to induce M1 or M2 polarization, respectively, and the cytokines were then removed before infection. Quite unexpectedly, we observed that both M1 and M2 polarization led to reduced levels of virus replication, although with different profiles. M1-MDM showed a more robust inhibition of virus replication (ca. 90% vs. control, unpolarized cells) whereas M2-MDM decreased HIV-1 production by ca. 50% although M2-induced inhibition of virus production lasted longer than that caused by M1 signals that vanished ca. 3 days after removal of the cytokines [104]. M1 polarization was associated with a profound downregulation of CD4 from the plasma membrane together with an upregulated secretion of some CCR5-binding chemokines. Therefore, we initially proposed that M1 polarization induced a potent, yet partial restriction of viral entry, as supported by the quantitative analysis of cell-associated HIV DNA [104]. Concerning M2-MDM we further demonstrated a significant role of DC-SIGN in the restricted patters observed [101].

We then investigated whether the M1 restriction could be also observed bypassing the cell entry step using a VSV-g pseudotyped virus. The results indicated that also this virus was profoundly restricted in its capacity to replicate in human MDM [66], suggesting a model whereby M1 polarization imposes different "hurdles" to virus replication both at the levels of viral entry and at one or more post-entry steps [104]. Of note, we observed a clear-cut upregulation of APOBEC-3A (A3A) in M1, but not in M2, MDM that returned to the levels observed in monocytes [66]. This observation, together independent reports from other research groups [67], suggested that A3A could be involved in the overall restricted profile of HIV-1 replication typical of M1-MDM. No significant modulation of A3G or SAMHD1 was observed in polarized vs. unpolarized macrophages.

These initial studies were finalized to explore whether M1/M2 polarization of macrophages before infection would affect their susceptibility to support virus replication in case of infection. Therefore, we next investigated the potential role of functional macrophage polarization in already infected cells; based on the observation of a more profound inhibitory effect of M1 polarization on virus replication, these experiments were not conducted with M2-MDM. A *caveat* to these experiments was based on the very well-known effects of both IFN-γ and, particularly, TNF-α on virus replication as a consequence of their activation of transcription factors acting on integrated proviruses by promoting or enhancing their transcription. In this regard, both STAT1, activated by IFN-γ, and NF-kB (induced by TNF-α) have well-defined binding sites in the 5'-LTR of the HIV-1 provirus. Therefore, we hypothesized that the restimulation of M1-MDM with IFN-γ and TNF-α several days after infection (allowing proviral integration to occur) could result in triggering virus replication as a consequence of the positive effects of STAT1 and NF-kB on those cells carrying integrated proviruses.

Quite surprisingly, we observed a very reproducible pattern whereby repolarization with M1-cytokines of HIV-1 infected M1-MDM (an experimental condition that we defined as "$M1^2$-MDM") drove virus replication to nearly undetectable levels (in the absence of cytopathic effects) (**Figure 2)**. The levels of HIV-1 DNA remained ca. 100-fold lower than those of control, unpolarized MDM whereas they returned to levels similar to those of control cells in the case of M1-MDM that were not restimulated by M1-cytokines. Finally, both unspliced and multiply spliced HIV-1 RNA remained at baseline levels when measured 8 days post-infection (i.e., 24 h after cytokine restimulation). As expected, A3A, and also A3G, were promptly upregulated in $M1^2$-MDM in comparison to control and M1-MDM that were not restimulated [43]. This quasi-silent pattern of HIV expression was not correlated with the lack of induction of STAT1 and NF-kB in these cells whereas other factors known to act as repressors of proviral transcription, namely TRIM22 and CIITA, were also upregulated [43].

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TRIM22, also known as Staf50, is an ISG whose expression is profoundly upregulated by IFN stimulation of different cell types [38]. Our group described it as the key factors differentiating U937 cell clones with a restrictive phenotype in terms of supporting HIV-1 replication ("Minus clones") in comparison to those fully permissive (Plus clones) [105]. TRIM22 does not possess a DNA binding domain and acts, at least in part, by preventing the binding of Sp1, a positive transcription factor constitutively expressed by many cell types, to the core promoter of the HIV-1 provirus [106]. CIITA was originally described to be the key transcription factor for MHC Class II antigen expression under the control of IFN-γ, as reviewed [44]. It was then demonstrated to play a significant role in the inhibition of both HTLV-1/HTLV-2 transcription and by competing with the regulatory protein Tat for binding to P-Tefb (a complex formed by Cyclin t1 and CDK9) in order to enhance HIV-1 proviral transcription also in myeloid cells [107].

Therefore, restimulation of HIV-1 infected M1-MDM by M1 cytokines induced both positive and negative restriction factors with the latter dominating over the former (**Figure 2**). Therefore, M1[2]-MDM could represent a potential model to investigate the hypothesis that, together with CD4+ T cells, also myeloid cells including macrophages could contribute to the establishment and maintenance of the viral reservoir resistant to cART. Indeed, cocultivation of M1[2]-MDM with allogeneic PBMC stimulated by the mitogen phytohemagglutinin (PHA) or incubation with their culture supernatants potently reverted virus infection into a productive one [43]. PHA-stimulated PBMC were not resuspended in a culture medium containing IL-2 to promote the formation of PHA blasts because M1[2]-MDM were incubated with PHA blasts they were readily eliminated by cell killing from the cell culture and/or diluted out by the proliferating cell [43]. Reversal from a state of quasi-latency to a fully productive infection induced by PHA-stimulated PBMC or their supernatant was selectively observed in M1[2]-MDM, but not in control or M1-MDM that had not been restimulated [43] perhaps because restimulation with M1 cytokines upregulated NF-kB and STAT1 (together with other transcription factors) that, in addition to the "kick" provided by allogeneic PHA-stimulated PBMC, tilted the balance of proviral transcription in favor of virus expression (**Figure 2**). These observations were confirmed after MDM infection with a VSV-g pseudotyped virus demonstrating that proviral reactivation occurred in a single HIV replicative cycle level [43].

Thus, M1[2]-MDM represent a robust model to investigate which factors can affect either positively or negatively HIV-1 infection, latency and replication in myeloid cells. It should be underscored, in this regard, that TRM, and not CD4+ T cells, have been shown to represent the viral reservoir in urethral tissue of HIV-infected men receiving cART and undergoing gender reassortment [20], whereas the existence of M1-polarized macrophages exhibiting a restricted profile compatible with our in vitro observations has been demonstrated *in vivo* at least in the case of decidual macrophages [18].

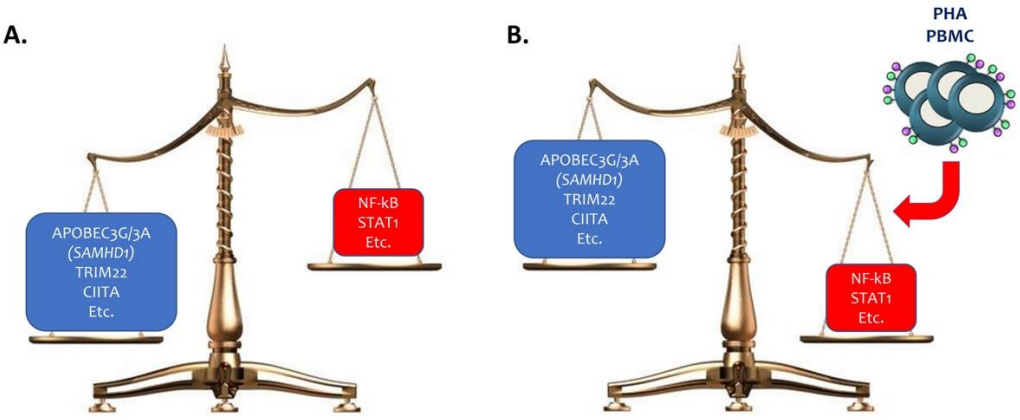

Figure 2. M1[2]-MDM: A model of reversible HIV-1 latency in primary human MDM. A. Restimulation of infected M1-MDM with M1 cytokines (IFN-γ and TNF-α) resulted in a very restricted profile of HIV-1 infection associated with the

expression of both negative (blue box) and positive (red box) restriction and transcription factors in terms of virus expression. **B.** coculture with PHA-stimulated allogeneic PBMC (in the absence of IL-2) tilted the balance of these factors resulting in a productive infection.

5. Conclusions

HIV-1 infection of CD4+ cells involves primarily a prominent subset of T lymphocytes and myeloid cells. These latter encompass very different cell types, such as myeloid dendritic cells, circulating monocytes, eventually extravasating to become MDM in inflammatory conditions, and TRM that acquire very distinctive features according to the anatomical site (from Kupffer cells in the liver to the microglia in the CNS). Although the prominent role of latently infected CD4+ T cells in establishing and diversifying the HIV reservoir of cells carrying replication-competent proviruses has been well established, a growing number of evidence indicates that myeloid cells could also contribute significantly to this unsolved issue preventing the eradication of HIV-1 infection from cART-treated individuals. The identification of specific extracellular and intracellular factors influencing the susceptibility of myeloid cells to become targets of either latent or productive infection could be a crucial goal in order to define effective strategies aiming at the curtailment of the HIV-1 reservoir or to its definitive silencing.

Author Contributions: GP designed the overall structure of the review; IP, SG and EV contributed to the literature analysis concerning restriction factors, PD contributed to the optimization of the M1-MDM model of HIV restriction. All authors reviewed and concurred with the hypotheses and interpretations of this article.

Funding: this article contains a description of original results funded in part by the PRIN project 2017TYTWZ3 to GP.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable..

Acknowledgments: the authors wish to thank previous collaborators on the characterization of TRIM22 restriction factor, and particularly Dr. Anna Kajaste-Rudnitski, and on the optimization of the M1-MDM model of HIV-1 restriction, and particularly Dr. Francesca Graziano.

Conflicts of Interest: the authors declare no conflict of interest.

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