REVIEW

Arch. Immunol. Ther. Exp., 2007, **55**, 131–138
PL ISSN 0004-069X

DOI 10.1007/s00005-007-0023-9

The innate cellular responses to HIV-1 invasion: emerging molecules of ancient defense mechanisms

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Received: 2006.12.14, **Accepted:** 2007.02.22, **Published online first:** 2007.06.08

Abstract

Along alternative protective pathways, human cells can synthesize biologically active proteins that interfere with HIV replication, but are not viral antigen specific. HIV is sensitive to several viral inhibitors of cellular origin, such as interferons or interferon-regulated proteins. With the progress of AIDS research it has become evident that the immune cells of some individuals are capable of restricting the virus by secretion of other, yet unidentified factor(s) that can be detected only by their potent antiviral activity. Research efforts to identify this inhibitor of HIV – a "magic bullet" released by our immune cells – have revealed the identity of several novel molecules and added to the knowledge of innate cellular responses to viral invasion.

Key words: innate immunity, antiviral factors, $CD4^+$ T cells, $CD8^+$ T cells.

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INTRODUCTION

Human beings possess multiple strategies to control viral replication and pathogenesis. Although the protective immunity in which exposure to virus elicits antigen-specific responses is more efficient in controlling later infections [31], the innate immunity is the first line of defense. Activated in multicellular organisms upon invasion by infectious microorganisms, immune cells can synthesize biologically active proteins that interfere with viral replication. For example, natural killer cells and γδ-T cells can kill infected cells through cytolytic mechanisms [7, 60, 69, 71], neutrophils produce antimicrobial defensins [73], and macrophages and dendritic cells take up and present viral antigens and also secrete several soluble cytokines and chemokines that augment the antiviral response. Interferons and interferon-regulated proteins are well-known exemplars of this class [35, 57]. Their contribution to antiviral defense (including anti-HIV-1 activity) and immune regulation has been well documented [28] and is not within the scope of this review.

With the progress of research it has become apparent that immune cells might be equipped with a more robust arsenal of active molecules regulating non-cytolytic anti-HIV-1 responses [1, 3, 8, 10, 11, 27, 29, 44, 51, 61, 76]. The first observations of such HIV-1 suppressor activity were made 20 years ago, soon after HIV-1 was discovered as an etiologic factor of AIDS [68]. This non-cytolytic anti-HIV-1 activity was carried out by soluble molecules secreted by $CD8^+$ T cells and was later named CAF (CD8 antiviral factor) [67]. Subsequent years of research showed that not only $CD8^+$ T cells secreted potent antiviral molecules, but the extracellular material from $CD4^+$ T cells also restricted virus replication through similar non-cytolytic mechanisms [1, 37, 51, 61, 62]. In the absence of effective treatments against HIV-1, all this brought much attention to these novel innate responses against HIV-1, particularly to their potential application in treating and preventing AIDS. It was particularly suspected that these unidentified molecules could play a major role in the antiviral defense of a small group of people who were infected with HIV-1 but did not progress to AIDS (long-term nonprogressors) and of others who were highly exposed to virus but who maintained seronegative status (highly exposed persistently negative). The long-term nonprogressors posed a challenge to AIDS researchers striving to understand the mechanism of this distinctive interference with the virus's replication, and most hypotheses were based on the existence of novel molecules that regulated cellular resistance to HIV.

The identification of three novel factors, RANTES and the macrophage inflammatory proteins 1α and 1β (MIP-1α, MIP-1β) [16], with non-cytolytic antiviral activity confirmed these scientific expectations and further challenged the conventional view of our immune system. Some researchers considered the notion that both the innate and adaptive responses to viral infection could be supplemented by these factors [20]. The discovery of viral secondary entry receptors and the restriction of viral entry by their natural ligands [16, 19, 22] gave only a partial explanation for the question of the protective mechanisms involving both $CD8^+$ and $CD4^+$ T lymphocytes, and the issue of the increased production of β--chemokines and their link to pathogenesis is still unsettled. The current status of the scientific quest to identify the novel molecules responsible for innate cellular responses to HIV-1 infection is discussed in this review.

NOVEL SOLUBLE PROTEINS MEDIATING ANTI-HIV-1 ACTIVITY IN $CD8^+$ T CELLS

The first observations of non-cytolytic activity against HIV-1 were obtained from peripheral blood mononuclear cells (PBMCs) isolated from long-term nonprogressors. Depletion of $CD8^+$ T cells from a total population of their PBMCs resulted in a rapid induction of viral expression [68], while reconstitution of depleted cultures with autologous $CD8^+$ T cells restored the antiviral activity [45, 72]. These data clearly suggested that the suppression of viral replication in this group of people was linked to a novel quality of their $CD8^+$ T lymphocytes. The concept that HIV-1 infection could be controlled by $CD8^+$ T cell-mediated non-cytolytic antiviral mechanisms was new at the time and brought much excitement to the AIDS field, particularly after subsequent studies revealed that the observed antiviral activity was mediated by soluble molecules [45, 67] suppressing a wide variety of primary HIV-1 isolates [4, 40].

In 1995, three novel factors, RANTES and the macrophage inflammatory proteins 1α and 1β (MIP-1α, MIP-1β), secreted by $CD8^+$ T cells were identified as novel HIV-1 suppressors [16]. These three cytokines are C-C (first two cysteines adjacent) or β-chemokines, first discovered on the basis of their chemotactic effect on lymphocytes and monocytes [58, 65, 66], RANTES, MIP-1α, and MIP-1β are known to exert their physiological functions through the ligation and activation of the G-protein-coupled cellular membrane receptor [54]. The natural receptor for these three chemokines is a seven-transmembrane-spanning G-protein-coupled surface protein called CCR5. Interestingly, CCR5 was also found to be used by HIV-1 as a secondary receptor for its cellular entry. In the presence of RANTES, MIP-1α, and MIP-1β, cellular invasion by the virus was severely limited and resulted in the interruption of its replication cycle [2, 5, 13, 18, 21].

This discovery marked a milestone in AIDS research and re-energized the immunobiological approach to HIV, tackling the emerging aspects of chemokine-mediated responses to the virus. For the first time, CAF activity was significantly reduced by specific neutralizing antibodies to RANTES, MIP-1α, and MIP-1β. However, this effect could be achieved only by a mixture of antibodies to all of these factors and suggested that CAF could be a collection of multiple molecules with redundant functions. Subsequent studies, however, showed that RANTES, MIP-1α, and MIP-1β suppressed only HIV-1 strains (called now R5) which utilized the CCR5 receptor and were inactive against X4 viruses, which utilize another surface protein, CXCR4, for cellular entry [16]. The X4 HIV-1 strains were blocked by a natural ligand of the CXCR4 receptor, SDF-1 [9]. Selective suppression of viral replication based on its glycoprotein composition was somewhat different from that described for CAF. CAF suppressed all viral isolates regardless of their receptor requirements, which suggested that some components of this antiviral activity were still unknown and were now referred to as any suppressive molecule other than RANTES, MIP-1α, and MIP-1β [10, 44].

Two years after the discovery of C-C chemokines and their contribution to antiviral responses, Pal et al. [51] identified another molecule with antiviral potential named MDC (macrophage-derived chemokine). MDC is a β-chemokine secreted into the extracellular compartment by activated mononuclear cells and acts on diverse cellular targets, including dendritic cells and T cell subsets [47]. The role of MDC protein in controlling HIV-1 infection has been controversial from the start. MDC tested by Pal was isolated from the supernatant of an immortalized $CD8^+$ T cell clone and lacked the first two NH_2 amino acids. This form of MDC was found to inhibit R5 and X4 HIV-1 isolates [51]. On the other hand, the synthetic form of the full-sized MDC used by Cota et al. [17] suppressed mainly R5 HIV-1 Bal in macrophages, but not in T cells. These discrepancies suggested that the inhibitory potential of MDC varied depending on the source and the methods of its preparation. The mechanism of MDC-mediated inhibition of HIV-1 replication was also different in various HIV-1 target cells. In primary T cells, for example, MDC inhibited HIV-1 at the level of reverse transcription, but in macrophages it inhibited the replication of R5 viruses at the post-reverse-transcription phase [17].

In 2002, a group led by David Ho announced the elimination of CAF activity by a cluster of small proteins: α-defensins-1, -2, and -3 [76]. Defensins are members of a large family of small cationic peptides characterized by a broad range of antimicrobial activity against bacteria, fungi, and viruses (including HIV) [36, 46, 59, 63, 74]. They were found to inhibit HIV by direct inactivation of viral particles, but also affected the ability of target cells to replicate the virus [46]. The claim that defensins could contribute to CAF activity was unjustified because they are expressed mainly by neutrophiles [36] and monocytes [46], but not by $CD8^+$ T cells. These reservations were later confirmed by subsequent studies

by the same group showing that the $CD8^+$ T cell cultures used for the initial studies were contaminated by neutrophils, the apparent source of the defensins, and this resulted in a retraction of the original interpretation [75]. Although defensins did not withstand the standards described for CAF, they have an antiviral potential and were enlisted into the array of factors contributing to innate responses to HIV invasion. Furthermore, their primary activity in the direct inactivation of the viral particle could be useful in the development of novel microbicidal therapies against virus.

Although the identity of CAF remained elusive, the mechanism of its antiviral activity pointed to inhibition of the transcription of viral genes through signal transduction pathways [10]. The mechanistic studies became possible due to technological advances allowing the development of the herpes virus saimiri and human T lymphotropic virus immortalized primary $CD8^+$ T cells producing CAF [16, 51]. Using now these immortalized CAF-producing cells, sufficient amounts of extracellular material were available to commence these studies. Based on this technology, Chang et al. [10] could establish that CAF induced cellular protection from HIV through the activation of signal transducer and activator of transcription 1 (STAT1) followed by the induction of interferon regulatory factor 1 (IRF-1) gene expression. IRF-1 is generally considered to be a positive regulator of expression [64] and, as shown in Chen's work, CAF treatment promoted binding of this protein to the HIV-1 promoter through the DBP/IRF binding site. On the other hand, the sibling of IRF-1, IRF-2, which is a general suppressor of transcription, also binds to the HIV-1 long terminal repeat (LTR) through the same DBP/IRF cognate sequence; however, the formation of the DNA/IRF-2 complex is not regulated by CAF, so the question as to how IRF-1 could be involved in HIV-1 LTR inhibition remained unresolved. Although it is highly speculative, it has been proposed that in the presence of CAF, both IRFs compete for the same DBP/IRF binding site on the HIV-1 promoter, which results in the repression of transcription of the virus's genes [10].

The first attempts to describe the biochemical characteristics of the unidentified component of CAF demonstrated that this heat-stable protein(s) maintained antiviral activity over a broad pH range and was less than 10 kDa and greater than 3 kDa in size [48] (Table 1). Ion exchange chromatography on $CD8^+$ T cell culture supernatants separated this material into six elution peaks [48]. CAF activity eluted in the high salt concentration and the high affinity of this protein to the strong anion resin suggested an overall negative charge. Six years later, using more sophisticated separation tools, the same group isolated and identified prothymosine α (Pro$T\alpha$) from CAF-positive supernatants of a herpes virus-transformed $CD8^+$ T cell line [49]. Pro$T\alpha$ is a small, hormone-like acidic protein with diverse intracellular and extracellular functions [30]. Although both native and recombinant forms of Pro$T\alpha$ inhibit HIV-1 by affecting the LTR, this protein demonstrated characteristics distinct from those described earlier for CAF. For example, the antiviral activity of native Pro$T\alpha$ was cell type dependent and the recombinant form of this protein had limited or no antiviral activity in both primary and transformed $CD4^+$ T cells. Furthermore, Pro$T\alpha$ did not induce the phosphorylation of STAT1, as was described for CAF [10], and lastly, the promoter inhibitory activity of Pro$T\alpha$ was not limited solely to the HIV-1 LTR. All this suggested that the unfractionated supernatants of CAF-expressing $CD8^+$ T cells still possess the unidentified molecule(s) exerting the anti-HIV activity of CAF, and continuation of these studies will complete the description of the novel mechanism through which cells of the immune system regulate the rapid responses to HIV infection.

NOVEL SOLUBLE PROTEIN FACTORS MEDIATING ANTI-HIV-1 ACTIVITY IN $CD4^+$ T CELLS

CD4-bearing lymphocytes are the primary targets of HIV-1 infection. The ligation of cellular receptors in these cells with the HIV envelope results in its fusion with the cell membrane and the initiation of the viral replication cycle. This viral envelope/cell membrane association also rapidly activates the expression of physiologically active proteins responsible for inflammatory responses, including NF-κB [14, 15]. Although $CD4^+$ T cells are equipped with a large arsenal of soluble non-cytolytic factors that are capable of controlling HIV, in most situations AIDS is still the ultimate end of this invasion. In isolated cases, however, $CD4^+$ T cells possess a quality that allows the suppression of viral infection and, similarly as in $CD8^+$ T cells, this resistance is mediated through potent soluble factor(s). The scientific evidence for these molecules is growing, particularly from studies involving multiply exposed uninfected individuals. For example, studies on a cohort of 25 subjects with histories of multiple high-risk sexual exposure to HIV-1 revealed that purified $CD4^+$ T lymphocytes were less susceptible to infection with primary isolates of HIV-1 than were $CD4^+$ T lymphocytes from unexposed controls [53]. The observed resistance was restricted by the envelope glycoprotein, not by a low cell surface density of CD4, and was associated with the activity of C-C chemokines. Another piece of evidence was published by Saha et al. [56], who showed that immortalized $CD4^+$ T cells isolated from long-term nonprogressors were resistant to HIV-1. This finding confirmed earlier observations made by Leith et al. [37] who demonstrated for the first time that supernatants of $CD4^+$ T cells suppressed HIV-1-LTR-mediated gene expression. The observed activity was not mediated by the C-C chemokines RANTES, MIP-1α, and MIP-1β, thus indicating the existence of novel soluble factors secreted by these primary targets of HIV-1.

Evidence for the secretion of novel factors produced by $CD4^+$ T cells resurfaced during our investigation of

cellular responses to long-term HIV-1 infection. We found that primary $CD4^+$ T cells exposed for an extended period of time to an attenuated form of HIV-1 acquired resistance to the virus and this resistance was maintained through an unknown soluble molecule, which we later named HRF (HIV-1 resistance factor) [61, 62]. Based on this observation, we devised a system for inducing resistance to HIV-1 by both primary and transformed $CD4^+$ T cells, enabling a model (named HRF(+)) for investigating the acquired resistance phenotype [61, 62]. Analysis of the virological aspects of resistant $CD4^+$ T cells and the HRF indicated that HIV-1 bound, entered, and reverse-transcribed efficiently, but, similarly as in CAF treatments, was blocked at transcription. The subsequent studies indicated that HRF(+) cells restricted the transcription of viral genes at the level of nuclear NF-κB/DNA binding [38, 39, 41, 61] and suggested that HRF could be related to some regulator of transcription.

Evaluation of the biochemical characteristics of the bioactive component in HRF(+) cell culture supernatant demonstrated that, like CAF, HRF is a heat-stable protein(s) active at wide pH, ranging from 4.5–10.5 (unpublished data; Table 1). Separation of HRF(+) cell culture supernatants through seven different ion exchange ligands showed that the active component of HRF bound with high affinity to the strong anion and, like CAF, eluted in the high salt concentration (Table 1). Subsequent mass spectrometric analysis (lc/ms/ms) of the HRF(+) protein peak identified the peptides derived from ubiquitin, histone H1 [38], and a novel pattern of peptides absent from human protein databanks (unpublished data).

The elution of monoubiquitinated histone at high salt concentration from a positively charged resin was unexpected and could be explained only by association of this protein with another active component of HRF. The subsequent removal of H1 from the HRF(+) cell culture supernatant (the extracellular compartment) did not affect its antiviral potential. Conversely, the siRNA interference with the expression of H1 abrogated HRF activity in both the intra- and extracellular compartments. These data provided evidence that histone H1 is indispensable for HRF activity only in resistant cells and suggested that histone H1 might have an auxiliary function in the HRF(+) system. Based on a thorough review of the multiple functions of extrachromosomal histones [6, 25, 33, 52, 55, 70], we hypothesized that monoubiquitinated histone H1 could serve as a chaperone for the secretion of HRF through the cell membrane. This observation also suggested that the antiviral HRF activity does not result from the function of just one protein, but represents a novel mechanism that might involve multiple cellular factors. This hypothesis was confirmed by our recent identification of another protein implicated in HRF-mediated activity, a transcription repressor: CTCF [32, 42].

CTCF has been called the first "truly multivalent" nuclear factor performing multiple regulatory tasks in gene expression [34, 50]. Through the binding of highly divergent DNA sequences and the formation of various protein complexes, this multifunctional protein might suppress or activate various genes [24]. As CTCF is a potent suppressor of transcription [23, 24, 26], a characteristic that is also the "landmark" of HRF antiviral activity [39, 41, 62], we explored the significance of CTCF and its link to the acquired resistance to HIV-1 in HRF(+) cells. This investigation demonstrated that the transcription of CTCF mRNA was increased in HIV-1--resistant HRF(+) cells and the elevated activity of this gene product could be correlated with the extent of antiviral protection [42]: higher CTCF transcriptional activity corresponded to stronger antiviral responses. The antiviral activity secreted to the extracellular compartment by HRF(+) cells could be eliminated by the RNAi-mediated depletion of CTCF mRNA, which suggested that CTCF protein is another intracellular effector of HRF activity. Most remarkably, the exposure of HIV-1-susceptible cells to HRF treatments induced the expression of CTCF mRNA also in these naïve virus target cells, thus providing a clear indication that HRF regulates the antiviral responses in an autocrine system, where the extracellular, active component of HRF induces the intracellular expression of its effector molecule, the transcription inhibitor CTCF (Fig. 1). At this time we do not have a clear explanation of how increased levels of CTCF protein restrict HIV replication. Based on our observations, however, we hypothesize that increased levels of CTCF protein might have an impact on the HIV-1 promoter (unpublished data).

Table 1. The comparison of known biochemical and functional characteristics of HRF and CAF

	HRF	CAF
Cell type	$CD4^+$ T lymphocytes	$CD8^+$ T lymphocytes
Cell compartment	Extracellular/soluble	Extracellular/soluble
Point of HIV-1 inhibition	LTR transcription inhibitor/interference with NF-κB/LTR binding	LTR transcription inhibitor/induction of IRF-1/LTR binding
Active pH range	4.5–10.5	2–12
Predicted charge based on ion affinity	Negative	Negative
Intracellular co-factors	Histone H1B; CTCF	STAT-1, IRF-1
Extracellular co-factors	??	MIP-1α and β, RANTES, MDC, ProTα

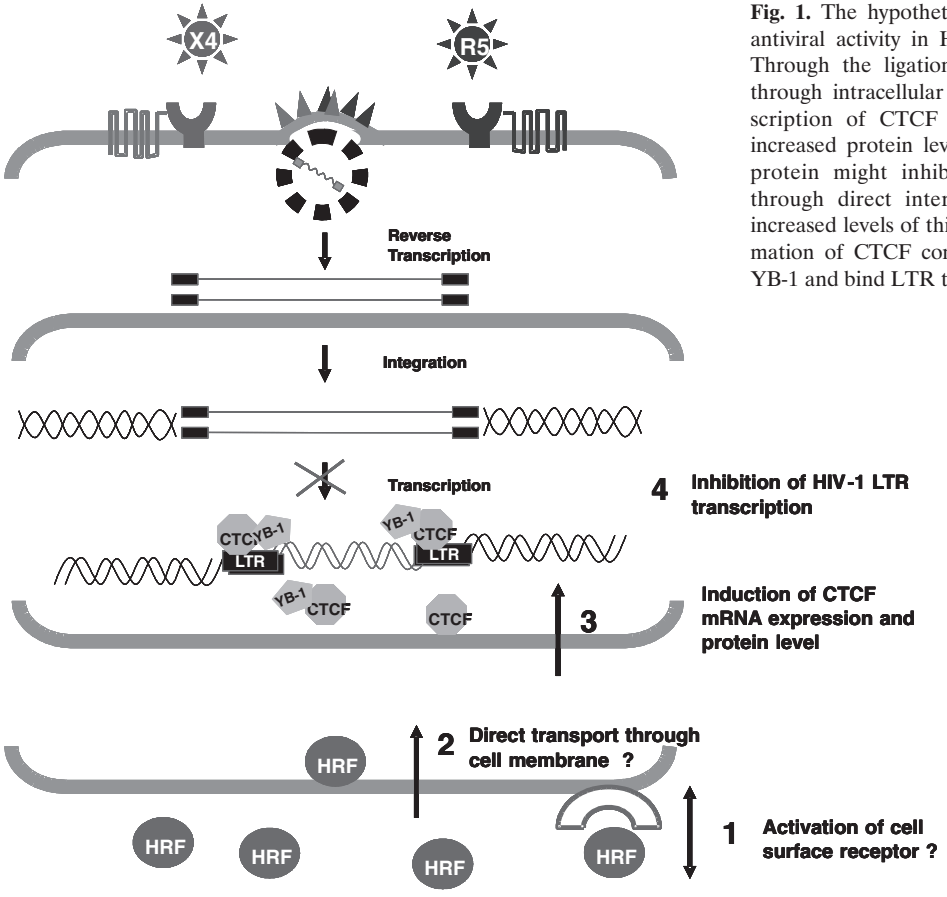

Fig. 1. The hypothetical scenario for HRF-mediated antiviral activity in HIV-1-susceptible CD4+ T cells. Through the ligation of a cell surface receptor or through intracellular uptake, HRF induces the transcription of CTCF mRNA, which is followed by increased protein levels. We hypothesize that CTCF protein might inhibit the transcription of HIV-1 through direct interaction with LTR, or that the increased levels of this protein might promote the formation of CTCF complexes with its protein partner YB-1 and bind LTR through the YB-1 binding site.

Although there is no scientific evidence of CTCF regulating HIV-1 transcription directly, its association with the YB-1 transcription regulator [12] might be vital for the observed activity.

CONCLUSIONS

Viruses have evolved mechanisms to hide from defensive measures of the host throughout the course of infection. At the same time, our immune system implements overlapping strategies to gain an advantage in this struggle, which becomes even more complicated when the target cell for viral invasion is the very cell of the immune system. Cellular responses to HIV are activated rapidly by the virus's invasion, and novel non-cytolytic responses to HIV represent a potent, but also complicated, array of multiple factors protecting cells from the virus's replication through targeting various steps of its replication cycle. As shown in Fig. 2 (p. 6), some of them inactivate the viral particles directly, while others interfere with their cellular entry or block the consecutive steps of their intracellular phase. Several of these antiviral molecules have been recently identified, but others, such as HRF and CAF, can be detected only by their potent antiviral activity. It is also quite reasonable to believe that factors mediating soluble non-cytolytic antiviral activity can be implemented to develop novel therapeutic strategies targeting HIV at the point of its cellular attack. Thus the scientific quest to uncover these "novel-ancient" molecule(s) secreted by our immune cells is important not only in the prevention and treatment of AIDS, but also in gaining a better understanding of our innate immune responses to HIV-1 at the initial stage of its cellular invasion.

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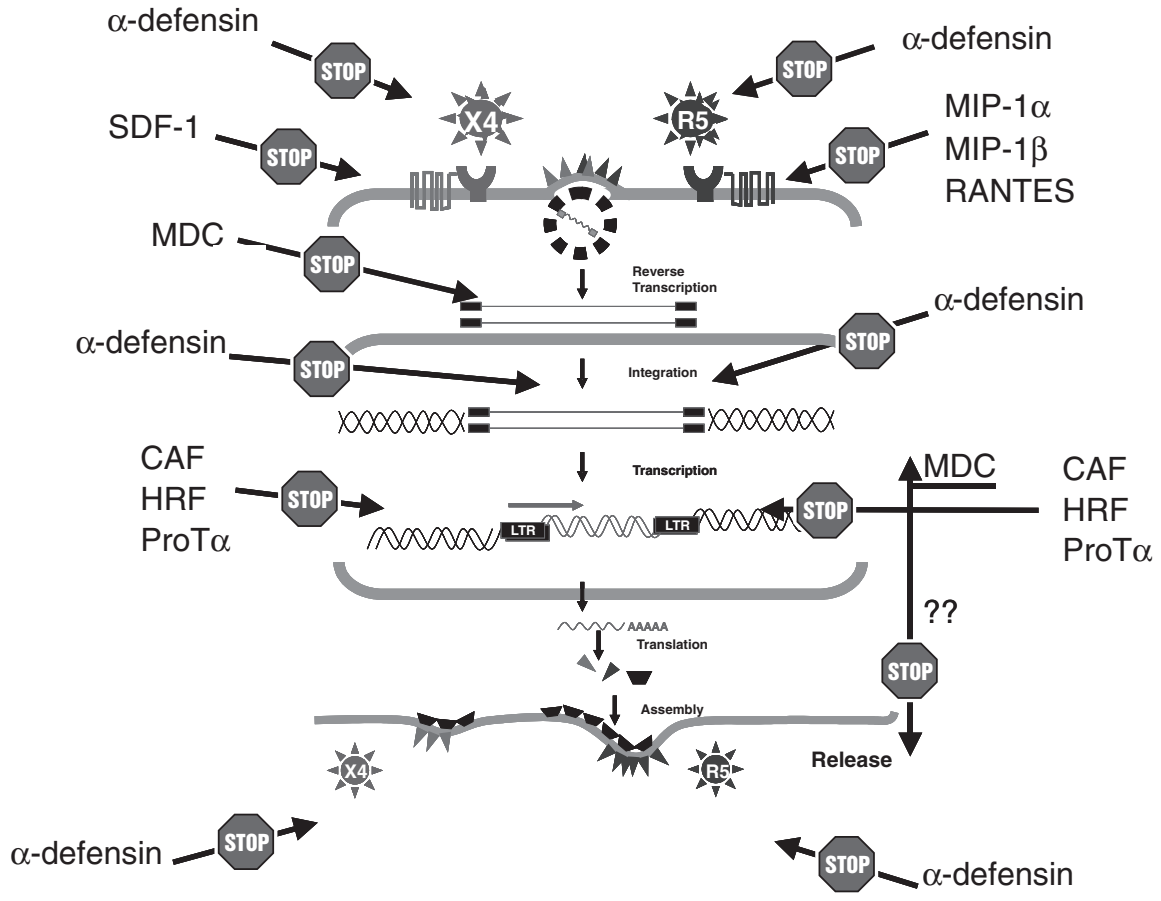

Fig. 2. A schematic representation of novel antiviral molecules targeting multiple steps of the HIV-1 replication cycle. SDF-1 – stromal derived factor 1, MDC – macrophage derived chemokine, CAF – CD8 antiviral factor, HRF – HIV-1 resistance factor, ProTα – prothymosine α, MIP-1α and -β – macrophage inflammatory protein α and β.

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