Mechanisms underlying activity of antiretroviral drugs in HIV-1-infected macrophages: new therapeutic strategies

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Abstract: Monocyte-derived macrophages (M/M) are considered the second cellular target of HIV-1 and a crucial virus reservoir. M/M are widely distributed in all tissues and organs, including the CNS, where they represent the most common HIV-infected cells. Differently from activated CD4+ T lymphocytes, M/M are resistant to the cytopathic effect of HIV and survive HIV infection for a long time. Moreover, HIV-1 replication in M/M is a key pathogenetic event during the course of HIV-1 infection. Overall findings strongly support the clinical relevance of anti-HIV drugs in M/M. Nucleoside RT inhibitors (NRTIs) are more active against HIV in M/M than in CD4+ T lymphocytes. Their activity is further boosted by the presence of an additional monophosphate group (i.e., a phosphonate group, as in the case of Tenofovir), thus overcoming the bottleneck of the low phosphorylation ability of M/M. In contrast, the antiviral activity of non-NRTIs (not affecting the DNA chain elongation) in M/M is similar to that in CD4+ T lymphocytes. Protease inhibitors are the only clinically approved drugs acting at a late stage of the HIV lifecycle. They are able to interfere with HIV replication in HIV-1 chronically infected M/M, even if at concentrations greater than those observed in HIV-1 chronically infected CD4+ T lymphocytes. Finally, several new drugs have been shown to interfere efficiently with HIV replication in M/M, including entry inhibitors. A better understanding of the activity of the anti-HIV drugs in M/M may represent a key element for the design of effective anti-HIV chemotherapy. *J. Leukoc. Biol.* 80: 1103–1110; 2006.

Key Words: *anti-HIV drugs · protease · reverse transcriptase · gp41 · CCR5 coreceptor*

INTRODUCTION

Introduction of the highly active antiretroviral therapy has provided an extraordinary clinical benefit in HIV-infected patients by lowering morbidity and mortality [1–3]. Despite this success, the eradication of HIV from the body is not achievable, and the main reason is the presence of virus reservoirs. Monocyte-derived macrophages (M/M) are one of the major cellular targets for HIV-1 infection and an important virus reservoir. M/M contribute to the transmission and the pathogenesis of HIV-1 infection throughout the progression of HIV-1 infection, especially at late stages when CD4+ T lymphocytes have been depleted extensively [4–6]. In fact, productively infected M/M can fuse with uninfected CD4+ T lymphocytes and transfer the virus to these cells, thus further contributing to depletion of CD4+ T lymphocytes [7]; in addition, HIV-1 infected M/M may induce the apoptosis on bystander uninfected cells, such as CD4+ and CD8+ T lymphocytes, neurons, and astrocytes by releasing cytotoxic factors [8–12]. Consistent with these results, it has been demonstrated that few HIV-infected M/M may completely deplete millions of autologous CD4+ T lymphocytes in a SCID mouse model [13].

HIV-infected M/M are commonly found in the blood and widely distributed in all tissues, organ and compartments [14–16]. In the CNS, M/M and microglia cells represent the most common cell lineages that support virus replication, thus being responsible for the onset of HIV-associated dementia and the neuropathological features of HIV encephalitis [17–19].

The dynamics of HIV-1 infection in M/M are substantially different from that in CD4+ T lymphocytes. In contrast to HIV-infected CD4+ T lymphocytes, which are rapidly killed by HIV-1 [20], M/M may survive to the cytopathic effect of HIV-1 and support long-term production of HIV-1 particles without a significant alteration of their homeostasis [21–24].

All these findings underline the relevance of identifying therapeutic strategies able to prevent HIV-1 replication in M/M. Thus, in this review, we reported the state of the art of the anti-HIV-1 drug activity in M/M, focusing also the attention on new and innovative compounds. As a result of their different cellular characteristics, the efficacy of the anti-HIV-1 drugs in M/M has been described in comparison with that observed in CD4+ T lymphocytes.

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RT INHIBITORS (RTIs)

As HIV-1 RT has a pivotal role in the HIV-1 replication cycle, it has become a central target for the development of anti-HIV drugs. In particular, to date, seven nucleoside RTIs (NRTIs: AZT-zidovudine, d4T-stavudine, 3TC-lamivudine, ddI-didanosine, ABC-abacavir, ddC-zalcitabine, FTC-emtricitabine), one acyclic nucleoside phosphonate (NtRTI: TDF-tenofovir), and three non-NRTIs (NNRTIs: nevirapine, efavirenz, and delavirdine) have been approved in clinical practice [25–28].

Analog NRTIs

All the NRTIs clinically available have been demonstrated to be more active in M/M than in CD4+ T lymphocytes in in vitro biological models [29–35]. This is a result of the fact that M/M are resting cells characterized by a poor deoxynucleotide metabolism and low endogenous nucleotides pool, which results in a competition by deoxy-unspecified nucleoside 5′-triphosphates (dNTPs) lower in M/M than in CD4+ T lymphocytes [36, 37]. The strong activity of NRTIs in M/M may have important clinical implications. In fact, despite the low concentration of NRTIs (such as AZT, d4T, 3TC, and ddI) in the cerebrospinal fluid, as a result of their low penetration in this compartment [38, 39] coupled with the high expression of p170 glycoprotein in M/M able to excrete NRTIs outside the cell [38], NRTIs have been shown to reduce the onset of HIV-associated dementia and the neuropathological features of HIV encephalitis in the CNS, where M/M represent the majority of cells infected by HIV [39–41].

It is interesting that the poor nucleoside/nucleotide metabolism in M/M may also slow down the activity of the RT and consequently the development of drug resistance. Indeed, a recent study shows that in vitro mutations conferring resistance to lamivudine (one of the most commonly used NRTI) do not emerge during treatment with lamivudine in HIV-infected M/M, even at time-points far longer than those sufficient to induce full-blown resistance in CD4+ T lymphocytes [42]. As a confirmation of these data, several studies show that the number of mutations present in HIV-1 from cerebrospinal fluid of NRTI-treated patients is lower than that detected in the corresponding plasma samples [43–47]. Under these conditions, recruitment of newly infected cells, transfer of the virus to lymphocytes, induction of apoptosis of bystander lymphocytes, and production of factors triggering virus replication and cell death (all phenomena attributed to M/M in the pathogenesis of HIV infection) may be slowed down under NRTI treatment, which is apparently no longer effective. This can in part contribute to the discordant results often seen in patients, where CD4+ T cell counts continue to increase, and general conditions improve (including enhancement of responses to opportunistic antigens), despite a rebound of viremia.

However, the same study showed that viruses carrying a M184V mutation in the RT enzyme are full-resistant to lamivudine (**Fig. 1**) and have a replicative capacity in M/M, sixfold lower than that observed in CD4+ T lymphocytes. Conversely, the wild-type viruses have a replication capacity threefold lower in M/M than CD4+ T lymphocytes. Again, this difference may be attributed to the resting status of M/M. In fact, it has been demonstrated that the M184V mutation, located in the conserved YMDD region near the polymerase active site, causes a reduced RT processivity in vitro compared with wild-type enzyme in primary cells and cell-free virions [48–50]. Such defects in RT processivity, likely as a result of an altered interaction of the enzyme with the primer/template

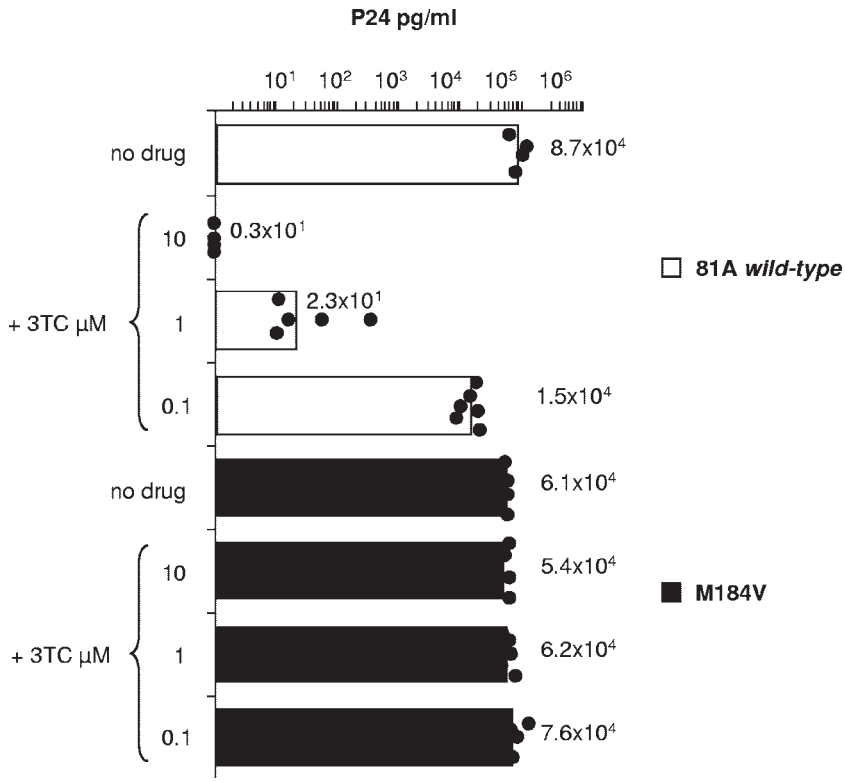

Fig. 1. Replication capacity of the wild-type 81A virus and its M184V RT virus variant in macrophages (M/M) under lamivudine treatment. The replication capacity of the wild-type HIV-1 81A (open columns) and its M184V RT virus variant (solid columns) was assessed in M/M, in the absence and presence of increasing concentration of lamivudine by measuring p24-antigen concentration in the culture supernatants 14 days post-infection when M/M release the highest amount of viral particles. The results shown are the means from at least four independent experiments. 3TC, Lamivudine. Data from ref. [42].

duplex [53], are correlated with reduced replication capacity, which is more pronounced in primary cells containing a low amount of dNTP levels (such as M/M) [51].

Further studies are necessary to clarify the development of resistance to the other drugs in M/M compared with CD4+ T lymphocytes.

NtRTIs

The majority of NRTIs is characterized by a low affinity for the cellular kinases, responsible of their activation. Thus, several attempts have been undertaken to deliver activated (phosphorylated) NRTIs directly into the virus-infected target cells. One of these approaches is represented by the NtRTIs, in which a phosphonate group is linked to the alkyl side-chain of purines or pyrimidines [52, 53].

Tenofovir disoproxil fumarate (TDF), the first NtRTI approved for clinical use, has a potent anti-HIV-1 activity in M/M, greater than that observed in CD4+ T lymphocytes (**Table 1**). The higher efficacy may be explained by the ability of TDF to overcome the first phosphorylation step coupled with the low dATP levels (poorly competing with phosphorylated TDF) in M/M [61, 62]. It has also been demonstrated that TDF may be converted more efficiently to its active diphosphate metabolite 9-[2-(phosphonomethoxy)propyl]adenine-pp in dendritic cells (DC) and Langherans cells than zidovudine and lamivudine. As Langerhans cells and interstitial DC are the earliest targets for HIV infection through sexual transmission of HIV, TDF has been proposed as a candidate drug for application in postexposure prophylactic treatment [63].

It is conceivable that the antiviral effect of TDF in M/M contributes to its excellent clinical efficacy, making it one of the most effective anti-HIV drugs currently available.

Taken together, these findings suggest that the DNA chain termination is the major mechanism able to impair HIV-1 replication in M/M.

TABLE 1. Activity of Anti-HIV Drugs in Acutely Infected T Lymphocytes (PBL), Acutely Infected Macrophages (M/M), and Chronically Infected Macrophages (M/M)

	EC_{50}[a] (μM) (acute infection)[b]		EC_{50}[a] (μM) (chronic infection)[c]	Cmax[d] (μM) range	
Drugs	PBL[e]	M/M[e]	M/M[e]	Plasma	CSF[f]
Nucleoside RT inhibitors					
Zidovudine	0.2	0.02	n.e.	4.49–6.64	0.12–0.41
Didanosine	0.5	0.05	n.e.	2.12–11.0	0.17–0.51
Zalcitabine	0.04	0.003	n.e.	0.05–0.18	0.003–0.03
Lamivudine	0.04	0.02	n.e.	4.37–8.74	0.05–1.14
Stavudine	0.8	0.24	n.e.	3.35–6.43	0.2–0.36
Abacavir	0.9	0.3	n.e.	5.2–10.9	0.5–1.83
Tenofovir	0.37	0.02	n.e.	0.35–0.38	–
Non-nucleoside RT inhibitors					
Nevirapine	0.04	0.05	n.e.	7.52–16.92	1.3–10.9
Delavirdine	0.006	0.01	n.e.	15–55	0.02–0.22
Efavirenz	0.01	0.01	n.e.	9.2–16.6	0.006–0.09
Protease inhibitors					
Saquinavir	0.01	0.05	0.5	1.85–3.23	0.003–0.008
Indinavir	0.05	0.06	0.4	12.2–13	0.03–0.66
Ritonavir	0.02	0.12	3.3	10.5–26	0.003–0.032
Nelfinavir	0.04	0.08	1.4	5.63–8.45	0.003–0.012
Amprenavir	0.03	0.01	0.72	10.6–19.2	0.003–0.36
Fusion inhibitor					
T-20[g]	0.01	0.02	n.d.	0.39–1.11	<0.005[h]
CCR5 antagonist[i]					
Vicriviroc (SCH-D)[j]	n.d.	0.001	n.d.	1.3	n.d.
Maraviroc (UK-427,857)[j]	n.d.	0.0005	n.d.	0.07–0.28	n.d.
Aplaviroc (GSK873140)[j]	n.d.	0.03	n.d.	0.04–0.17	n.d.

Adapted from C.F. Perno et al. on antiviral research [54]. Data from refs. [36, 46, 55–60]. [a] Effective concentration 50%. [b] Acutely infected PBL and M/M: Antiviral treatment started before virus challenge; before HIV-DNA integration. [c] Chronically infected M/M: Antiviral treatment started after virus challenge, when HIV-DNA is already integrated within the cellular genome. Drug treatment was added at 14 days after infection. This is the time in which virus production reaches a plateau, thus determining the assessment of the chronic infection. Virus production was measured at regular time-points starting from Day 3 from the addition of the drug at a time in which the production of viral particles is already ongoing. [d] The maximal concentration of drugs. [e] All the EC_{50} values reported in the table derived from primary cell cultures of PBL and M/M. Data from HIV-1 chronically infected CD4+ T lymphocytes are not reported, as they derived from the CD4+ T cell line chronically infected by HIV_{LAI}. In fact, it is not possible to establish a primary CD4+ T cell culture, as these cells are rapidly killed by HIV. [f] Cerebrospinal fluid. [g] EC_{50} values have been calculated by using the virus strain HIV_{SF162}. [h] This value is below the assay detection limit. [i] EC_{50} values have been calculated by using the virus strain HIV_{BaL}. [j] S. Aquaro (personal communication) data on file. n.e., Not effective; n.d., not defined.

NNRTIs

As the NNRTI activity is not affected by the intracellular dNTP pools, the anti-HIV-1 activity of the currently available NNRTI showed no substantial differences between M/M and CD4+ T lymphocytes (Table 1). It is interesting that TMC125, a second generation NNRTI, efficiently inhibits HIV replication in M/M, even if at a concentration similar to those observed in CD4+ T lymphocytes [64]. In particular, it has been shown that the EC_{50} of TMC125 against the laboratory strain HIV_{Bal} is in the lower nanomolar range [2.0 (0.8–4.0)] nm and highly similar to that observed for efavirenz {2.0 (0.9–3.0) nm [64]}. The HIV_{Bal} replication in M/M is also inhibited efficiently by the pyrrolobenzoxazepinones, a new class of NNRTIs designed to target the highly conserved primer grip within the α12-α13 hairpin. It is interesting that 15c, the most promising pyrrolobenzoxazepine investigated, inhibits HIV-1 replication at a concentration that is 40-fold lower than that necessary to inhibit HIV-1 replication in CD4+ T lymphocytes [55]. Similarly, 15f inhibits HIV-1 replication in M/M at a concentration sixfold lower than that required in CD4+ T lymphocytes.

PROTEASE INHIBITORS

The dynamics of the HIV-1 lifecycle in M/M underline the importance of drugs able to interfere with HIV replication at a post-integrational level. Indeed, once the proviral DNA is integrated into the host genome, the production of viral particles is independent of the RT enzyme and thus, is not affected by the RTIs. For these reasons, the activity of several drugs acting at a late stage of HIV replication (anti-rev, anti-tat, transcription inhibitors, IFN-α, IFN-γ, ampligen) has been tested in chronically infected M/M [64]. However, results were not encouraging, as all these drugs failed to suppress HIV replication. Protease inhibitors (PIs) represented the only exception. All clinically available PIs and also the next generation TMC114 are able to inhibit HIV replication in chronically infected M/M, although at EC_{50} values greater than those required in chronically infected CD4+ T lymphocytes [56, 65–68]. It should be noted that such data derived from primary M/M cell culture and from the CD4+ T cell line chronically infected with $HIV\text{-}1_{LAI}$. This is a result of the fact that in vivo, HIV-1 chronically and productively infected CD4+ T lymphocytes are not present. Thus, it is not possible to establish in vitro a culture of primary CD4+ T lymphocytes chronically infected by HIV-1.

The difference in the anti-HIV-1 activity of PIs in HIV-1 chronically infected M/M and CD4+ T lymphocytes may be explained by the high and sustained RNA metabolism in M/M, which affords a great production of virus particles, even from a limited amount of proviral DNA in these cells. Consistent with this hypothesis, HIV-RNA production from chronically infected M/M is not at all affected by PIs, even when protein maturation and release of infectious virus particles are inhibited significantly [69].

This may have important clinical implications. In fact, the high concentration of PIs required to suppress HIV-1 replication in chronically infected M/M is often higher than through the PI concentration in plasma of treated patients. This situation is even more pronounced in patients with a poor compliance to therapy or an altered drug absorption or metabolism. This limitation may be overcome by boosting PIs with ritonavir, which enhances PI concentration in plasma to levels able to maintain full suppression of virus replication, thus creating conditions for consistent and long-lasting HIV inhibition. At the same time, PIs do not affect proviral DNA in chronically infected cells. Therefore, it is conceivable that virus replication resumes after drug removal. This phenomenon, clearly demonstrated in in vitro experiments [70, 71], may explain (at least in part) the rapid reappearance of virus replication after therapeutic interruptions, thus further supporting the role of macrophages as a key target of antiviral interventions.

Despite their antiretroviral activity, treatment of HIV-1-infected patients with PIs is unfortunately associated with a number of clinically significant, metabolic abnormalities and an increased risk of premature atherosclerosis and myocardial infarction. It has been shown that M/M are the most prominent cell types present in atherosclerotic lesions and play an essential role in early lesion development and late lesion complications [70]. In particular, a major role in atherosclerotic lesion development in vivo seems to be played by the M/M scavenger receptor CD36 [57].

TOWARD NEW THERAPEUTIC STRATEGY

Fusion inhibitors

T-20 (Fuzeon/Enfuvirtide) is the prototype of a new drug class: the entry inhibitors. In particular, T-20 is a fusion inhibitor recently approved for clinical practice. This drug targets the HIV-1 glycoprotein gp41, thus preventing the fusion between the viral and the host cell membrane. It has been demonstrated by using different lab-adapted HIV-1 strains, that T-20 may efficiently prevent the entry of HIV-1 into PBMC, M/M, and immature DC [73]. It is interesting that a recent study showed, by using T-20 naïve, primary isolates, that the T-20 susceptibility may be modulated by coreceptor specificity [74]. In particular, it has been demonstrated that CCR5-using strains are characterized by an intrinsic resistance to T-20, and thus, their replication is suppressed at concentrations of T-20, higher than those required for CXCR4-using strains [74]. Moreover, the clinical efficacy of T-20 is also strengthened by the fact that the development of drug resistance [58, 75–78] may be associated with immunological success, despite virological failure [79]. Thus far, data about the activity of T-20 in M/M are limited (Table 1). Studies are ongoing to define this point of obvious clinical relevance.

CCR5 chemokine inhibitors

CCR5 (belonging to the G protein-coupled receptor) is a β-chemokine receptor, mainly expressed by activated CD4+ T cells and M/M and involved in chemotaxis. During the entry of HIV in the target cell, CCR5 is the main coreceptor, which allows HIV to enter M/M. CCR5 also plays a crucial role in the transmission of HIV strains, which establish initial infection, remain the dominant form in 50% of late stage HIV-1-infected

patients, and predominate in the brain, where HIV causes HIV-associated dementia complex [80–83]. The natural CCR5Δ32 omozigosity confers high protection against HIV infection [83], and this concept may not be applied to other pathologies such as West Nile and Hepatitis C [84, 85]. Thus, the rate of disease progression associated with different viral infections may vary according to the genetic of the host. Based on these assumptions, CCR5 have represented an attractive target for anti-HIV-1 chemotherapy. However, the use of CCR5 inhibitors may render HIV-1-infected patients vulnerable to West Nile or Hepatitis C infection.

To date, various CCR5 ligands with antiviral properties have been described including modified chemokines, small-molecule inhibitors with potential for oral administration, and mAb. However, none of them is in clinical practice. The CCR5 inhibitors vicriviroc (SCH-D, Schering-Plough, Kenilworth, NJ), maraviroc (UK-427,857, Pfizer, Groton, CT), and aplaviroc (873140, Glaxo-SmithKline, UK) showed an excellent potency in vitro (Table 1) [59, 86, 87] and a good pharmacokinetic profile [60, 87, 88] in vivo. Unfortunately, Aplaviroc has been withdrawn from further clinical development as a result of liver toxicity in clinical trials, and Vicriviroc has been associated with high rates of drug failure. Despite this, the antiviral activity of other CCR5 inhibitors is ongoing in investigation [89]. It is interesting that novel, small molecules derived from the UK-427,857 discovery program showed no cross-reactivity against alternative HIV coreceptors and have good efficacy against a diverse range of R5 and R5X4 HIV-1 isolates as well as HIV-2 and SIV strains. Inhibition was also observed in cell lines as well as primary PBMCs and M/M, even if the extent of inhibition is dependent on cell type and on cell surface CCR5 concentration.

Recently, the antiviral activity of a number of synthetic peptides mimicking the short region of the V2 or V3 loop of gp120 has been investigated [90–93]. Among them, Peptide T is a synthetic peptide corresponding to eight amino acids (185–192) of the gp120 V2 region, which binds CCR5. This compound has been shown to block HIV-1 entry in M/M and microglia and to prevent the M/M-mediated apoptosis of neuronal cells at nanomolar/subnanomolar concentrations [91–93].

Another antiviral approach targeting CCR5 selectively is represented by mAb. To date the mAb Pro-140 (Progenics, Tarrytown, NY), directed against CCR5, have been shown to inhibit HIV replication efficiently in CD4+ T lymphocytes and M/M [94]. The antiviral activity of other types of molecules directed against the CCR5 is in current investigation. Among them, shikonin, a major component of zicao (purple gromwell, the dried root of *Lithospermum erythrorhizon*), a Chinese herbal medicine with various biological activities, has been shown to inhibit HIV replication in M/M by down-regulating the expression of the CCR5 [95].

Plant lectins as potential anti-HIV compounds with microbicidal action

Recently, plant-derived carbohydrate-binding lectins have been proposed as potential anti-HIV microbicide drugs, which target the glycans present on the surface of the HIV-1 gp120. These compounds may inhibit HIV infection and also efficiently prevent HIV transmission from virus-infected cells to uninfected CD4+ T lymphocytes [96–98]. It is interesting that these compounds were found active against CCR5-using HIV-1 strains in M/M [97].

INTEGRASE INHIBITORS

To date, a number of new drugs belonging to the class of aril diketo acids have been demonstrated to inhibit HIV-1 replication by inhibiting the activity of the integrase enzyme.

Despite this, there are no data regarding the efficacy of such inhibitors in interfering with HIV-1 replication in M/M. This is a gap that should be filled rapidly, as some of them are in advanced clinical investigation.

DRUGS AGAINST NONCLASSICAL TARGET

In the last years, a number of new molecules have been proposed to become lead compounds for the development of new drug classes. Some of them are directed against the accessory proteins. In particular, fumagillin was shown to have a potent anti-HIV activity in M/M by a direct interaction with Vpr and a consequent down-regulation of Vpr-dependent genes expression [99]. The identification of drugs able to interfere with HIV-1 replication in M/M by targeting Vpr selectively is crucial, as this accessory protein is essential for HIV-1 productive infection in resting cells, which represent the major obstacle for viral eradication.

It is different that the amiloride analogs (5-[*N*,*N*-hexamethylene] amiloride and 5-[*N*,*N*-dimethyl] amiloride), whose target is HIV-1 accessory protein Vpu, were shown to inhibit the replication of HIV in M/M at micromolar concentration [100].

Another promising compound is Ozadiasol, targeting the process of nuclear translocation of the HIV preintegration complex. This compound shows potent anti-HIV activity in cultures of CD4+ T lymphocytes and M/M and also inhibited HIV-1 replication in ex vivo-cultured lymphoid tissue by inhibiting the nuclear import of viral DNA [101]. The development of these compounds is still in preclinical stages; thus, further studies are required to assess whether these interesting characteristics have implications in clinical practice.

CONCLUSIONS

As a consequence of the peculiar characteristics of the HIV lifecycle, the RTIs and the protease inhibitors are able to inhibit HIV replication in M/M, even if at concentrations different than those required in actively replicating CD4+ T lymphocytes. The limited development of drug resistance suggests that these drugs may efficiently suppress HIV replication in M/M (the main cellular reservoir of HIV infection) for a long time.

Moreover, the crucial role of M/M in the pathogenesis of HIV infection, especially in the CNS, underlines the importance of testing in M/M the antiviral efficacy of new drugs designed to target different stages of the HIV lifecycle. Particular attention has been dedicated to drugs able to target the CCR5 coreceptor selectively, the main coreceptor used by HIV

to enter M/M. The CCR5 antagonists also represent a promising approach for their ability to synergize with T-20, the first fusion inhibitor in clinical use.

Taken together, overall findings support the clinical relevance of interfering with HIV replication in M/M. In particular, the inherent properties of HIV infection of M/M should be taken into account in designing therapeutic strategies aimed at achieving an optimal, therapeutic effect in all tissue compartments where the virus hides and replicates.

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