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Review

Macrophages and HIV infection: therapeutical approaches toward this strategic virus reservoir

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Abstract

Cells of macrophage lineage represent a key target of human immunodeficiency virus (HIV) in addition to CD4-lymphocytes. The absolute number of infected macrophages in the body is relatively low compared to CD4-lymphocytes. Nevertheless, the peculiar dynamics of HIV replication in macrophages, their long-term survival after HIV infection, and their ability to spread virus particles to bystander CD4-lymphocytes, make evident their substantial contribution to the pathogenesis of HIV infection. In addition, infected macrophages are able to recruit and activate CD4-lymphocytes through the production of both chemokines and virus proteins (such as nef). In addition, the activation of the oxidative pathway in HIV-infected macrophages may lead to apoptotic death of bystander, not-infected cells. Finally, macrophages are the most important target of HIV in the central nervous system. The alteration of neuronal metabolism induced by infected macrophages plays a crucial role in the pathogenesis of HIV-related encephalopathy. Taken together, these results strongly support the clinical relevance of therapeutic strategies able to interfere with HIV replication in macrophages. In vitro data show the potent efficacy of all nucleoside analogues inhibitors of HIV-reverse transcriptase in macrophages. Nevertheless, the limited penetration of some of these compounds in sequestered districts, coupled with the scarce phosphorylation ability of macrophages, suggests that nucleoside analogues carrying preformed phosphate groups may have a potential role against HIV replication in macrophages. This hypothesis is supported by the great anti-HIV activity of tenofovir and other acyclic nucleoside phosphonates in macrophages that may provide a rationale for the remarkable efficacy of tenofovir in HIV-infected patients. Non-nucleoside reverse transcriptase inhibitors (NNRTI) do not affect HIV–DNA chain termination, and for this reason their antiviral activity in macrophages is similar to that found in CD4-lymphocytes. Interestingly, protease inhibitors (PIs), acting at post-integrational stages of virus replication, are the only drugs able to interfere with virus production and release from macrophages with established and persistent HIV infection (chronically-infected cells). Since this effect is achieved at concentrations and doses higher than those effective in

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de-novo infected CD4-lymphocytes, it is possible that lack of adherence to therapy, and/or suboptimal dosage leading to insufficient concentrations of PIs may cause a resumption of virus replication from chronically-infected macrophages, ultimately resulting in therapeutic failure. For all these reasons, therapeutic strategies aimed to achieve the greatest and longest control of HIV replication should inhibit HIV not only in CD4-lymphocytes, but also in macrophages. Testing new and promising antiviral compounds in such cells may provide crucial hints about their efficacy in patients infected by HIV.

Keywords: CD4-lymphocytes; HIV infection; Macrophages; Therapy; Drugs; Antiviral

1. Introduction

The introduction of highly active antiretroviral therapy (HAART), a combination therapy of at least three antiretroviral drugs, has led to a dramatic decrease of both the morbidity and the mortality of patients with human immunodeficiency virus (HIV-1) infection (Palella et al., 1998; Murphy et al., 2001; De Martino et al., 2000). This result has to be ascribed to the prolonged suppression of viral load to detectable levels, and to the consequent reconstitution (though partial) of the immune system.

Despite this success, the eradication of HIV-infection is not achievable; the main reason is the presence of virus reservoirs in the body of infected patients.

The concept of reservoirs has been brought to the attention of scientists and clinicians, yet a thorough definition of their role in the pathogenesis of HIV infection is still missing. From a practical point of view, we can define two types of HIV-1 reservoirs: cellular and anatomical (Schrager and D'Souza, 1998). Three types of cells are representative of cellular reservoirs: (i) quiescent $CD4^+$ lymphocytes (non-productive HIV-1-infected lymphocytes); (ii) macrophages (M/M) and dendritic cells; (iii) follicular dendritic cells (FDC). The central nervous system (CNS), and the male genital tract are considered the major anatomical reservoirs (or 'sanctuaries'), and in addition, other body areas (e.g. intestinal tract) can act as reservoirs of the infection.

2. Macrophages as main cellular reservoir of HIV-1

$CD4^+$ lymphocytes latently infected by HIV-1 have been indicated as a major virus reservoir (Finzi and Siliciano, 1998; Chun and Fauci, 1999; Blankson et al., 2002). These cells carry HIV provirus integrated within cellular genome, non-replicating or producing incomplete strands of HIV–RNA (see below). This suggests that HIV–DNA in resting lymphocytes is fully quiescent; resumption of virus replication may therefore occur only after activation of resting lymphocytes by exogenous stimuli. Recent observations show that virus quasispecies reappearing in plasma of patients undergoing interruption of a successful antiviral therapy are often genetically different from those present, at the same time, in $CD4^+$ lymphocytes latently infected by HIV-1 (Chun et al., 2000; Zhang et al., 2000). These data strongly suggest that other reservoirs may also be implicated in the rebound of HIV-1 replication.

HIV-1-infected monocytes and M/M are commonly found in blood and tissues of seropositive patients receiving HAART (Lambotte et al., 2000; Sharkey et al., 2000). Their distribution is widespread in all tissues, organs and compartments (Koenig et al., 1986; Tschachler et al., 1987; McElrath et al., 1989; Meltzer et al., 1990). Furthermore, circulating monocytes harbour infectious provirus both in untreated patients and in those undergoing successful HAART (Sonza et al., 2001; Innocenti et al., 1992; Williams et al., 2001). Therefore, M/M may represent not only a primary target for HIV-1 infection, but also a persistently-infected cellular reservoir whose virus production is difficult to control by currently available antiviral therapy.

The role of M/M as agents for virus dissemination is well known: indeed, productively-infected M/M can fuse with $CD4^+$ lymphocytes and transfer the virus to these cells (Crowe et al., 1990); in addition, infected M/M are able to trigger apop-

tosis of lymphocytes (either $CD4^+$ or $CD8^+$) (Mastino et al., 1993; Badley et al., 1997; Herbein et al., 1998), astrocytes, and neurons even without directly infecting these cells (bystander effect) (Aquaro et al., 2000a).

Productively-infected M/M are reported to be relatively rare in lymph nodes (Chun et al., 1997). Nevertheless, few HIV-infected M/M are sufficient to induce the recruitment and activation of HIV-infected resting $CD4^+$ lymphocytes (Swingler et al., 1999). In addition, M/M are insensitive to the cytopathic effect of the virus. As a consequence, it is conceivable that the long life span of infected M/M may in part compensate for their relatively low number, and therefore substantiate their contribution to the overall daily virus production and release during the slow-phase of virus decay in HAART experienced patients. This supports the role of M/M as an important source of HIV-1 and as a real cellular reservoir able to challenge the attempts to eradicate the virus from patients (Orenstein et al., 1997; Perelson et al., 1997; Schrager and D'Souza, 1998).

2.1. HIV-1 replication profile in macrophages

The virus life cycle in HIV-1-infected M/M is quite different than that in $CD4^+$ lymphocytes. The large majority of activated $CD4^+$ lymphocytes once infected are rapidly killed by HIV-1 (Perelson et al., 1997); by contrast, M/M are poorly affected by the cytopathic effect of HIV-1 (Gendelman et al., 1988; Orenstein et al., 1988; Garaci et al., 1999). Short-term dynamics (up to day 14) studies in activated $CD4^+$ lymphocytes shows a rapid exponential increase of virus replication (expressed also as viral multiply-spliced, MS-, and unspliced, US-, RNA during the first hours/days after the infection), followed by massive cell death. By contrast, non-productively (latently) infected $CD4^+$ lymphocytes exhibit an aberrant pattern of viral RNA expression, with production of non-infectious singly spliced (SS) or MS-RNA, but not full-length genomic (infectious) HIV–RNA (Pomerantz et al., 1990). The same study in M/M shows a linear increase of production of full MS-RNA and US-RNA (Bagnarelli et al., 1996) (Fig. 1). Plateau of virus replication is not reached even 14 days after virus challenge (that is when the lymphocyte cultures are completely destroyed by the virus). More recent studies extended this observation, and showed that M/M can produce and release high levels of HIV-1 particles during a very long period of time, with a plateau of virus

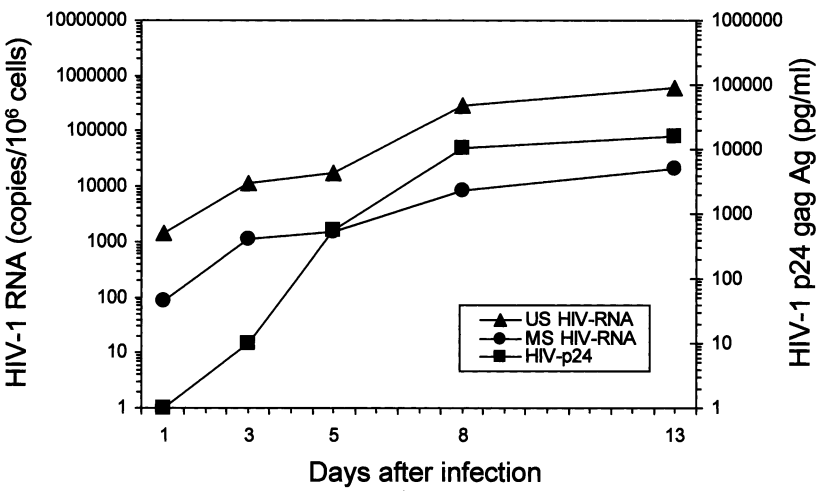

Fig. 1. Viral dynamics of HIV-1 replication in human macrophages. Macrophages were infected by a monocytropic strain of HIV-1; virus replication was monitored by measuring unspliced (US) (▲) HIV–RNA, multiply-spliced (MS) (●) HIV–RNA, and p24 gag Ag (■) production at different time points (Aquaro et al., 1998).

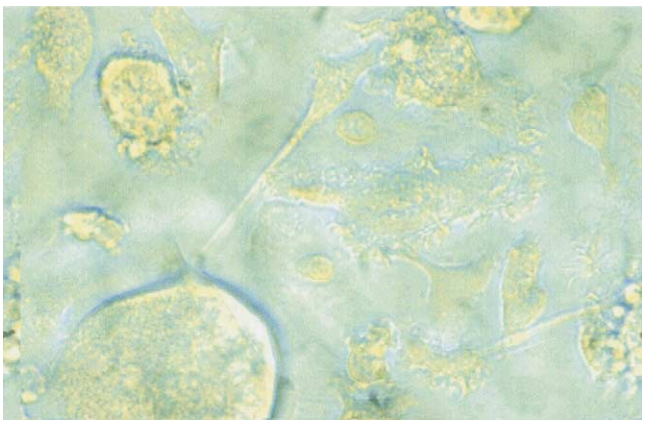

Fig. 2. HIV-infected macrophages. Macrophages are poorly affected by HIV-related cytopathic effects. After HIV infection, giant cells and syncytia are frequently depicted in M/M cultures (× 40).

production lasting at least until 60 days after virus challenge (Aquaro et al., 2001a). Giant cells constituted by M/M are frequently observed; interestingly, and in contrast to $CD4^+$ lymphocytes, the viability of such cells is similar to that of non-infected M/M (Fig. 2). Ultrastructural studies show many virus particles frequently located within intracytoplasmic vacuoles in HIV-1-infected M/M. This is a typical feature of infected M/M, since intracytoplasmic vacuoles filled with HIV particles cannot be found in HIV-1-infected $CD4^+$ lymphocytes (Fig. 3).

Overall results further substantiate the characteristics of M/M as cells chronically- and persistently-infected by HIV-1, compared to quiescent $CD4^+$ lymphocytes whose infection does not produce significant amount of virus particles unless activated by exogenous stimuli. Moreover, the expression of both receptor and coreceptors molecules for HIV-1 on the cellular membrane is quite different in M/M and $CD4^+$ lymphocytes. This can influence both virus tropism and (more importantly) the antiviral activity of compounds and chemokines acting at the stage of viral entry.

Taken together, latently-infected $CD4^+$ lymphocytes remain the main cause of failure of virus eradication from the body, while persistently-infected M/M (able to spread virus to bystander cells) represent a key challenge for therapeutic approaches aimed at decreasing residual virus replication.

The dynamics of virus replication, quite different in M/M and lymphocytes, strongly suggests that anti-HIV-1 drugs act differently in these cells. On this basis, we reviewed the characteristics of the activity in M/M of antiviral compounds of clinical interest, as well as the factors affecting their efficacy. In addition, new and innovative

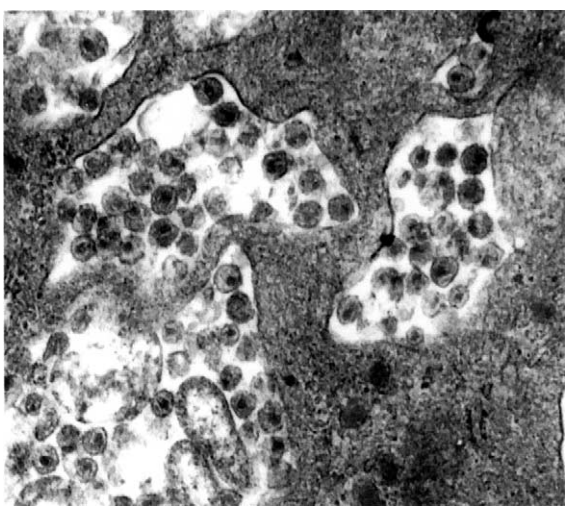

Fig. 3. Electron microscopy of chronically-HIV-infected macrophages. The microphotograph shows the presence of numerous virus particles contained in cytoplasmic vacuoles of infected macrophages (× 64 000).

Table 1
2′-Deoxynucleoside-tryphosphate levels in resting macrophages and lymphocytes

dNTP	pmoles/10^6 cells	
	M/M[a]	PBL[b]
dATP	5.13	99.11
dCTP	14.21	88.45
dGTP	8.65	127.30
dUTP	4.34	106.40
dTTP	19.15	451.55

Table reports data from Aquaro et al. (1998).
[a] Macrophages: human primary M/M obtained from the blood of healthy seronegative donors.
[b] Peripheral blood lymphocytes.

anti-HIV-1 therapeutic approaches directed to HIV-1-infected M/M are briefly described.

3. Antiviral activity of reverse transcriptase inhibitors (RTIs) in macrophages

The nucleoside-RTI (NRTIs) require triphosphorylation by cellular kinases to act as competitors of the natural 2′-deoxy-nucleoside triphosphates (dNTPs). For this reason, the antiviral activity of NRTIs depends both on the intracellular concentrations of their triphosphorylated moiety, and on the concentration of endogenous dNTP pools in that particular cell type.

All resting cells, such as M/M, are characterized by low dNTP concentrations; this in turn impairs the catalytic activity of HIV-1 RT in M/M (O'Brien et al., 1994). For these reasons, NRTIs show remarkable activity against HIV replication in M/M, usually greater than that found in replicating cells such as activated lymphocytes, whose intracellular levels of dNTPs are 6–20-fold greater than those found in M/M (Table 1) (Aquaro et al., 1997, 1998). These metabolic characteristics can explain why NRTI approved for clinical use are more active in M/M than in lymphocytes.

3.1. Acyclic nucleosides phosphonates

Acyclic nucleosides phosphonates (ANP) represent an atypical class of viral polymerase- (including RT-) inhibitors, in which a phosphonate group is linked to the alkyl side chain of purine and pyrimidines (De Clercq et al., 1986, 1987). Due to their monophosphate moiety, ANP skip the first phosphorylating step catalyzed by cellular enzymes. 9-2(Phosphonylmethoxyethyl)adenine (PMEA, adefovir) and 9-2(phosphonylmethoxypropyl)adenine (PMPA, tenofovir) are able to inhibit 50% replication of HIV-1 (and other retroviruses) and DNA-viruses, like HSV-1 (Perno et al., 1996) in M/M at concentrations (EC_{50}) of 0.02 and 0.04 μM, respectively (Table 2); the cytotoxic concentration (CC_{50}) is in the range of ≥ 300 μM for both drugs. As a consequence, the selectivity index (SI) of tenofovir and adefovir in M/M (Table 2) is markedly higher than that achieved in activated lymphocytes (15 500 and 8750 for ade-

Table 2
Efficacy of ANP against HIV-1 in macrophages and lymphocytes

Compound	EC_{50}[a] (μM)		CC_{50}[b] (μM)		SI[c]	
	M/M (Ba-L)[d]	PBL (IIIB)[e]	M/M (Ba-L)	PBL (IIIB)	M/M	PBL
PMEA	0.02	2.5	310	50	15 500	20
PMPA	0.04	0.37	350	300	8750	810

Table was modified according to Balzarini et al. (1996a).
[a] Effective concentration 50%.
[b] Cytotoxic concentration 50%.
[c] SI: CC_{50}/EC_{50}.
[d] Monocytotropic strain of HIV-1.
[e] Lymphocytrotopic strain of HIV-1.

fovir and tenofovir, respectively, about 700- and 10-fold higher than in $CD4^+$ lymphocytes) (Table 2).

The potent effect of ANP in M/M is related to two different phenomena: (i) the low dATP levels (competing with tenofovir and adefovir) in M/M (Table 1); (ii) the bypass by ANP of the first phosphorylation step (notoriously slow in M/M), due to the presence of a preformed phosphate group. For these reasons, PMPApp/dATP ratio is at least 100-fold greater in M/M than in lymphocytes, and explains the high activity of tenofovir in M/M.

Adefovir showed limited efficacy in clinical trials (toxicity was the main limiting factor), while the bis(isopropyloxycarboxymethyl) ester of (*R*)-9-2(phosphonylmethoxypropyl)adenine [Bis(-POC)-PMPA] exhibited potent antiviral activity in HIV-infected patients. Tenofovir disoproxil represents now one of the key alternatives to other anti-HIV drugs approved for clinical use (Naesens et al., 1998; Balzarini et al., 1996a). It is conceivable that the antiviral effect of tenofovir in M/M may be a factor for its excellent clinical efficacy.

In conclusion, NRTIs are more effective in M/M than in lymphocytes. This is true for all NRTIs of clinical interest, that is AZT, ddC, ddI, d4T, 3TC, abacavir, and tenofovir, as well as for the large majority of NRTI under development.

3.2. Non-nucleoside reverse transcriptase inhibitors

NNRTIs (the most clinically relevant compounds of this class being nevirapine, delavirdine, and efavirenz) do not act as chain terminators, thus their antiviral effect is related only to their direct inhibition of HIV-1 RT (De Clercq, 1996, 2000, 2002), and it is not affected by dNTP pool. For this reason, major differences in antiviral activity of NNRTI between M/M and lymphocytes are not expected. Consistent with this hypothesis, all NNRTI tested are substantially equiactive in both cell types. Their activity is not modulated by macrophage-colony stimulating factor, a cytokine enhancer of virus replication, that affects the activity of NRTI by increasing dNTP pools, and thus decreasing the chain termination induced by these drugs (Aquaro et al., 1997). This further confirms that the chain termination represents a major mechanism regulating the antiviral effect of NRTIs (but not NNRTIs) in M/M.

3.3. Protease inhibitors

The remarkable antiviral effect of reverse transcriptase inhibitors in M/M (NRTI more than NNRTI) is clinically relevant, due to the importance of inhibiting new rounds of virus replication continuously occurring in HIV-infected patients. At the same time, the peculiar characteristics of the HIV lifecycle in M/M (long-term replication of the virus without a remarkable cytopathic effect) stresses the importance of drugs interfering with virus replication at stages later than integration and transcription. Indeed, reverse transcription occurs after entry and uncoating of a virus particle by transforming the genomic RNA encapsulated within the virion into double-stranded DNA. Once, proviral DNA is integrated within the host genome, reverse transcriptase is no longer active, and the production of virus particles is independent on this enzyme. As a consequence, all RT inhibitors are completely ineffective in cells chronically-infected by the virus (such as persistently-infected M/M). By contrast, drugs acting at late (post-integrational/post-transcriptional) stages of virus replication can maintain at least part of their activity.

The characteristics of persistently-infected cells prompted the assessment of a large number of compounds in M/M chronically-infected by HIV. The results initially were not very encouraging: all late stage inhibitors (LSIs) of HIV-1 replication tested (anti-rev, anti-tat, antisense oligonucleotides, transcription inhibitors, interferon-α, interferon-γ, ampligen, etc.) completely failed in chronically-infected M/M (Perno et al., 1994). The only exception was protease inhibitors (PIs). All PIs currently in clinical use showed remarkable antiviral activity in M/M chronically-infected by HIV (i.e. treated after viral integration, when transcription/translation processes are fully active). Nevertheless, their activity occurs at concen-

Table 3
Anti-HIV-1 activity of protease inhibitors in chronically-infected macrophages (M/M) and in acutely-infected T-lymphocytes (PBL)

Compound	EC_{50}[a] (μM)	
	M/M (chronically-infected)[b]	PBL (acutely-infected)[c]
Saquinavir	0.5	0.01
Ritonavir	3.3	0.05
Indinavir	0.4	0.02
KNI-272[d]	0.8	0.059
U-75875	0.7	0.03

Data from Perno et al. (1993, 1994), Aquaro et al. (1997), Perno et al. (1998).

[a] Effective concentration 50%.

[b] Chronically-infected M/M: antiviral treatment started after virus challenge, i.e. when HIV–DNA is already integrated within cellular genome.

[c] Acutely-infected PBL: antiviral treatment started before virus challenge, i.e. before HIV–DNA integration.

[d] KNI-272: kynostatin-272.

trations greater than those required in $CD4^+$ lymphocytes (Table 3). A complete explanation of the lower activity of PIs in M/M versus lymphocytes (opposite of that described for NRTI in acutely-infected M/M and lymphocytes, see above) is still missing. It is conceivable that the high RNA metabolism in M/M, which affords a great production of virus particles even from a limited amount of proviral DNA present in such cells, may account at least in part for the limited effect of PIs against HIV replication in chronically-infected M/M.

There are practical consequences to these findings. The high concentrations of PIs required to effect high-level suppression of HIV-1 production in M/M are often at the upper limit, or even beyond, the through PIs concentrations achievable in plasma of treated patients; for this reason, tissue M/M chronically-infected by HIV may be likely to escape complete HIV-1 suppression, particularly in patients with poor compliance to therapy or altered drug absorption and metabolism. In addition, since antiviral treatment is unable to affect the proviral DNA in such cells, the production of infectious virus from chronically-infected M/M in the body may rapidly resume in the absence of PIs are withheld from treatment. This is the situation in patients with poor compliance and/or treated with suboptimal doses of PIs, or in patients with interruption of therapy (e.g. structured interruptions of HAART) (Ortiz et al., 2001; Lori and Lisziewicz, 2001).

Taken together, these results underline the difficulty to achieve results against virus replication in chronically-infected cells, but at the same time suggest the importance of PI as drugs able to interfere with late-stages of virus lifecycle. Therefore, PI represent a crucial tool in the therapeutic armamentarium, whose activity against HIV replication in chronically-infected cells may contribute to the achievement of good therapeutic results if used at the appropriate dose (Lavalle et al., 2000).

4. New therapeutic approaches to HIV-infection in M/M

4.1. Masked NRTIs monophosphate derivatives

The resting status of M/M is characterized by limited DNA synthesis not requiring, for physiological functions, high intracellular levels of dNTP (Table 1). This factor overcomes the low affinity of most NRTI for kinases acting at their first phosphorylation step (thymidine kinase, 2′deoxycitidine kinase, adenosine kinase, etc.) (Ahluwalia et al., 1987; Balzarini et al., 1987, 1988, 1989; Johnson and Fridland, 1989; Hao et al., 1990). Consequently, the ratio of triphosphate forms of NRTIs to their natural dNTP counterparts is higher than that found in lymphocytes (Aquaro et al., 1997). However, under special circumstances (such as sequestered compartments), the intracellular concentrations of phosphorylated moieties of NRTIs may be suboptimal for a number of reasons, including their incomplete penetration in sanctuaries (Haworth et al., 1998; Lewis et al., 1996) and the high expression of p170 glycoprotein in M/M (Malorni et al., 1998) able to excrete NRTIs across the cellular membrane.

In order to circumvent the dependence of NRTI from the activation by nucleoside kinases, a number of prodrugs of the 5′monophosphate

forms of nucleoside analogues have been synthesized, including phosphoramidate triesters, cyclic saligenyl phosphotriester (Balzarini et al., 1999, 2000), and *S*-acyl-2-thioethyl (SATE) (Puech et al., 1993; Perigaud et al., 1994). In particular, phosphoramidate triesters are characterized by a monophosphate-NRTI backbone containing an aryl group linked to the phosphorous through an ester bond, and a methyl ester of L-alanine linked to the phosphorous through a phosphoramidate bond with the primary amino moiety. The potent anti-HIV effect in M/M of d4T-MP phosphoramidate (SO324, a prototype compound of this class of prodrugs) is particularly promising from a clinical standpoint (Balzarini et al., 1996b), and is extended to phosphoramidate derivatives of all NRTIs whose limiting step of activation is the first phosphorylation (i.e. d4A, ddA) (Aquaro et al., 2000b). In some cases, their antiviral activity in M/M is dramatically greater than that achieved by their parent compound (Table 4). By contrast, the monophosphate approach is less appealing in the case of phosphoramidates of NRTI whose first phosphorylation step is not crucial (such as AZT, 3TC, ddC). Under these circumstances, their antiviral activity is not greater (or in some cases even lower) than that shown by their parent compounds. Taken together, the remarkable anti-HIV activity of selected monophosphorylated NRTIs in acutely-infected M/M suggests the potential advantage pending the achievement of a desirable pharmacokinetic profile in vivo.

Table 4
Anti-HIV-1 activity of d4T, AZT, ddA, d4A, 3TC, ddC, and their aryloxyphosphoramidate prodrugs derivatives

Compound	M/M[a]	
	EC_{50}[b] (μM)	EC_{90}[c] (μM)
d4T	0.20	0.78
So324	0.008	0.03
AZT	0.005	0.02
So221	0.012	0.1
DdA	1	n.a.[d]
Cf-1093	0.004	0.02
d4A	5	n.a.
Cf-1001	0.008	0.03
3TC	0.016	0.013
Cf-1109	0.032	0.18
ddC	0.003	0.01
Cf-1221	0.09	0.48

Data from Aquaro et al. (2000b).
[a] Resting macrophages.
[b] Effective concentration 50%.
[c] Effective concentration 90%.
[d] Not achieved.

4.2. Selective delivery of antiviral compounds to macrophages

The peculiar metabolic characteristics of M/M have suggested the importance of selectively delivering drugs to these cells, by taking advantage of some of their functions. Among them, phagocytosis is the most suitable to this purpose. Loading substances and compounds into carriers that can be phagocytized by M/M may increase the chance to reach substantial concentrations without affecting the metabolism of non-phagocyting cells (such as lymphocytes).

Erythrocytes (red blood cells, RBC) are recognized as useful carriers for the encapsulation of drugs, enzymes and other molecules, because of the following properties: (i) they contain large aqueous volume, are biodegradable, and have a long lifespan in the blood; (ii) drug encapsulation in RBC is relatively easy and achieves a relevant yield; (iii) RBC contain a number of enzymes (kinases, pyrophosphatases, etc.) potentially able to metabolize the encapsulated drugs (Magnani et al., 1989; De Flora et al., 1993); (iv) their phagocytosis can be enhanced by promoting the clustering of band 3, that is the predominant RBC transmembrane protein that functions as an anion transport system (Wieth and Brahm, 1985; Jennings, 1984). Band 3 is randomly distributed over the RBC membrane, and its clustering can be induced by several agents (Lelkes et al., 1986; Lutz et al., 1987; Hui et al., 1990; Turrini et al., 1993; Rettig et al., 1999). Once the clusters are formed and stabilized by a cross-linking agent, they are viewed by the immune system as non-self and opsonized by autologous antibodies (Turrini et al., 1991). Subsequently, the Fc region of the autoantibody is recognized and bound by M/M, which phagocytize the complex antibody–RBC.

Hydrophilic compounds are unable to cross cytoplasmic membranes. For this reason, phosphorylated moieties of NRTI (that bypass the phosphorylation steps mediated by cellular enzymes) cannot be used as free drugs. Their encapsulation within RBC, however, affords remarkable concentrations in M/M, coupled with a dramatic enhancement of antiviral activity. In several circumstances, even a single treatment of M/M with drug-loaded RBC before HIV challenge affords substantial inhibition of virus replication over a long period of time. This latter result shows the efficacy of RBC as trojan horses carrying drugs within M/M, and supports the hypothesis that drugs released by loaded RBC within M/M are slowly transformed to their active triphosphate moieties (Perno et al., 1997; Rossi et al., 1998; Franchetti et al., 2000; Rossi et al., 2001). These results are also confirmed in in-vivo models of retroviral infection (Fraternale et al., 2000, 2001), and open the possibility of the utilization of biological carriers to target phagocyting cells of HIV-infected patients with drugs aimed to selectively hit (or even kill) them without interfering with the overall metabolism of non-phagocyting cells.

4.3. Viral entry inhibition through blockade of the viral coreceptor CCR5

The chemokine receptor CCR5 is expressed by M/M, and represents the most important coreceptor for M-tropic R5 HIV-1 strains to enter the cells (Alkhatib et al., 1996; Li et al., 1999; Tuttle et al., 1998; Wang et al., 1998; Weissman et al., 1997; Wu et al., 1997). In the CNS more than 90% of HIV-1-infected cells are M/M (Gabuzda et al., 1986; Koenig et al., 1986; Lipton and Gendelman, 1995; Tyor et al., 1993): the presence of CCR5 Δ32 heterozygosity is a factor that prevents the development of the AIDS dementia complex (Van Rij et al., 1999).

Several evidences show that the downregulation of CCR5 expression by CC-chemokines in M/M is associated to a reduction of virus entry and replication (Jiang and Jolly, 1999). These data demonstrate the important role of CC-chemokines in reducing HIV entry and hence virus replication through their interaction with CCR5. The CC-chemokines MIP-1α, RANTES, and MIP-1β are natural ligands for the CC-chemokine receptor CCR5, and are inhibitors of M-tropic HIV strains (Cocchi et al., 1995). Recently, a non-allelic isoform of MIP-1α, LD78β (which differ in three amino acids form the isoform LD78α) (Irving et al., 1990; Nakao et al., 1990; Obaru et al., 1986) has been reported as the most potent chemokine in inhibiting virus entry and replication of CCR5-using HIV-1 strains (both lab adapted strains and clinical isolates) in M/M. The antiviral activity of LD78β is mainly related to its high affinity for CCR5 and thus to its efficiency in binding/downregulating CCR5 expressed on cell membrane of M/M (Aquaro et al., 2001b). This is in agreement with previous studies demonstrating that coreceptor internalization of CXCR4 and CCR5 contributes to the inhibition of HIV-1 entry by chemokines (Amara et al., 1997; Mack et al., 1998). Interestingly, the potent anti-HIV-1 activity of LD78β, as compared to LD78α (the other isoform of MIP-1α) is conferred by the difference with LD78α of only three amino acids (Menten et al., 1999), therefore showing how the NH_2-terminal dipeptide is extremely important for receptor affinity (Struyf et al., 1998a).

The biological relevance, in terms of antiviral activity, of the NH_2-terminal residues of CXC- and CC-chemokines has been well demonstrated (Proost et al., 1998a,b; Schols et al., 1998; Simmons et al., 1997; Struyf et al., 1998b; Wuyts et al., 1999), and represents an interesting issue for the development of oligopeptides able to block HIV-1 entry in M/M by binding CCR5. One of the most relevant compounds of this class is TAK-779, the first non-peptide antagonist directed against CCR5 so far identified. TAK-779 (a molecule with small molecular weight, 531.13) has a high affinity for CCR5, and is a potent inhibitor of HIV-1 replication at concentrations of about 10 nM; by contrast, it is totally inactive against lymphocytotropic strains (Baba et al., 1999). Its potency, coupled with the absolute specificity for CCR5-using strains of HIV, makes TAK-779 as a crucial candidate for virus inhibition in M/M, and suggests the importance of preclinical an clinical studies aimed to better

define its anti-HIV activity (Baba et al., 1999; Takashima et al., 2001; D'Souza et al., 2000; De Clercq and Schols, 2001).

4.4. Drugs affecting the bystander killing effect of HIV-infected macrophages

4.4.1. Superoxide dismutase-mimetics

The ability of M/M to transfer virus particles to other cells, as well as to produce and release factors able to kill bystander, non-infected cells, stresses the importance of identifying drugs able to interfere with the M/M-derived factors involved in these phenomena, mainly (but not only) caused by oxygen catabolites provided with great pro-oxidative activity.

Recently, a class of non-peptidic low-molecular weight compounds (whose prototypic drug is M40403) proved to possess comparable catalytic activity to that of the native superoxide dismutase (SOD), a superoxide anion scavenger. Therefore, the use of these compounds has been suggested to counteract the damage induced by the superoxide overproduction (Salvemini et al., 1998, 1999). These new SOD-mimetics represent an advance in chemical design (Cuzzocrea et al., 2001). Indeed, SOD-mimetics are stable both in vitro biological system, and in vivo animal models, show high activity, and are selective for superoxide with no activity toward H_2O_2, peroxynitrite, nitric oxide, or hypochlorite. This selectivity is due to the nature of the manganese(II) center in these low molecular weight complexes. The resting oxidation state of the complex is the reduced state, Mn(II). For this reason, the complex has no reactivity with reducing agents until it is oxidized to Mn(III) by superoxide. Therefore, many oxidants will not oxidize these complexes, including nitric oxide and oxygen (that operate via a simple one-electron oxidation pathway), as well as other two-electron non-radical oxidants (e.g. $OONO^-$, H_2O_2, OCl^-).

The selectivity of these complexes for superoxide in the presence of other ROS enables the elucidation of the role of superoxide in disease models in which ROS are implicated. A new SOD-mimetic, named M40401, has been recently synthesized. M40401 possesses much higher catalytic activity at pH 7.4 than the native enzyme, and about 100 times the activity of M40403. M40401 has no catalase activity or reactivity with peroxynitrite. On the other hand, M40401 has been shown to produce central effects counteracting peroxidative processes in brain tissues of rats undergoing ischemia/reperfusion brain damage (Mollace et al., 2001). When tested in a macrophage model infected by HIV, M40401 has shown a remarkable antiviral activity against HIV-1 in M/M (Aquaro et al., 2002), thus indirectly confirming previous data showing that pro-oxidative status enhances virus replication in M/M (Palamara et al., 1996; Garaci et al., 1997).

Additional to the direct anti-HIV effect, M40401 reverts the apoptotic death of astroglial cells induced by HIV-1-infected M/M, which is driven by overproduction of superoxide anions (Mollace et al., 2002) (Fig. 4). Apoptosis of cells of CNS origin (such as neurons and astrocytes) is a crucial event in the pathogenesis of HIV-related encephalopathy. Therefore, the unique and potent activity profile of M40401 makes it as an interesting candidate for clinical trials aimed to assess the possibility to inhibit the HIV-M/M driven bystander phenomenon of apoptosis.

5. Conclusions

The characterization of macrophages as infected cells able to spread virus to bystander cells, and to interfere with the homeostasis of the immune system and of the neural compartment, strongly supports the importance of inhibiting virus replication in such cells. The dynamics of virus replication in macrophages, and their intrinsic biochemical and metabolic characteristics suggest that reverse transcriptase- and protease-inhibitors are able to affect virus replication in HIV-infected macrophages, yet at concentrations different than those effective in activated CD4-lymphocytes. Generally, the relevance of M/M in the pathogenesis of HIV infection underlines the importance of testing the antiviral efficacy of new compounds inhibitors of different stages of virus life-cycle (inhibitors of entry, integrase, nuclear transport, etc.) in M/M, early in development.

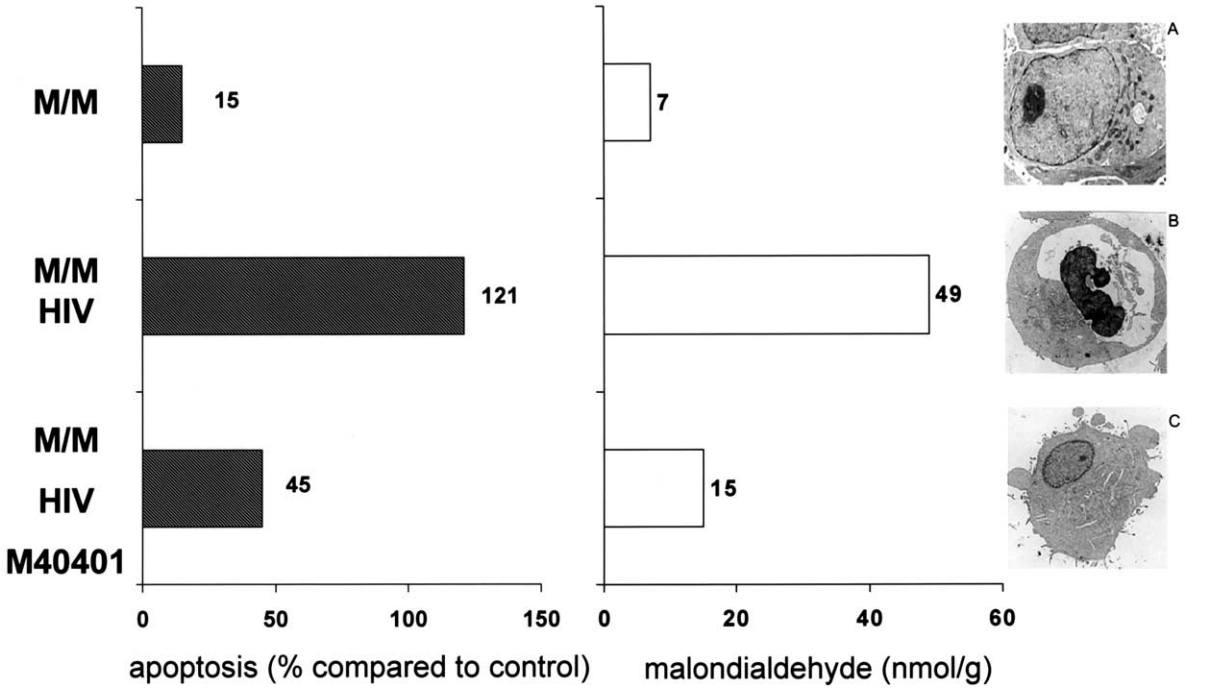

Fig. 4. The SOD-mimetic M40401 prevents astrocyte damage induced by HIV-1-infected macrophages. Formation of malondialdehyde and apoptosis of astrocytes induced by HIV-1-infected macrophages was inhibited by M40401. Panel A shows human astrocytes exposed to supernatant from mock-infected macrophage cultures (control). Exposure to HIV-infected macrophages supernatants induced apoptotic cell death in astrocytes (Panel B). This effect was dramatically inhibited by treatment with M40401 (Panel C). (Data from Mollace et al., 2002.)

Therapeutic attempts to interfere with the viability of infected macrophages may result in a deprivation of virus reservoirs in the body that, in turn, could contribute to HIV eradication process (not achievable with the current therapeutic drugs and strategies). Preliminary results seem to suggest the possibility of selectively delivering toxins and cytotoxic drugs to long-term infected cells (Perno et al., 2001; Alfano et al., 2001). These results however need to be further substantiated in experimental models before being considered for clinical studies.

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