ELSEVIER

Antiviral Research 33 (1997) 141–152

Antiviral Research

Mini review

Cellular factors as alternative targets for inhibition of HIV-1[1]

Masanori Baba*

Division of Human Retroviruses, Center for Chronic Viral Diseases, Faculty of Medicine, Kagoshima University, Kagoshima 890, Japan

Received 21 August 1996; accepted 24 August 1996

Keywords: Cofactor; Heat shock; HIV-1; NF-κB; OM-10.1; Signal transduction

1. Introduction

A number of compounds have been reported to inhibit the replication of human immunodeficiency virus type 1 (HIV-1) in vitro (De Clercq, 1995a,b). Among them, HIV-1 reverse transcriptase (RT) and protease inhibitors have shown efficacy in vivo. At present, six RT inhibitors (zidovudine, didanosine, zalcitabine, stavudine, lamivudine, and nevirapine) and three protease inhibitors (saquinavir, ritonavir, and indinavir) have been formally licensed for clinical use in the treatment of HIV-1 infections. Since these compounds interact with the viral-specific enzymes HIV-1 RT or protease, emergence of drug-resistant viruses caused by amino acid mutations of the enzymes may not be avoidable during long-term treatment with monotherapy. In particular, rapid emergence of drug-resistance is a serious concern in the treatment with non-nucleoside RT inhibitors, since they have high specificity to HIV-1 RT (Baba et al., 1995; De Clercq, 1996). Thus, it is generally considered that the virus-specific inhibitors have potent activity, weak toxicity, narrow antiviral spectrum, and high risk for drug-resistance. In contrast, the inhibitors that interact with host cellular factors may have low selectivity (little difference between their effective concentration and cytotoxic threshold), broad antiviral spectrum, and low risk for drug-resistance.

Several events in the viral replicative cycle have been identified gas possible targets for inhibition of HIV-1 replication, namely, viral adsorption, virus-cell fusion, penetration, uncoating, reverse transcription, integration, transcription, translation, maturation, assembly, and budding (Fig. 1A). Virus-specific structural and non-structural proteins play an important role in fulfilling each event. In addition to the viral proteins, several

* Corresponding author. Tel.: +81 99 2755930; fax: +81 99 2755932.

[1] This review is based on the presentation originally made at the Ninth International Conference on Antiviral Research in Urabandai, Fukushima, Japan, May 19–24, 1996.

0166-3542/97/$17.00 © 1997 Elsevier Science B.V. All rights reserved
PII S0166-3542(96)01010-8

known and unknown cellular factors are assumed to be essential for or involved in the replication of HIV-1 (Fig. 1B). For instance, murine cell lines expressing human CD4 are resistant to the fusogenic effect of HIV-1 envelope, suggesting that the cells lack human-specific cofactors necessary for the CD4/envelope-mediated membrane fusion (Dragic et al., 1992). A novel cellular protein 'fusin' and the β-chemokine receptor CC-CKR-5 have recently been identified as cofactors of HIV-1 fusion and entry (Feng et al., 1996; Alkhatib et al., 1996; Deng et al., 1996; Dragic et al., 1996). Furthermore, with the exception of the viral trans-activator protein Tat, the transcription of HIV-1 is mainly regulated by cellular transcriptional factors, such as nuclear factor-κB (NF-κB) and Sp1 (Jones et al., 1986; Nabel and Baltimore, 1987). Thus, effective anti-HIV-1 chemotherapy might also be accomplished by the compounds that interact with such host cellular factors. The present review will focus on the host cellular factors as a possible approach to the intervention in HIV-1 replication.

2. Agents targeted at cellular factors

2.1. Inhibitors of cellular factors for viral entry

Several but not many agents (or compounds) have been identified as possible inhibitors of HIV-1 replication in vitro through their interacting with cellular factors. They could be classified according to the putative target molecules (Table 1). The first group includes bovine β-lactoglobulin modified by 3-hydrophthalic anhydride (3HP-β-LG) (Neurath et al., 1996), secretory leukocyte protease inhibitor (SLPI) (McNeely et al., 1995), and C-C chemokines released by $CD8^+$ T cells (RANTES, MIP-1α, and MIP-1β) (Cocchi et al., 1995). These substances interact with the CD4 molecule or cellular co-factors for viral entry and interfere with HIV-1 infection. 3HP-β-LG blocks the binding of monoclonal antibodies specific for the HIV-1 binding site on CD4 and inhibits viral replication at nanomolar concentrations (Neurath et al., 1996). SLPI appears to be targeted at a host cell-associated molecule (McNeely et al., 1995). However, another study has demonstrated that SLPI did not exert anti-HIV-1 activity under any experimental conditions (Turpin et al., 1996). Thus, exact nature of this molecule still remains to be elucidated. The C-C chemokines have proved to be highly potent inhibitors of HIV-1 and HIV-2 infections (Cocchi et al., 1995). Furthermore, they are assumed to confer relative resistance to HIV-1 infection in persons who are hyper-exposed to the virus but remain uninfected (Paxton et al., 1996).

2.2. Inhibitors of cellular transcription factors

The compounds that interact with the cellular transcriptional factors NF-κB and Sp1 may become promising anti-HIV-1 agents, since the HIV-1 long terminal repeat (LTR) contains two tandem κB elements followed by three tandem Sp1-binding sites and TATA box sequence (Luciw and Shacklett, 1993; Roulston et al., 1995). Tran-

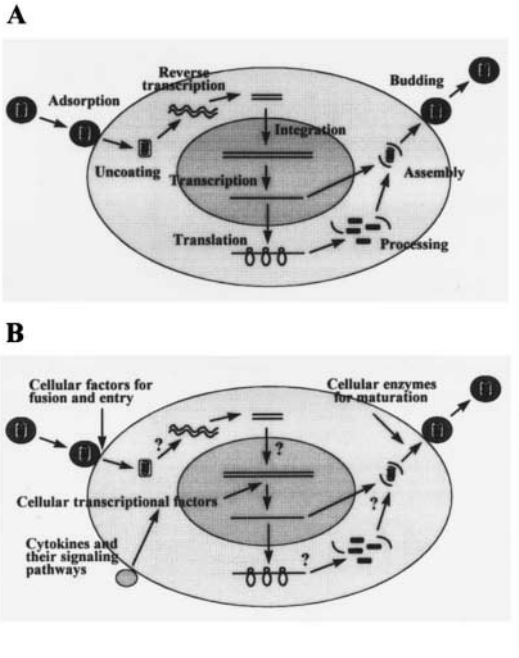

Fig. 1. Essential steps in the HIV-1 replicative cycle (A) and possible cellular factors that affect viral replication (B). To simplify the figures, complex structures of viral RNA with reverse transcriptase and proviral DNA with integrase are not taken into account for drawing.

Table 1
Agents targeted at cellular factors

1.	Inhibitors of cellular factors for viral entry (CD4 and co-factors) Bovine β-lactoglobulin modified by 3-hydrophthalic anhydride (3HP-β-LG), Secretory leukocyte protease inhibitor (SLPI), C-C chemokines
2.	Inhibitors of cellular transcription factors (NF-κB and Sp1) Pentoxyfylline, Sodium salicylate, Topotecan, β-Lapachone, Curcumin, 3′-O-methylnordihydroguaiaretic acid (Mal.4)
3.	Antioxidants *N*-acetyl-L-cysteine (NAC), Glutathione (GSH), Oltipraz, Ebselen
4.	Inhibitors of cytokine production (TNF-α and IL-6) Pentoxifylline, Thalidomide, RP 55778 (Acopafant), OPC-8212 (Vesnarinone)
5.	Inhibitors of second messengers (phosphatidic acid and PKC) CT-2576, Gö 6976, Staurosporine
6.	Inhibitors of cellular factors for viral maturation (*N*-myristoyltransferase and gp160 processing enzyme) 13-Oxatetradecanoic acid, Brefeldin A
7.	Others SDZ NIM 811 (cyclosporin A derivative), Benzothiophene derivatives, Flavonoids (chrysin, acacetin, apigenin)

scription of the HIV-1 genome is regulated by these transcriptional factors cooperating with the viral regulatory protein Tat (Berkhout et al., 1990; Kamine et al., 1991; Alcamí et al., 1995). Therefore, inhibition of NF-κB and Sp1 leads to effective suppression of Tat-induced transactivation as well as basal transcription of HIV-1. Pentoxifylline, 1-(5-oxohexyl)-3,7-dimethylxanthine, has been shown to suppress HIV-1 replication through the inhibition of either tumor necrosis factor-α (TNF-α) production or NF-κB activation or both (Biswas et al., 1993; Fazely et al., 1991), yet existence of another mechanism of action is also suggested (Navarro et al., 1996). Although recent clinical trials in AIDS patients have demonstrated that pentoxifylline certainly decreases the expression of TNF-α without significant adverse effects, its antiviral efficacy has not yet been fully established yet (Dezube and Lederman, 1995).

Fig. 2 schematically shows the intracellular signaling events after stimulation of HIV-1-infected cells with TNF-α (Schutze et al., 1992; Hannun, 1994). In general, NF-κB exists in an inactive form in the cytoplasm, where it is bound to the inhibitory molecule IκBα. Stimulation of the cells with several cytokines including TNF-α leads to the immediate degradation of IκBα and activates NF-κB through the indicated pathways, resulting in the translocation of NF-κB from the cytoplasm to the nucleus (Thanos and Maniatis, 1995; Roulston et al., 1995). Sodium salicylate and aspirin have been reported to prevent the degradation of IκBα and inhibit the activation of NF-κB (Kopp and Ghosh, 1994). This study has also demonstrated that these compounds can inhibit κB-dependent transcription from HIV-1 LTR in Jurkat T cells at high concentrations (2–5 mM). Furthermore, salicylic acid interferes with ultraviolet- and *cis*-platinum-induced HIV-1 expression in HeLa cells (Woloschak et al., 1995). Topotecan, β-lapachone, and curcumin have been identified as potent and selective inhibitors of HIV-1 LTR-directed gene expression at concentrations that have minor effects on the host cells (Li et al., 1993). They inhibit p24 antigen production in the cells either acutely or chronically infected with HIV-1. Their target is transcriptional function of the LTR. In addition to these compounds, a plant

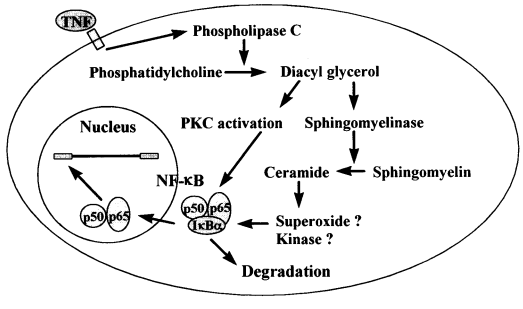

Fig. 2. Intracellular signaling pathways of NF-κB activation by TNF-α. Ceramide, a breakdown product of sphingomyelin, is the second messenger-like molecule, and it leads to the phosphorylation of IκBα by protein kineases followed by its degradation. IκBα is also phosphorylated directly by PKC in vitro.

lignan, 3′-*O*-methylnordihydroguaiaretic acid (Mal.4) is able to suppress HIV-1 replication by blocking the promotor activity of HIV-1 LTR. Gel mobility-shift studies have revealed that Mal.4 does not affect NF-κB binding but does strongly inhibit Sp1 binding (Gnabre et al., 1995).

2.3. Antioxidants

In addition to the cytokine stimulation, HIV-1 gene expression and viral replication can be induced by oxidative stress, suggesting that the signaling pathways leading to NF-κB activation are redox regulated (Legrand-Poels et al., 1990; Staal et al., 1990; Schreck et al., 1991). Thus, various antioxidants have been examined for their inhibitory effects on HIV-1 activation and found to suppress viral replication. Among these, *N*-acetyl-L-cysteine (NAC) has been most extensively studied. NAC inhibits cytokine-stimulated HIV-1 replication in both acutely and chronically infected cell systems (Roederer et al., 1990, 1991). Furthermore, it has been demonstrated that NAC also inhibits the activation of NF-κB induced by TNF-α, phorbol myristate acetate (PMA) or oxidative stress (Mihm et al., 1991; Staal et al., 1990, 1993). Since NAC exerts its anti-HIV-1 activity by increasing the intracellular level of glutathione (GSH) (Burgunder et al., 1989), administration of GSH itself and its monoester also increases the intracellular GSH level and inhibits HIV-1 expression in chronically infected monocytic cells (Kalebic et al., 1991; Ho and Douglas, 1992).

Other anti-oxidative molecules recently identified as HIV-1 inhibitors are oltipraz (5-pyrazinyl-4-methyl-1,2-dithiole-3-thione) and ebselen [2-phenyl-1,2-benzisoselenazol-3(*2H*)-one]. Oltipraz, an anti-carcinogenic agent, and its metabolite are known to inhibit HIV-1 replication in chronically infected cells as well as acutely infected cells (Prochaska et al., 1993, 1995). Although the metabolite has recently proved inhibitory to HIV-1 LTR-directed gene expression (Prochaska et al., 1996), the mechanism of inhibition has not been fully elucidated yet. Ebselen is a selenium-containing heterocyclic compound (Fig. 3), which exhibits GSH peroxidase-like activity and anti-inflammatory activity (Parnham and Graf, 1987). Ebselen is now under phase III clinical trials for cerebrovascular diseases as an anti-stroke agent in Japan. We have recently found that ebselen selectively inhibits HIV-1 replication in MOLT-4 cells at a concentration of 5-10 μM (data not shown). In addition, it proved also inhibitory to PMA-induced NF-κB activation in MOLT-4 cells at this concentration, as determined by the gel mobility shift assay (Fig. 4). Although it remains to be determined whether the anti-HIV-1 activity of ebselen can be attributed to the inhibition of NF-κB activation, ebselen may be a new candidate drug for treatment of HIV-1 infections in vivo.

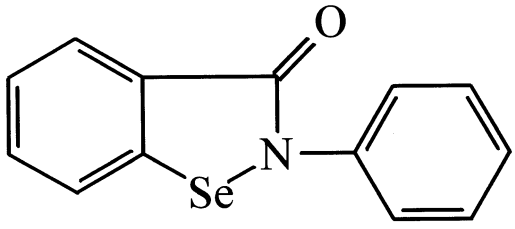

Fig. 3. Chemical structure of ebselen.

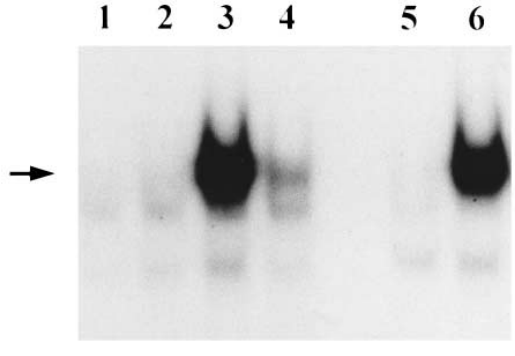

Fig. 4. Inhibitory effect of ebselen on PMA-induced NF-κB activation determined by gel mobility shift assay. MOLT-4 cells were cultured for 2 h in the absence (lanes 1, 3, 5, and 6) or presence of ebselen (10 μM) (lanes 2 and 4). The cells were stimulated with PMA (10 ng/ml) (lanes 3–6) and further incubated for 60 min. Nuclear proteins were extracted and examined for their NF-κB binding activity to the [γ-^{32}P]ATP-labeled NF-κB-specific DNA probe. One hundred-fold molar excess of the unlabeled specific DNA probe (lane 5) or the random DNA probe (lane 6) was added for competition.

2.4. Inhibitors of cytokine production

Several cytokines, such as TNF-α, interleukin (IL)-1β, and IL-6, are potent inducers of HIV-1 replication, and they are overexpressed in the lymphoid tissues of HIV-1-positive individuals (Fauci, 1993; Fauci et al., 1996). Blocking these factors may suppress their action to enhance viral replication. Thalidomide, a selective inhibitor of TNF-α synthesis (Moreira et al., 1993), has been shown to suppress the activation of HIV-1 in U1 cells (a monocyte cell line latently infected with HIV-1) and peripheral blood mononuclear cells (PBMCs) isolated from HIV-1-infected patients (Makonkawkeyoon et al., 1993). This compound seems also effective to the wasting syndrome in AIDS patients (Tramontana et al., 1995). A phase I clinical study of thalidomide in HIV-1-infected patients is currently enrolling. A platelet-activating factor antagonist, (+)-3-(3-pyridyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-7-carboxamide (RP 55778, Acopafant) is also capable of inhibiting the expression of HIV-1 in U1 cells through the inhibition of endogenous TNF-α production (Weissman et al., 1993). However, RP 55778 is not effective in monocyte-derived macrophages infected with a primary isolate of HIV-1 (Naour et al., 1994). Another interesting compound is 3,4-dihydro-6-[4-(3,4-dimethoxybenzoyl)-1-piperazinyl]-2(1*H*)-quinolinone (OPC-8212, Vesnarinone), which is an oral inotropic agent used for treatment of congestive heart failure in Japan. OPC-8212 suppresses HIV-1 replication in acutely infected PBMCs and chronically infected macrophages at clinically achievable concentrations (Maruyama et al., 1993). It inhibits the production of TNF-α and IL-6 from lipopolysaccharide (LPS)-stimulated PBMCs.

2.5. Inhibitors of second messengers

Second messengers involved in the intracellular signaling pathways after stimulation with cytokines, mitogens, and antigens might also be the targets for suppression of HIV-1 activation, if they could be effectively blocked without affecting cellular functions (Fig. 1B and Fig. 2). 1-(11-Octylamino-10-hydroxyundecyl)-3,7-dimethylxanthine (CT-2576) is a potent inhibitor of phosphatidic acid metabolism, which plays an important role in cellular activation and mitogenesis (Rice et al., 1994). CT-2576 has been proven to suppress Tat-induced phosphatidic acid generation and HIV-1 LTR-directed gene expression in response to Tat or TNF-α at a posttranscriptional step (Leung et al., 1995). CT-2576 displays the selective inhibition of HIV-1 replication in both acutely and chronically infected cell systems. Gö 6976 is structurally related to staurosporine, the most potent inhibitor of protein kinase C (PKC), and has been shown to block HIV-1 transcription induced by PKC activators and cytokines in chronically infected cells at nanomolar concentrations (Qatsha et al., 1993). Since the PKC isoform responsible for viral activation in HIV-1-infected cells has not been clarified yet (Diaz-Meco et al., 1994; Folgueira et al., 1996), the specificity of PKC inhibitors to this isoform seems to be a crucial factor for this class of compounds.

2.6. Inhibitors of cellular factors for viral maturation

The Gag and Gag-Pol precursor proteins need to be myristoylated by the cellular enzyme *N*-myristoyltransferase for their maturation (Göttlinger et al., 1989). Therefore, this enzyme seems to be a target for inhibition of HIV-1. Several myristic acid derivatives, such as 13-oxatetradecanoic acid (Bryant et al., 1991), have been found to inhibit HIV-1 replication in both acutely and chronically infected cells, yet their selectivity is rather small. The envelope glycoproteins are initially synthesized as a precursor polyprotein (gp160) and are cleaved to gp120 and gp41 by the cellular enzyme(s) in the Golgi complex (Willey et al., 1988; Pal et al., 1989). Inhibition of this processing leads to the formation of non-infectious viral particles (McCune et al., 1988). However, the enzyme(s) responsible for the gp160 processing has not been fully characterized. Therefore, with the exception of glycosylation inhibitors (De Clercq, 1995a), few compounds have been reported as the selective inhibitors of envelope maturation. Brefeldin A, which is known

to block intracellular transport of proteins, inhibits the processing of gp160 (Pal et al., 1991). In fact, the infectivity of HIV-1 particles released from brefeldin A-treated cells is markedly lower than that obtained from untreated cells.

2.7. Other inhibitors

The immunosuppressive drug cyclosporin A has previously been shown to inhibit HIV-1 replication (Karpas et al., 1992). Recently, a nonimmunosuppressive cyclosporin A analog (SDZ NIM 811) has been identified as a highly potent and selective inhibitor of HIV-1 (Rosenwirth et al., 1994). Since it has been shown that cyclophilin A, a major receptor molecule for cyclosporin A, specifically binds to the HIV-1 Gag precursor proteins and is necessary for the formation of infectious virus particles (Franke et al., 1994; Thali et al., 1994), the mechanism of action of SDZ NIM 811 may be attributed to the interference with Gag-cyclophilin A interaction. Other agents that probably interact with cellular factors are benzothiophene derivatives and certain flavonoids (chrysin, acacetin, and apigenin) (Butera et al., 1995; Critchfield et al., 1996). Although benzothiophene and flavonoid totally differ in their chemical structures, they share several characteristic features. Both derivatives are effective in inhibiting HIV-1 replication in chronically infected cells. They can prevent HIV-1 mRNA accumulation, yet they do not inhibit the activity of Tat or NF-κB (Butera et al., 1995; Critchfield et al., 1996). The molecular basis for their antiviral activity remains to be determined.

3. Screening systems for inhibitors of cellular factors

To specifically detect the agents that can interact with the cellular factors involved in HIV-1 replication, novel screening assay systems need to be established. The cytopathicity inhibition assay by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in MT-2 or MT-4 cells (Pauwels et al., 1988), which is now widely used for screening of anti-HIV-1 compounds, may not be suitable for this purpose, since it is prone to detect the virus-specific inhibitors, such as viral adsorption inhibitors, RT inhibitors, and protease inhibitors. In addition, the Tat inhibitors Ro5-3335 and Ro24-7429 have proved ineffective against HIV-1 replication in MT-2 and MT-4 cells (Witvrouw et al., 1992; Baba, unpublished data). This may be due to the high level expression of NF-κB in these cell lines (Luznik et al., 1995). Thus, it appears that the MTT assay in MT-2 or MT-4 cells cannot pick up the compounds inhibitory to cellular transcription factors, cytokine production, or second messengers (Table 1).

We have recently described a novel anti-Tat screening system by using the CEM cells stably transfected with a plasmid (Kira et al., 1995). The plasmid contains the hygromycin B phosphotransferase gene under the control of HIV-1 LTR and constitutively expressed tat gene. This system is also based on the quantitative determination of cell viability by the MTT method. We have found that it is applicable to the screening of the agents inhibitory to cellular transcription factors as well as Tat, if the construction of the plasmid is slightly modified (Merin et al., 1996). Another possible approach is the viral induction assay by using the cell line latently infected with HIV-1, such as OM-10.1 and U1 cells. These cell lines produce little or no HIV-1 under basal conditions (Fig. 5A and 5C) but do induce significant level of virus after stimulation with various substances, including TNF-α (Butera et al., 1991; Folks et al., 1987). In fact, OM-10.1 cells became HIV-1 antigen-positive by more than 90% after stimulation with 1 ng/ml TNF-α (Fig. 5B). However, only 20–25% of the U1 cells expressed HIV-1 antigens under the same experimental conditions (Fig. 5D), indicating that OM-10.1 cells are more sensitive to TNF-α stimulation than U1 cells. Furthermore, OM-10.1 cells have a unique property. Unlike other chronically infected cell lines, OM-10.1 cells remain $CD4^+$ unless the activation of HIV-1 occurs (Feorino et al., 1993).

It has been reported that heat shock induces the activation of HIV-1 in U1 and ACH-2 (T cell line latently infected with HIV-1) cells and that κB sequence is necessary for maximal heat-induced

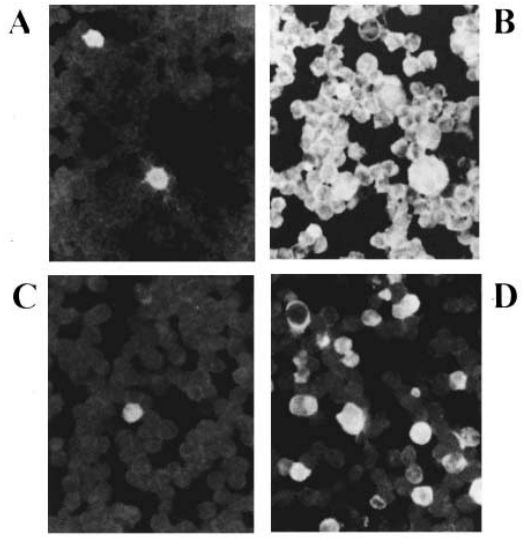

Fig. 5. TNF-α-induced activation of HIV-1 in OM-10.1 and U1 cells. OM-10.1 (panels A and B) and U1 (panels C and D) were incubated in the absence (panels A and C) or presence (panels B and D) of TNF-α (1 ng/ml). After a 3-day incubation at 37°C, the cells were subjected to the indirect immunofluorescence microscopy by using an anti-HIV-1 polyclonal antibody as the probe.

viral production (Stanley et al., 1990). We have also examined whether the heat shock treatment activates the latent HIV-1 in OM-10.1 cells and found that the heat shock (42°C for 2 h) results in a high level of HIV-1 production without addition of any cytokines (Hashimoto et al., 1996). Interestingly, addition of anti-TNF-α antibody into culture medium was able to partially suppress the heat shock-induced viral activation (Fig. 6). To elucidate the mechanism of HIV-1 activation by heat shock, we examined the effect of heat shock on NF-κB activation in OM-10.1 cells. No NF-κB binding activity was observed in the nuclear proteins extracted at 30 min after heat shock treatment (Fig. 7), indicating that the heat shock does not directly induce the activation of NF-κB. This result is consistent with the previous report in heat shock-treated HeLa cells (Kretz-Remy and Arrigo, 1994).

Fig. 8 shows putative signaling pathways of heat shock-induced HIV-1 activation in OM-10.1 cells. In this scheme, it appears that the heat shock treatment induces HIV-1 replication without direct activation of NF-κB and that the addition of anti-TNF-α antibody suppresses viral production. Furthermore, any other substances that interfere with one of these pathways may also be able to reduce the production of HIV-1. We have examined the inhibitory effects of anti-TNF-α antibody, staurosporine, Ro5-3335, and pentox-

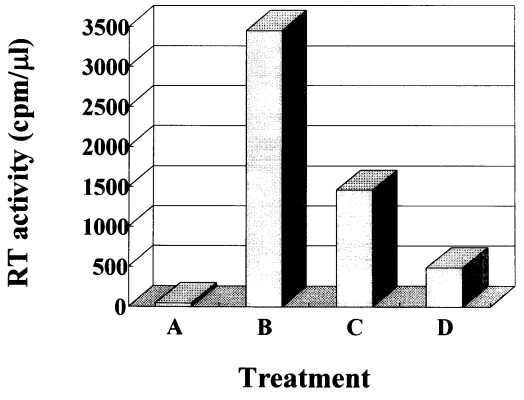

Fig. 6. Activation of HIV-1 by TNF-α and heat shock in OM-10.1 cells. OM-10.1 cells were untreated (A) or treated with 50 U/ml TNF-α (B) or heat shock at 42°C for 2 h (C and D) and cultured in the absence (A–C) or presence (D) of anti-TNF-α antibody. After a 3-day incubation at 37°C, the culture supernatants were examined for their RT activity. Data are taken from Hashimoto et al. (1996).

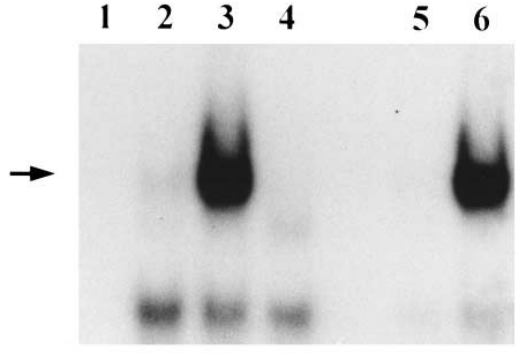

Fig. 7. Effect of heat shock on NF-κB determined by gel mobility shift assay. OM-10.1 cells were untreated (lane 2) or treated with 1 ng/ml TNF-α (lanes 3, 5, and 6) or heat shock at 42°C for 2 h (lane 4). Nuclear proteins were extracted and examined for their NF-κB binding activity to the [γ-^{32}P]ATP-labeled NF-κB-specific DNA probe. One hundred-fold molar excess of the unlabeled specific DNA probe (lane 5) or the random DNA probe (lane 6) was added for competition. Lane 1 is the control with no nuclear proteins.

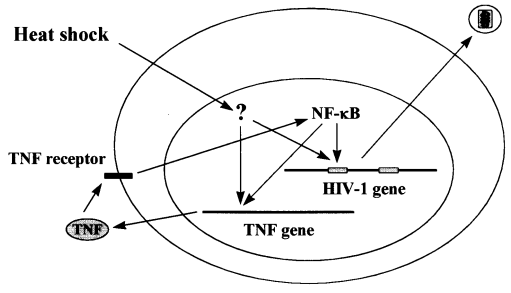

Fig. 8. Putative mechanism of heat shock-induced HIV-1 activation in OM-10.1 cells. Heat shock induces unknown cellular factors, presumably heat shock proteins and heat shock factors (Baler et al., 1992; Abravaya et al., 1992). The heat shock factors interact with the consensus heat shock element existing in the promotor region of TNF-α gene and bring about TNF-α production and NF-κB activation. The activated NF-κB induces TNF-α production as well as HIV-1 replication (autocrine loop). The heat shock factors may also be able to interact directly with the κB elements of HIV-1 LTR and induce HIV-1 replication. Furthermore, it cannot be excluded that cytokines other than TNF-α also generate a similar autocrine loop and play a considerable role in the heat shock-induced HIV-1 activation.

ifylline on HIV-1 production in the heat shock-treated OM-10.1 cells and compared to those in the TNF-α-treated cells. With the exception of anti-TNF-α antibody, all compounds were more inhibitory to HIV-1 antigen expression in the heat shock-treated cells than in the TNF-α-treated cells (Table 2). In particular, pentoxifylline achieved 71.4% inhibition at a concentration of 1 mM in the heat shock-treated cells, whereas it displayed little, if any, inhibition in TNF-α-treated cells, suggesting that the suppression of TNF-α production is a primary mechanism of this compound. Thus, the heat shock-treated OM-10.1 cells are considered as a useful system for detection of anti-HIV-1 agents that modulate host cellular factors including cytokine production.

4. Conclusion

The recent progress of the combination chemotherapy with HIV-1 RT inhibitors and protease inhibitors has achieved more than 2 $\log_{10}$ reduction of viral RNA level in plasma within several weeks of starting treatment (Gulick et al., 1996; Condra et al., 1996; Markowitz et al., 1996), indicating that the repetitive acute HIV-1 infection accounts for $\geq 99\%$ of the plasma viruses in infected individuals (Perelson et al., 1996). On the other hand, several lines of evidence suggest that long-survived chronically infected cell populations such as tissue macrophages considerably affect the disease progression. These cell populations continuously produce or start producing HIV-1 particles in response to various stimuli. It is, therefore, clear that viral production from these cell populations needs to be suppressed until they have been totally eradicated. Furthermore, secondary bacterial or viral infections induce unregulated immune cell activation through their antigens thereby enhancing the replication of HIV-1 (Nelson et al., 1990; Wallis et al., 1993; Hashimoto et al., 1995). Thus, a rationale exists for the inhibition of excessive production of certain cytokines and subsequent activation of cellular transcriptional factors. In this regard, the approach described herein should be undertaken as an effective intervention in the treatment of HIV-1 infections.

Table 2
Inhibition of heat shock- or TNF-α-induced HIV-1 activation in OM-10.1 cells

Treatment	Inhibition of HIV-1 antigen expression[a] (%)	
	Heat shock	TNF-α
None	0	0
Anti-TNF-α antibody (1 μg/ml)	80.5	100
Staurosporine (10 nM)	92.0	48.3
Ro5-3335[b] (15 μM)	62.6	34.9
Pentoxifylline (1 mM)	71.4	11.2

[a]OM-10.1 cells were treated with heat shock at 42°C for 2 h or 50 U/ml TNF-α. After a 36 h incubation at 37°C, the cells were subjected to the indirect immunofluorescence and analyzed for their HIV-1 antigen expression by FACScan. All compounds did not show significant cytotoxicity at the indicated concentrations, as determined by the MTT method (data not shown).

[b]7-Chloro-5-(2-pyrryl)-3*H*-1,4-benzodiazepine-2(*H*)-one (Hsu et al., 1991).

Data are taken from Hashimoto et al. (1996).

Acknowledgements

I greatly thank Dr. M. Okamoto (Kagoshima University, Kagoshima, Japan) for her excellent technical assistance and Dr. K. Hashimoto (Fukushima Medical College, Fukushima, Japan) for his useful discussion. OM-10.1 cells were obtained through the AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, Bethesda, MD (Contributor: Dr. S. Butera). This work was supported in part by a grant from the Japan Human Science Foundation.

References

Abravaya, K., Myers, M.P., Murphy, S.P. and Morimoto, R.I. (1992) The human heat shock protein hsp70 interacts with HSF, the transcription factor that regulates heat shock gene expression. Genes Dev. 6, 1153–1164.

Alcamí, J., de Lera, T.L., Folgueira, L., Pedraza, M.-A., Jacqué, J.-M., Bachelerie, F., Noriega, A.R., Hay, R.T., Harrich, D., Gaynor, R.B., Virelizier, J.-L. and Arenzana-Seisdedos, F. (1995) Absolute dependence on κB responsive elements for initiation and Tat-mediated amplification of HIV transcription in blood CD4 lymphocytes. EMBO J. 14, 1552–1560.

Alkhatib, G., Combadiere, C., Broder, C.C., Feng, Y., Kennedy, P.E., Murphy, P.M. and Berger, E.A. (1996) CC CKR5: A RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 272, 1955–1958.

Baba, M., Balzarini, J., Pauwels, R. and De Clercq E. (1995) HIV-1-specific reverse transcriptase inhibitors. In: P. Mohan and M. Baba, (Eds.), Anti-AIDS Drug Development: Challenges, Strategies and Prospects, pp. 239–267, Harwood, Chur, Switzerland.

Baler, R., Welch, W.J. and Voellmy, R. (1992) Heat shock gene regulation by nascent polypeptides and denatured proteins: hsp70 as a potential autoregulatory factor. J. Cell. Biol. 117, 1151–1159.

Berkhout, B., Gatignol, A., Rabson, B. and Jeang, K.-T. (1990) TAR-independent activation of the HIV-1 LTR: evidence that Tat requires specific regions of the promoter. Cell 62, 757–767.

Biswas, D.K., Dezuke, B.J., Ahlers, C.M. and Pardee, A.B. (1993) Pentoxifylline inhibits HIV-1 LTR-driven gene expression by blocking NF-κB action. J. Acquir. Immune Defic. Syndr. 6, 778–786.

Bryant, M.L., Ratner, L., Duronio, R.J., Kishore, N.S., Devadas, B., Adams, S.P. and Gordon, J.I. (1991) Incorporation of 12-methoxydodecanoate into the human immunodeficiency virus 1 gag polyprotein precursor inhibits its proteolytic processing and virus production in a chronically infected human lymphoid cell line. Proc. Natl. Acad. Sci. USA 88, 2055–2059.

Burgunder, J.M., Varriale, A. and Lauterburg, B.H. (1989) Effect of *N*-acetylcysteine on plasma cysteine and glutathione following paracetamol administration. Eur. J. Clin. Pharmacol. 36, 127–131.

Butera, S.T., Perez, P.L., Wu, B.-Y., Nabel, G.J. and Folks, T.M. (1991) Oscillation of the human immunodeficiency virus surface receptor is regulated by the state of viral activation in a $CD4^+$ cell model of chronic infection. J. Virol. 65, 4645–4653.

Butera, S.T., Roberts, B.D., Critchfield, J.W., Fang, G., McQuade, T., Gracheck, S.J. and Folks, T.M. (1995) Compounds that target novel cellular components involved in HIV-1 transcription. Mol. Med. 1, 758–767.

Cocchi, F., DeVico, A.L., Garzino-Demo, A., Arya, S.K., Gallo, R.C. and Lusso, P. (1995) Identification of RANTES, MIP-1α, and MIP-1β as the major HIV-suppressive factors produced by $CD8^+$ T cells. Science 270, 1811–1815.

Condra, J.H., Holder, D.J., Schleif, W.A., Chodakewitz, J.A., Massari, F.E., Blahy, O.M., Danovich, R.M., Gabryelski, L.J., Laird, D., Quintero, J.C., Rhodes, A., Robbins, H.L., Roth, E., Shivaprakash, M., Yang, T. and Emini, E.A. (1996) Bi-directional inhibition of HIV-1 drug resistance selection by combination therapy with indinavir and reverse transcriptase inhibitors. XI International Conference on AIDS, Abstract Th.B.932.

Critchfield, J.W., Butera, S.T. and Folks, T.M. (1996) Inhibition of HIV activation in latently infected cells by flavonoid compounds. AIDS Res. Hum. Retroviruses 12, 39–46.

De Clercq, E. (1995a) Antiviral therapy for human immunodeficiency virus infections. Clin. Microbiol. Rev. 8, 200–239.

De Clercq, E. (1995b) Toward improved anti-HIV chemotherapy: therapeutic strategies for intervention with HIV infections. J. Med. Chem. 38, 2491–2517.

De Clercq, E. (1996) Non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of human immunodeficiency virus type 1 (HIV-1) infections: strategies to overcome drug resistance development. Med. Res. Rev. 16, 125–157.

Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R.E., Hill, C.M., Davis, C.B., Peiper, S.C., Schall, T.J., Littman, D.R. and Landau, N.R. (1996) Identification of a major co-receptor for primary isolates of HIV-1. Nature 381, 661–666.

Dezube, B.J. and Lederman, M.M. (1995) Pentoxifylline for the treatment of HIV infection and its complications. J. Cardiovasc. Pharmacol. 25, S139–S142.

Diaz-Meco, M.T., Dominiguez, I., Sanz, L., Dent, P., Lozano, J., Municio, M.M., Berra, E., Hay, R.T., Sturgill, T.W. and Moscat, J. (1994) ζ PKC induces phosphorylation and inactivation of IκB in vitro. EMBO J. 13, 2842–2848.

Dragic, T., Charneau, P., Clavel., F. and Alizon, M. (1992) Complementation of murine cells for human immunodeficiency virus envelope/CD4-mediated fusion in human/murine heterokaryons. J. Virol. 66, 4794–4802.

Dragic, T., Litwin, V., Allaway, G.P., Martin, S.R., Huang, Y., Nagashima, K.A., Cayanan, C., Maddon, P.J., Koup, R.A., Morre, J.P. and Paxton, W.A. (1996) HIV-1 entry into $CD4^+$ cells is mediated by the chemokine receptor CC-CKR-5. Nature 381, 667–673.

Fauci, A.S. (1993) Multifactorial nature of human immunodeficiency virus disease: implication for therapy. Science 262, 1011–1018.

Fauci, A.S., Pantaleo, G., Stanley, S. and Weissman, D. (1996) Immunopathogenic mechanism of HIV infection. Ann. Intern. Med. 124, 654–663.

Fazely, F., Dezube, B.J., Allen-Ryan, J., Perdee, A.B. and Ruprecht, R.M. (1991) Pentoxifylline (Trental) decreases the replication of the human immunodeficiency virus type 1 in human peripheral blood mononuclear cells and in cultured T cells. Blood 77, 1653–1656.

Feng, Y., Broder, C.C., Kennedy, P.E. and Berger, E.A. (1996) HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 272, 872–877.

Feorino, P.M., Butera, S.T., Folks, T.M. and Schinazi, R.F. (1993) Prevention of activation of HIV-1 by antiviral agents in OM-10.1 cells. Antiviral Chem. Chemother. 4, 55–63.

Folgueira, L., McElhinny, J.A., Bren, G.D., MacMorran, W., Diaz-Meco, M, Moscat, J. and Paya, C.V. (1996) Protein kinase C-ζ mediates NF-κB activation in human immunodeficiency virus-infected monocytes. J. Virol. 70, 223–231.

Folks, T.M., Justement, J., Kinter, A., Dinarello, C.A. and Fauci, A.S. (1987) Cytokine-induced expression of HIV-1 in a chronically infected promonocytic cell line. Science 238, 800–802.

Franke, E.K., Yuan, H.E.H. and Luban, J. (1994) Specific incorporation of cyclophilin A into HIV-1 virions. Nature 372, 359–362.

Gulick, R.M., Mellors, J., Havlir, D., Eron, J., Gonzalez, C., McMahon, D., Richman, D., Valentine, F., Rooney, J., Jonas, L., Meibohm, A., Emini, E. and Chodakewitz, J. (1996) Potent and sustained antiretroviral activity of indinavir (IDV), zidovudine (ZDV) and lamivudine (3TC). XI International Conference on AIDS, Abstract Th.B.931.

Gnabre, J.N., Brady, J.N., Clanton, D.J., Ito, Y., Dittmer, J., Bates, R.B. and Huang, R.C.C. (1995) Inhibition of human immunodeficiency virus type 1 transcription and replication by DNA sequence-selective plant lignans. Proc. Natl. Acad. Sci. USA 92, 11 239–11 243.

Göttlinger, H.G., Sodroski, J.G. and Haseltine, W.A. (1989) Role of capsid precursor processing and myristoylation in morphogenesis and infectivity of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA 86, 5781–5785.

Hannun, Y.A. (1994) The sphingomyelin cycle and the second messenger function of ceramide. J. Biol. Chem. 269, 3125–3128.

Hashimoto, K., Baba, M., Gohnai, K., Sato, M. and Shigeta, S. (1996) Heat shock induces HIV-1 replication in chronically infected promyelocyte cell line OM 10.1. Arch. Virol. 141, 439–447.

Hashimoto, K., Shigeta, S. and Baba, M. (1995) Superantigen toxic shock syndrome toxin-1 (TSST-1) enhances the replication of HIV-1 in peripheral blood mononuclear cells through selective activation of $CD4^+$ T lymphocytes. J. Acquir. Immune Defic. Syndr. Hum. Retrovir. 10, 393–399.

Ho, W.-Z. and Douglas, S.D. (1992) Glutathione and *N*-acetylcysteine suppression of human immunodeficiency virus replication in human monocyte/macrophages in vitro. AIDS Res. Hum. Retroviruses 8, 1249–1253.

Hsu, M.-C., Schutt, A.D., Holly, M., Slice, L.W., Sherman, M.I., Richman, D.D., Potash, M.J. and Volsky, D.J. (1991) Inhibition of HIV replication in acute and chronic infections in vitro by a Tat antagonist. Science 254, 1799–1802.

Jones, K.A., Kadonaga, J.T., Luciw, P.A. and Tjian, R. (1986) Activation of the AIDS retrovirus promoter by the cellular transcription factor Sp1. Science 232, 755–759.

Kalebic, T., Kinter, A., Poli, G., Anderson, M.E., Meister, A. and Fauci, A.S. (1991) Suppression of human immunodeficiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester, and *N*-acetylcysteine. Proc. Natl. Acad. Sci. USA 88, 986–990.

Kamine, J., Subramanian, T. and Chinnadurai, G. (1991) Sp1-dependent activation of a synthetic promoter by human immunodeficiency virus type 1 Tat protein. Proc. Natl. Acad. Sci. USA 88, 8510–8514.

Karpas, A., Lowdell, M., Jacobson, S.K. and Hill, F. (1992) Inhibition of human immunodeficiency virus and growth of infected T cells by the immunosuppressive drugs cyclosporin A and FK-506. Proc. Natl. Acad. Sci. USA 89, 8351–8355.

Kira, T., Merin, J.P., Baba, M., Shigeta, S. and Okamoto, T. (1995) Anti-Tat MTT assay: a novel anti-HIV drug screeinig system using the viral regulatory network of replication. AIDS Res. Hum. Retroviruses 11, 1359–1366.

Kopp, E. and Ghosh, S. (1994) Inhibition of NF-κB by sodium salicylate and aspirin. Science 265, 956–959.

Kretz-Remy, C. and Arrigo, A.-P. (1994) The kinetics of HIV-1 long terminal repeat transcriptional activation resemble those of hsp70 promoter in heat shock treated HeLa cells. FEBS Lett. 351, 191–196.

Legrand-Poels, S., Vaira, D., Pincemail, J., Van de Vorst, A. and Piette, J. (1990) Activation of human immunodeficiency virus type 1 by oxidative stress. AIDS Res. Hum. Retroviruses 6, 1389–1397.

Leung, D.W., Peterson, P.K., Weeks, P., Gekker, G., Chao, C.C., Kaplan, A.H., Balantac, N., Tompkins, C., Underiner, G.E., Bursten, S., Harris, W., Bianco, J.A. and Singer, J.W. (1995) CT-2576, an inhibitor of phospholipid signaling, suppresses constitutive and induced expression of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA 92, 4813–4817.

Li, C.J., Zhang, L.J., Dezube, B.J., Crumpacker, C.S. and Pardee, A.B. (1993) Three inhibitors of type 1 human immunodeficiency virus long terminal repeat-directed gene expression and virus replication. Proc. Natl. Acad. Sci. USA 90, 1839–1842.

Luciw, P.A. and Shacklett, B.L. (1993) Molecular biology of the human and simian immunodeficiency viruses. In: W.J.W. Morrow and N.L. Haigwood, (Eds.), Molecular organization, pathogenicity and treatment, pp. 123–219, Elsevier, Amsterdam.

Luznik, L., Kraus, S., Gutatelli, J., Richman, D. and Wong-Staal, F. (1995) Tat-independent replication of human immunodeficiency virus. J. Clin. Invest. 95-328–332.

Makonkawkeyoon, S., Limson-Pobre, R.N.R., Moreira, A.L., Schauf, V. and Kaplan, G. (1993) Thalidomide inhibits the replication of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA 90, 5974–5978.

Markowitz, M., Cao, Y., Hurley, A., O'Donovan, R., Heath-Chiozzi, M., Leonard, J., Smiley, L., Keller, A., Johnson, D., Johnson, P. and Ho, D.D. (1996) Triple therapy with AZT, 3TC, and ritonavir in 12 subjects newly infected with HIV-1. XI International Conference on AIDS, Abstract Th.B.933.

Maruyama, I., Maruyama, Y., Nakajima, T., Kitajima, I., Osame, M., Zhao, J.-Q., Chen, I.S.Y., Nakai, S., Ikeda, M., Yabu-uchi, Y. and Adachi, M. (1993) Vesnarinone inhibits production of HIV-1 in cultured cells. Biochem. Biophys. Res. Commun. 195, 1264–1271.

McCune, J.M., Rabin, L.B., Feinberg, M.B., Lieberman, M., Mosek, J.C., Reyes, G.R. and Weissman, L.L. (1988) Endoproteolytic cleavage of gp160 is required for the activation of human immunodeficiency virus. Cell 53, 55–67.

McNeely, T.B., Dealy, M., Dripps, D.J., Orenstein, J.M., Eisenberg, S.P. and Wahl, S.M. (1995) Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. J. Clin. Invest. 96, 456–464.

Merin, J.P., Matsuyama, M., Kira, T., Baba, M. and Okamoto, T. (1996) α-Lipoic acid blocks HIV-1 LTR-dependent expression of hygromycin resistance in THP-1 stable transformants. FEBS Lett. 394, 9–13.

Mihm, S., Ennen, J., Pessara, U., Kurth, R. and Dröge, W. (1991) Inhibition of HIV-1 replication and NF-κB activity by cysteine and cysteine derivatives. AIDS 5, 497–503.

Moreira, A.L., Sampaio, E.P., Zmuidzinas, A., Frindt, P., Smith, K.A. and Kaplan, G. (1993) Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation. J. Exp. Med. 177, 1675–1680.

Nabel, G. and Baltimore, D. (1987) An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature 326, 711–713.

Naour, R.L., Clayette, P., Henin, Y., Mabondzo, A., Raoul, H., Bousseau, A. and Dormont, D. (1994) Infection of human macrophages with an endogenous tumor necrosis factor-α (TNF-α)-independent human immunodeficiency virus type 1 isolate is unresponsive to the TNF-α synthesis inhibitor RP 55778. J. Gen. Virol. 75, 1379–1388.

Navarro, J., Punzón, M.C., Pizarro, A., Fernández-Cruz, E., Fresno, M. and Muñoz-Fernández, M.A. (1996) Pentoxifylline inhibits acute HIV-1 replication in human T cells by a mechanism not involving inhibition of tumor necrosis factor synthesis or nuclear factor-κB activation. AIDS 10, 469–475.

Nelson, J.A., Ghazal, P. and Wiley, C.A. (1990) Role of opportunistic viral infections in AIDS. AIDS 4, 1–10.

Neurath, A.R., Jiang, S., Strick, N., Lin, K., Li, Y.-Y. and Debnath, A.K. (1996) Bovine β-lactoglobulin modified by 3-hydroxyphthalic anhydride blocks the CD4 cell receptor for HIV. Nature Med. 2, 230–234.

Pal, R., Hoke, G.M. and Sarngadharan, M.G. (1989) Role of oligosaccharides in the processing and maturation of envelope glycoproteins of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA 86, 3384–3388.

Pal, R., Mumbauer, S., Hoke, G.M., Takatsuki, A. and Sarngadharan, M.G. (1991) Brefeldin A inhibits the processing and secretion of envelope glycoproteins of human immunodeficiency virus type 1. AIDS Res. Hum. Retroviruses 7, 707–712.

Parnham, M.J. and Graf, E. (1987) Seleno-organic compounds and the therapy of hydroperoxide-linked pathological conditions. Biochem. Pharmacol. 36, 3095–3102.

Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewijn, P., Desmyter, J. and De Clercq E. (1988) Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J. Virol. Methods 20, 309–321.

Paxton, W.A., Martin, S.R., Tse, D., O'Brien, T.R., Skurnick, J., VanDevanter, N.L., Padian, N., Braun, J.F., Kotler, D.P., Wolinsky, S.M. and Koup, R.A. (1996) Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposures. Nature Med. 2, 412–417.

Perelson, A.S., Neumann, A.U., Markowitz, M., Leonard, J.M. and Ho, D.D. (1996) HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science 271, 1582–1586.

Prochaska, H.J., Bornmann, W.G., Baron, P. and Polsky, B. (1995) Inhibition of human immunodeficiency virus type 1 replication by 7-methyl-6,8-bis(methylthio)pyrrolo-[1,2-α]pyrazine, an in vivo metabolite of oltipraz. Mol. Pharmacol. 48, 15–20.

Prochaska, H.J., Fernandes, C.L., Pantoja, R.M. and Chavan, S.J. (1996) Inhibition of human immunodeficiency virus type 1 long terminal repeat-driven transcription by an in vivo metabolite of oltipraz: implications for antiretroviral therapy. Biochem. Biophys. Res. Commun. 221, 548–553.

Prochaska, H.J., Yeh, Y., Baron, P. and Polsky, B. (1993) Oltipraz, an inhibitor of human immunodeficiency virus type 1 replication. Proc. Natl. Acad. Sci. USA 90, 3953–3957.

Qatsha, K.A., Rudolph, C., Marmé, D., Schächtele, C. and May, W.S. (1993) Gö 6976, a selective inhibitor of protein

kinase C, is a potent antagonist of human immunodeficiency virus 1 induction from latent/low-level-producing reservoir cells in vitro. Proc. Natl. Acad. Sci. USA 90, 4674–4678.

Rice, G.C., Brown, P.A., Nelson, R.J., Bianco, J.A., Singer, J.W. and Bursten, S. (1994) Protection from endotoxic shock in mice by pharmacologic inhibition of phosphatidic acid. Proc. Natl. Acad. Sci. USA 91, 3857–3861.

Roederer, M., Raju, P.A., Staal, F.J.T., Ela, S.W., Herzenberg, L.A. and Herzenberg, L.A. (1991) *N*-Acetylcysteine inhibits latent HIV expression in chronically infected cells. AIDS Res. Hum. Retroviruses 7, 563–567.

Roederer, M., Staal, F.J.T., Raju, P.A., Ela, S.W., Herzenberg, L.A. and Herzenberg, L.A. (1990) Cytokine-stimulated human immunodeficiency virus replication is inhibited by *N*-acetyl-L-cysteine. Proc. Natl. Acad. Sci. USA 87, 4884–4888.

Rosenwirth, B., Billich, A., Datema, R., Donatsch, P., Hammerschmid, F., Harrison, R., Hiestand, P., Jaksche, H., Mayer, P., Peichl, P., Quesniaux, V., Schatz, F., Schuurman, H.-J., Traber, R., Wenger, R., Wolff, B., Zenke, G. and Zurini, M. (1994) Inhibition of human immunodeficiency virus type 1 replication by SDZ NIM 811, a nonimmunosuppressive cyclosporine analog. Antimicrob. Agents Chemother. 38, 1763–1772.

Roulston, A., Lin, R., Beauparlant, P., Wainberg, M.A. and Hiscott, J. (1995) Regulation of human immunodeficiency virus type 1 and cytokine gene expression in myeloid cells by NF-κB/Rel transcription factors. Microbiol. Rev. 59, 481–505.

Schreck, R, Rieber, P. and Baeuerle, P.A. (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of NF-κB transcription factor and HIV-1. EMBO J. 10, 2247–2258.

Schutze, S., Potthoff, K., Machleidt, T., Berkovic, D., Wiegmann, K. and Kronke M. (1992) TNF activation NF-κB by phosphatidylcholine-specific phospholipase C-induced acidic sphingomyelin breakdown. Cell 71, 765–776.

Staal, F.J.T., Roederer, M., Herzenberg, L.A. and Herzenberg, L.A. (1990) Intracellular thiols regulate activation of nuclear factor κB and transcription of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA 87, 9943–9947.

Staal, F.J.T., Roederer, M., Raju, P.A., Anderson, M.T., Ela, S.W., Herzenberg, L.A. and Herzenberg, L.A. (1993) Antioxidants inhibit stimulation of HIV transcription. AIDS Res. Hum. Retroviruses 9, 299–306.

Stanley, S.K., Bressler, P.B., Poli, G. and Fauci, A.S. (1990) Heat shock induction of HIV production from chronically infected promonocytic and T cell lines. J. Virol. 145, 1120–1126.

Thali, M., Bukovsky, A., Kondo, E., Rosenwirth, B., Walsh, C.T., Sodroski, J. and Göttlinger, H.G. (1994) Functional association of cyclophilin A with HIV-1 virions. Nature 372, 363–365.

Thanos, D. and Maniatis, T. (1995) NF-κB: a lesson in family values. Cell 80, 529–532.

Tramontana, J.M., Utaipat, U., Molloy, A., Akarasewi, P., Burroughs, M., Makonkawkeyoon, S., Johnson, B., Klausner, J.D., Rom, W. and Kaplan, G. (1995) Thalidomide treatment reduces tumor necrosis factor alpha production and enhances weight gain in patients with pulmonary tuberculosis. Mol. Med. 1, 384–397.

Turpin, J.A., Schaeffer, C.A., Bu, M., Graham, L., Buckheit Jr., R.W., Clanton, D. and Rice, W.G. (1996) Human immunodeficiency virus type-1 (HIV-1) replication is unaffected by human secretory leukocyte protease inhibitor. Antiviral Res. 29, 269–277.

Wallis, R.S., Vjecha, M., Amir-Tahmasseb, M., Okwera, A., Byekwaso, F., Nyole, S., Kabengera, S., Mugerwa, R.D. and Ellner, J.J. (1993) Influence of tuberculosis on human immunodeficiency virus (HIV-1): enhanced cytokine expression and elevated β2-microglobulin in HIV-1-associated tuberculosis. J. Infect. Dis. 167, 43–48.

Weissman, D., Poli, G., Bousseau, A. and Fauci, A.S. (1993) A platelet-activating factor antagonist, RP 55778, inhibits cytokine-dependent induction of human immunodeficiency virus expression in chronically infected promonocyte cells. Proc. Natl. Acad. Sci. USA 90, 2537–2541.

Willey, R.L., Bonifacino, J.S., Potts, B.J., Martin, M.A. and Klausner, R.D. (1988) Biosynthesis, cleavage, and degradation of the human immunodeficiency virus 1 envelope glycoprotein gp160. Proc. Natl. Acad. Sci. USA 85, 9580–9584.

Witvrouw, M., Pauwels, R., Vandamme, A., Schols, R., Reymen, D., Yamamoto, N., Desmyter, J. and De Clercq E. (1992) Cell type-specific anti-human immunodeficiency virus type 1 activity of the transactivation inhibitor Ro5-3335. Antimicrob. Agents Chemother. 36, 2628–2633.

Woloschak, G.E., Panozzo, J., Schreck, S. and Libertin, C.R. (1995) Salicylic acid inhibits ultraviolet- and cis-platinum-induced human immunodeficiency virus expression. Cancer Res. 55, 1696–1700.