Antiviral Research, 17 (1992) 289–304

AVR 00543

Mini Symposium – HIV Infections*

Different pattern of activity of inhibitors of the human immunodeficiency virus in lymphocytes and monocyte/macrophages

Carlo-Federico Perno[1], Robert Yarchoan[3], Jan Balzarini[4], Alberto Bergamini[2], Giuseppe Milanese[2], Rudi Pauwels[4], Erik De Clercq[4], Giovanni Rocchi[2] and Raffaele Calio[1]

[1]*Chair of Virology, Department of Experimental Medicine and Biochemical Sciences, and* [2]*Chair of Infectious Diseases, Department of Public Health, University of Rome, Tor Vergata, Italy,* [3]*Clinical Oncology Program, National Cancer Institute, NIH, Bethesda, MD, U.S.A. and* [4]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgium*

Summary

Monocyte/macrophages (M/M) are important targets for HIV in the body, and represent the majority of cells infected by the virus in some body compartments such as the central nervous system (CNS). M/M can be different from T-lymphocytes in terms of surface antigens, cell replication and drug metabolism. Thus, we evaluated, in M/M and in T-lymphocytes, the pattern of viral inhibition induced by various anti-HIV drugs, and assessed some of the mechanisms of action related to such antiviral activity.

Inhibitors of HIV binding on CD4 receptors have similar activity in M/M and T-lymphocytes, while AZT and other dideoxynucleosides (ddN) are in general more active against HIV in M/M than in T-lymphocytes. This phenomenon can be related to the increased ratio in M/M of ddN-triphosphate/deoxynucleoside-triphosphate, and can at least in part explain the ability of zidovudine and didanosine in improving neurological dysfunctions in AIDS patients. Moreover, the antiviral activity of AZT (but not of other ddN- or HIV-binding inhibitors) is potently enhanced by cytokines like granulocyte-macrophage colony stimulating factor (GM-CSF) in M/M, while

*Based on a presentation originally made at the Fourth International Conference on Antiviral Research held in New Orleans, Louisiana, U.S.A., April 21–26, 1991.

Correspondence to: Dr. Carlo-Federico Perno, Dept. of Experimental Medicine, University of Rome,Tor Vergata, Via Orazio Raimondo, 00173 Roma, Italy.

anti-HIV activity of TIBO compounds in M/M is not down-modulated by GM-CSF and other cytokines. Finally, non-toxic concentrations of adriamycin, an anticancer drug reported to be active against DNA viruses, can inhibit HIV replication in M/M (but not in T-lymphocytes).

Taken together, these results suggest that M/M are selective targets for HIV with peculiarities different from those of T-lymphocytes. Thus, promising anti-HIV compounds should be evaluated both in T-cells and in M/M before reaching clinical trials. This may help in selecting drugs with good chances of being effective in patients with HIV-related disease.

HIV; Macrophage; Lymphocyte; Anti-HIV drug; Cytokine

Introduction

The search for drugs able to inhibit the replication of human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), is now a major research effort, and substantial progress has been made since 1985, when 3′-azido-2′,3′-dideoxythymidine (AZT) was discovered as the first drug active in patients with HIV-related disease (Mitsuya et al., 1985; Yarchoan et al., 1986, 1988, 1990; Fischl et al., 1987). Since then, many compounds have proven to have at least some activity against HIV replication in several in vitro models, and a few have been shown to induce clinical improvement in patients (Yarchoan et al., 1989a; Mitsuya et al., 1990). However, a number of compounds which had activity in vitro have failed to show clinical activity (Mitsuya et al., 1990). This discrepancy is the cause of serious concern regarding both the reliability of antiviral drug screening, and the extent to which such in vitro models are representative of in vivo pathological events linked to HIV replication.

The pathogenesis of HIV infection is a complex process which is still only imperfectly understood. The percentage of cells infected by HIV varies in different tissues. In addition, HIV recognizes a variety of cellular targets in addition to CD4-bearing T-lymphocytes (Amstrong et al., 1984; Gartner et al., 1986a; Klatzmann et al., 1984; Tschachler et al., 1987; Montagnier et al., 1984); each of these target cells may potentially play a role in the pathogenesis and progression of the disease (Meltzer et al., 1990; Gabudza and Hirsch, 1987). In particular, cells belonging to the monocyte/macrophage lineage (M/M) are widely recognized as a major target for HIV infection in the body (Gartner et al., 1986a; Meltzer et al., 1990; Ho et al., 1986; McElrath et al., 1989; Perno et al., 1989). There is evidence that infection of M/M may represent an early event in the in vivo infection (Popovic and Gartner, 1987). Tissue M/M are a selective target for HIV, as shown by prominent infection of Langerhans cells of the skin, Kupffer cells of the liver, lung alveolar M/M, peritoneal M/M, reticulo-endothelial cells in lymphnodes and in the bone marrow compartment

(Amstrong et al., 1984; Gartner et al., 1986a; Tschachler et al., 1987; Meltzer et al., 1990; McElrath et al., 1989). More importantly, M/M-derived cells account for the large majority of cells infected by HIV in the central nervous system (CNS), and M/M infection and consequent neuronal dysfunction in the brain play a crucial role in the pathogenesis of HIV-related dementia complex (Gabuzda and Hirsch, 1987; Koenig et al., 1986; Gartner et al., 1986b). Generally M/M are resistant to the cytopathic effect of the virus (Gartner et al., 1986a; Perno et al., 1988), and as a consequence, viral production by HIV-infected M/M is quite abundant and persists for long time. Due to these characteristics, M/M are able to spread HIV to other cells such as CD4-lymphocytes that have a specific interaction with M/M in the antigen presentation and the activation of immune response (Chantal-Petit et al., 1988).

CD4-lymphocytes require activation (and cell replication) in order to produce substantial amounts of the virus (Popovic et al., 1984; Klatzmann et al., 1984). In contrast, M/M produce a large number of infectious virus particles even under resting conditions (Gartner et al., 1986a; Perno et al., 1988). At the same time, such virus production could be further enhanced by some cytokines able to modulate both immunological functions and maturation of M/M (Perno et al., 1989; Koyanagi et al., 1988; Gendelman et al., 1988).

Mature M/M are non-replicating cells, thus they have a limited DNA machinery: as a consequence, concerns were at one time raised that antiviral drugs, such as AZT, which require activation by cellular enzymes involved in DNA synthesis, might have lower antiviral activity in cells of M/M lineage. However, several studies demonstrated that AZT improves neurological dysfunctions and HIV-related abnormalities of glucose metabolism in the brain of patients with AIDS-related dementia (Yarchoan et al., 1987). Since M/M-derived cells are the majority of HIV-target cells in the brain, it was conceivable that the clinical efficacy of AZT in these patients was at least in part due to the inhibition of viral replication in M/M of the CNS. Indeed, our group and others have shown that dideoxynucleosides (including AZT) are effective inhibitors of HIV infection of M/M. Whether this is in fact related to the inhibition of HIV replication in M/M-derived cells in the CNS, however, is still a matter for further research.

Infection of M/M by HIV can be enhanced by cytokines such as granulocyte-macrophage colony stimulating factor (GM-CSF) and macrophage-colony stimulating factor (M-CSF) (Perno et al., 1989; Koyanagi et al., 1988; Gendelman et al., 1988). Such enhancement of viral replication in M/M may have important implications in vivo. Indeed, Pluda and coworkers recently reported that six patients with AIDS and neutropenia experienced a substantial increase in HIV p24 antigenemia when they were given GM-CSF (Pluda et al., 1990). GM-CSF does not affect HIV replication in vitro in T-lymphocytes (Perno et al., 1989). While the number of patients studied was small, these data suggest that the increase in p24 antigenemia in these patients may be related to the modulation of viral production in cells of M/M lineage.

Taken together, these reports suggest that the infection of M/M by HIV has clinical implications, and that it needs to be considered in therapeutic strategies for HIV. A failure to address the infection of HIV in M/M could potentially be a cause of a failure of chemotherapy in HIV-infected patients. Evaluation of the efficacy of antiviral drugs in the M/M system is thus important in designing clinical regimens for the therapy of HIV infection. For these reasons, we studied several anti-HIV drugs in different normal M/M populations in the presence or absence of cytokines, and we compared the activity of these drugs with that achieved in T-lymphocytes. The results are discussed in this paper.

Materials and Methods

Cells

Fresh M/M and M/M-depleted lymphocytes (PBL) were obtained from peripheral blood mononuclear cells of normal, HIV-negative donors by short-term adherence or by countercurrent centrifugal elutriation, as previously described (Perno et al., 1988). U937 is a monocytoid cell line obtained from patients with hystiocytic leukemia. H9 is a T4-lymphocytic cell line, as is ATH8, a T4-lymphocytic cell clone immortalized by human T-lymphotropic virus type 1 and highly sensitive to the cytopathic effect of HIV-1. T-Ly and B-Ly were T-lymphocytes (obtained by E-rosette depletion) and EBV-transformed B-lymphocytes, respectively, obtained from the same healthy HIV-negative donor.

Test compounds

AZT was obtained from Wellcome (Pomezia, Italy), 2′,3′-dideoxycytidine (ddC), 2′,3′-dideoxyadenosine (ddA) and 2′,3′-dideoxyinosine (ddI) from Pharmacia (Italy), and recombinant soluble CD4 from Genentech Inc. (San Francisco, CA, U.S.A.). 9-(2-phosphonylmethoxyethyl)adenine (PMEA) was synthesized by Dr. A. Holy and I. Rosenberg (Praha, Czeckoslovakia), and tetrahydroimydazol-benzodiazepin-one (TIBO) derivatives were provided by Janssen Pharmaceuticals (Beerse, Belgium). Adriamycin was purchased from Farmitalia (Italy). Anti-HIV concentrated antibodies (HIVIG), a kind gift of Dr. L. Cummins (Abbott, IL, U.S.A.) were purified from asymptomatic, HIV-seropositive donors according to a previously described procedure (Cummins et al., 1991). HIVIG preparation contains high levels of anti-gp120 antibodies.

Cytokines

GM-CSF and M-CSF were obtained from Genetics Institute (Cambridge, MA, U.S.A.).

Virus

HTLV-III_{Ba-L} (HIV-BaL) and HTLV-III_B (HIV-IIIB), monocytotropic and lymphocytotropic strains of HIV-1, respectively, were a gift of Drs. M. Popovic, S. Gartner and R.C. Gallo (NCI, Bethesda, MD, U.S.A.). HIV-BaL and HIV-IIIB virus strains were titrated in M/M and lymphocytes respectively, and stored at −80°C until used. Details about these virus strains are described elsewhere (Gartner et al., 1986a; Popovic et al., 1984).

Antiviral assay

Details on the viral infection of each cell type were described previously (Perno et al., 1988, 1989, 1990a) Briefly, 10^5 cells (of each type listed above) were exposed to various concentrations of drugs for 20 min, and then challenged with 300 50% minimum infectious doses (MID_{50}) of HIV-BaL or HIV-IIIB. In experiments involving fresh M/M and cytokines, 100 U/ml GM-CSF or 1000 U/ml M-CSF were added 5 days prior to viral challenge and were maintained in culture during the whole length of the experiments. Virus excess was extensively washed off two days after viral challenge. Cells were replenished with fresh medium and drugs, and then cultured in a CO_2-enriched humidified atmosphere. Virus replication was assessed in M/M and lymphocytes by a commercially available HIV-p24 antigen assay and, in selected experiments, by reverse transcriptase activity and/or by evaluation of syncytia formation. Cytopathic effect of HIV-IIIB was assessed in T-cell lines by viable cell counting in a haemocytometer chamber. Cell toxicity was evaluated by the trypan blue dye-exclusion method in mock-infected cell cultures. Culture medium used in these experiments was RPMI 1640 supplemented with 20% fetal calf serum, 2 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin. In experiments involving PBL, T-Ly or ATH8 cells, the medium was enriched with phytohemoagglutinin 1:200 (v/v) and 5 U/ml of recombinant interleukin-2 (in the case of PBL or T-Ly) or with 50 U/nl of recombinant interleukin-2 (in the case of ATH8).

Drug metabolism

Details of the procedure for the evaluation of dideoxynucleoside metabolism have been previously described (Cooney et al., 1986). Briefly, 5×10^6 cells were cultivated for 24 h with 0.5 μM [^{3}H]ddC. Cells were then harvested, counted, and washed three times in cold RPMI. Cell pellets were then extracted with 0.5 ml of 10% trichloroacetic acid, neutralized and analyzed via HPLC for metabolites. Results were given as picomoles of ddCTP per 10^6 cells.

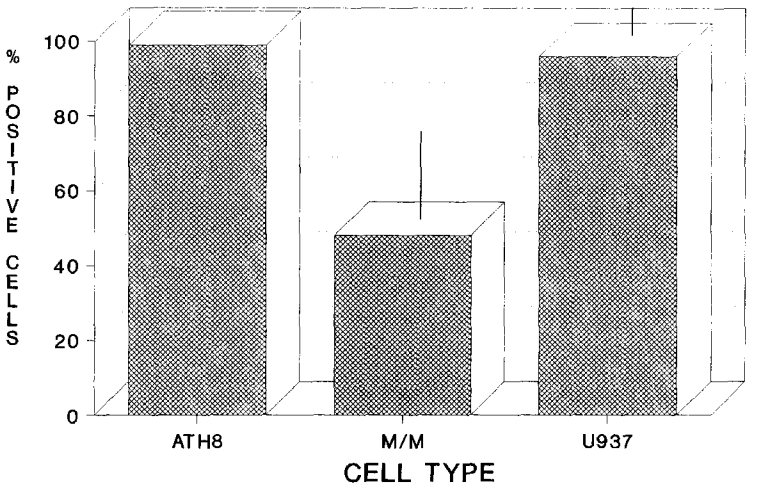

Fig. 1. Expression of CD4 antigens on the surface of different cells. ATH8 T4-lymphocytes, resting M/M and U937 monocytoid cells were stained with fluorescein-labeled OKT4A monoclonal antibody, and evaluated by FACS analyzer. Results shown in this figure represent the average of 3–8 different experiments.

Results

Preliminary experiments were devoted to study whether some peculiar M/M characteristics could affect the replication of HIV and the activity of antiviral drugs. As we have previously published (Perno et al., 1990a,b), the density of CD4-positive cells is substantially lower in M/M than in ATH8 or U937 cell lines (Fig. 1). Moreover, M/M have lower levels of CD4 receptors (on a per cell basis) than U937 monocytoid cell line, and far less CD4 than ATH8 (data not shown). However, the presence of CD4 antigen on M/M surface suggests its involvement in the attachment and entry of HIV within M/M, as well the

TABLE 1

Anti-HIV activity of inhibitors of CD4-gp120 binding in lymphocytes and monocyte/macrophages

Cell	Drug			
	sCD4 (μg/ml)		HIVIG (μg/ml)	
	ID_{50}	ID_{99}	ID_{50}	ID_{99}
ATH8	2	10	90	500
M/M	0.9	2	15	70

ATH8 and M/M were exposed to 300 minimum infectious doses of HIV-III_B and HIV_{Ba-L}, respectively, in the presence of various concentration of drugs. Data shown in this table were evaluated by HIV-p24 *gag* production in the supernatants, and confirmed by evaluation of the cytophatic effect (for ATH8) and syncytia formation (for M/M). Data represent the average of three experiments. Preliminary experiments showed that HIVIG concentrated antibody does not interfere with the ELISA method used to assess viral production.

possibility of inhibiting HIV entry in M/M by CD4-gp120-binding inhibitors. Indeed, this is the case for sCD4 and HIVIG (Table 1). More than 99% inhibition of HIV replication was achieved in M/M by 2 μg/ml sCD4, while >99% viral inhibition in ATH8 T-cells was obtained with 10 μg/ml. Similarly, >99% protection was achieved in M/M by 70 μg/ml HIVIG, with an ID_{50} of 15 μg/ml. In ATH8 cells, complete block of viral replication was achieved by 500 μg/ml HIVIG, with an ID_{50} of 90 μg/ml. Similar results were obtained with OKT4A (a monoclonal antibody that binds CD4 receptor within the epitope recognized by HIV-gp120) at similar concentrations in both M/M and T-lymphocytes (data not shown). Thus, specific inhibitors of CD4-HIV gp120 binding are able to prevent HIV replication in M/M with a similar, or even greater activity, than in T-cells. It should be stressed that a clear comparison between the levels of HIV inhibition in T-cells and those achieved in M/M by inhibitors of CD4-gp120 binding cannot be made at this point. Experiments described in this paper were done with two different viral strains ($HTLV\text{-}III_B$ and $HTLV\text{-}III_{Ba\text{-}L}$) in T-cells and M/M, respectively. Recent work by Daar and coworkers and Gomatos and coworkers (Daar et al., 1991; Gomatos et al., 1990) suggests that certain fresh or monocytotropic isolates of HIV are less sensitive to inhibition by sCD4, but not by OKT4A or Leu-3a monoclonal antibody (another monoclonal antibody similar to OKT4A). Thus, further studies will be needed to sort out these issues.

The anti-HIV activity of reverse transcriptase inhibitors, such as dideoxynucleosides, showed substantial variations when tested in different cell systems. As shown in Table 2, the ID_{50} of AZT in H9 T-cell line and in purified normal T-Ly is 0.2 and 0.4 μM respectively, and TD_{50} is 20 μM. These values are similar to the results achieved in U937, a monocytoid cell line that can be easily infected by HIV-IIIB (a lymphocytotropic strain), but not by HIV-BaL (monocytotropic). However, the AZT activity in normal M/M is substantially greater, with an ID_{50} of 0.01 μM, and ID_{99} of 0.1 μM. No toxicity was found, in resting M/M, at concentrations of AZT up to 50 μM. Similar

TABLE 2

Comparison of anti-HIV activity of AZT and DDI in T-cells, M/M and a monocytoid cell line

Cell	Drug (μM)					
	AZT			ddI		
	ID_{50}	ID_{99}	TD_{50}	ID_{50}	ID_{99}	TD_{50}
H9	0.2	2	20	0.5	10	>100
T-Ly	0.4	5	20	0.5	10	95
U937	0.2	5	20	1	50	>100
M/M	0.01	0.1	>50	0.01	0.1	>100

H9, T-Ly and U937 were exposed to $HIV\text{-}III_B$, and M/M to $HIV_{Ba\text{-}L}$ (300 minimum infectious doses for each cell type) in the presence of various concentrations of AZT and DDI. Data obtained with dideoxyadenosine (ddA, a prodrug immediately converted to ddI by the ubiquitous enzyme adenosine deaminase) completely paralleled those obtained with ddI.

TABLE 3

Anti-HIV activity of DDC in different cells correlated with the intracellular phosphorylation of DDC and with the endogenous levels of deoxycytidine-triphosphate

Cell	[DDC] (μM)			ddCTP (pmol/10^6 cells)	dCTP (pmol/10^6 cells)	ddCTP/dCTP
	ID_{50}	ID_{99}	TD_{50}			
H9	0.05	0.2	20	1.6	15.4	0.082
T-Ly	N.D.	0.5	10	N.D.	N.D.	–
U937	0.001	0.2	0.5	2.3	N.D.	
M/M	0.003	0.01	>10	0.64	2.45	0.165

Infection of target cells was performed as described in Table 2. In all cases, virus production was assessed by HIV-p24 *gag* production in the culture supernatants, and toxicity was evaluated by trypan blue exclusion test. Evaluation of intracellular ddCTP and dCTP was done as described in Materials and Methods.

results were achieved with ddA and ddI: anti-HIV activity was obtained in normal M/M at concentrations 50–100-fold lower than those needed to block HIV replication in T-Ly (or PBL) or U937. Also ddC is more active against HIV in M/M than in T-Ly (Table 3). Interestingly, in the case of ddC, the antiviral activity in U937 is similar to that achieved in M/M, but toxicity was detected in U937 cells at concentrations at least 20-fold lower than in M/M. In comparison, the ID_{50} of ddC in H9 T-cells is 0.05 μM, and the ID_{99} in H9 and in T-Ly is 0.2 and 0.5 μM, respectively. Thus, under our experimental conditions, dideoxynucleosides (ddN) are generally more active and/or less toxic against HIV in M/M than in T-lymphocytes or U937 cells. In this respect, the monocytoid line U937 behaves more like replicating T-lymphocytes than M/M.

Additional studies suggested that there is a correlation between the antiviral activity and the pharmacokinetics of ddC in target cells. As shown in Table 3, the phosphorylation of ddC to its active moiety ddC-5′-triphosphate (ddCTP) is about 1/2 the level observed in H9 T-cells. However, the endogenous levels of 2′-deoxycytidine-triphosphate (dCTP, the natural competitor of ddCTP on viral reverse transcriptase) is only 1/6 of the levels found in H9 cells. Thus, M/M have a substantially higher ratio of ddCTP/dCTP than do H9 cells, which may explain the more pronounced antiviral activity of ddC in M/M.

TABLE 4

Inhibition of HIV replication by PMEA in T-cells and monocyte/macrophages

Cells	ID_{50} (μM)	TD_{50} (μM)	Therapeutic index
H9	0.4	81	203
ATH8	2.5	80	32
PBL	3.7	50	14
M/M	0.022	>100	>4545

Results shown in this table represent the average of at least three different experiments performed, in the case of M/M, in 5-day adherent M/M (mature M/M), and confirmed in elutriated M/M (blood monocytes).

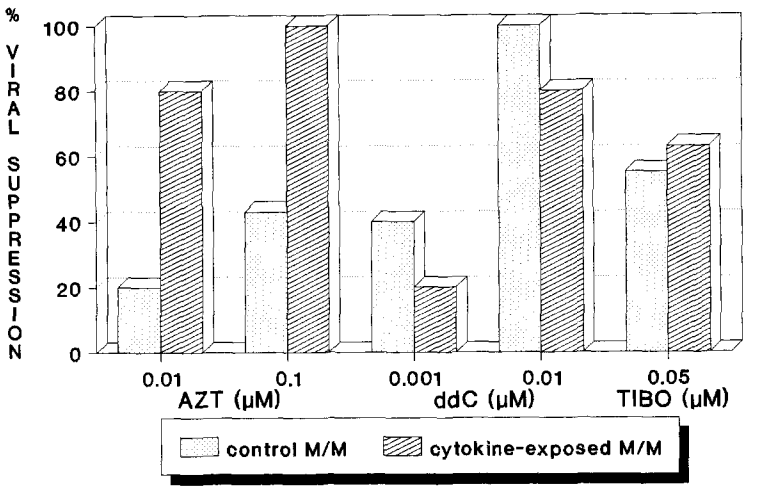

Fig. 2. Antiviral activity of AZT, ddC and TIBO in M/M exposed to cytokines enhancer of HIV replication. M/M were exposed to GM-CSF or M-CSF, and then challenged with HIV_{Ba-L} in the presence of AZT, ddC or TIBO. Results are expressed as % of suppression of HIV-p24 *gag* production in the supernatants of drug-exposed M/M compared to M/M not exposed to drugs.

Preliminary experiments also suggest that ddC has a relatively low anti-HIV efficacy in two EBV-transformed B-cell lines, one of them from the same donor as T-Ly (data not shown). This area can be matter of further research, in view of the evaluation of the metabolism of ddC in such cells.

We also evaluated the antiviral activity of PMEA (an acyclic nucleoside phosphonate) in both lymphocytes and M/M. As shown in Table 4, the ID_{50} of PMEA in M/M is 0.022 μM (with an ID_{99} of 0.1 μM) (data not shown). No toxicity was detected up to 100 μM. The ID_{50} and TD_{50} of PMEA in peripheral blood lymphocytes is 3.7 μM and 50 μM, respectively. Similar results were achieved in H9 and ATH8 T-cells (Table 4). PMEA-diphosphate (PMEA-DP), the active moiety of PMEA, is believed to compete with dATP at the level of viral reverse transcriptase, and to induce chain termination of viral DNA in formation (Balzarini et al., 1991a,b). Preliminary data suggest an increased ratio of PMEA-DP/dATP in M/M. Thus, also in this case, it is conceivable that relatively decreased dATP pools may contribute to the increased activity of this drug in M/M. PMEA is so far one of the most active anti-HIV agents in M/M, and deserves further studies in view of its potential clinical application.

We finally assessed whether cytokines that enhance viral replication could affect the activity of antiviral drugs in M/M. As previously noted (Perno et al., 1989; Koyanagi et al., 1988), GM-CSF enhances viral replication in M/M. At the same time, this cytokine enhances the anti-HIV activity of AZT by more than 10-fold (Fig. 2). For example, 20% inhibition of HIV replication was achieved in control M/M by 0.01 μM AZT, while the same concentration gave 80% viral inhibition in the presence of GM-CSF. In contrast with AZT, the antiviral activity of ddC was reduced in GM-CSF-exposed M/M compared

with control M/M. An evaluation of the metabolism of AZT, as well of its natural counterpart 2'-deoxythymidine-triphosphate (dTTP), revealed that GM-CSF enhances by more than 10-fold the ratio AZTTP/dTTP compared to control M/M, thus accounting, at least in part, for the increased antiviral activity of AZT in GM-CSF-exposed M/M (Perno et al., 1989). Preliminary data also suggest that, in contrast to the results with GM-CSF, the antiviral activity of both AZT and ddC was reduced in M/M exposed to M-CSF.

The antiviral activity of TIBO has also been assessed in our M/M system (Fig. 2). 0.05 μM TIBO afforded about 50% inhibition of viral replication in M/M, a concentration similar to that active in T-cells. In contrast with AZT and ddC, antiviral activity of TIBO in M/M was not modulated by cytokines and, in the presence of M-CSF, the ID_{50} in M/M is virtually unchanged.

Modulation of anti-HIV activity by cytokines could be of some importance from the clinical standpoint, since GM-CSF and M-CSF are present in the tissues at concentrations similar to those used in these experiments. It is thus conceivable that they may affect viral production and the activity of antiviral drugs in patients with HIV infection.

Discussion

The results reported in this paper suggest that the activity of certain inhibitors of HIV replication may be affected in vitro (and perhaps in vivo) by several factors, one of the most important being the characteristics of the cellular targets. Nevertheless, we also show that a number of the drugs under study are able to maintain or even increase their anti-HIV activity under different experimental conditions. This is a desirable quality in the consideration of agents for clinical use.

HIV can infect a variety of cells in the body, of which the most important are believed to be CD4-lymphocytes and the cells of M/M lineage (Gartner et al., 1986a; Klatzmann et al., 1984). The infection of both cells may be crucial for the progression of the disease. Thus, it is desirable that putative anti-HIV drugs be active against HIV in both lymphocytes and M/M. Drugs active in vitro in few selected cell targets or under non-stringent conditions (i.e. low multiplicity of infection, limited number of viral strains, etc.) have conceivably less chance of being effective in patients (Mitsuya et al., 1984, 1988; Broder et al., 1985; Jasmin et al., 1974; McCornick et al., 1984; Anand et al., 1986). Furthermore, a lack of testing antiviral compounds in M/M may determine an incomplete (and perhaps wrong) estimation of the real possibility of such drugs being effective in patients.

In the case of M/M, they are a peculiar target for HIV in the central nervous system (CNS), and are involved somewhat in the development of the damage to brain functions (Gabuzda and Hirsch, 1987; Koenig et al., 1986; Gartner et al., 1986b). It is then conceivable that the potential ability of anti-HIV drugs to restore the normal CNS functions is thus related to two factors: the anti-HIV

activity of such drugs in cells of M/M lineage, and their concentration in the cerebrospinal fluid (CSF) (that is usually lower than that in the plasma) (Klecker et al., 1987, 1988; Hartman et al., 1990; Yarchoan et al., 1989b). In the case of AZT and ddI (the most active anti-HIV compounds so far given to patients), drug concentrations in the CSF are indeed 2–10 times lower than in the blood (Klecker et al., 1988; Hartman et al., 1990). Even in this case, the in vitro anti-HIV activity of such drugs in M/M is >10-fold greater than in T-lymphocytes (Perno et al., 1988). Thus, despite the relatively low concentrations of AZT and ddI in the CSF, the levels are still sufficient to achieve a substantial HIV inhibition in M/M of the CNS. This may explain, at least in part, the ability of AZT and ddI to improve the neurological dysfunctions in patients with AIDS-dementia complex (Yarchoan et al., 1987). However, as noted above, the pathogenesis of HIV-related dementia is still incompletely understood, and it is possible that the beneficial effect of these drugs on dementia occurs by different mechanisms.

Monocytoid cells such as U937 are easier to test in the laboratory than normal resting M/M. However, there are profound differences between U937 cells and M/M, and in fact, at least from this point of view, they do not provide a good model system for studying the effects of anti-HIV drugs on M/M. U937 are replicating cells (easily infected by a lymphocytotropic strain of HIV-1, but not by a monocytotropic strain) (Perno, Broder, and Yarchoan, unpublished data). Thus, their intracellular 2′-deoxynucleoside-triphosphate (DNA) pools are higher than those of resting M/M (Perno et al., 1987), and are quite comparable with those of replicating T-cells (similar results, not reported in this paper, were obtained with another replicating monocytoid cell line). In considering the metabolism of ddN, it is important to remember that their activity is influenced by the levels of the competing endogenous 2′-deoxynucleotide-triphosphate pools (dNTP) (Hao et al., 1988). Low levels of endogenous dNTP pools in M/M necessarily result in decreased competition with ddN-triphosphate on viral reverse transcriptase, and may therefore allow more efficient viral DNA chain termination. This may explain why ddN are more potent antiviral agents in M/M than in T-cells and U937 monocytoid cells, in spite of their decreased phosphorylation. In a similar fashion, sCD4 and other inhibitors of the CD4-gp120-binding process show different patterns of activity in M/M than in T-lymphocytes. In the case of sCD4, the activity appears to be highly dependent on the strain of the virus (Gomatos et al., 1990).

These data demonstrate that there are differences in the activity of anti-HIV drugs in various target cells. In addition, as we have seen, cytokines may affect the replication of HIV and activity of certain drugs (especially ddN) in M/M. Certain cytokines may have clinical utility in improving bone marrow damage induced by antiviral drugs (such as AZT) or by HIV per se (Pluda et al., 1990; Miles et al., 1990; Fischl et al., 1990; Groopman et al., 1987). However, as noted above, certain cytokines may enhance HIV replication in vitro, and at least one study has suggested that this may occur in patients (Pluda et al.,

1990). However, various trials have yielded conflicting results in this area, and any potential risk for HIV replication must be balanced against the potential benefit of the cytokine. Moreover, at least one cytokine, GM-CSF, may enhance the entry and intracellular metabolism of AZT to its active triphosphate moiety (Perno et al., 1989; Dhawan et al., 1990). Such enhancement appears to be sufficient to overcome the cytokine-induced enhancement of viral replication and to induce greater inhibition of viral replication. For this reason, the combination of AZT and GM-CSF deserves further clinical studies to evaluate the potential clinical application of their therapeutic combination.

In the case of other ddNs, GM-CSF-induced increase in ddN metabolism is not sufficient to improve the anti-HIV efficacy, and either no effect or a net loss of antiviral activity was found (Perno et al., 1989). Interestingly, the antiviral activity of TIBO derivatives, a new class of compounds with potent anti-HIV activity, is not down-modulated by M-CSF and other cytokine enhancers of HIV replication. TIBO are believed to inhibit HIV-1 (but not HIV-2) reverse transcriptase through a non-competitive mechanism with dNTP pools (Debyser et al., 1991). Inhibition of HIV-1 replication takes place at nanomolar doses in both T-lymphocytes and M/M (Pauwels et al., 1990). The activity of such drugs in M/M, in conjunction with their penetration into the brain, suggests that they may have utility in the treatment of HIV-related dementia, and this issue should be explored in clinical trials of these drugs. However, with their exquisite specificity to HIV-1, it will be important that the potential for viral resistance is carefully evaluated in such patients.

Toxicity of a number of drugs is related to the selective inhibition of nucleotide metabolism or cellular DNA replication. In such a case, a weak antiviral activity cannot be detected, unless a cellular system less sensitive to toxic effect of the compound is used. This is indeed the case of adriamycin, an anticancer drug of some interest in HIV infection, because it is commonly used in AIDS patients with Kaposi sarcoma, lymphomas and other cancers. We found substantial anti-HIV activity of adriamycin in M/M at concentrations toxic for T-lymphocytes, but still in the range of those achievable in cancer patients (Bergamini et al., 1991). Its mechanism of action, still under investigation, seems to be related to an inhibition of postretrotranscriptional step(s) of viral replication, similar to that recently published for another class of compounds inhibiting DNA topoisomerase I (Priel et al., 1991). The replication of HIV utilizes the machinery of host cells, thus it is possible that a non-specific effect resulting from the cellular toxicity of ADR may play a role in viral inhibition. With this concept in mind, we performed various experiments demonstrating that anti-HIV activity of ADR takes place in M/M at concentrations non-toxic for the cells. However, our data cannot completely rule out the possibility that minor toxicity, not detected by our standard assays, can be relevant to viral replication. For these purposes, studies are now under way to evaluate whether other derivatives of the adriamycin family may display more specific anti-HIV activity and reduced toxicity.

In conclusion, our data strongly suggest the importance of verifying the activity of putative anti-HIV drugs in M/M as a step in their preclinical development. This can aid in selecting drugs with a greater chance of being effective in patients with HIV-related disease. Automatization of drug screening in M/M would be desirable, but for the moment this remains more labor-intensive than testing in T-lymphocytes. Studies are now underway to achieve this goal and make such screening available to the majority of laboratories involved in HIV research.

Acknowledgements

We gratefully acknowledge Mrs. Franca Serra, Ms. Patrizia Saccomandi and Mr. Vincenzo Serra in preparing the manuscript, and Ms. Marcella Capozzi for her excellent technical assistance. We also acknowledge Drs. David G. Johns, David A. Cooney for technical advice, Dr. Michael W. Baseler for FACS analysis, and Dr. Hiroaki Mitsuya for providing the ATH8 cell line.

This work has been supported by the Italian Istituto Superiore di Sanitá, Progetto AIDS, and by the Italian Consiglio Nazionale delle Ricerche, Progetto FATMA.

References

Anand, R., Moore, J. and Feorino, P. (1986) Rifabutine inhibits HTLV-III. Lancet 2, 97–98.

Armstrong, J.A. and Horn, P. (1984) Follicular dendritic cells and virus-like particles in AIDS-related lymphadenopathy. Lancet 2, 370–72.

Balzarini, J., Hao, Z., Herdewijn, P., Johns, D.G. and De Clercq, E. (1991a) Intracellular metabolism and mechanism of anti-retrovirus action of 9-(2-phosphonylmethoxyethyl)adenine a potent anti-human immunodeficiency virus compound. Proc. Natl. Acad. Sci. USA 88, 1499–1503.

Balzarini, J., Perno, C.F., Schols, D. and De Clercq, E. (1991b) Activity of acyclic nucleoside phosphonate analogues against human immunodeficiency virus in monocyte/macrophages and peripheral blood lymphocytes. Biol. Biophys. Res. Comm. (in press).

Bergamini, A., Perno, C.F., Balzarini, J., Capozzi, M., Caliò, R. and Rocchi, G. (1991) Adriamycin selectively inhibits HIV replication in resting macrophages. Proc. VII Int. Conf. AIDS, Florence, June 16–21, Vol. II, p. 109.

Broder, S., Yarchoan, R. and Collins, J.M. (1985) Effects of suramin on HTLV-III/LAV infection presenting as Kaposi's sarcoma or AIDS-related complex: clinical pharmacology and suppression of virus replication in vitro. Lancet 2, 627–630.

Chantal-Petit, A.J., Tersmette, M., Terpstra, F.G., de Goede, R.E.J., van Lier, R.A.W. and Miedema, F. (1988) Decreased accessory cell function by human monocytic cells after infection with HIV. J. Immunol. 140, 1485–1493.

Cooney, D.A., Dalal, M., Mitsuya, H., McMahon, J.B., Nadkarni, M., Balzarini, J., Broder, S. and Johns, D.G. (1986) Initial studies on the cellular pharmacology of 2',3'-dideoxycytidine, an inhibitor of HTLV-III infectivity. Biochem. Pharmacol. 35, 2065–2071.

Cummins, L.M., Weinhold, K.J., Matthews, T.J., Langlois, A.J., Perno, C.F., Condie, R.M. and Allain J.P. (1991) Preparation and characterization of an intravenous solution of IgG from human immunodeficiency virus-seropositive donors. Blood 77, 1111–1117.

Daar, E.S., Moudgil, T., Meyer, R.D. and Ho, D.D. (1991) Transient high levels of viremia in patients with primary human immunodeficiency virus type-1 infection. N. Engl. J. Med. 324, 961–964.

Debyser, Z., Pauwels, R., Andries, K., Desmyter, Y., Kukla, M., Janssen, P.A.J. and De Clercq, E. (1991) An antiviral target on reverse transcriptase of human immunodeficiency virus type-1 revealed by tetrahydroimidazol-[4,5,1-jk][1,4]-benzodiazepin-2(1H)-one and -thione derivatives. Proc. Natl. Acad. Sci. USA 88, 1451–1455.

Dhawan, R.K., Kharbanda, S., Nakamura, M., Ohno, T. and Kufe, D. (1990) Effect of granulocyte-macrophage colony-stimulating factor on 3′-azido-3′-deoxythymidine uptake, phosphorylation and nucleotide retention in human U-937 cells. Biochem. Pharmacol. 40, 2695–2701.

Fischl, M.A., Richman, D.D., Grieco, M.H., Gottlieb, M.S., Volberding, P.A., Laskin, O.L., Leedom, J.M., Groopman, J.E., Mildvan, D., Schooley, R.T., Jackson, G.G., Durack, D.T., King, D. and the AZT collaborative working group (1987) The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-treated complex. A double-blind placebo-controlled trial. N. Engl. J. Med. 317, 185–190.

Fischl, M.G.J.E., Levine, J.D., Groopman, J.E., Henry, D.H., Kennedy, P., Miles, S., Robbins, W., Starret, B., Zalusky, R., Abels, R.I., Tsai, H.C. and Rudnick, S.A. (1990) Recombinant human erythropoietin for patients with AIDS treated with zidovudine. N. Engl. J. Med. 322, 1488–1494.

Gabuzda, D.H. and Hirsch, M.S. (1987) Neurologic manifestation of infection with human immunodeficiency virus. Ann. Int. Med. 107, 383–389.

Gartner, S., Markovits, P., Markovitz, D.M., Kaplan, M.H., Gallo, R.C. and Popovic, N. (1986a) The role of mononuclear phagocytes in HTLV-III/LAV infection. Science 233, 215–19.

Gartner, S., Markovits, P., Markovitz, D.M., Betts, R.F. and Popovic, M. (1986b) Virus isolation from and identification of HTLV-III/LAV-producing cells in brain tissue from a patient with AIDS. J. Am. Med. Assoc. 256, 2365–2372.

Gendelman, H.E., Orenstein, J.M., Martin, M.A., Ferrua, C., Mitra, R., Phipps, T., Wahl, L.A., Lane, H.C., Fauci, A.S., Burke, D.S., Skillman, D. and Meltzer, M.S. (1988) Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. J. Exp. Med. 167, 1428–1440.

Gomatos, P.J., Stamatos, N.M., Gendelman, H.E., Fowler, A., Hoover, D.L., Kalter, D.C., Burke, D.S., Tramont, E.C. and Meltzer, M.S. (1990) Relative inefficiency of soluble recombinant CD4 for inhibition of infection by monocyte-tropic HIV in monocytes and T-cells. J. Immunol. 144, 4183–4188.

Groopman, J.E., Mitsuyasu, R.T., DeLeo, M.J., Oette, D.H. and Golde, D.W. (1987) Effect of human granulocyte-macrophage colony-stimulating factor on myelopoiesis in the acquired immunodeficiency syndrome. N. Engl. J. Med. 321, 593–598.

Hao, Z., Cooney, D.A., Hartman, N.R., Perno, C.F., Fridland, A., De Vico, A.L., Sarngadharan, M.G., Broder, S. and Johns, D.G. (1988) Factors determining the activity of 2′-3′-dideoxynucleosides in suppressing human immunodeficiency virus in vitro. Mol. Pharmacol. 34, 431–435.

Hartman, N.R., Yarchoan, R., Pluda, J.M., Thomas, R.V., Marczyk, K.S., Broder, S. and Johns, D.G. (1990) Pharmacokinetics of 2′-3′-dideoxyadenosine and 2′-3′-dideoxyinosine in patients with severe HIV infection. Clin. Pharmacol. Ther. 47, 647–654.

Ho, D.D., Rota, T.R. and Hirsch, M.S. (1986) Infection of monocytes/macrophages by human T lymphotropic virus type III. J. Clin. Invest. 77, 1712–1715.

Jasmin, C., Chermann, J.C. and Raybaud, M. (1974) In vivo inhibition of murine leukemia and sarcoma viruses by heteropolyanion 5-tungsto-2-antimoniate. J. Natl. Canc. Inst. 53, 469–474.

Klatzmann, D., Barré-Sinoussi, F., Nugeyre, M.T., Dauguet, C., Vilmer, E., Griscelli, C., Brun-Vezinet, F., Rouzioux, C., Gluckman, J.C., Chermann, J.C. and Montagnier, L. (1984) Selective tropism of lymphadenopathy-associate virus (LAV) for helper-inducer T-lymphocytes. Science 225, 59–63.

Klecker, R.W. Jr., Collins, J.M., Yarchoan, R., Thomas, R., Jenkins, J.F., Broder, S. and Myers, C.E. (1987) Plasma and cerebrospinal fluid pharmacokinetics of 3′-azido-3′-deoxythymidine: a

novel pyrimidine analog with potential application for the treatment of patients with AIDS and related diseases. Clin. Pharmacol. Ther. 41, 407–415.

Klecker, Jr. R.W., Collins, J.M., Yarchoan, R., Thomas, R., McAtee, N., Broder, S. and Myers, S. (1988) Pharmacokinetics of 2′-3′-dideoxycytidine in patients with AIDS and related disorders. J. Clin. Pharm. 28, 837–842.

Koenig, S., Gendelman, H.E., Orenstein, J.M., Dal Canto, J., Pezeshkpour, G.H., Yungbluth, M., Janotta F., Aksamit, A., Martin, M.A. and Fauci, A.S. (1986) Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science 233, 1089–1092.

Koyanagi, Y., O'Brien, W.A., Zhao, J.Q., Golde, D.W., Gasson, J.C. and Chen, I.S.Y. (1988) Cytokines alter production of HIV-1 from primary mononuclear phagocytes. Science 241, 1673–1677.

McCornick, J.B., Getchell, J.P., Mitchell, S.W. and Hicks, D.R. (1984) Ribavirin suppresses replication of lymphadenopathy-associated virus in cultures of human adult T-lymphocytes. Lancet 2, 1367–1369.

McElrath, M.J., Pruett, J.E. and Cohn, Z.A. (1989) Mononuclear phagocytes of blood and bone marrow: comparative roles as viral reservoirs in human immunodeficiency virus type 1 infection. Proc. Natl. Acad. Sci. USA 86, 675–679.

Meltzer, M.S., Nakamura, M., Hansen, B.D., Turpin, J.A., Kalter, D.C. and Gendelman, H.E. (1990) Macrophages as susceptible targets for HIV infection, persistant viral reservoir in tissue, and key immunoregolatory cells that control levels of virus replication and extent of disease. AIDS Res. Hum. Retroviruses 6, 967–975.

Miles, S.A., Mitsuyasu, R.T., Lee, K., Moreno, J., Alton, K., Egrie, J.C., Souza, L. and Glaspy, J.A. (1990) Recombinant human granulocyte colony-stimulating factor increases circulating burst forming unit-erythroid and red blood cell production in patients with severe human immunodeficiency virus infection. Blood 75, 2137–2146.

Mitsuya, H., Popovic, M. and Yarchoan, R. (1984) Suramin protection of T-cells in vitro against infectivity and cytophatic effect of HTLV-III. Science 266, 172–174.

Mitsuya, H., Weinhold, K.J., Furman, P.A., St. Clair, M.H., Nusinoff Lehrman, S., Gallo, R.C., Bolognesi, D., Barry, D.W. and Broder, S. (1985) 3′-Azido-3′-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytophatic effect of human T-lymphotropic virus type III-lymphadenopathy-associated virus in vitro. Proc. Natl. Acad. Sci. USA 82, 7096–7101.

Mitsuya, H., Looney, B.J., Kuno, S., Ueno, R., Wong-Staal, F. and Broder, S. (1988) Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to CD4+ cells. Science 240, 646–650.

Mitsuya, H., Yarchoan, R. and Broder, S. (1990) Molecular targets for AIDS therapy. Science 249, 1533–1540.

Montagnier, L., Gruest, J., Chamaret, S., Dauguet, C., Axler, C., Guetard, D., Nugeyre, M.T., Barré-Sinoussi, F., Chermann, J.C., Brunet, J.B., Klatzmann, D. and Gluckman, J.C. (1984) Adaptation of lymphadenopathy-associated virus (LAV) to replication in EBV-transformed B-lymphoblastoid cell lines. Science 225, 63–67.

Pauwels, R., Andries, K., Desmyters, J., Schols, D., Kukla, M.J., Breslin, H.J., Raeymaecks, A., Van Gelder J., Woestenborghs, R., Heykants, J., Schellekens, K., Janssen, M.A.C., De Clercq, E. and Janssen, P.A.J. (1990) Potent and selective inhibition of HIV-1 replication in vitro by a novel series of TIBO derivates. Nature 343, 470–473.

Perno, C.F., Yarchoan, R., Tosato, G., Cooney, D., Mitsuya, H. and Broder, S. (1987) III Int. Conference on AIDS, Washington DC. (USA), June 1–5, p. 165.

Perno, C.F., Yarchoan, R., Cooney, D.A., Hartman, N.R., Gartner, S., Popovic, M., Hao, Z., Gerrard, T.L., Wilson, Y.A., Johns, D.G. and Broder, S. (1988) Inhibition of human immunodeficiency virus (HIV-1/HTLV-III$_{Ba-L}$) replication in fresh and cultured human peripheral blood monocytes/macrophages by azidothymidine and related 2′,3′-dideoxynucleosides. J. Exp. Med. 168, 1111–1125.

Perno, C.F., Yarchoan, R., Cooney, D.A., Hartman, N.R., Webb, D.S.A., Hao, Z., Mitsuya, H., Johns, D.G. and Broder, S. (1989) Replication of human immunodeficiency virus in monocytes: granulocyte/macrophage colony-stimulating factor (GM-CSF) potentiates viral production yet

enhances the antiviral effect mediated by 3′-azido-2′,3′-dideoxythymidine AZT) and other dideoxynucleoside congeners of thymidine. J. Exp. Med. 169, 933–951.

Perno, C.F., Baseler, M.W., Broder, S. and Yarchoan, R. (1990a) Infection of monocytes by human immunodeficiency virus type-1 is blocked by inhibitors of CD4-gp120 binding, even in presence of enhancing antibodies. J. Exp. Med. 171, 1043–1056.

Perno, C.F., Cooney D.A., Currens, M.J., Rocchi G., Johns D.G., Broder S. and Yarchoan R. (1990b) Ability of anti-HIV agents to inhibit HIV replication in monocyte/macrophages or U937 monocytoid cells under conditions of enhancement by GM-CSF or anti-HIV antibody. AIDS Res. Hum. Retroviruses 6, 1051–1058.

Pluda, J.M., Yarchoan, R., Smith, P.D., McAtee, N., Shay, L.E., Oette, D., Maha, M., Wahl, S.M., Myers, C.E. and Broder, S. (1990) Subcutaneous recombinant granulocyte-macrophage colony stimulating factor used as single agent and in an alternative regimen with azidothymidine in leukopenic patient with severe human immunodeficiency virus infection. Blood 76, 463–471.

Popovic, M., Sangaradharan, M.G., Read, E. and Gallo, R.C. (1984) Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 224, 497–501.

Popovic, M. and Gartner, S. (1987) Isolation of HIV-1 from monocytes but not T-lymphocytes. Lancet 2, 916–917.

Priel, E., Showalter, S.D. and Blair, D.G. (1991) Inhibition of human immunodeficiency virus (HIV-1) replication by non-cytotoxic doses of camptothecin, a topoisomerase I inhibitor. Proc. VII Int. Conf. AIDS, Florence, June 16–21, Vol. II, p. 40.

Tschachler, E., Groh, V., Popovic, M., Mann, D.L., Konrad, K., Safai, B., Eron, L., Di Marzo Veronese, F., Wolff, K. and Stingl, G. (1987) Epidermal Langerhans cell: a target for HTLV-III/LAV infection. J. Invest. Dermatol. 88, 233–241.

Yarchoan, R., Klecker, R.W., Weinhold, K.J., Markham, P.D., Lyerly, H.K., Durack, D.T., Gelmann, E., Nusinoff Lehrmann, S., Blum, R.M., Barry, D.W., Shearer, G.M., Fischl, M.A., Mitsuya, H., Gallo, R.C., Collins, J.M., Bolognesi, D.P., Myers, C.E. and Broder, S. (1986) Administration of 3′-azido-3′-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. Lancet 1, 575–582.

Yarchoan, R., Berg, G., Browers, P., Fischl, M.A., Spitzer, A.R., Wichman, A., Grafman, J., Thomas, R.V., Safai, B., Brunetti, A., Perno, C.F., Schmidt, P.J., Larson, S.M., Myers, C.E. and Broder, S. (1987) Response of human immunodeficiency virus-associated neurological disease to 3′-azido-3′-deoxythymidine. Lancet 1, 132–136.

Yarchoan, R., Perno, C.F., Thomas, R.V., Klecker, R.W., Allain, J.P., Wills, R.J., McAtee, N., Fischl, M.A., Dubinsky, R., McNeely, M.C., Mitsuya; H., Pluda, J.M., Lawley, T.J., Leuther, M., Safai, B., Collins, J.M., Myers, C.E. and Broder, S. (1988) Phase I studies of 2′,3′-dideoxycytidine in severe human immunodeficiency virus infection as a single agent and alternating with zidovudine (AZT). Lancet 1, 76–81.

Yarchoan, R., Mitsuya, H., Myers, C.E. and Broder, S. (1989a) Clinical pharmacology of 3′-azido-2′,3′-dideoxythymidine (zidovudine) and related dideoxynucleosides. N. Engl. J. Med. 321, 726–738.

Yarchoan, R., Mitsuya, H., Thomas, R.V., Pluda, J.M., Hartman, N.R., Perno, C.F., Marczyk, K.S., Allain, J.P., Johns, D.G. and Broder, S. (1989b) In vivo activity against HIV and favorable toxicity profile of 2′-3′-dideoxyinosine. Science 245, 412–417.

Yarchoan, R., Pluda, J.M., Perno, C.F., Mitsuya, H., Thomas, R.V., Wyvill, K.M. and Broder, S. (1990) Initial clinical experience with dideoxynucleosides as single agents and in combination therapy. Ann. N.Y. Acad. Sci. 616, 328–343.