Antiviral Research 14 (1990) 87–98
Elsevier

AVR 00428

In vitro screening for antiretroviral agents against simian immunodeficiency virus (SIV)

Che-Chung Tsai[1], Kathryn E. Follis[1], Michele Yarnall[1], Larry E. Deaver[1], Raoul E. Benveniste[2] and Polly R. Sager[3]

[1]*Regional Primate Research Center, University of Washington, Seattle, Washington,* [2]*Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland, and* [3]*Developmental Therapeutics Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, U.S.A.*

(Received 31 January 1990; accepted 13 April 1990)

Summary

Simian immunodeficiency virus (SIV), which causes an acquired immunodeficiency syndrome in macaques, is a lentivirus that is morphologically, antigenically, genetically, and biologically similar to the human immunodeficiency virus (HIV). Because of these similarities, the SIV model represents a unique opportunity for in vitro and in vivo testing of antiretroviral agents. Since antiretroviral agents may exhibit different properties in different cells in vitro, more than one cell line may be necessary to evaluate the efficacy and modes of action of an antiretroviral agent. Initially we tested ten cell lines for their permissiveness to five SIV isolates. One B-cell line (AA-2) and one T-cell line (HuT 78) were selected to test antiretroviral agents since both were extremely permissive for SIV_{mac251}, an isolate with a high rate of infectivity. Using this optimized in vitro testing protocol, we screened ten antiretroviral agents for their ability to inhibit SIV replication. Six of the compounds completely inhibited SIV viral antigen expression. Based on the selectivity index, 3′-azido-3′-dideoxythymidine, 3′-azido-2′,3′-dideoxyuridine, and 3′-fluoro-3′-deoxythymidine appear to be the most efficacious antiretroviral agents against SIV_{mac251}. Several different assays for determining viral antigen inhibition were conducted and the results of these assays were comparable. Our results demonstrate that the SIV in vitro model is a valuable screening tool for determining the efficacy and toxicity of new antiretroviral agents.

SIV; Acquired immunodeficiency syndrome; Antiretroviral agent

Correspondence to: C-C. Tsai, University of Washington, Primate Field Station, Medical Lake, WA 99022-0536, U.S.A.

Introduction

In 1984, a simian immunodeficiency virus (SIV_{mac}) was isolated from immunosuppressed rhesus macaques at the New England Regional Primate Research Center (Daniel et al., 1985). Subsequently, several SIV strains were isolated from immunosuppressed rhesus and pigtailed macaques at other primate centers (Kornfeld et al., 1987; Benveniste et al., 1986) and from asymptomatic sooty mangabey macaques (Fultz et al., 1986; Lowenstine et al., 1986) and African green monkeys (Fukasawa et al., 1988). SIV, a nonhuman primate lentivirus, is closely related to the human immunodeficiency virus (HIV) (Kanki et al., 1985; Hirsch et al., 1986; Benveniste et al., 1988). SIV possesses a CD4+ cellular tropism and induces an immunodeficiency disease in macaques that is strikingly similar to acquired immunodeficiency syndrome (AIDS) in humans. SIV also closely parallels HIV in genomic structure and biologic properties (Kanki et al., 1985; Hirsch et al., 1986; Franchini et al., 1987; Benveniste et al., 1988). Because of these similarities and since there is no animal system to evaluate pathogenesis by HIV directly, SIV models provide an important alternative for testing potential antiretroviral agents.

Although a previous in vitro study indicates that SIV can grow in certain nonhuman primate cells (Benveniste et al., 1986), SIV has a broad tropism for many human cell lines which are also tropic to HIV (Benveniste et al., 1986; Mitsuya and Broder, 1988; Wu et al., 1988). This similarity in cellular tropism allows for examination and comparison of antiviral agents against both SIV and HIV (Schinazi et al., 1988; Mitsuya and Broder, 1988). In this paper we describe the infectivity and pathogenicity of five SIV isolates in ten human cell lines. We also describe the development and optimization of an in vitro model for screening antiretroviral agents against SIV, and the inhibitory activity of ten antiretroviral agents using this in vitro drug assay system.

Materials and Methods

SIV isolates

Five different SIV isolates were established in our laboratory. The following three isolates were obtained through the AIDS Research and Reference Reagent Program, AIDS Program, NIAID, NIH: SIV_{mac251} from Dr R. Desrosiers (Daniel et al., 1985), SIV_{mac186} from Dr R. Desrosiers (Kestler et al., 1988), and SIV_{BK28} from Dr J.I. Mullins (Kornfeld et al., 1987). The isolate SIV_{mne} (Benveniste et al., 1986) was isolated from a pigtailed macaque at the Washington Regional Primate Research Center, and $SIV_{DeltaB670}$ (Baskin et al., 1986) was obtained from Dr Murphey-Corb of the Delta Regional Primate Research Center.

Cell lines and cell cultures

The following human cell lines were obtained through the AIDS Research and

Reference Reagent Program, AIDS Program, NIAID, NIH: A3.01 (T-cell line) from Dr T. Folks (Folks et al., 1985), CEM-T4 (T-lymphoblastoid cell line) from Dr P.J. Jacobs (Foley et al., 1965), MT-2 and MT-4 (T-cell leukemia cell line) from Dr D. Richman (Harada et al., 1985), H9 (T-cell line) from Dr R. Gallo (Popovic et al., 1984), and AA-2 (splenic EBV+ B-lymphoblastoid cell line) from Dr M. Hershfield (Chafee et al., 1988). The remaining four cell lines tested were HuT 78 (cutaneous T-cell lymphoma cell line), C-8166 (transformed umbilical cord blood lymphocytes) (Salahuddin et al., 1983), Raji (Burkitt lymphoma, B-cell line), and U937 (histiocytic lymphoma) (American Type Culture Collection, Rockville, MD).

The cell lines were propagated in RPMI-1640 supplemented with 10–15% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin at 37°C in an atmosphere of 5% CO_2 in air. The AA-2 and C-8166 cell lines were supplemented with 1% nonessential amino acids and 1% sodium pyruvate.

Actively growing cell lines were subcultured (5×10^5 cells/ml) into 25 cm^2 flasks. Cells were exposed to 1 ml of filtered supernatant from SIV-infected stock cultures for 2 h. All infections were performed with stocks that had a titer of 10^5 cell culture infectious dose (CCID) per milliliter. The multiplicity of infection (MOI) was approximately 0.2 (10^5 CCID per 5×10^5 cells). The cells were washed twice with 0.01 M phosphate-buffered saline (PBS, pH 7.2), resuspended in 8 ml of culture medium, and incubated at 37°C in an atmosphere of 5% CO_2 in air. On incubation days 4, 7, 10, and 14, cultures were examined for syncytial cell formation (cytopathic effect: pathomorphologic change of cells induced by SIV) with an inverted microscope. Under 200× magnification, the number of syncytial cells from five random fields in each culture were counted and recorded. Four ml of cell suspension was also harvested from each culture and centrifuged at 1300 rpm for 15 min. These cell pellets were assayed for viral antigens by immunofluorescence (IF) and immunoblotting (IB), and the cell-free supernatants were tested for viral antigens by an antigen capture (AC) assay and for reverse transcriptase (RT) activity by an RT assay. Four ml of fresh culture medium containing noninfected cells (5×10^4 cells/ml) were added to each flask on respective test days.

Antiretroviral agents

AZT (3′-azido-3′-deoxythymidine), ddC (2′,3′-dideoxycytidine), ddA (2′,3′-dideoxyadenosine), ddI (2′,3′-dideoxyinosine), D4T (2′,3′-dideoxy-2′,3′-didehydrothymidine), FdT (3′-fluoro-3′-deoxythymidine), CAS (castanospermine), PFA (phosphonoformic acid) and IDU (5-iodo-2′-deoxyuridine) were obtained from the Developmental Therapeutics Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID). AZdU (3′-azido-2′,3′-dideoxyuridine) was obtained from Triton Biosciences Inc., Alameda, CA.

IF assay for SIV antigen expression

Viral antigen expression in cell cultures was determined by an IF assay. Cells

were pelleted and resuspended in PBS at 10^6 cells/ml. The cell suspension was smeared on a slide, air dried, and fixed in methanol-acetone (1:1). Fixed cells were reacted with macaque antibody (1:20) against SIV_{mne} and washed in PBS. Cells were then reacted with fluorescein-conjugated goat anti-monkey IgG (Organon Teknika-Cappel, Malvern, PA). The stained slides were washed in PBS, mounted in PBS-buffered glycerol, and examined with a fluorescent microscope. Fluorescing cells were indicative of the presence of SIV antigen; uninfected cells did not fluoresce. Approximately 200 to 500 cells were examined and the percentage of IF-positive cells was determined for each culture.

Viral antigen preparation, gel electrophoresis, and IB

Viral antigens were extracted from infected cell pellets (approximately 8×10^6 cells) by incubation for 30 min at 37°C with 50 μl of 1% Triton X-100, 0.5% sodium deoxycholate and 0.1% sodium dodecyl sulfate (SDS) in PBS. The soluble cell lysate containing viral antigens was obtained by centrifugation at $10\,000 \times g$ for 15 min. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of each cell lysate (30 μl) was performed on 12% gels under reducing conditions (Laemmli et al., 1970). The separated viral antigens in the cell lysates were transferred to nitrocellulose paper (Towbin et al., 1979) and probed with monkey anti-SIV serum (1:200). The nitrocellulose blots were developed with peroxidase-conjugated goat anti-monkey IgG followed by chloronaphthol plus H_2O_2. Purified SIV antigens were used as positive controls.

AC assay

Anti-SIV IgG was isolated from monkey anti-SIV_{mne} serum by affinity chromatography on protein A-sepharose (Zymed Laboratories, San Francisco, CA). The purity of the immunoglobulin preparation was monitored by SDS-PAGE stained with Coomassie blue. The purified anti-SIV IgG was labeled with biotin via a modification of the procedure described by Yolken et al. (1983). Wells of 96-well flat-bottomed microtiter plates (Costar, Cambridge, MA) were coated with 50 μl of 5 μg/ml monkey anti-SIV IgG and incubated at 4°C overnight. After the plates were washed with saline-Tween 20, 100 μl of culture supernatant (pretreated with Triton X-100 at a final concentration of 0.5% for 2 h at room temperature) were added to the wells and diluted in PBS containing 0.1% Tween 20. Following incubation and washing, biotinylated monkey anti-SIV IgG (5 μg/ml) was added and incubated at 37°C for 2 h. The plates were washed and 100 μl of horseradish peroxidase-conjugated streptavidin (1:4000) was added to each well and incubated for 15 min at room temperature. After an additional washing, 100 μl of orthophenylenediamine substrate solution was added to each well and incubated for 30 min at room temperature in the dark. The chromogenic reaction was stopped by the addition of 50 μl of 2 N H_2SO_4 and the plate was read at 492 nm with the Titertek Multiskan (Flow Laboratories, Inglewood, CA). The amount of p28 core protein per ml was calculated on the basis of a standard curve of p28.

RT assay

Cell cultures in 25 cm^2 flasks were clarified and 6 ml of culture fluid was assayed for RT activity as previously described (Benveniste et al., 1986). [^{3}H] TMP incorporation into radioactive poly (dT) was measured after a 60-min incubation at 37°C.

In vitro screening of antiretroviral agents

Routine assays for screening the antiretroviral activity of test compounds were performed in 25-cm^2 T flasks or 96-well flat-bottomed microtiter plates. Various concentrations of each antiretroviral agent were tested in duplicate in two or more cell lines and tests were repeated a minimum of three times. Cells (5×10^5) were first exposed to 1 ml of filtered SIV_{mac251} supernatant (approximately 10^5 CCID, 0.2 MOI) for 2 h. The CCID was based on the titer of SIV_{mac251} in AA-2 cells after a 7-day incubation. The cells were washed twice with PBS, and then resuspended in culture medium containing the desired concentration of antiretroviral agent. Additional medium containing the antiretroviral agent was not added during the 7-day incubation period. Positive and negative controls were present for each experiment. These controls consisted of untreated cells that were SIV-infected and noninfected, and SIV-infected cells treated with an effective dose of AZT. The final volume was 8 ml for flasks and 200 μl per well for 96-well plates.

At the end of the 7-day incubation, the cell cultures were examined for cytotoxic and cytopathic effects resulting from the drug treatment. The presence of syncytial cells was determined, the total number of cells in each culture was counted, percent viability was determined by the trypan blue exclusion method, and the 50% cytotoxic dose (CD_{50}) was established for each antiretroviral agent based on the inhibition of cell viability in drug-treated cultures compared with infected control cultures. The pellets from experimental and control cell cultures were tested via IB and IF assay for viral antigen expression. The 50% effective dose (ED_{50}) of the compounds tested was determined on the basis of the IF results. The 100% effective dose (ED_{100}), the lowest drug dose to completely inhibit SIV antigen expression, was also determined. The selectivity index (S.I.), which is the ratio of CD_{50} to ED_{50}, was calculated to show the effectiveness of each compound in cell culture. Theoretically, the greater the S.I., the more effective the compound. Culture supernatants were tested for SIV antigen via the AC assay.

Results

Permissiveness of cell lines for SIV infection

Ten human cell lines were used to examine permissiveness of SIV infectivity and to determine susceptibility to viral cytopathic effects in vitro. Permissiveness of cell lines for SIV infection was evaluated for 14 days by syncytial cell (SC) formation,

TABLE 1
Cell lines infected with SIV isolates 10–14 days after initiation of culture

Cell line	SIV_{mac186}			SIV_{mac251}			SIV_{BK28}			SIV_{mne}			SIV_{delta}		
	%IF[a]	ACA[b]	IB[c]	%IF	ACA	IB	%IF	ACA	IB	%IF	ACA	IB	%IF	ACA	IB
HuT 78	36	180	+++	34	155	+++	23	175	+++	26	49	+++	28	140	+++
H9	0	24	–	49	75	+++	0	29	+	10	48	++	13	51	++
CEM-T4	1	7	+	3	6	+/–	0	7	+	9	83	++	0	6	–
MT-4	0	9	–	0	7	–	0	6	–	0	42	–	0	6	–
A3.01	0	6	–	0	3	–	0	7	–	1	33	–	0	3	–
C-8166	28	51	+++	34	105	+++	36	244	+++	20	55	+++	53	370	+++
MT-2	0	14	–	80[d]	46[d]	++[d]	0	24	+	0	14	–	15	34	++
AA-2	42	42	+++	57	110	+++	27	98	+++	44	66	+++	58	110	+++
Raji	0	9	–	0	8	–	0	13	–	0	6	–	0	9	–
U937	0	9	–	0	11	–	0	10	–	0	12	–	0	6	–

[a]Immunofluorescence assay; the percentage of IF was based on the ratio of fluorescing to nonfluorescing cells.
[b]Antigen capture assay; the relative amount of p28 core protein (ng/ml) was calculated based on a standard curve of p28.
[c]Immunoblot assay; –, no viral antigens detected; +/–, weak reactivity detected against one viral protein (p28); +, one viral protein detected (p28); ++, two viral proteins detected (p28, gp 120, or p16); +++, three or more viral proteins detected.
[d]Results were observed 7 days after initiation of cultures. Cells were completely lysed at 10–14 days.

and by the IF, AC and IB assays. The cell lines HuT 78, C-8166, and AA-2 were highly susceptible to infection by all five SIV isolates. In addition, H9 and MT-2 cell lines were highly susceptible to SIV_{mac251} (Table 1). The remaining cell lines were either slightly permissive or nonpermissive to SIV. Syncytial cell formation was consistently observed in AA-2, C-8166, and MT-2 cells after infection with permissive SIV isolates. Syncytia did not occur in any of the other cell lines. The course of viral infection and the production of viral antigens depended on the SIV isolate tested. In four cell lines (HuT 78, H9, AA-2, MT-2) infected with SIV_{mac251}, viral antigens were detected in as few as 7 days (data not shown); however, for the other SIV isolates, peak virus production usually occurred at 10–14 days after infection. These results remained consistent from experiment to experiment.

Comparison of different assays for detection of SIV

Five different assays were compared for determining the inhibition of SIV_{mac251} by AZT in AA-2 and HuT 78 cell lines (Table 2). On day 7 of cell culture, the inhibitory effects of AZT were measured by syncytial, IF, IB, AC and RT assays. The percent inhibition was based on the ratio of the results from drug-treated cultures to untreated cultures. Both IF and IB assays detected SIV antigens in infected cells, while the AC assay detected SIV antigens and the RT assay detected RT activity in cell-free supernatants. The inhibition of SIV viral antigens in cell-free supernatant compared closely with inhibition of viral antigen expression in cell pellets. In addition, the inhibition of syncytia in AA-2 cells was consistent with the other assays.

Screening of antiretroviral agents

The ten compounds screened for antiretroviral activity against SIV_{mac251} were tested in HuT 78 and AA-2 cells. SIV_{mac251} was considered the most suitable SIV isolate for testing antiretroviral agents since SIV_{mac251} was capable of inducing infection in both cell lines after 7 days in culture. IDU was tested at concentrations of 0.28 to 28 μM, AZT was tested at 0.01 to 10 μM, and AZdU was tested at 1 to 1000 μM. The remaining compounds (ddA, ddC, ddI, D4T, FdT, PFA, CAS) were tested at concentrations of 0.1 to 100 μM. The in vitro efficacy of these ten compounds is summarized in Table 3. Based on CD_{50}, ddC was the most cytotoxic of the compounds tested in either cell line. AA-2 cells appeared to be more susceptible to the cytotoxic effects of the compounds than HuT 78 cells.

Six compounds, FdT, ddA, ddC, ddI, AZdU, and AZT, completely inhibited SIV_{mac251} viral antigen expression (100% IF inhibition, ED_{100}) in both cell lines

TABLE 2
Comparison of assays in AZT-treated cultures

Cell line	Concentration (μM)	Syncytia[a]	% IF inhibition[b]	IB[c]	% ACA inhibition[d]	% RT inhibition[e]
AA-2	10	–	100	–	100	100
	1	–	100	–	97	100
	0.1	–	100	–	93	100
	0.01	++	50	+	94	76
	0	++++	0	+++	0	0
	uninfected	–	NA	–	NA	NA
HuT78	10	–	100	+/–	100	98
	1	–	56	+	56	94
	0.1	–	0	+++	27	22
	0.01	–	0	+++	5	0
	0	–	0	+++	0	0
	uninfected	–	NA	–	NA	NA

[a]The number of syncytia in cell cultures were counted in five random fields (200×) and scored as follows: over 30 (++++), 20–30 (+++), 10–19 (++), 1–9 (+), and 0 (–).

[b]Immunofluorescence assay; the percent of fluorescing cells in infected untreated cultures was 52% in AA-2 cells and 29% in HuT 78 cells. The percentage of IF inhibition by an antiretroviral agent was based on the ratio of fluorescing cells in drug-treated cultures to fluorescing cells in infected-untreated cultures.

[c]Immunoblot assay; –, no viral antigens detected; +/–, weak reactivity detected against one viral protein; +, one viral protein detected (p28); ++, two viral proteins detected (p28, gp 120, or p16); +++, three or more viral proteins detected.

[d]Antigen capture assay; the relative amount of p28 core protein (ng/ml) was calculated based on a standard curve of p28. The amount of p28 in infected untreated culture supernatant was 123 ng/ml in AA-2 cells and 110 ng/ml in HuT 78 cells. The percent of ACA inhibition was based on the ratio of p28 in drug-treated cultures to p28 in infected-untreated cultures.

[e]Reverse transcriptase assay; the amount of RT activity in infected-untreated culture supernatants was 4.6×10^6 cpm in AA-2 cells and 1.6×10^6 cpm in HuT 78 cells. The percent of RT inhibition was based on the ratio of RT in drug-treated cultures to RT in infected-untreated cultures. Polymerase activities in uninfected cells varied from 2.8×10^3 to 3.6×10^3 cpm in this assay.

NA, not applicable.

TABLE 3
Summary of in vitro efficacy of antiretroviral agents against SIV_{mac251} in different cell lines

Cell line	Antiretroviral agent	CD_{50}[a] (μM)	ED_{50}[b] (μM)	ED_{100}[c] (μM)	Selectivity index[d]
AA-2	AZT	4.0	0.01	0.1	400
	ddA	20.0	10.0	100.0	2
	ddC	0.3	0.05	0.1	6
	ddI	65.0	2.2	100.0	30
	AZdU	>1000.0	4.4	1000.0	227
	D4T	27.0	0.1	NA	270
	FdT	>100.0	<0.1	1.0	>1000
	PFA	>100.0	33.0	NA	3
	IDU	5.0	2.8	NA	2
	CAS	>100.0	>100.0	NA	1
HuT 78	AZT	>100.0	0.8	10	125
	ddA	>100.0	13.0	100.0	8
	ddC	28.0	0.1	1.0	280
	ddI	>100.0	11.7	100.0	8
	AZdU	500.0	0.03	10.0	16667
	D4T	>100.0	11.0	NA	9
	FdT	>100.0	<0.1	10.0	>1000
	PFA	>100.0	42.0	NA	2
	IDU	>28.0	2.0	NA	14
	CAS	>100.0	>100.0	NA	1

[a]CD_{50}: 50% cytotoxic dose, based on the reduction of cell viability in drug-treated cultures compared with infected control cultures.
[b]ED_{50}: 50% antiviral effective dose, based on the inhibition of viral antigen expression in an IF assay.
[c]ED_{100}: 100% antiviral effective dose, the lowest dose of compound required to achieve complete inhibition of SIV, based on the inhibition of viral antigen expression in an IF assay.
[d]Selectivity index is the ratio of CD_{50} to ED_{50}.
NA, not applicable; complete inhibition did not occur at the highest concentration tested.

tested (Table 3). D4T did not completely inhibit SIV_{mac251} in either cell line at the concentrations tested, although the selectivity index was high. IDU, CAS, and PFA had minimal inhibitory effects in both cell lines and low selectivity indexes. The antiretroviral agents FdT, AZdU, and AZT appear to be the most efficacious compounds against SIV.

Discussion

Distinct SIV isolates have been shown to exhibit different pathogenic properties, possibly owing to specific viral factors (Zhang et al., 1988; Baskin et al., 1988). Antiretroviral agents may also exhibit different properties in different cells. Therefore, several SIV isolates and more than one cell line may be necessary to evaluate the efficacy and modes of action of a drug in vitro. In this paper we tested five SIV isolates in ten human cell lines. These SIV isolates exhibited different rates of infection in different cell lines. In addition, the time of production of viral antigens

varied with cell type. These observations are similar to results reported recently for HIV, where the rate and course of infection varied both with cell type and viral isolate (Cloyd and Moore, 1990). Although differences existed in the permissiveness of certain cell lines for the various SIV isolates, we developed a reliable in vitro drug screening system for testing new antiretroviral compounds. This drug screening system can be modified to use any isolate of SIV and any permissive B or T cell line. The standardization of in vitro antiviral testing against SIV depends heavily on the cell type employed. For our experiments we optimized the drug screening system with SIV_{mac251} in one B cell line (AA-2) and one T cell line (HuT 78).

The in vitro drug screening system is a simple, reliable, and flexible method for testing potential antiretroviral agents against SIV. Five assays that measured different parameters of SIV infection were comparable in the detection of SIV inhibition. Therefore, only one or two assays appear to be sufficient for measuring SIV inhibition in initial antiretroviral studies. By testing antiretroviral agents in two or more cell lines against different isolates of SIV, this system can accurately reflect the efficacy of compounds in vitro.

We evaluated the efficacy and toxicity of ten potential antiretroviral agents. Six of these compounds (AZT, ddA, ddC, ddI, AZdU, FdT) completely inhibited SIV replication, two compounds (D4T, PFA) were moderately inhibitory, and the remaining two compounds (IDU, CAS) were slightly inhibitory. The selectivity indexes of the ten compounds suggest that AZT, AZdU, and FdT are the most efficacious antiretroviral agents. With the exception of AZdU, all antiretroviral agents tested were more cytotoxic in the AA-2 cells than in the HuT 78 cells. Also, there was significant variability in the ED_{50} values of certain compounds with the different host cell lines. Therefore, it is necessary to test an antiretroviral agent in more than one cell line to ensure the accuracy of the results.

Antiretroviral drugs work by interacting directly with the retrovirus, with a retroviral-encoded protein, or with a cellular factor required for retroviral replication or pathogenesis. The 5′-triphosphate forms of the nucleosides tested (AZT, ddA, ddC, ddI, AZdU, D4T, FdT, and PFA) inhibit the retrovirus by interfering with the virally encoded gene product, RT (Baba et al., 1987; Cooney et al., 1986; Hamamoto et al., 1987). IDU inhibits DNA replication (Dolin, 1985). CAS targets a cellular enzyme (a-glucosidase I) that blocks late steps in the retroviral life cycle by inducing structural and functional alterations in the retroviral glycoprotein envelope (Ruprecht et al., 1989). To date, it appears that drugs which affect the retroviral enzymes and proteins have been the most effective antiretroviral treatments for AIDS (Yarchoan et al., 1989). Our data also suggest that the RT inhibitors are the most efficacious compounds against SIV in vitro.

Recent studies have shown that SIV-infected cells and monkeys are relevant models of AIDS and may be suited to the development of antiretroviral agents (Wu et al., 1988; Fultz, 1989). We have found six compounds that completely inhibit SIV in our in vitro drug screening system. The ED_{50} values of these compounds against SIV are consistent with the ED_{50} values for HIV in vitro (Mitsuya and Broder, 1986; Schinazi et al., 1988; Polsky et al., 1989). As information becomes available from clinical trials using these drugs in HIV-infected individuals, an examination

of the predictive value of the SIV model will be possible.

Acknowledgements

We thank Dr W.R. Morton and LaRene Kuller for providing monkey anti-SIV serum and Kate Elias for reviewing the manuscript. This work was supported by NIAID contract NO1-AI-62526 and NIH grant RR00166.

References

Baba, M., Pauwel, R., Herdewijn, P., De Clercq, E., Desmyter, J. and Vandeputte, M. (1987) Both 2′,3′-dideoxythymidine and its 2′,3′-unsaturated derivative (2′,3′-dideoxythymidinene) are potent and selective inhibitors of human immunodeficiency virus replication in vitro. Biochem. Biophys. Res. Commun. 142, 128–134.

Baskin, G.B., Martin, L.N., Rangan, S.R.S., Gormus, B.J., Murphey-Corb, M., Wolf, R.H. and Soike, K.F. (1986) Transmissible lymphoma and simian acquired immunodeficiency syndrome in rhesus monkeys. J. Natl. Cancer Inst. 77, 127–139.

Baskin, G.B., Murphey-Corb, M., Watson, E.A. and Martin, L.N. (1988) Necropsy findings in rhesus monkeys experimentally infected with cultured SIV/Delta. Vet. Pathol. 25, 456–457.

Benveniste, R.E., Arthur, L.O., Tsai, C-C., Sowder, R., Copeland, T.D., Henderson, L.E. and Oroszlan, S. (1986) Isolation of a lentivirus from a macaque with a lymphoma: comparison to HTLV-III/LAV and other lentiviruses. J. Virol. 60, 483–490.

Benveniste, R.E., Morton, W.R., Clark, E.A., Tsai, C-C., Ochs, H.D., Ward, J.M., Kuller, L., Knott, W.B., Hill, R.W., Gale, M.J. and Thouless, M.E. (1988) Inoculation of baboons and macaques with simian immunodeficiency virus/Mne, a primate lentivirus closely related to human immunodeficiency virus type 2. J. Virol. 62, 2091–2101.

Chafee, S., Leeds, J.M., Matthews, T.J., Weinhold, K.J., Skinner, M., Bolognesi, D.P. and Hershfield, M.S. (1988) Phenotypic variation in the response to the human immunodeficiency virus among derivatives of the CEM T and WIL-2 B cell lines. J. Exp. Med. 168, 605–621.

Cloyd, M.W. and Moore, B.E. (1990) Spectrum of biological properties of human immunodeficiency virus (HIV-1) isolates. Virol. 174, 103–116.

Cooney, D.A., Dalal, M., Mitsuya, M., 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, 2065–2068.

Daniel, M.D., Letvin, N.L., King, N.W., Kannage, M., Sehgal, P.K., Hunt, R.D., Kanki, P.J., Essex, M. and Desrosiers, R.C. (1985) Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. Science 228, 1201–1204.

Dolin, R. (1985) Antiviral chemotherapy and chemoprophylaxis. Science 227, 1296–1303.

Foley, G.E., Lazarus, H., Farber, S., Uzman, B.G., Boon, B.A. and McCarthy, R.E. (1965) Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. Cancer 18, 22–529.

Folks, T., Benn, S., Rabson, A., Theodore, T., Hoggan, M.D., Martin, M., Lightfoote, M. and Sell, K. (1985) Characterization of a continuous T-cell line susceptible to the cytopathic effects of the acquired immunodeficiency syndrome (AIDS)-associated retrovirus. Proc. Natl. Acad. Sci. USA 82, 4539–4543.

Franchini, G., Gurgo, C., Guo, H-G., Gallo, R.C., Collati, E., Fargnoli, K.A., Hall, L.F., Wang-Staal, F. and Reitz, M.S. Jr. (1987) Sequence of simian immunodeficiency virus and its relationship to the human immunodeficiency virus. Nature (London) 328, 539–542.

Fukasawa, M., Mlura, T., Haseqawa, A., Morikawa, S., Tsujimoto, H., Miki, K., Kitamura, T. and Iiayami, M. (1988) Sequence of simian immunodeficiency virus from African green monkeys, a new member of the HIV/SIV group. Nature (London) 333, 457–461.

Fultz, P. (1989) Nonhuman primates and the acquired immunodeficiency syndrome: a union of necessity. J. Med. Primatol. 18, 73–83.

Fultz, P.N., McClure, H.M., Anderson, D.C., Swenson, R., Anand, R. and Srinivasan, A. (1986) Isolation of T-lymphotrophic virus from naturally infected sooty mangabey monkeys (*Cercocebus atys*). Proc. Natl. Acad. Sci. USA 83, 5286–5290.

Hamamoto, Y., Nakashima, H., Matsui, T., Matsuda, A., Ueda, T. and Yamamoto, N. (1987) Inhibitory effect of 2′,3′-didehydro-2′,3′-dideoxynucleosides on infectivity, cytopathic effects, and replication of human immunodeficiency virus. Antimicrob. Agents Chemother. 31, 907–910.

Harada, S., Koyanagi, Y. and Yamamoto, N. (1985) Infection of HTLV-III/LAV in HTLV-I-carrying cells MT-2 and MT-4 and application in a plaque assay. Science 229, 563–566.

Hirsch, V., Riedel, N., Kornfeld, H., Kanki, P.J., Essex, M. and Mullins, J.I. (1986) Cross-reactivity to HTLV-III/LAV and molecular cloning of simian T-cell lymphotropic virus type III from African green monkeys. Proc. Natl. Acad. Sci. USA 83, 9754–9758.

Kanki, P.J., McLane, M.F., King, N.W. Jr., Letvin, N.L., Hunt, R.D., Sehgal, P., Daniel, M.D., Desrosiers, R.C. and Essex, M. (1985) Serologic identification and characterization of a macaque T-lymphotropic retrovirus closely related to HTLV-III. Science 228, 1199–1201.

Kestler III, H.W., Li, Y., Naidu, Y.M., Butler, C.V., Ochs, M.F., Jaenel, G., King, N.W., Daniel, M.D. and Desrosiers, R.C. (1988) Comparison of simian immunodeficiency virus isolates. Nature (London) 331, 619–622.

Kornfeld, H., Riedel, N., Viglianti, G.A., Hirsch, V. and Mullins, J.I. (1987) Cloning of HTLV-4 and its relation to simian and human immunodeficiency viruses. Nature (London) 326, 610–613.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227, 680–685.

Lowenstine, L.J., Pedersen, J., Higgins, K.C., Pallis, K.C., Uyeda, A., Marx, P., Lerche, N.W., Munn, R.J. and Gardner, M.B. (1986) Seroepidemiologic survey of captive Old World primates for antibodies to human and simian retroviruses and isolation of a lentivirus from sooty mangabeys (*Cercocebus atys*). Int. J. Cancer 38, 563–575.

Mitsuya, H. and Broder, S. (1986) Inhibition of the in vitro infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV III/LAV) by 2′,3′-dideoxynucleosides. Proc Natl. Acad. Sci. USA 83, 1911–1915.

Polsky, B., Baron, P.A., Gendelman, H.E., Gold, J.W.M., Chou, T.-C. and Armstrong, D. (1989) Development of an in vitro human monocyte-derived macrophage (MDM)-based system for drug screening against HIV-1. Abstract M.C.P 131. In: Abstracts of the V Int. Conf. AIDS, Montreal, Canada.

Popovic, V., Sarngadharan, M.G., Read, E. and Gallo, R.C. (1984) Detection, isolation, and continuous production for cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 224, 497–500.

Ruprecht, R.M., Mullaney, S., Andersen, J. and Bonson R. (1989) In vivo analysis of castanospermine, a candidate antiretroviral agent. J.A.I.D.S. 2, 149–157.

Salahuddin, S.Z., Markham, P.D., Wong-Staal, F., Franchini, G., Kalyanaraman, V.S. and Gallo, R.C. (1983) Restricted expression of human T-cell leukemia-lymphoma virus (HTLV) in transformed human umbilical cord blood lymphocytes. Virol. 129, 51–64.

Schinazi, R.F., Arnold, B.H. and Chu, C.K. (1988) Activity of 2′,3′-dideoxypyrimidine nucleosides against human immunodeficiency virus type II. In: Abstracts of the IV Int. Conf. AIDS, Stockholm, Sweden.

Towbin, H., Staehelin, T. and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76, 4350–4354.

Wu, J.C., Chernow, M., Boehme, R.E., Suttmann, R.T., McRoberts, M.J., Prisbe, E.J., Matthew, T.R., Marx, P.A., Chuang, R.Y. and Chen, M.S. (1988) Kinetics and inhibition of reverse transcriptase from human and simian immunodeficiency viruses. Antimicrob. Agents Chemother. 32, 1887–1890.

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

Yolken, R.H., Leister, F.J., Whitcomb, L.S. and Sanotsham, M. (1983) Enzyme immunoassays for the detection of bacterial antigens utilizing biotin-labeled antibody and peroxidase biotin-avidin complex. J. Immunol. Methods 56, 319–327.

Zhang, J., Martin, L.M., Watson, E.A., Montelaro, R.C., West, M., Epstein, L. and Murphey-Corb, M. (1988) Simian immunodeficiency virus/delta-induced immunodeficiency disease in rhesus monkeys: relation of antibody response and antigenimia. J. Infect. Dis. 158, 1277–1286.