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Antiviral Research 28 (1995) 265–279

Antiviral Research

In vivo drug screening applications of HIV-infected cells cultivated within hollow fibers in two physiologic compartments of mice

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Received 20 April 1995; accepted 6 July 1995

Abstract

Previous studies demonstrated that human cell lines can be cultivated in hollow fibers in the subcutaneous and intraperitoneal compartments of mice. We have extended the range of cell lines to include cells infected with the human immunodeficiency virus (HIV). Furthermore, these HIV-infected cells have been shown to replicate in the hollow fibers located in both physiologic compartments (intraperitoneal and subcutaneous) of SCID mice. Treatment of the host mice with antiviral agents can suppress virus replication in these hollow fiber cultures. The potential use of this system for early in vivo screening of anti-HIV compounds is discussed.

Keywords: Animal model; Human immunodeficiency virus (HIV); Hollow fiber

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SSDI 0166-3542(95)00055-0

1. Introduction

Human immunodeficiency virus (HIV) infection is an ever-growing health crisis, the treatment of which is dependent upon therapeutic agents effective against the infectious processes as well as the immunologic dysfunctions associated with the disease. The development of new antiviral therapies depends, at least in part, upon demonstration of in vivo efficacy in one or more appropriate models. The studies described here were initiated to develop a low cost, high throughput, in vivo screen for preliminary anti-HIV efficacy evaluations which could serve to direct the best lead compounds into additional animal model testing. This approach combines features of the previously described rodent models (e.g., McCune et al., 1990; Wetherall, 1990; Mosier et al., 1991) with the capacity of human cells to grow in mice in biocompatible hollow fibers (Hollingshead et al., 1995).

Development of animal models to evaluate antiretroviral therapies has been actively pursued since the discovery of HIV and its pathogenesis in humans. There are distinct advantages associated with evaluating chemotherapeutics in small laboratory rodents including cost, housing space requirements, the quantities of compound needed for testing and the number of animals required per experiment. Since HIV does not produce infection and disease in rodents (Canivet et al., 1990; Chesebro et al., 1990), a variety of systems have been described using severe combined immune deficient (SCID), triple deficient (bg/xid/nu; NIH-3) or athymic (nu/nu) mice. With the exception of transgenic mice (Leonard et al., 1988), these models depend upon the growth of human cell xenografts which can support HIV replication. The human xenografts can consist of fetal tissue (Namikawa et al., 1988; McCune et al., 1990), peripheral blood leukocytes (Mosier et al., 1989, 1991; Ussery et al., 1994), bone marrow cells (Kamel-Reid and Dick, 1988), tumor cell lines (Wetherall, 1990), or chronically infected lymphoid and monocytoid cell lines (Puddu et al., 1991). For these types of studies, the xenografts can be implanted intraperitoneally, intravenously, subcutaneously or under the renal capsule. While these models are useful for evaluating antiviral and immunomodulating therapies, they are often costly, time consuming, technically complex and, in some cases, require specialized equipment (e.g., PCR, flow cytometry) and materials (e.g., fetal tissues) that are not readily available to all laboratories.

We previously described a method for in vivo cultivation of human tumor cells in hollow fibers which led to the development of an in vivo assay system for evaluating compounds with possible antitumor activity (Hollingshead et al., 1995). We have now expanded this hollow fiber assay to include HIV-infected human cell lines cultivated in SCID mice. The results demonstrate that HIV infection can be established, monitored and treated in hollow fiber cultures of human cells cultivated in two physiologic compartments of SCID mice. The methodology allows complete recovery of the implanted cells making it possible to evaluate test agent cytotoxicity as well as antiviral activity. The methods and the results obtained with clinically relevant agents are described here.

2. Materials and methods

2.1. Cell culture

CEM-SS human lymphoid $CD4^+$ cells (NIH AIDS Research and Reference Reagent Program, Bethesda, MD) were cultivated in continuous passage as suspension cultures in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) containing 2 mM glutamine (Life Technologies, Inc., Grand Island, NY), 10 μg/ml gentamicin, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% fetal bovine serum (FBS) (HyClone Laboratories, Inc., Logan, UT). Cells were maintained in log phase growth by serial subculture when the cell density reached 1×10^6 cells/ml of culture medium.

2.2. Virus stocks

Virus stocks were prepared as described previously (Buckheit and Swanstrom, 1991). Briefly, CEM-SS cells were acutely infected with HTLV-IIIb (NIH AIDS Research and Reference Reagent Program, Bethesda, MD) and infectious supernatants were collected at 4, 5, 6 and 7 days postinfection. The infectivity titers were determined by titration of the supernatants on CEM-SS cells in 96-well tissue culture plates with reverse transcriptase activity measured in the well supernatants on day 6. The tissue culture infectious dose was calculated by the method of Reed and Muench (1938).

2.3. Hollow fiber conditioning

Polyvinylidene fluoride hollow fibers (Spectrum Medical Corp., Houston, TX) with a molecular weight cut-off of 500,000 and an internal diameter of 1 mm were conditioned to optimize cell growth as described previously (Hollingshead et al., 1995). The conditioned fibers were kept at 37°C in 5% CO_2 until use.

2.4. Fiber sample preparation

The conditioned hollow fibers were filled with the cell inoculum (uninfected, acutely or chronically infected cells) on a sterile field within a biological safety cabinet using a 20-gauge teflon catheter in the previously described methodology (Hollingshead et al., 1995). The fibers were heat sealed at 2 cm intervals with hot needleholders (270–300°F) and the samples were separated at the center of the heat seal. The fiber samples were kept in medium at 4°C until implanted into mice. Each 2-cm sample contained approximately 20 μl of inoculum.

2.5. Stable endpoint MTT viability assay

The 'stable-endpoint' MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma Chemical Co., St. Louis, MO) assay was conducted as described by Alley et al. (1991) using the modifications of Hollingshead et al. (1995). The optical

density of the MTT formazan was read at 570 nm in a microplate reader following extraction into dimethylsulfoxide (DMSO).

2.6. Sample lysis

Fibers for reverse transcriptase (RT) assay and p24 antigen quantitation were placed into 1.5 ml microtest tubes and stored at −20°C until ready to assay, allowing all samples collected at multiple experimental time points to be assayed simultaneously. To assay the fibers, 250 μl of lysing buffer (0.02 M Tris-HCl (pH 7.5), 0.05 M NaCl, 0.5% Triton X-100) were added to each tube, the fibers were cut in half and incubated in the lysing buffer at 37°C for 30 min. After incubation the samples were vortexed and 150 μl of the lysate was transferred to 96-well round-bottom plates to serve as the sample source for the RT and p24 antigen assays.

2.7. Reverse transcriptase assays

Complete RT reaction mixture was prepared on the day of assay by mixing 1 part [methyl-1′-2′-^{3}H] dTTP (100 Ci/mM) (2.5 mCi/ml specific activity in 1.5 M Tris buffer; NEN, Wilmington, DE), 2.5 parts rAdT (0.5 mg/ml poly rA and 1.6 U/ml oligo dT) (LKB Biotech., Piscataway, NJ), 2.5 parts 5 × reaction mix (125 μl 1 M EGTA, 125 μl dH_2O, 125 μl 20% Triton X-100, 50 μl 1 M Tris (pH 7.4), 50 μl 1 M DTT, 40 μl 1 M $MgCl_2$) and 4 parts water. Ten μl of the complete RT reaction mixture was placed into each well of a 96-well round-bottom microtiter plate and 15 μl of the fiber lysate was added to the test wells and mixed. The samples were incubated for 1 h at 37°C. Each sample (25 μl) was spotted onto DE81 chromatography paper (Whatman Intl., Maidstone, UK). The filters were washed sequentially in 5% sodium phosphate buffer, distilled water and 95% ethanol and then placed into plastic scintillation vials to dry. Each vial received 3 ml of Opti-Fluor O (Packard Instrument Co., Meriden, CT) and the radioactivity was quantitated in a liquid scintillation counter (Packard Tri Carb 1900 TR, Packard Instrument Co., Meriden, CT). Results are given as counts per minute (cpm) per 25-μl spot which contains 15 μl of the fiber lysate.

2.8. p24 Antigen assay

Antigen quantitation assay kits were obtained commercially (Coulter Corp., Hialeah, FL) and conducted according to the manufacturer's instructions.

2.9. Animal husbandry

SCID mice (SCID/NCr) were obtained from the NCI Animal Production Facility at NCI-FCRDC (Frederick, MD). Mice were housed in filter top polycarbonate cages (Allentown Caging Co., Allentown, NJ) with sterilized food and hyperchlorinated water provided ad libitum. All animal studies were conducted in AAALAC approved animal research facilities in compliance with the USPHS Policy for the Humane Care and Use of Laboratory Animals. Biosafety level 3 precautions were observed.

2.10. In vivo implantation

Hollow fiber samples were implanted into mice anesthetized by methoxyflurane (Pitman-Moore, Inc., Mundelein, IL) inhalation as described previously (Hollingshead et al., 1995). The subcutaneous (s.c.) fibers were implanted via a tumor implant trocar (Popper and Sons, Inc., New Hyde Park, NY) through a small incision in the dorsal cervical skin. The hollow fibers for intraperitoneal (i.p.) implantation were introduced into the peritoneal cavity through a small incision in the dorsolateral skin and abdominal wall. Each mouse received 3 s.c. fibers and 3 i.p. thus providing 6 experimental data points.

2.11. Drug treatment

Mice receiving drug treatments were dosed by the i.p., s.c. or oral routes (p.o.) at the doses and schedules described in the text. Generally, the drugs were administered in two daily doses in an effort to maintain adequate blood levels.

3. Results

In vitro studies demonstrated that HIV-infected CEM-SS cells could be successfully grown in PVDF hollow fibers while maintaining active virus replication. It was possible to monitor cell viability via MTT dye conversion assay and to measure p24 antigen and RT activity in fiber sample lysates (data not shown). To determine the capacity of an antiviral agent to diffuse into the fiber and alter virus replication, CEM-SS cells (5×10^6 cells/ml) were acutely infected with HTLV-IIIb (2.2×10^3 $TCID_{50}$/ml). Fiber samples (infected and uninfected) were exposed to 2′,3′-dideoxycytidine (NSC 606170; ddC) at concentrations of 0.01, 0.1, 1.0 and 10 μM. Samples were assayed for RT activity and for viable cell mass. The results (Table 1) indicated that an antiviral agent could alter the infectious process occurring in hollow fiber cultures and the effective concentration range was comparable to results obtained with cells in standard 96-well plate cultures (data not shown).

Assays similar to that described for ddC were conducted to evaluate a non-nucleoside RT inhibitor (NSC 629243; UC38; benzoic acid, 2-chloro-5-[[(1-methyl-ethoxy) thioxomethyl] amino]-,1-methylethyl ester) (McMahon et al., 1995) and an attachment/fusion inhibitor (NSC 631567; Chicago Sky Blue 60) (Clanton et al., 1992) as well as 3′-azido-3′-deoxythymidine (NSC 602670; AZT). All 3 agents suppressed virus production as measured by RT activity and by viable cell mass at concentrations equivalent to those having activity in standard 96-well plate assays (data not shown).

The capacity of AZT as well as a proprietary protease inhibitor (NSC 654021) to inhibit virus replication following a delay in drug exposure was evaluated. For these studies, CEM-SS cells (1×10^7 cells/ml) were acutely infected with HTLV-IIIb (2.2×10^3 $TCID_{50}$/ml) and loaded into PVDF hollow fibers. Representative samples were exposed to serial 10-fold dilutions of the test agent either immediately or following a 24-h incubation at 37°C in a 5% CO_2 in air atmosphere. Samples were assayed for RT

Table 1
Evaluation of the effect of ddC on the replication of HIV in CEM-SS cells cultivated in hollow fibers in vitro

Fiber contents	MTT (OD_{570})		RT activity (cpm)	
	Day 0	Day 6	Day 0	Day 6
Assay control samples				
5×10^6 cells/ml	0.421	> 2.773 [a]	285	369
5×10^6 cells/ml/HIV	ND	1.739	ND	98,015
HIV-infected CEM-SS cells				
10 mM ddC	ND	2.188 [b]	ND	369 [c]
1.0 mM ddC	ND	2.407 [d]	ND	295 [e]
0.1 mM ddC	ND	2.613 [f]	ND	10,960 [g]
0.01 mM ddC	ND	1.649 [h]	ND	135,745 [i]

[a] One sample read offscale resulting in the calculation of a greater than value.
[b] Uninfected sample average = 2.734.
[c] Uninfected sample average = 640 cpm.
[d] Uninfected sample average > 2.649.
[e] Uninfected sample average = 366 cpm.
[f] Uninfected sample average > 2.339.
[g] Uninfected sample average = 258 cpm.
[h] Uninfected sample average = 2.268.
[i] uninfected sample average = 339 cpm

activity and viable cell mass on day 7. Both compounds were able to inhibit virus replication even when treatment was delayed for 24 h postinfection, thereby indicating that multiple rounds of infection were occurring in the hollow fiber culture (data not shown). The shift in RT activity relative to drug concentration following a 24-h treatment delay was more noticeable with AZT than with the protease inhibitor NSC 654021; however, the 50% inhibitory concentration remained in the range of 0.01–0.1 μM.

The morphology of uninfected and HTLV-IIIb-infected (m.o.i. = 0.0001) CEM-SS cells (1×10^7 cells/ml) was assessed by transferring samples to 10% buffered formalin at 2-day growth intervals. The samples were paraffin-embedded, sectioned at 5 μm and stained with hematoxylin and eosin. As seen in Fig. 1, the virus infected cells (Fig. 1a) developed syncytia and underwent massive cell lysis by day 8 while the uninfected cells formed a growing cell mass (Fig. 1b) with little evidence of non-viable cells being present.

To assess in vivo growth, a cell density of 1×10^7 CEM-SS cells/ml was selected based on previous experience with CEM-SS cells grown in hollow fibers in vivo (Hollingshead et al., 1995). Hollow fiber samples were prepared for uninfected CEM-SS cells and for HTLV-IIIb infected cells (m.o.i. = 0.0001). Groups of mice (10 mice/group) received: (1) samples containing HTLV-IIIb infected cells; (2) samples containing uninfected CEM-SS cells; or (3) samples containing medium only (blank controls). One group of mice bearing infected samples received 50 mg ddC/kg i.p. twice daily while the second group of mice received 1 mg AZT/ml of drinking water. Treatments (ddC or AZT) were discontinued the evening preceding sample collection. The third group of mice bearing infected samples as well as the groups of mice with uninfected

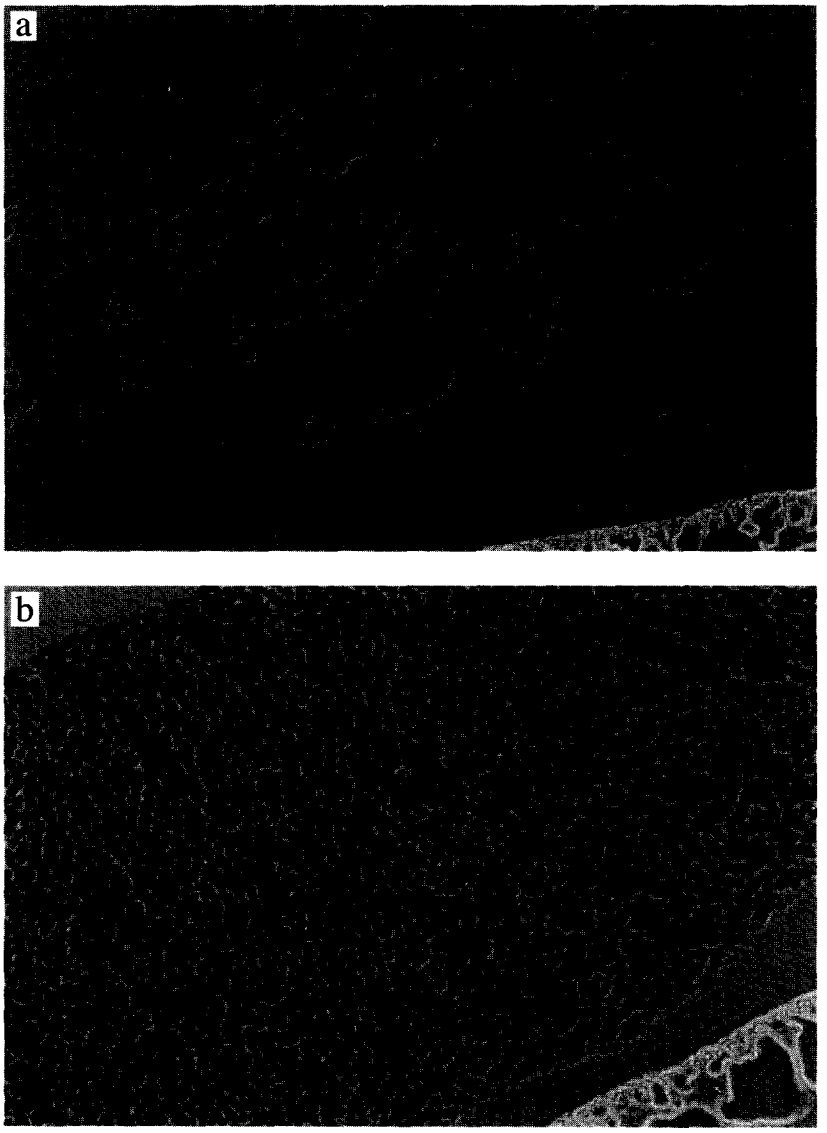

Fig. 1. Histology of CEM-SS cell hollow fiber cultures. a: histologic appearance of CEM-SS cells growing in PVDF hollow fibers in vitro is shown for day 8 cultures of HIV-infected CEM-SS cells in a. b: section of hollow fiber containing uninfected CEM-SS cells cultured in parallel to the virus infected culture shown in a. Note the dense solid growth of uninfected cells in b compared to the severe cytopathicity present in the HIV-infected cell sample where syncytia and pyknotic cells are evident. The wall of the hollow fiber is evident in the lower right portion of a and b.

cells and with blank fibers served as untreated controls. The samples were collected from half of the mice on day 9 with the remainder collected on day 15. In vitro controls were prepared for viable cell mass and for RT activity on days 0, 9 and 15. The results

Table 2
Effects of AZT treatment on day 12 samples of HIV-infected cells cultivated in hollow fibers in vivo

	Saline	AZT			Saline	AZT		
		600 mg/kg [a]	300 mg/kg	150 mg/kg		600 mg/kg	300 mg/kg	150 mg/kg
	i.p. uninfected CEM-SS cells RT activity (cpm)				*i.p. HIV / CEM-SS cells RT activity (cpm)*			
Average	550	662	797	799	76,308	26,707	29,799	41,455
S.D. [b]	204	UE [c]	4	UE	50,820	31,765	48,603	57,228
t-test	NA [d]	ND [e]	ND	ND	NA	0.268	0.207	0.338
	s.c. uninfected CEM-SS cells RT activity (cpm)				*s.c. HIV / CEM-SS cells RT activity (cpm)*			
Average	617	457	453	494	176,115	37,803	137,913	106,137
S.D.	121	UE	84	UE	98,109	52,684	48,151	72,336
t-test	NA	ND	ND	ND	NA	0.128	0.503	0.235
	i.p. uninfected CEM-SS cell viability (MTT OD_{570})				*i.p. HIV / CEM-SS cell viability (MTT OD_{570})*			
Average	1.968	2.418	2.707	2.778	0.874	1.186	1.045	0.921
S.D.	0.869	0.824	0.435	0.048	0.332	0.353	0.454	0.681
t-test	NA	ND	ND	ND	NA	0.145	0.370	0.849
	s.c. uninfected CEM-SS cell viability (MTT OD_{570})				*s.c. HIV / CEM-SS cell viability (MTT OD_{570})*			
Average	2.919	2.681	2.356	2.972	0.951	2.298	1.489	1.169
S.D.	0.169	0.361	0.386	0.040	0.359	1.122	0.705	0.517
t-test	NA	ND	ND	ND	NA	0.004	0.052	0.297

[a] AZT administered by oral gavage at the doses listed on a twice daily on days 0–11 with sample collection occurring on day 12.
[b] S.D., standard deviation of the average for each group.
[c] UE, unevaluable, only a single sample was used for these background measurements.
[d] ND, not done.
[e] NA, not applicable.

indicated that HTLV-IIIb could replicate in CEM-SS cells in vivo in the i.p. and s.c. hollow fibers (data not shown). This was measured as increases in RT activity and decreases in viable cell mass compared to uninfected cell controls. In addition, there was evidence of antiviral activity in the mice receiving ddC, and to a lesser extent, in mice receiving AZT treatment as well. These results indicated the feasibility of this approach for evaluating antiviral activity as a preliminary in vivo assay system. The ddC was more active against the i.p. fiber cultures than the s.c. fiber cultures which was expected since ddC treatment was administered i.p. taking advantage of the generally greater sensitivity of a 'same-site' model (unpublished observations, DTP, DCT, NCI). The ddC treatments protected against decreased cell viability and prevented increases in RT activity while AZT was only effective against decreases in cell viability.

The failure of AZT to markedly suppress RT activity while appearing to improve cell viability, particularly in the i.p. samples, led to an evaluation of AZT by oral gavage at doses of 600, 300, and 150 mg/kg in mice bearing infected or uninfected hollow fiber samples of CEM-SS cells. The AZT was administered twice daily starting immediately after fiber implantation (within 15 min) and continued through day 11 with sample collection on day 12. As seen in Table 2, AZT decreased RT activity at all dose levels with an accompanying increase in cell viability particularly for the s.c. fiber samples.

Table 3
Effects of ddC treatment on day 12 samples of HIV-infected cells cultivated in hollow fibers in vivo

	Saline	ddC			Saline	ddC		
		500 mg/kg [a]	250 mg/kg	125 mg/kg		500 mg/kg	250 mg/kg	125 mg/kg
	i.p. uninfected CEM-SS cells RT activity (cpm)				*i.p. HIV / CEM-SS cells RT activity (cpm)*			
Average	424	305	328	260	13,478	487	223	343
S.D. [b]	76	41	10	16	25,668	409	36	242
t-test	NA [d]	ND [e]	ND	ND	NA	0.546	0.342	0.282
	s.c. uninfected CEM-SS cells RT activity (cpm)				*s.c. HIV / CEM-SS cells RT activity (cpm)*			
Average	564	205	280	220	44,460	360	215	221
S.D.	428	UE	187	UE	32,805	214	53	32
t-test	NA	ND	ND	ND	NA	0.008	0.009	0.009
	i.p. uninfected CEM-SS cell viability (MTT OD_{570})				*i.p. HIV / CEM-SS cell viability (MTT OD_{570})*			
Average	2.378	2.088	1.913	2.932	1.292	0.972	1.582	1.370
S.D.	0.743	0.678	0.591	0.130	0.652	0.520	0.742	0.777
t-test	NA	ND	ND	ND	NA	0.250	0.398	0.825
	s.c. uninfected CEM-SS cell viability (MTT OD_{570})				*s.c. HIV / CEM-SS cell viability (MTT OD_{570})*			
Average	2.208	1.957	2.094	2.235	0.732	0.894	1.530	1.426
S.D.	0.663	0.624	0.785	0.537	0.523	0.322	0.576	0.640
t-test	NA	ND	ND	ND	NA	0.450	0.014	0.048

[a] ddC was administered twice daily by oral gavage at the doses listed on days 0–11 with sample collection occurring on day 12.
[b] S.D., standard deviation of the average for each group.
[c] UE, unevaluable, only a single sample was used for these background measurements.
[d] NA, not applicable.
[e] ND, not done.

However, the effect was not statistically significant except for the improved cell viability that occurred in the s.c. fibers of the mice receiving the high test dose ($P = 0.004$). The question of whether an alternate treatment schedule would improve efficacy remains open since AZT treatments were given only twice daily while the preferred schedule is 4–6 times daily.

Dideoxycytidine (ddC) was evaluated by s.c. administration at doses of 500, 250 and 125 mg/kg to mice bearing hollow fiber cultures of uninfected or HTLV-IIIb infected CEM-SS cells. ddC was administered twice daily starting immediately after fiber implantation (within 5 min) and continuing through day 11 with sample collection on day 12. As shown (Table 3), ddC markedly decreased RT activity at all dose levels. This effect was statistically significant ($P < 0.01$) for the s.c. fiber samples at all dose levels tested. The i.p. fiber samples also were reduced to background levels for all doses of ddC; however, these results did not achieve statistical significance because of variability in the RT activity present in the infected control samples. There was an increase in cell viability at the intermediate and low test doses, particularly for the s.c. fiber samples. Samples from the high-dose ddC group had decreased cell viabilities compared to the untreated CEM-SS cell controls, which presumably reflects cytotoxic effects associated with ddC treatment. This was also noted in the infected cell samples from the high-dose

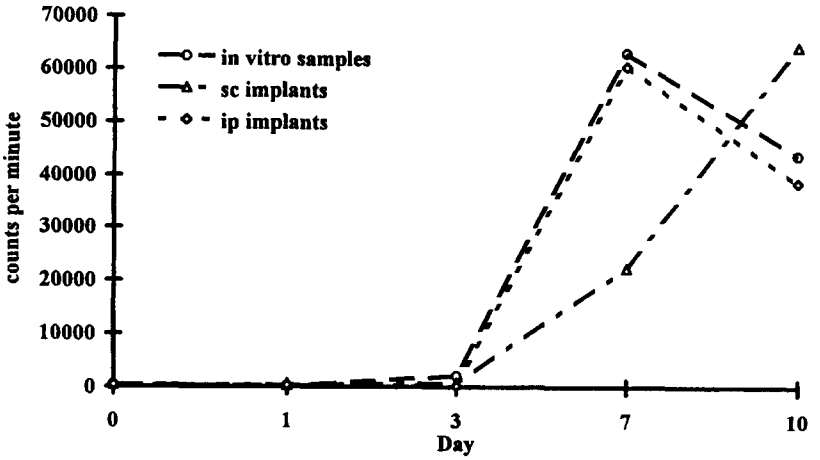

Fig. 2. Average reverse transcriptase activity in hollow fiber cultures of HIV-infected CEM-SS cells: 3–8 replicate hollow fiber cultures were collected from each site shown in the key and the fibers were assayed for RT activity as described in the text using a total hollow fiber lysate as the enzyme activity source.

ddC group where cytotoxicity may have been a contributing factor since samples from the lower dose ddC groups had increased cell viability compared to the controls.

A study was conducted to determine whether male or female mice were superior hosts for the CEM-SS cells cultivated in hollow fibers. Except for a somewhat greater viable uninfected CEM-SS cell mass in the s.c. fibers from male versus female mice, no notable differences existed between the samples from the male and the female mice (data not shown).

The experiments conducted to this time relied upon an experimental observation period of 10–12 days, so a small study was conducted to determine whether a shorter experimental period was feasible. For this, groups of mice received hollow fiber cultures of uninfected or HTLV-IIIb infected (m.o.i. = 0.0001) CEM-SS cells. Representative mice from each group were sacrificed on days 1, 3, 7 and 10 postimplantation for sample collection. Blood samples were collected from the mice sacrificed on days 1, 3 and 7. The hollow fibers were assayed for RT activity and the peripheral blood samples were assayed for p24 antigen concentration. As shown (Fig. 2), the RT activity in fibers containing HIV-infected CEM-SS cells were near baseline through day 3. At days 7 and 10, RT activity was markedly increased compared to the controls for all 3 groups of infected samples. Serum p24 antigen was undetectable in any of the blood samples from mice bearing uninfected samples. Serum p24 antigen was not detectable in mice bearing infected cell samples at days 1 and 3; however, 3 of 3 mice were positive for serum p24 at day 7 postimplantation. The mean serum p24 concentration in these 3 mice was 204 ± 32 pg/ml.

An in vivo evaluation was conducted to confirm the activity of ddC and to extend the evaluation to the closely related RT inhibitor, 2′,3′-dideoxyinosine (NSC 612049; ddI). For this, hollow fiber samples of uninfected and of HTLV-IIIb (m.o.i. = 0.0001) CEM-SS cells were prepared in parallel. Treatment groups included: (1) saline (vehicle)

controls; (2) 300 mg ddI/kg/injection; and (3) 125 mg ddC/kg/injection. All treatments were administered twice daily by the subcutaneous route on days 0–9 with samples collected on day 10. Blood was collected on day 7 for determination of the serum p24 antigen concentration. The serum p24 antigen concentration of the vehicle control mice at day 7 was 165 ± 47 pg/ml ($n = 6$). No p24 antigen was detected in the serum of ddI- or ddC-treated mice ($n = 4$ mice/drug). No p24 antigen was detected in the serum of any uninfected control mice. The average RT activity in i.p. hollow fiber samples collected from ddI-treated (7111 cpm) and ddC-treated (426 cpm) mice were significantly reduced ($P \leq 0.001$) compared to the vehicle control group that had an average RT activity of 47,855 cpm. The s.c. fibers from ddI- and ddC-treated mice were markedly reduced compared to the control samples; however, statistical significance was not achieved. The control s.c. samples had an average RT activity of 10,964 cpm ($n = 8$) while the ddI and ddC had average RT activities of 2541 ($n = 6$) and 1413 ($n = 6$), respectively.

4. Discussion

The need for a simple, expedient, sensitive in vivo efficacy assay to evaluate new antiviral agents cannot be overstated. The capacity of in vitro assays to screen and identify new lead compounds presently exceeds the capability of most systems to evaluate these agents. These limits are due, in part, to the time and expense required to conduct in vivo assays as well as the time, expense and difficulty of obtaining test compounds in quantities sufficient for in vivo evaluations. While in vitro assays can be conducted with a few milligrams of test agent, the in vivo evaluation of compounds generally requires hundreds of milligrams of compound. This often results in drug development delays due to the time and costs required to synthesize test agents in quantities adequate for full in vivo evaluation. A rodent-based model for preliminary efficacy evaluations that requires a small quantity of compound would allow the current drug development resources to be invested in those compounds which pose the greatest probability of having demonstrable in vivo activity. The system described here has the potential to identify those agents selected from in vitro antiviral assays which meet the minimum pharmacodynamic requirements for activity. Since multiple hollow fiber cultures can be implanted into two physiologic compartments (i.p. and s.c.) in each mouse, the number of samples obtained from each experiment is maximized.

We have demonstrated that CEM-SS cells are capable of in vitro and in vivo replication within PVDF hollow fibers and that this growth can be accompanied by replication of HIV. This indicates that the hollow fiber material is compatible with the cells and with virus replication. Furthermore, virus replication in cells within hollow fibers can be suppressed by known anti-HIV agents. In vitro activity by compounds of varying mechanisms of action, including nucleoside and non-nucleoside RT inhibitors as well as a protease inhibitor and an attachment/fusion inhibitor, has demonstrated the capacity of various chemical agents to impact the hollow fiber cultures. Replication of HIV in CEM-SS cells in hollow fibers implanted in the s.c. and the i.p. compartments

has been demonstrated using cell viability, RT activity and serum p24 antigen concentration as markers of virus replication. The virus replication/cytopathicity occurring in both physiologic compartments (i.p. and s.c.) can be suppressed by treating the host mice with effective antiviral agents (e.g., ddI, ddC, AZT).

Hollow fibers composed of PVDF were selected after evaluation of fibers composed of various other materials with molecular weight cut-offs ranging from 2000 to greater than 1,000,000 Da and internal diameters of 0.1 –1.0 mm (Hollingshead et al., 1995). The molecular weight cut-off of the selected hollow fiber material is large (500,000 Da), so the risk of missing an active agent because it is excluded from the fiber is very low. Furthermore, this high molecular weight cut-off does not restrict interaction of protein-bound compounds with the target cells which may be important when testing agents that are bound by serum albumins. Additionally, the high molecular weight cut-off allows one to evaluate immunoglobulin-directed therapies since IgG can cross the fiber membrane. To date, in vitro/in vivo studies with in excess of 350 anticancer agents has not identified a problem with drug distribution to the fiber cultures (Hollingshead et al., 1995). In addition to the previously described endpoints, cells can be monitored by histopathologic evaluations of the hollow fiber samples or by expelling the cells from the fiber samples (Casciari et al., 1994) for visual observation or for quantitation of infectious virus.

Since the hollow fiber material provides a physical barrier between the host animal and the target cells, the reason for selection of immunocompromised mice as hosts requires comment. We have demonstrated that human cells can be cultivated in these fibers in immunocompetent mice as well as a variety of other species (data not shown). Selection of the SCID mouse as host was based upon several factors. First, we have found that drug-induced toxicity is dependent upon strain as well as species of experimental animal. Therefore, we have elected to maintain strain continuity between the mice used in this preliminary screening assay and the mice used in subsequent evaluations (e.g., McCune et al., 1990; Mosier et al., 1991). Additionally, evaluation of alternate strains of mice has demonstrated superior growth of human hematopoietic cells in SCID mice compared to other strains, including athymic (nu/nu) mice. Whether this is due to an absence of an undefined activity in SCID mice or due to the presence of elevated cytokine/lymphokine levels remains unknown.

This methodology allows HIV-infected human cells to grow in two distinct and separate physiologic compartments (i.p. and s.c.) which permits evaluation of drug distribution and delivery to multiple tissue sites. Because of the difficulty often associated with formulating compounds early in their development, the identification of appropriate compound dose, route and schedule can prove formidable. The ability to administer a test agent at one site (e.g., i.p. injection) and observe its effect on the same site (i.p.) and a distant site (s.c.) aids in determining the effects of distribution and metabolism on a compound without requiring development of an accurate quantitative pharmacologic assay for the test agent. Information gained from the preliminary evaluation of a test agent in this system may aid in making optimal dose, route and schedule selections for use in additional in vivo evaluations.

Because of the ease of sample preparation and the technical simplicity of sample implantation, the size of a given experiment can be limited by the desired size and

number of test groups rather than by the feasibility of conducting an experiment of significant size. A skilled operator can prepare in excess of 500 hollow fiber cultures in several hours. If a 6 fiber/mouse approach is adopted, then experiments consisting of 80–100 mice are routinely feasible. Thus, the limitation on testing becomes an issue of the technical difficulty associated with dosing hundreds of mice a day rather than an issue of how many mice can be prepared with HIV-infected cell cultures. This is of particular significance when drug combination testing is desired since combination tests require multiple drug exposure regimens for each test agent, both singly and in combination. The hollow fiber methodology described here will allow for multiple treatment permutations in a single assay. Another feature of this methodology is the capacity to assess anticellular toxicity of the test agent. Since the hollow fiber culture contains human cells as well as the virus, it is possible to determine compound-related toxicity to the human cells, a feature missing from several of the previously described rodent-based HIV models.

While this assay system does not reproduce the complex intercellular reactions occurring during HIV infections, it does allow evaluation of the pharmacology of the test agent as demonstrated with the clinically approved agents. Compounds that pass this pharmacologic 'pre-screen' can then be evaluated more extensively in other models of HIV infection including the xenograft-based SCID mouse systems as well as the primate models. By selecting only the pharmacologically active agents for additional testing, the resources required for compound resynthesis as well as in vivo testing can be minimized. This system offers several advantages over other xenograft-based mouse models including the time required to evaluate a compound, the number of mice required for preliminary evaluation, the potential for improved safety by sequestering virus in controlled packets, the simplicity of the assay endpoints, the retrievable nature of the implant and the reduced requirement for compound during preliminary evaluations due to the short assay length and the limited experimental group size. Other microcapsule/macrocapsule models for antiviral evaluations have been described previously. The methodology described here is superior to the alginate microencapsulation methods (Gorelik et al., 1987; McMahon et al., 1990), because the sample can be retrieved in its entirety, the implant is very non-reactive in the host (Hollingshead et al., 1995), and an implant site other than the peritoneal cavity can be used. This method is superior to the agar-based macroencapsulation approach (Allen et al., 1992) because the implants are non-reactive in the host, the implants can be placed outside the peritoneal cavity, the hollow fiber implants are not susceptible to breakdown like the agar implants and active cell replication occurs in the hollow fiber cultures. Not only acutely infected CEM-SS cells can be used as targets in the hollow fiber assay, a variety of acutely and chronically infected cell lines also have demonstrable growth in this PVDF hollow fiber methodology (data not shown). Furthermore, the methodology is amenable to human peripheral blood mononuclear cells (PBMCs) since we have demonstrated that HIV can replicate in human PBMCs cultivated in hollow fibers in vitro (data not shown). This, in conjunction with the capacity of human PBMCs to grow in PVDF hollow fiber cultures in vivo (data not shown), indicates that PBMCs can serve as the target in the in vivo hollow fiber cultures. We are optimistic that others will find this method amenable to growth of a wide variety of cell types as well as other virus families.

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