Presence of Human Immunodeficiency Virus DNA in Laser Smoke

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Concentrated tissue culture pellets infected with human immunodeficiency virus (HIV) containing 1×10^7 cells/ml were vaporized by means of a carbon dioxide laser. The vaporous debris resulting from the laser's impact were evacuated through sterile silastic tubing, then bubbled through sterile culture medium (RPMI) positioned in series with a commercial smoke evacuator. No HIV DNA was detected in the culture medium flask. Tissue culture studies of the silastic collection tubing revealed p24 HIV gag antigen in 3 of 12 tube segments at the end of 1 week and in 1 of 12 tube segments at 2 weeks. No sustained infection of HIV cultured cells was observed at the 28th day. Polymerase chain reaction (PCR) analysis of particulate debris obtained from the silastic collection tubing was positive from proviral HIV DNA in both immediately sampled and day 14 cultured material.

Key words: smoke evacuation, CL-30, bi-pure filters, laser engineering

INTRODUCTION

Laser vaporous by-products (plume) have been shown to contain fine particulate matter which creates anatomically identifiable lesions when chronically inspired [1]. The particulates are commonly minuscule with mean aerodynamic diameters of 0.1–0.3 μm [2]. Careful and continuous evacuation of the smoke with high flow rate evacuation systems equipped with sophisticated filtration systems capable of trapping particles of 0.3 μm have been shown to protect animal models breathing filtered plume [3]. Equally important, the nozzle of the smoke evacuator hose must be kept within 1–2 in from the source of the vaporization, i.e., origin of the smoke [4].

Laser vaporization of cutaneous and mucous membrane warts instigated by the human papilloma virus have proved to be highly efficacious and yield superior results compared to other methods of therapy [5]. Garden et al. [6] studied laser vapor emanating from bovine fibropapillomas and human verrucae to determine whether HPV DNA could be recovered. These investigators found intact viral DNA in the smoke of 4 fibropapillomas and in the vapor collected from 2 of 7 human warts. An unanswered question was whether intact virion capable of transmitting infection was present [7]. To date this question has not been answered [8].

There will be more than 270,000 cases of AIDS in the USA by 1991 [9]. A substantially higher number of human immunodeficiency virus (HIV) infections without clinical AIDS are predicted. Those afflicted individuals are likely prey for other viral infections, including warts as well as intraepithelial neoplasias of the lower genital tract and anus. Both laser treatment and/or electrocautery are advantageous methods of treatment [10,11] because they control bleeding, but both are likely to produce smoke by-products. It was considered important to determine whether this laser vapor contained HIV DNA or intact virus capable of infection.
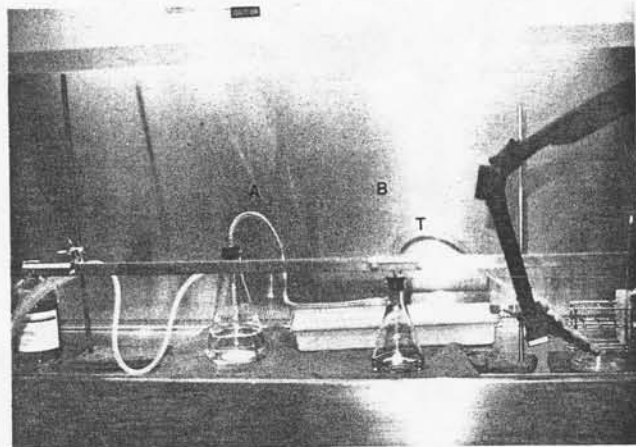
Fig. 1. The experimental model is illustrated and was carried out under a hood enclosure. The laser articulated arm is seen on the far right. Proximal silastic suction tubing (T) is connected to a flask (B) containing RPMI culture medium. Another section of tubing leads from flask B to a flask (A) containing household bleach and finally to a smoke evacuation pump.
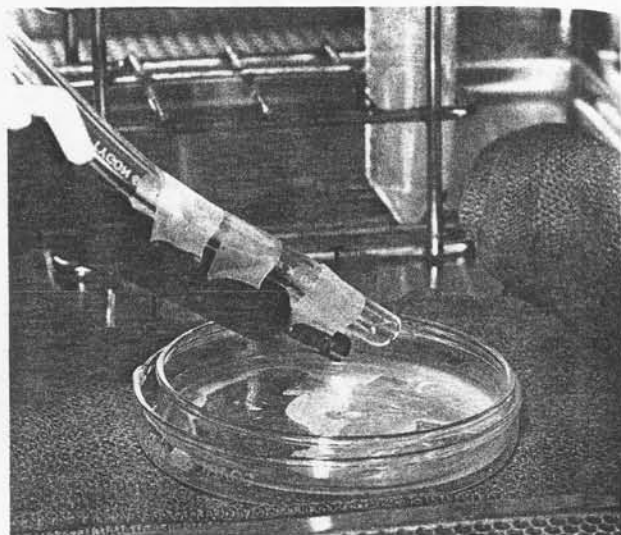

Fig. 2. Closeup of the laser's handpiece (L) targeted on a Petri dish filled with concentrated human immune deficiency virus infected cells. A smoke evacuation tube (T) protrudes 1 cm beyond the terminus of the laser handpiece.

MATERIALS AND METHODS

An in vitro experiment was performed in the AIDS viral laboratory at the SUNY Health Science Center in Syracuse, New York. The experiment was performed under a laminar flow hood and within a laboratory equipped with positive air flow.

The articulated arm and handpiece of a carbon dioxide laser were brought into the hood and held in position by two adjustable clamps (Fig. 1). Sterile 11-mm diameter silastic tubing measuring 110 cm long was attached to the handpiece such that it protruded approximately 1 cm above and beyond the laser beam's exit port (Fig. 2). The tube was connected to a sterile flask containing HIV culture medium (RPMI medium). The tubing was numbered at 1-in intervals beginning with the handpiece end and terminating with the point of entry into the flask containing the culture medium. From the culture media flask a second length of tubing ran to a second, larger sterile flask containing household bleach. This in turn was connected to a commercial laser smoke evacuator (Laser Engineering and Stackhouse Associates). The smoke evacuator was adjusted to draw the vapor from the laser target through the tubing, bubbling it in turn through the culture media and then through the bleach. Additionally a fluoropore Milex-FG 50 filter (Millipore, Bedford, MA) capable of collecting particles as small as 0.05 μm was inserted into the tubing before it entered to smoke evacuators filter system.

Ten milliliters of HIV infected cells (10^7 cells per milliliter) containing 1 copy of HIV/cell were decanted into a sterile glass petri dish (Fig. 3). The dish was positioned at a distance from the laser handpiece to allow a spot diameter of between 1.5 and 2.5 cm to focus on the cell concentrate.

Laser power was adjusted to deliver 20 W of power; continuous time setting; average power density 500 W/cm^2.

During the experiment the laser was fired intermittently until the pool of cells was vaporized; then the petri dish was rotated clockwise, exposing a new pool; when this was vaporized the dish was again rotated until the entire concentrate had been vaporized (Fig. 3).

At the termination of the vaporization the proximal tube was disconnected, and cut into 1.5-cm numbered segments with separate sterile scissors. The segments were then divided into 2 portions. One portion was placed into culture; the second portion was prepared for polymerase chain reaction. The flask containing HIV culture medium (RPMI medium) was similarly divided.

Prior to the performance of the above experiment a petri dish containing uninfected HUT 78 cells was vaporized and placed into culture and evaluated by the polymerase chain reaction.
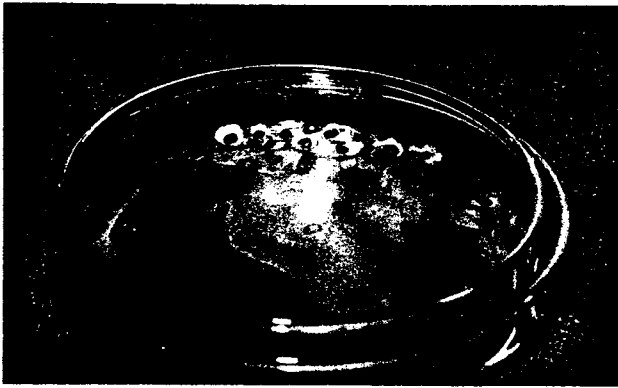
Fig. 3. The laser vaporized the pool of infected cells. The Petri dish was rotated until the contents of the dish were vaporized.

TABLE 1. Typical Standard Curve

HIV p24 antigen standard concentration (pg/ml)	Optical density		
	Well		
	1	2	Mean
0	.020	.021	.021
7.8	.149	.151	.150
15.6	.274	.278	.276
31.25	.487	.523	.512
62.5	.897	.896	.896
125.0	1.759	1.805	1.782

Culture of Tubing Segments

Fourteen milliliters of RPMI 1640 culture medium (GIBCO Laboratory) containing 10% fetal calf serum and 1% penicillin-stretomycin was added to individual tubing sections contained in 50 ml conical tubes. HUT 78 cells (1.5×10^6) were added. These were incubated at 37°C in an atmosphere of 5% CO_2 and air. After 3 days the tubing was removed and the cell culture was transferred to a 25 cm² culture flask and incubated at 37°C. Cultures were fed by pipetting off 5 ml of the condtioned media and adding 5 ml of fresh media. On day seven 5×10^5 HUT 78 cells were added. All cultures were fed and sampled for p24 protein on days 3, 7, 10, 14, 18, 21, and 24. On days 14 and 28, cells were frozen for polymerase chain reaction studies. All cultures were terminated on day 28.

Determination of p24 Antigen

An enzyme-linked immunosorbent assay for detecting the presence of human immunodeficiency virus (HIV) p24 antigen (Retro-Tek, Cellular Products, Inc., Buffalo, NY) was used. Tissued cultured HUT 78 cells incubated with the laser vaporous debris were assayed between days 3 and 28 as noted in the preceding section.

Twenty-five liters of lysing buffer, containing 10% Triton X-100 in phosphate buffered saline, was added to 225 μl of each tissue culture sample. After washing the HIV p24 antibody coated microplate, containing 96 wells (12×8 well strips) coated with a mixture of purified murine p24 monoclonal antibodies, each well was filled with 300 μl of wash buffer (phosphate buffered saline and Tween 20) and aspirated. Six fill/aspirate cycles were performed. After the final wash cycle the microplate was thoroughly blotted. Two hundred microliters of diluent (normal goat serum, phosphate buffered saline, Triton X-100) was micropipetted into one empty well, which was used for background determination (substrate blank). Next, 200 μl of standard, containing detergent disrupted, heat inactivated viral antigen at a concentration of 2.5 ng of p24 per ml was micropipetted into a duplicate well. Then, 200 μl of each test specimen was placed into separate wells of the microplate. The microplate was then covered with a plate sealer and incubated overnight at 37°C. The contents of each well were aspirated and 200 μl of HIV detecter antibody (biotin-labelled human antibody to heat inactivated HIV containing 50% glycerol/PBS and normal goat serum; tested negative for hepatitis B surface antigen) was added to each well of the plate except the substrate blank, which received 200 μl of diluent. The contents of each well were again aspirated and the plate washed as described above. Next, 200 μl of streptavidin-peroxidase reagent was added into each well except the substrate blank, which received 200 μl of diluent. The microplate was covered and incubated for 30 minutes at 37°C. Aspiration was again repeated. 200 μl of tetramethylbenzidine (TMB) solution in dimethyl sulfoxide was added into each well and incubated uncovered for 30 minutes at 18–25°C. A dense blue color developed in wells containing viral antigen. At the end of 30 minutes 50 μl of 2 M sulfuric acid was dispensed into each well. A color change from blue to yellow resulted within 15 minutes, the absorbance of each well at 450 nm was read and compared to a standard curve (Table 1).

DNA Directed Polymerase Chain Reaction

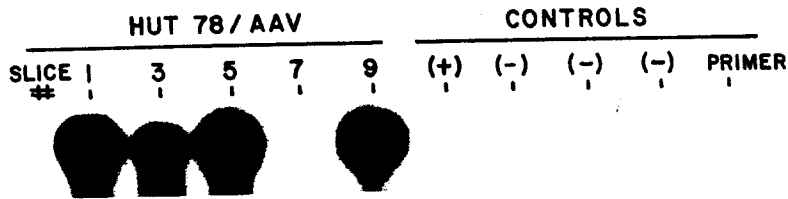**Rapid lysis.** Polymerase chain reactions (PCR) were performed on sterile sections of silas-

Fig. 4. Polymerse chain reaction of tubing segments containing vaporous debris from laser vaporization in Figure 3. The HUT 78 infected cells show strongly positive reaction in slices 1, 3, 5, and 9, indicating presence of proviral human immune deficiency virus DNA. The controls are negative.
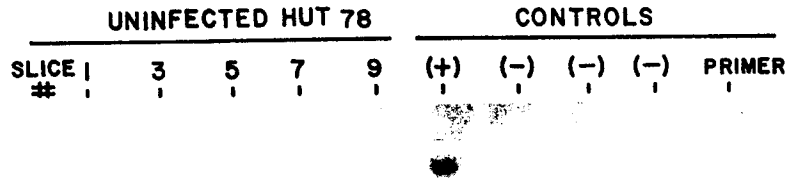

Fig. 5. Vaporization of uninfected HUT 78 cells reveal no presence of human immune deficiency virus proviral DNA (polymerase chain reaction).

tic tubing through which laser vapor passed before reaching the flask containing the culture medium. Additionally similar studies were performed on control tubing as well as medium obtained from flask B (Fig. 1). The 1.5-cm numbered sections of tubing were placed in 15-ml conical tubes and covered with lysis buffer (100 mM KCl, 10 mM TRIS [pH 8.3], 2.5 mM Mg Cl_2.) A second series of polymerase chain reaction were done on cultured cells obtained and frozen on days 14 and 28 as described under Culture of Tubing Segments. To 1×10^6 cells, 25 µl of solution A, containing 100 mM KCl, 10 mM TRIS (pH 8.3), 2.5 mM Mg Cl_2, was added. Next, 25 µl of solution B, containing 100 mM TRIS (pH 8.3), 2.5 mM Mg Cl_2, 1% Tween 20, 1% NP40 (containing 2.4 µl self-digested proteinase K, 5 mg/ml) was added. The sample was incubated for 1 hour at 60°C and the proteinase K was then inactivated by boiling the sample for 30 minutes.

Polymerase chain reaction. Fifty liters of cell lysates from 1 and 2 were transferred to a 500-µl microcentinfuge tube for amplification of the gag region of HIV-1 (Fig. 4). Immediately prior to amplification for gag, a common reaction mixture was prepared containing 10 1 of 10× Taq buffer/sample; 6 µl of 3.75 mM d NTP's/sample, 1 µl 50 pm of each primer/sample, 0.2 µl 1U of Taq enzyme/sample, and the final volume was brought up to 50 µl/sample with Gene screen fil-

tered sterile distilled water. Fifty microliters of the reaction mixture was added to each cell or tubing sample, a negative DNA, and a reaction mixture control tube.

Amplification. The reaction tubes were placed in a Perkin Elmer Cetus DNA thermal cycler. The tubes were heated to 95°C for 30 seconds to denature the DNA and at a 1 second cycled interval cooled to 53°C for 30 seconds to allow the primer to anneal. The temperature was then raised to 68°C for 30 seconds to allow extension of the new strand of DNA. The heating and cooling cycles were repeated 30 times; the entire process encompassing approximately 2½ hours.

Liquid hybridization. After amplification 150 µl chloroform: amylisoalcohol (24:1) were added to the tubes, which were vigorously shaken. Two layers were then visible (The amplified DNA was in the top layer) 30 µl of the PCR-DNA was removed and placed in another 0.5 ml Eppendorf tube to which 20 µl of a liquid hybridization cocktail was added (probe 250,000 cpm/sample, 1.5 M NaCl, TE was added to allow 20 µl aliquots per sample). The probe/amplified DNA samples were boiled for 5 minutes, and placed in a 55°C water bath for 30 minutes 1/10 volume (5 µl), then a gel loading dye/sample was added. Samples were run on 8% polyacrylamide gel at 200 V for approximately 60 minutes. The gel was then covered with Saran Wrap, individually wrapped.
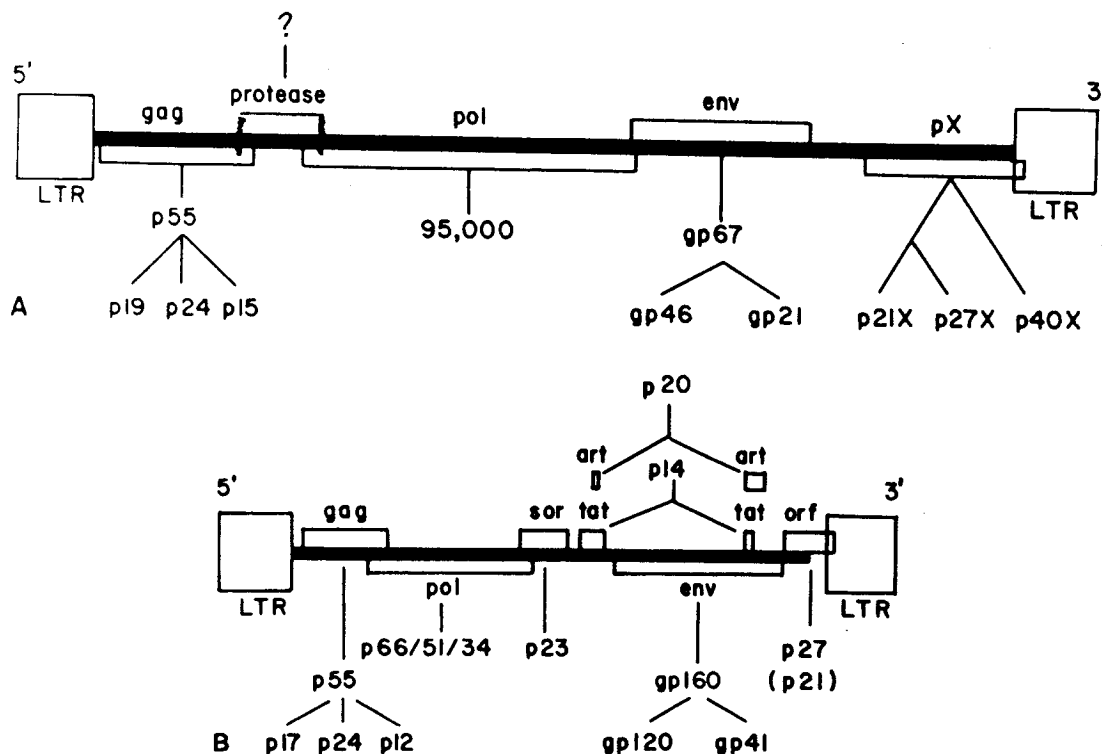
Fig. 6. Proviral DNA genomes and their known protein products of HTLV-1 (A) and HTLV-11 (B) p 24 protein is a product of the gag portion of the genome. Reprinted with permission from Poiesz BJ, Byrne BC: Retroviruses and anti-retroviral therapy in AIDS in Gynecology. Osborne NG (ed). Clinical Practice of Gynecology, Elsevier, New York, 1989 1:81–107.

Kodak X-A-R-5 film was placed over the gel and exposed at room temperature for 2 hours—overnight.

RESULTS

Polymerase chain reaction (PCR) studies of sterile, proximal tube segments 1, 3, and 5, were strongly positive for HIV proviral DNA (Fig. 4). Uninfected HUT 78 cells showed no evidence of proviral DNA by PCR (Fig. 5). Likewise material from the flask containing RPMI (HIV) culture medium through which the laser smoke bubbled was PCR negative for HIV proviral DNA.

Tube segments containing laser vaporous debris were placed in culture for 28 days and analyzed for gag gene group specific antigen, i.e., p24 protein (Fig. 6). A summation of the culture results is seen in Table 2. The cultures were considered positive if >30 pg ml of p24 protein was recovered. Tube segments 7 and 8 were positive for p24 protein for 7 days, then became negative. Segment 6 was positive for p24 protein until day 14 but was negative from day 18 to day 28 (Fig. 7).

Control tubing and culture flask contents were negative for p24 protein from day 1 to 28.

Polymerase chain reactions (PCR) were run on cells obtained from culture on days 14 and 28. Figure 8 shows PCR proviral DNA obtained from tube segments 6 and 8 on day 14 specimens.

No proviral DNA was identified by PCR in any day 28 cultured cell samples (Fig. 9).

DISCUSSION

This study has clearly shown that HIV provirall DNA is present in laser smoke. The results of the culture experiment showed that HIV p24 protein was detected for at least 14 days in one case. Additionally the cultured cells were also PCR positive for proviral DNA. Less clear is the fact that productive infection with HIV cultured cells was not sustained to the 28th day. The PCR's were all negative for proviral DNA in the 28 day culture samples. The most plausible explanation for the aforesaid events could be that the laser action somehow may have impaired the HIV integrity to such an extent that long-term replication was precluded.
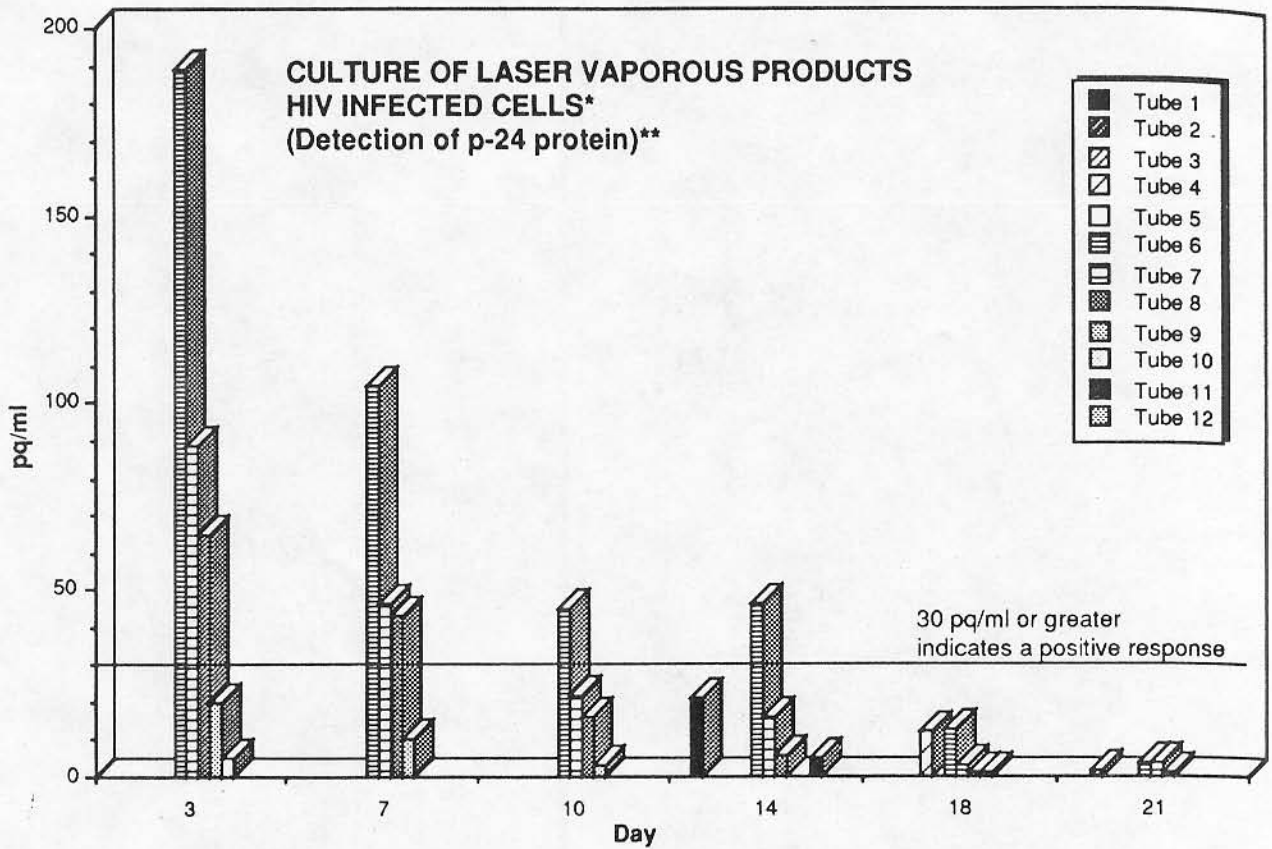
CULTURE OF LASER VAPOROUS PRODUCTS HIV INFECTED CELLS*
(Detection of p-24 protein)**

Fig. 7. *1 copy virus/cell; ** a positive result is greater ≥ 30 pq/ml of p-24 protein.
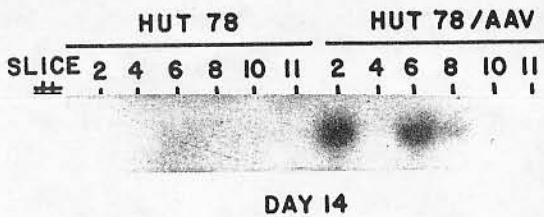
DAY 14

Fig. 8. Polymerase chain reaction of cultured HUT 78 cells vaporized by the CO_2 laser. The infected cell culture was positive for HIV proviral DNA in segments 2 and 6 on day 14. Uninfected HUT 78 cells are negative.

PCR FOR HIV-I DNA IN HUT 78 CELLS EXPOSED TO EITHER HUT 78 OR HUT 78/HIV AAV LAZER GENERATED SMOKE
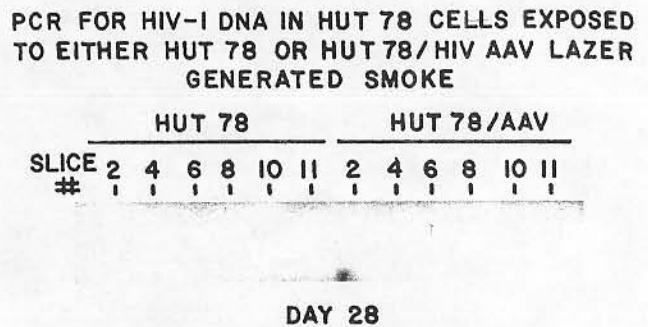
DAY 28

Fig. 9. Polymerase chain reaction was negative for uninfected and infected cultured cells at day 28.

Although the concentration of HIV positive cells vaporized by the laser in this experiment exceeded manyfold the number present in a patient with clinical AIDS, the results of this study are nonetheless sobering. Clearly the smoke must be dealt with carefully and compulsively. Efficient smoke evacuation must be maintained close to the operative field in order to remove the vapor before it is inhaled by operating room personnel. It appears from this study that most if not all potentially infectious debris will accumulate in the collection tubing. This collection tubing should be considered hazardous and should be disposed of appropriately.

Although we did not examine electrocautery by-products for the presence of HIV, one could surmise that the smoke would likely contain HIV DNA. An alarming fact is the smoke evacuators are not commonly employed in conjunction with electrocautery and electrosurgical operations.

This smoke is therefore breathed by the operating team and support staff. Clearly, in the presence of an HIV positive patient this practice is risky. Universal precautions should be observed with smoke by-products in all patients.

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