BRIEF COMMUNICATION

PHOTODYNAMIC INDUCTION OF AN ONCOGENIC VIRUS IN VITRO

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ABSTRACT Infectious simian virus 40 (SV40) was induced from SV40-transformed hamster kidney cells by treatment with proflavine and visible fluorescent light. The optimum levels of SV40 induced were about three orders of magnitude above spontaneous background levels observed with untreated cells. No virus induction above background levels was found by treatment of cells with either proflavine or light alone.

INTRODUCTION

Simian virus 40 (SV40), a DNA-containing virus of monkeys, produces tumors in newborn hamsters and can transform certain mammalian cells in tissue culture (2). The virus can be induced from some lines of SV40-transformed hamster cells by treating the cells with physical and chemical agents such as UV- or gamma-radiation and mitomycin C (3). The complete viral genome of this virus is integrated into the host cell DNA of inducible lines. Data exist indicating that one of the early events in induction of SV40 is excision of viral DNA from host cell DNA, as occurs during the induction of bacteriophage lambda DNA from the *Escherichia coli* genome (4).

Bacteriophage lambda can be induced from lysogenic *E. coli* by photodynamic treatment of cells with acridine orange, or methylene blue, plus visible light (5). The purpose of this study was to determine whether photodynamic induction of virus can occur with a mammalian virus-host cell system. The results show that SV40 can be induced by photodynamic treatment of SV40-transformed hamster cells.

MATERIALS AND METHODS

Photodynamic Induction

Clone E line of SV40-transformed inbred Syrian hamster kidney cells, established and characterized by Kaplan et al. (3), was obtained from J. C. Kaplan, Harvard Medical School. The cells were propagated in 25 cm^2 plastic flasks (Falcon Plastics, Div. BioQuest, Oxnard, Calif.)[1] at 37°C in

Preliminary results were reported at the fourth annual meeting of the American Society for Photobiology, Denver, Colo., 16–20 February 1976, and described in a Health, Education, and Welfare (Food and Drug Administration) technical report (1).

[1] Representative products and manufacturers are named for identification only and listing does not imply endorsement by the Public Health Service and the U.S. Department of Health, Education, and Welfare.

Eagle's minimal essential medium (MEM) (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% fetal bovine serum, a fourfold concentration of essential amino acids and vitamins, 4 mM L-glutamine, and 100 U/ml each of penicillin and streptomycin, pH 7.0, (4X MEM). For photodynamic treatment, nearly confluent cultures were rinsed with Dulbecco's phosphate-buffered saline (PBS), incubated for 1 h at 37°C in the dark with filter-sterilized proflavine sulfate (a gift of Mead Johnson & Co., Evansville, Ind.) in PBS, and rinsed with PBS to remove unbound dye. Fresh medium was added and cultures were irradiated with visible fluorescent light as described below. Since the cells were attached to the flask inside bottom surface, and the flasks were illuminated from below, medium did not shield cell monolayers from the light. At the time of irradiation, cell counts (ranging from 1 to 3 $\times$ 10^6 cells per flask) on replicate samples were determined by using an electronic cell counter (Coulter Electronics Inc., Hialeah, Fla.). After irradiation, the cultures were incubated in the dark for 3 d at 37°C to permit virus expression, and then frozen (−70°C). Preliminary experiments (L. Bockstahler, unpublished results) showed optimum virus virus induction for post-irradiation incubation times of 3–4 d and no induction for 1 d or less. Incubation times of 3–4 d also resulted in optimum SV40 induction after UV- or gamma-irradiation of cells, when utilizing the same induction system (3).

Procedures were carried out under red safety lamp illumination. Cells were routinely tested for the presence of *Mycoplasma*, by using a commercial detection procedure (Flow Laboratories, Inc., Rockville, Md.), and contaminated cultures were discarded. No attempt was made to control oxygen concentration.

Assay of Induced Virus

Cell-free extracts of the frozen, photodynamically treated clone E cells were prepared by two cycles of freeze-thawing, followed by sonification with a model W140D Branson sonifier-cell disruptor (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.). Extracts (5 ml) were assayed for induced SV40 infectivity by plaque determination on permissive CV-1 (TC7) monkey kidney cells by using a modified standard agar overlay procedure (3). Aliquots of the cell extracts were diluted in PBS and added to monolayers of CV-1 (TC7) cells in 25 cm^2 plastic flasks. After virus adsorption (2 h at 37°C), the inoculum was removed, and each monolayer was overlaid with 4 ml of MEM (supplemented with essential amino acids [twice], vitamins [twice], and antibiotics) containing 0.9% agar and 6% fetal bovine serum. 6 d later and again at 11 d, an additional 4 ml of the MEM-agar overlay was added. The last overlay contained 0.01% neutral red. Plaque determinations were made on days 12 and 13. The results are expressed as average plaque-forming units (PFU) per 10^6 clone E cells at the time of irradiation.

Visible Light Source

Twelve parallel standard 40-W F40T12-D-LT Sylvania "Daylight" fluorescent lamps (Sylvania Lighting Products Div., Hillsboro, N.H.) mounted 4.5 cm apart (center-center distance) on a metal frame were covered with two pieces of plate glass (5 mm each) to provide a heat filter and UV filtration below 320 nm. Samples in plastic flasks were placed on the glass at a distance of 5.2 cm above the lamps. Fans were used to aid in removing heat from the lamps. The temperature in the dishes during the time of irradiation was 30 $\pm$ 2°C.

Light Source Calibration

Calibration of the light source was performed by O. Ellingson, Food and Drug Administration, who used the above filtration (including plastic flask) and exposure conditions. The multiple lamp source was characterized by measuring the spectral irradiance (watts per square centimeter per nanometer) of an individual lamp over the wavelength range of 280–750 nm by using a Cintra spectroradiometer (Cintra Inc., Mountain View, Calif.) that incorporates a Bausch & Lomb quarter-meter double-grating monochromator and broad-band photomultiplier tube with an S-20 window (Bausch & Lomb Inc., Analytical Systems Div., Rochester, N.Y.). A UDT 40A optometer with diffuser (United De-

tector Technology Inc., Santa Monica, Calif.) was used as a reference instrument to determine the irradiance of the multiple lamp source at the sample exposure distance of 5.2 cm.

Another UDT 40A optometer was used to monitor the source output for any variations from experiment to experiment. The calibration procedures have been described (6). Uncertainty of the spectral irradiance measurement-calibration process due to systematic and random errors was estimated to be approximately ±25%. The irradiance corrected to the position of the sample exposure was 26.7 W/m^2 (350–750 nm). Of this incident radiation, 37% (approximately 10 W/m^2) was in the wavelength range (380–500 nm) relevant for photodynamic action with proflavine bound to DNA (7). The exposure values reported were calculated by multiplying the corrected irradiance of 26.7 W/m^2 (350–750 nm) by the exposure time in minutes.

RESULTS

Cultures of SV40-transformed hamster cells were treated with different concentrations of proflavine, exposed to visible light for 1 h (10^5 J/m^2), and incubated for virus expression. The SV40 infectivity of cell extracts was determined (Fig. 1A). For the range of dye concentrations examined, induction of SV40 was observed between 0.5 and 3 μM. The optimum level of SV40 induced (1 μM proflavine) represented an increase in virus production of three orders of magnitude above the spontaneous background level. The decrease in SV40 production that occurred for dye concentrations greater than 1 μM may represent a decrease in capacity of treated cells to support the growth of induced virus, resulting from cell damage by photodynamic action.

Fig. 1 B shows SV40 induction under conditions in which the concentration of proflavine was held constant (1.1 μM) and light exposure was varied. The amount of virus induced

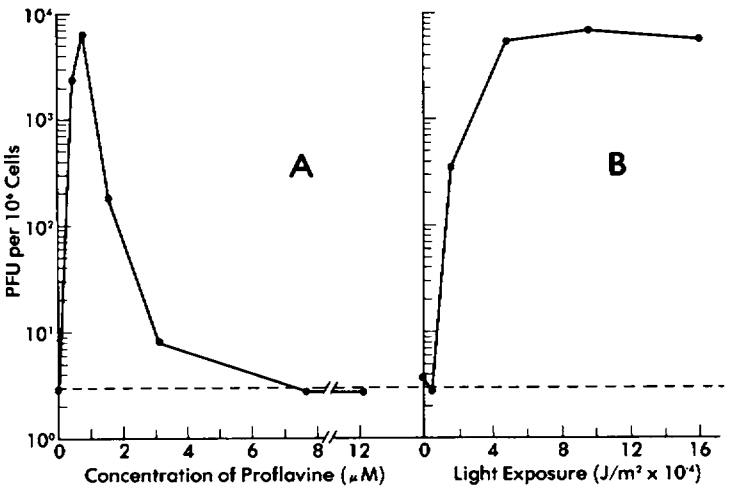

FIGURE 1 Induction of infectious simian virus 40 from SV40-transformed hamster cells after photodynamic treatment. Nearly confluent cultures of cells were pretreated with proflavine (A, different concentrations; B, 1.1 μM) for 1 h, rinsed, exposed to visible light (A, 1 h $\cong$ 10^5 J/m^2; B, different exposures), and incubated (72 h) for virus expression. Induced virus was harvested and assayed for infectivity on permissive monkey cells, as described in Materials and Methods. The induced virus yield is expressed as the average plaque-forming units (PFU) per 10^6 transformed cells. Each point represents the average of three determinations (cell extracts from three replicate samples each assayed once). Values lying below the dashed line are lower than the detection limit of the assay and are indicated by points with attached arrows.

increased with increasing light exposures up to about 5×10^4 J/m^2 and remained essentially constant for the larger exposures examined. Significant decreases in SV40 induction for exposures of 10^5 J/m^2 and above were observed when the proflavine concentration was increased to values above 1.1 μM (L. Bockstahler, manuscript in preparation).

The background level of spontaneous SV40 production varied from experiment to experiment, as reported previously (3). No virus induction above background levels was observed when cultures were treated with either proflavine alone (0.5–20 μM) or light alone.

DISCUSSION

This study demonstrates that photodynamic induction of virus can occur with a mammalian virus-host cell system. The optimum levels of SV40 induced were approximately equal to those observed with 254 nm UV- or gamma-irradiation of cells, or treatment with 5-bromodeoxyuridine plus visible light, when utilizing the same induction system (3). The results shown in Fig. 1 (A and B) suggest the relationship between the responses to the variables of dye concentration and visible light exposure is complex. When dye concentration was held constant and light exposure varied (Fig. 1 B), exposures greater than that sufficient for optimum induction did not result in decreased virus expression as observed in Fig. 1 A. Further studies are in progress to establish the relationship between these variables in SV40 photodynamic induction.

The percentage of cells induced by optimum photodynamic treatment is presently under investigation. Preliminary studies employing immunofluorescent detection of SV40 virus (V) antigen and electron microscopy indicate the number of virus-producing cells is small (less than 1%) (A. Lubiniecki and L. Bockstahler, unpublished results). Kaplan et al. (3) found by V antigen determination that only 2% of clone E cells were induced after optimum treatment with mitomycin C.

The mechanism of photodynamic induction of SV40 is unknown. Proflavine localizes preferentially in the nuclei of mammalian cells (8), binds to DNA by intercalation between bases, and damages DNA in the presence of light and oxygen. This major effect of photodynamic treatment, damage to cellular DNA, is shared by other inducers of SV40 including UV- and gamma-radiation (3). However, proflavine plus light treatment of cells also inactivates RNA, protein, and other cellular constituents (9). Thus, the mechanism(s) of induction could involve excision of viral DNA resulting from direct damage to the host cell genome, or occurring during repair of DNA damage, or may involve indirect effects on the cellular or viral DNA.

The idea for performing this study came from a review of benefits and potential risks of clinical photodynamic therapy for herpes simplex virus (9). In this review we suggested that one risk of photodynamic treatment might be induction of possible latent tumor virus, if harbored in infected or surrounding uninfected cells of patients. It has been hypothesized (10, 11) that cells of humans may contain integrated genetic information for tumor viruses; however, relatively little supporting experimental evidence is presently available.

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