INFECTION AND IMMUNITY, June 1981, p. 1216-1226
0019-9567/81/061216-11$02.00/0

Vol. 32, No. 3

Psoralen Inactivation of Influenza and Herpes Simplex Viruses and of Virus-Infected Cells

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Psoralen compounds covalently bind to nucleic acids when irradiated with long-wavelength ultraviolet light. This treatment can destroy the infectivity of deoxyribonucleic acid and ribonucleic acid viruses. Two psoralen compounds, 4'-hydroxymethyltrioxsalen and 4'-aminomethyltrioxsalen, were used with long-wavelength ultraviolet light to inactivate cell-free herpes simplex and influenza viruses and to render virus-infected cells noninfectious. This method of inactivation was compared with germicidal (short-wavelength) ultraviolet light irradiation. The antigenicity of the treated, virus-infected, antigen-bearing cells was examined by immunofluorescence and radioimmunoassay and by measuring the capacity of the herpes simplex virus-infected cells to stimulate virus-specific lymphocyte proliferation. The infectivity of the virus-infected cells could be totally eliminated without altering their viral antigenicity. The use of psoralen plus long-wavelength ultraviolet light is well suited to the preparation of noninfectious virus antigens and virus antigen-bearing cells for immunological assays.

In vitro assays of virus-specific cellular immunity generally involve the exposure of viable effector lymphocytes to virus antigens, followed by the measurement of some presumably relevant cellular response, such as proliferation, lymphokine release, or cytotoxicity (29, 30). Because virus infection of the effector lymphocytes may alter these measured responses, the virus antigens used should be noninfectious (23-25). Moreover, since in vivo cell-mediated immunity to many viruses, especially to members of the herpesvirus group, may involve the interaction of effector lymphocytes with virus-infected cells rather than with cell-free virus or soluble antigens (22), it may be important to use antigen-bearing virus-infected cells rather than cell-free virus or soluble virus antigens in in vitro assays of cellular immunity. Accordingly, we have attempted to develop a practical method that will eliminate the infectivity of virus-infected cells without affecting their antigenicity. In preliminary experiments we observed that, whereas cell-free herpes simplex virus (HSV) was rapidly inactivated by germicidal (short-wavelength) ultraviolet light (GUV), virus-infected cells were relatively resistant to GUV inactivation. We therefore investigated the capacity of long-wavelength ultraviolet light (LWUV) and psoralens to eliminate the infectivity of virus-infected cells.

Psoralen compounds are polycyclic planar molecules with an affinity for nucleic acids (26, 31). When a psoralen-nucleic acid complex is irradiated with LWUV, a photochemical reaction occurs which results in the covalent addition of the psoralen to a pyrimidine (3, 18) or purine (11) base. Psoralens plus LWUV irradiation (psoralen-LWUV) have been used to inactivate both deoxyribonucleic acid and ribonucleic acid viruses (4, 8, 19) to investigate the secondary structure (27), repair mechanisms (6, 13), and histone binding (7) of virus deoxyribonucleic acid, to probe the interaction of polymerases with viral ribonucleic acid (20, 21), and to investigate the structure of viral deoxyribonucleic acid-ribonucleic acid hybrids (28).

We present here a comparison of the ability of psoralen-LWUV and GUV to inactivate cell-free HSV and influenza virus and to eliminate the infectivity of HSV- and influenza virus-infected cells. The antigenicity of the treated virus-infected cells was examined by immunofluorescence, by radioimmunoassay, and in a virus-specific lymphocyte proliferation assay. The results of these studies demonstrate that psoralen-LWUV can effectively eliminate the infectivity of virus-infected cells without measurably altering their antigenicity.

MATERIALS AND METHODS

Cells. Rabbit kidney (RK13) cells were obtained from Charles Scott and Stewart Sell, University of California, San Diego. Madin-Darby canine kidney cells were obtained from Brian Murphy and Robert Chanock, National Institutes of Health, Bethesda, Md.

A strain of diploid human fibroblasts (350Q) initiated from newborn foreskin at the Virus Research Unit, Children's Hospital, Boston, Mass., was used between passages 18 and 30. Cells were grown in 150-cm^2 plastic cell culture bottles (Costar, Cambridge, Mass.) in an atmosphere of 5% CO_2 in air at 37°C, using Dulbecco-modified Eagle medium plus 100 U of penicillin and 100 μg of streptomycin per ml (DMEM), supplemented with 10% fetal bovine serum (FBS) for the RK13 and 350Q cells and with 5% FBS for the Madin-Darby canine kidney cells.

Virus. The MacIntyre strain of HSV type 1 (no. VR-539) was obtained from the American Type Culture Collection, Rockville, Md. Influenza A/Hong Kong/68(H3N2) virus was obtained from B. Murphy and R. Chanock.

Preparation of cell-free virus and virus-infected cells. (i) HSV. Confluent monolayer cultures of RK13 cells were infected with HSV at a multiplicity of infection of 1 plaque-forming unit (PFU) per cell and incubated at 36°C in DMEM plus 2% FBS. At 24 h postinfection, when more than 90% of the cells showed cytopathic effects, the culture medium was harvested and clarified by centrifugation at 800 × *g* for 10 min. This clarified medium, which contained 9 × 10^5 PFU of cell-free HSV per ml, was stored in aliquots at −70°C. The infected cells remaining in the monolayer were rinsed with Puck saline A and dispersed with 5 ml of 0.05% trypsin (Difco)–0.02% ethylenediaminetetraacetate at 36°C. When the cells were detached, 1 ml of FBS was added, and cell aggregates were dispersed by gentle pipetting. The cells were then pelleted by centrifugation at 400 × *g* for 10 min, resuspended at 2.0 × 10^6 cells per ml in DMEM plus 8% dimethylsulfoxide and 30% FBS, and frozen in aliquots at −70°C.

(ii) Influenza virus. Confluent monolayer cultures of Madin-Darby canine kidney cells were infected with influenza virus at multiplicity of infection of 0.04 PFU/cell and incubated at 37°C in DMEM containing 0.5 μg of tolysulfonyl phenylalanyl chloromethyl ketone-trypsin (Worthington Diagnostics, Freehold, N.J.) per ml. At 48 h postinfection, when more than 90% of the cells showed cytopathic effects, the medium was harvested, clarified by centrifugation, and stored in aliquots at −70°C. The titer of cell-free influenza virus was 1.2 × 10^8 PFU/ml. The infected cells remaining in the monolayer were dispersed with 0.5% trypsin–0.2% ethylenediaminetetraacetate, pelleted, resuspended at 2.8 × 10^7 cells per ml in DMEM plus 8% dimethylsulfoxide and 30% FBS, and frozen in aliquots at −70°C.

Ultraviolet light sources. The GUV source was a horizontally suspended 30-W General Electric (G30 T8) mercury lamp which emitted 85% of its radiant energy between 240 and 260 nm. The energy output, measured with a model J225 ultraviolet light meter from UV Products Inc., San Gabriel, Calif., was 5 × 10^3 ergs/s per cm^2 at a distance of 15 cm. The LWUV source was a horizontally mounted 15-W General Electric (F15 T8) black light which emitted 90% of its radiant energy between 355 and 380 nm. The energy output was 1.2 × 10^4 ergs/s per cm^2 when measured within the wells of a 24-well flat-bottomed tissue culture dish (Costar) held directly over the light source. A model J221 ultraviolet light meter (UV Products, Inc.) was used for this measurement.

Psoralen compounds. 4′-Aminomethyl-4,5′,8-trimethylpsoralen (AMT) and 4′-hydroxymethyl-4,5′,8-trimethylpsoralen (HMT) were obtained from Calbiochem-Behring, LaJolla, Calif. Stock solutions containing 1 mg of each psoralen per ml were prepared in dimethyl sulfoxide and stored at −20°C. The concentrations were confirmed spectrophotometrically (15). Working solutions containing 10× the desired final concentration of psoralen were prepared in sterile distilled water. The final concentration of dimethyl sulfoxide was always <1%. Psoralen solutions were always protected against exposure to light.

GUV inactivation. A 2-ml portion of DMEM supplemented with 2% FBS, containing cell-free virus or virus-infected cells (2.0 × 10^6 cells per ml), was exposed to GUV in an uncovered 35-mm plastic petri dish (Falcon Plastics, Oxnard, Calif.) at a distance of 15 cm at 24°C. The petri dish was agitated manually to ensure mixing and uniform exposure.

Psoralen-LWUV inactivation. Just before irradiation, 1 part of a 10× solution of psoralen was mixed with 9 parts of DMEM supplemented with 2% FBS, containing cell-free virus or virus-infected cells (2.2 × 10^6 cells per ml). One milliliter of this mixture was placed in a 1.6-cm-diameter well of a 24-well, flat-bottomed tissue culture dish (Costar). The bottom of the well was held directly over the LWUV lamp, and the plate was agitated manually to ensure mixing and uniform exposure.

Psoralen binding and photoreactivity with nucleic acid are reduced in the presence of cations (14, 21, 28). Thus, the use of DMEM may reduce the photoinactivation of cell-free virus compared with results obtained with a low-salt or nonionic solution. Likewise, the high intracellular concentration of cations would be expected to reduce the efficiency of psoralen photoinactivation of intracellular virus and virus nucleic acids.

Virus plaque assays. (i) HSV. Tenfold serial dilutions of cell-free virus or virus-infected cells were inoculated onto monolayer cultures of 350Q cells in six-well plastic cell culture dishes (Costar). After 1 h of adsorption at room temperature, the cultures were overlaid with Eagle basal medium containing 0.5% agarose, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. They were incubated in a humidified atmosphere of 5% CO_2 in air at 35°C for 3 days. Plaques were counted with an inverted microscope at ×40 magnification.

(ii) Influenza virus. Tenfold serial dilutions of cell-free virus or virus-infected cells were inoculated onto monolayer cultures of Madin-Darby canine kidney cells in six-well plastic cell culture dishes (Costar). After 1 h of absorption at room temperature, the cultures were overlaid with Liebowitz 15 medium containing 0.9% agarose, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 0.5 μg of tolylsulfonyl phenylalanyl chloromethyl ketone-trypsin per ml. They were incubated in air at 35°C for 3 days. Plaques were counted directly without staining.

Preparation of antisera. Rabbit antiserum to influenza A/Hong Kong/68(H3N2) was prepared in New Zealand White rabbits immunized by subcutaneous inoculation of 10^9 PFU of egg-grown virus (purified in

a 10 to 40% potassium tartrate density gradient) in 1 ml of complete Freund adjuvant, followed by two subcutaneous booster injections of 10^9 PFU of virus without adjuvant. Serum was prepared from blood obtained by cardiac puncture 4 weeks after the second booster injection. This antiserum had a hemagglutination inhibition titer of 512 against the immunizing virus. Rabbit antiserum to HSV was prepared in New Zealand White rabbits immunized by 3 monthly intramuscular inoculations of approximately $10^{5.5}$ PFU of virus. Serum was prepared from blood obtained by cardiac puncture 1 week after the last inoculation. This antiserum had a complement fixation titer of 1,024 against the immunizing virus.

Immunofluorescent staining of virus-infected cells. HSV-infected cells were stained by the direct fluorescent-antibody technique, using fluorescein-conjugated rabbit antiserum to HSV type 1 (M. A. Bioproducts, Bethesda, Md.). A total of 10^5 cells were suspended in 0.025 ml of the conjugated antiserum diluted 1:10 in phosphate-buffered saline (PBS) in wells of plastic U-plates (Cooke Laboratory Products, Alexandria, Va.) and incubated at room temperature. After 30 min, 0.15 ml of PBS was added to each well, and the plates were centrifuged at 400 × *g* for 10 min. The supernatant was carefully removed by aspiration, and the cells were washed twice with 0.15 ml of PBS per well. Washed, pelleted cells were resuspended in 0.020 ml of buffered glycerol (90% glycerol–10% PBS, pH 8.5), and 0.010 ml was placed under a glass cover slip for fluorescent microscopy.

Influenza virus-infected cells were stained by the indirect fluorescent-antibody technique, using rabbit antiserum to influenza virus and fluorescein-conjugated goat anti-rabbit globulin (Calbiochem-Behring). A total of 10^5 cells were incubated with rabbit antiserum to influenza virus diluted 1:100 in PBS and then washed three times as above. The washed, pelleted cells were then resuspended in 0.025 ml of fluorescein-conjugated goat anti-rabbit globulin diluted 1:10 in PBS and incubated for 30 min. The cells were then washed three times with PBS and resuspended in 0.020 ml of buffered glycerol, and 0.010 ml was placed under a glass cover slip for fluorescent microscopy. The cells were examined at a magnification of ×400 with a Zeiss Photomicroscope II equipped for epifluorescence. Control (uninfected) cells were included in each assay, and all samples were coded before examination. Only isolated intact cells were counted, and at least 100 consecutive cells were evaluated for each sample. Membrane fluorescence appeared as a bright rim at the periphery of the infected cells. Uninfected control cells never exhibited discernible membrane fluorescence.

[^{125}I]SPA radioimmunoassay. HSV antigens in HSV-infected cells were measured by means of a ^{125}I-labeled staphylococcal protein A ([^{125}I]SPA) immunofiltration assay as previously described (2). Briefly, 12,500 or 25,000 cells were incubated with a 1:4,000 dilution of rabbit antiserum to HSV type 1 (or of control rabbit serum) in the wells of the immunofiltration device for 30 min at 37°C. The cells were then washed three times to remove unbound antibody and incubated with 25,000 or 50,000 cpm of [^{125}I]SPA per well for 60 min at 35°C. The cells were again washed three times to remove unbound [^{125}I]SPA, and the bound [^{125}I]SPA was measured with a Searle model 1195 automatic gamma counter. Virus-specific counts per minute was defined as the counts per minute bound to cells incubated with antiserum minus those bound to the same number of cells incubated with control serum. The counts per minute bound to infected cells incubated with control serum (background counts) was never more than 10 to 15% of those bound to infected cells incubated with antiserum. The reported values represent the means ± standard deviations of four replicates.

Influenza antigens on 12,500 infected cells were measured similarly, using a 1:1,000 dilution of rabbit antiserum against influenza A/Hong Kong/68(H3N2) and 50,000 cpm of [^{125}I]SPA.

Lymphocyte proliferation assay. Lymphocytes were prepared from defibrinated rabbit blood (5) or heparinized human blood (1) as previously described. Lymphocytes were washed twice in Puck saline A and then suspended at a concentration of 2×10^6 cells per ml in RPMI 1640 medium supplemented with 100 U of penicillin per ml, 100 μg of streptomycin per ml, and either 5% heat-inactivated (56°C for 30 min) pooled normal rabbit serum or 10% heat-inactivated pooled human AB-positive serum (both serum pools prepared in this laboratory). A total of 2×10^5 lymphocytes in 0.10 ml were dispensed into wells of 96-well, flat-bottom plastic tissue culture dishes together with 0.05 ml of RPMI 1640 medium per well containing the various mitogens or antigens. The lymphocyte cultures were incubated for 6 days at 35°C in a humidified atmosphere of 5% CO_2 in air, and 0.25 μCi of [^{3}H]thymidine (New England Nuclear Corp., Boston, Mass.) in 0.01 ml of RPMI 1640 was added to each well at the beginning of the last 24 h. The lymphocytes were then harvested onto glass fiber filters, using an automatic harvester (Titertek; Flow Laboratories, Inglewood, Calif.). The filters were dried and placed in scintillation vials containing 5 ml of 0.5% 2,5-diphenyloxazole and 0.04% *p*-bis-[2-(5-phenyloxazolyl)]benzene in toluene, and the incorporated ^{3}H was determined in a Packard Tri-Carb liquid scintillation spectrometer. The stimulation index was calculated by dividing the counts per minute incorporated by lymphocytes cultured with each mitogen or antigen by those incorporated by lymphocytes cultured with medium alone (in the case of the mitogens) or with uninfected control cells (in the case of the antigen-bearing infected cells).

RESULTS

Inactivation of cell-free virus by GUV. Cell-free HSV and influenza virus were exposed to GUV (delivered dose rate, 5×10^3 ergs/s per cm^2) for various periods of time, and residual infectivity was measured by plaque assay. Both viruses were rapidly inactivated, although influenza virus was more sensitive than HSV (Fig. 1 and 2).

Inactivation of cell-free virus by psoralen-LWUV. Cell-free HSV and influenza virus were exposed to LWUV (delivered dose rate, 1.2×10^4 ergs/s per cm^2) for various periods of time

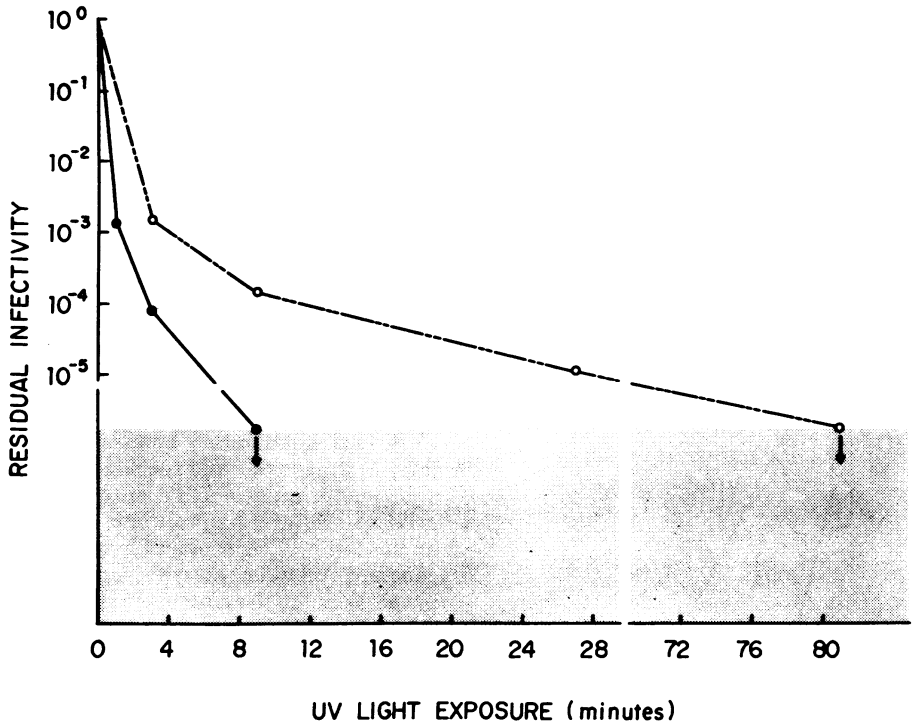

FIG. 1. *GUV inactivation of the infectivity of cell-free HSV (●) and HSV-infected RK-13 cells (○). Shaded area indicates the lower limit of measurable infectivity. Arrows indicate time points at which virus infectivity was less than that detectable by the plaque assay.*

in the presence of AMT or HMT, each at 0.1, 1.0, and 10 μg/ml. For each virus, the rate of inactivation was proportional to both psoralen concentration and total LWUV dose (Fig. 3 and 4). At equivalent psoralen concentrations the rate of inactivation of both viruses was greater with AMT than with HMT. A 10^4-fold reduction of HSV infectivity required 6.5×10^5 ergs of LWUV per cm^2 (0.9 min of LWUV exposure) in the presence of 10 μg of either psoralen per ml, compared with 9.1×10^5 ergs of GUV per cm^2 (2.7 min of GUV exposure). A 10^4-fold reduction of influenza virus infectivity required 5.0×10^5 ergs of LWUV per cm^2 (0.7 min of LWUV exposure) in the presence of 10 μg of AMT per ml, compared with 2.1×10^5 ergs of GUV per cm^2 (0.7 min of GUV exposure).

Elimination of the infectivity of virus-infected cells by GUV. HSV- and influenza virus-infected cells were exposed to GUV (5×10^3 ergs/s per cm^2) for various periods of time. As with cell-free virus, the infectivity of influenza virus-infected cells was more sensitive to GUV inactivation than was that of HSV-infected cells (Fig. 1 and 2). The infectivity of virus-infected cells, however, was considerably more resistant to inactivation than was that of cell-free virus. This difference was more pronounced with HSV-infected cells than with influenza virus-infected cells. It required 2.4×10^7 ergs of GUV per cm^2 (81 min of GUV exposure) to eliminate all measurable infectivity of HSV-infected cells. Moreover, only 60% of the HSV-infected cells that were initially present could be recovered intact at the end of this prolonged period of GUV irradiation.

Elimination of the infectivity of virus-infected cells by psoralen-LWUV. HSV- and influenza virus-infected cells were exposed to LWUV (1.2×10^4 ergs/s per cm^2) for various periods of time in the presence of the same three concentrations of AMT and HMT used with cell-free virus. As in the case of cell-free virus, elimination of the infectivity of virus-infected cells was proportional to both the psoralen concentration and the delivered dose of LWUV (Fig. 5 and 6). The infectivity of virus-infected cells was, in general, more rapidly abolished in the presence of AMT than in the presence of the same concentration of HMT. A 10^4-fold reduction in the infectivity of HSV-infected cells required 1.4×10^6 ergs of LWUV per cm^2 (2 min of LWUV exposure) in the presence of 10 μg of either psoralen per ml, compared with 3.3×10^6

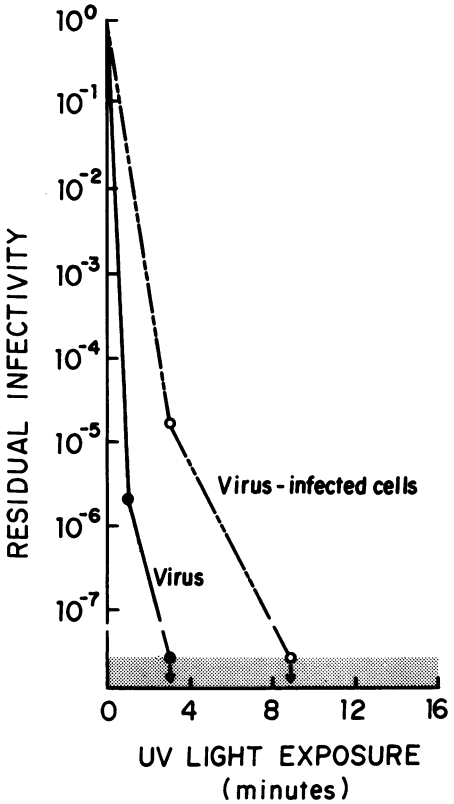

FIG. 2. *GUV inactivation of the infectivity of cell-free influenza virus (●) and influenza virus-infected Madin-Darby canine kidney cells (○). Shaded area indicates the lower limit of measurable infectivity. Arrows indicate time points at which virus infectivity was less than that detectable by the plaque assay.*

ergs of GUV per cm^2 (11 min of GUV exposure). A 10^4-fold reduction in the infectivity of influenza virus-infected cells also required 1.4×10^6 ergs of LWUV per cm^2 (2 min of LWUV exposure) in the presence of 10 μg of AMT per ml, compared with 9×10^5 ergs of GUV per cm^2 (3 min of GUV exposure). Recovery of intact virus-infected cells after all tested periods of exposure to LWUV in the presence of either psoralen was always >90% of the cells initially present.

Retention of antigenicity by virus-infected cells after GUV and psoralen-LWUV inactivation, as determine by immunofluorescence and [^{125}I]SPA radioimmunoassay. Since the object of these experiments was the preparation of noninfectious cells bearing virus antigens, we examined the effects of these inactivation procedures on the antigenicity of virus-infected cells.

(i) Immunofluorescence. The retention of virus antigens by virus-infected cells treated with GUV or psoralen-LWUV was examined by immunofluorescence as described in Materials and Methods. Both HSV- and influenza virus-infected cells exhibited membrane fluorescence, which appeared as a bright rim at the periphery of the cells. Neither GUV or psoralen-LWUV inactivation resulted in any discernible reduction in the percentage of cells positive for viral antigens or in the intensity or morphology of fluorescent staining (Tables 1 to 3). This was true even with conditions of inactivation that eliminated all measurable infectivity.

(ii) [^{125}I]SPA radioimmunoassay. The retention of viral antigens by virus-infected cells treated with GUV or psoralen-LWUV was examined by [^{125}I]SPA radioimmunoassay as described in Materials and Methods. Previous work in this laboratory has shown this method to be both sensitive and specific for the detection and quantitation of HSV antigens on infected cells (2). Under appropriate conditions, the amount of [^{125}I]SPA bound in the presence of a constant amount of antiviral antibody is directly proportional to the amount of virus antigen present. After incubation with viral antibody and then with [^{125}I]SPA, equivalent amounts of [^{125}I]SPA were bound by the GUV, psoralen-LWUV, and untreated infected cells. There was no reduction in the antigenicity detectable by this method, even with cells which had been rendered completely noninfectious (Tables 4 to 6).

Use of noninfectious virus-infected cells as antigen in a lymphocyte proliferation assay. The goal of these inactivation procedures was to prepare noninfectious virus antigens for in vitro assays in a form similar to that in which these antigens are presented to cells of the immune system in vivo. Therefore, we prepared intact virus-infected cells and utilized photoinactivation with GUV or psoralen-LWUV to eliminate their infectivity. However, we were concerned that the procedures used to eliminate infectivity might compromise the capacity of the virus-infected cells to stimulate antigen-specific lymphocyte responses. To assess this possibility, we examined the proliferative response of immune and nonimmune lymphocytes to HSV-infected cells which were uninactivated or which had been inactivated by psoralen-LWUV or GUV. Lymphocytes were prepared from rabbits and adult volunteers with and without prior HSV infection, as determined by clinical history and serum antibody testing. Lymphocytes were cultured alone, in the presence of mitogen (concanavalin A or phytohemagglutinin), in the presence of uninactivated or inactivated HSV-infected cells, or in the presence of similarly treated uninfected control cells. Lymphocyte proliferation, as measured by [^{3}H]thymidine in-

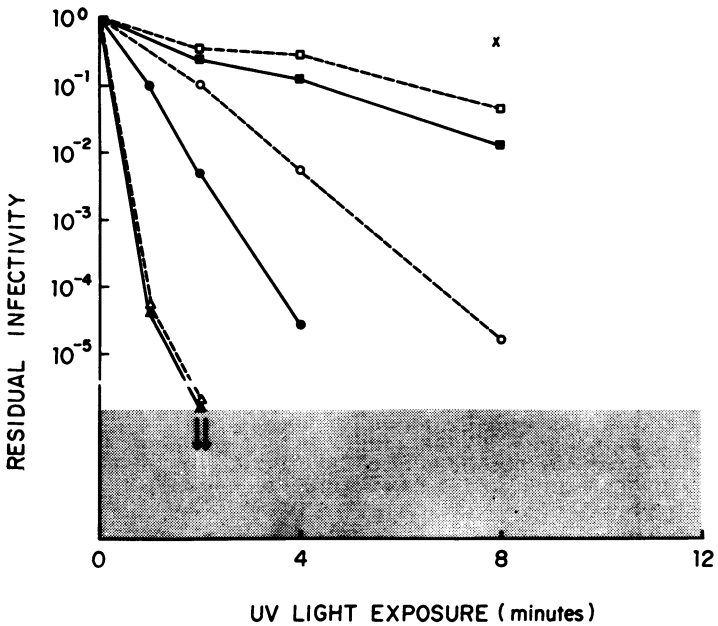

FIG. 3. *Psoralen-LWUV inactivation of HSV at three concentrations of HMT and AMT (in micrograms per milliliter): HMT, 0.1 (□); AMT, 0.1 (■); HMT, 1.0 (○); AMT, 1.0 (●); HMT, 10 (△); AMT, 10 (▲). (×) Titer of virus exposed to LWUV in the absence of psoralen. Shaded area indicates the lower limit of measurable infectivity. Arrows indicate time points at which virus infectivity was less than that detectable by the plaque assay.*

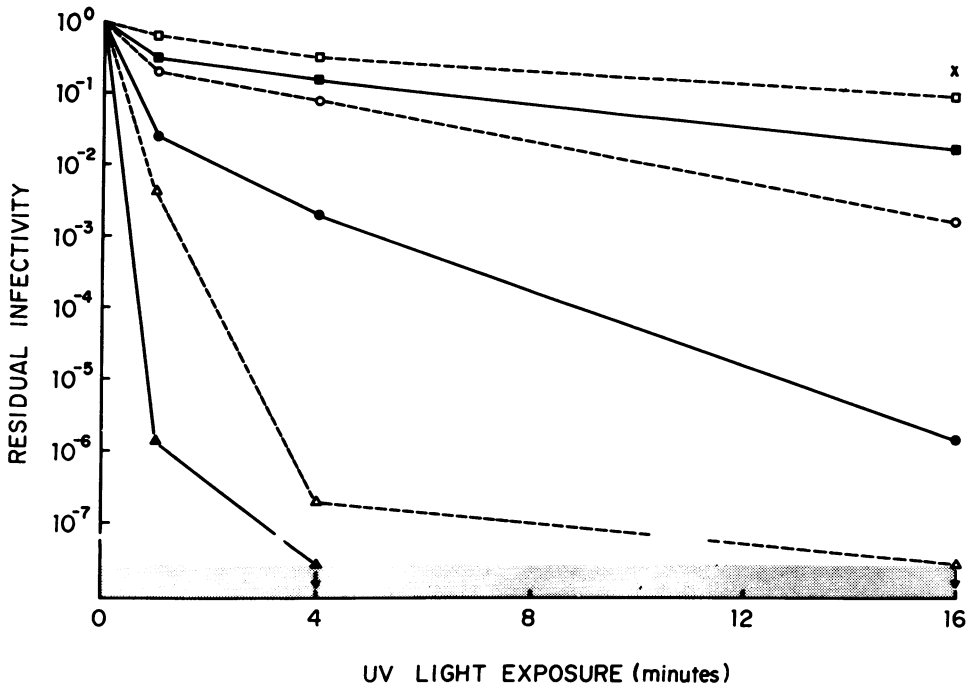

FIG. 4. *Psoralen-LWUV inactivation of influenza virus at three concentrations of HMT and AMT (in micrograms per milliliter): HMT, 0.1 (□); AMT, 0.1 (■); HMT, 1.0 (○); AMT, 1.0 (●); HMT, 10 (△); AMT, 10 (▲). (×) Titer of virus exposed to LWUV in the absence of psoralen. Shaded area indicates the lower limit of measurable infectivity. Arrows indicate time points at which virus infectivity was less than that detectable by the plaque assay.*

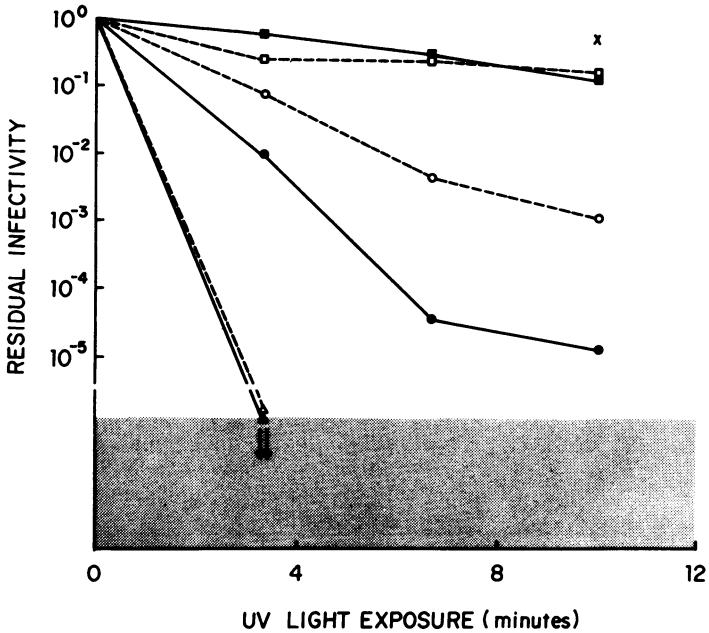

FIG. 5. *Psoralen-LWUV inactivation of the infectivity of HSV-infected RK13 cells at three concentrations of HMT and AMT (in micrograms per milliliter): HMT, 0.1 (□); AMT, 0.1 (■); HMT, 1.0 (○); AMT, 1.0 (●); HMT 10 (△); AMT, 10 (▲). (×) Titer of virus exposed to LWUV in the absence of psoralen. Shaded area indicates the lower limit of measurable infectivity. Arrows indicate time points at which virus infectivity was less than that detectable by the plaque assay.*

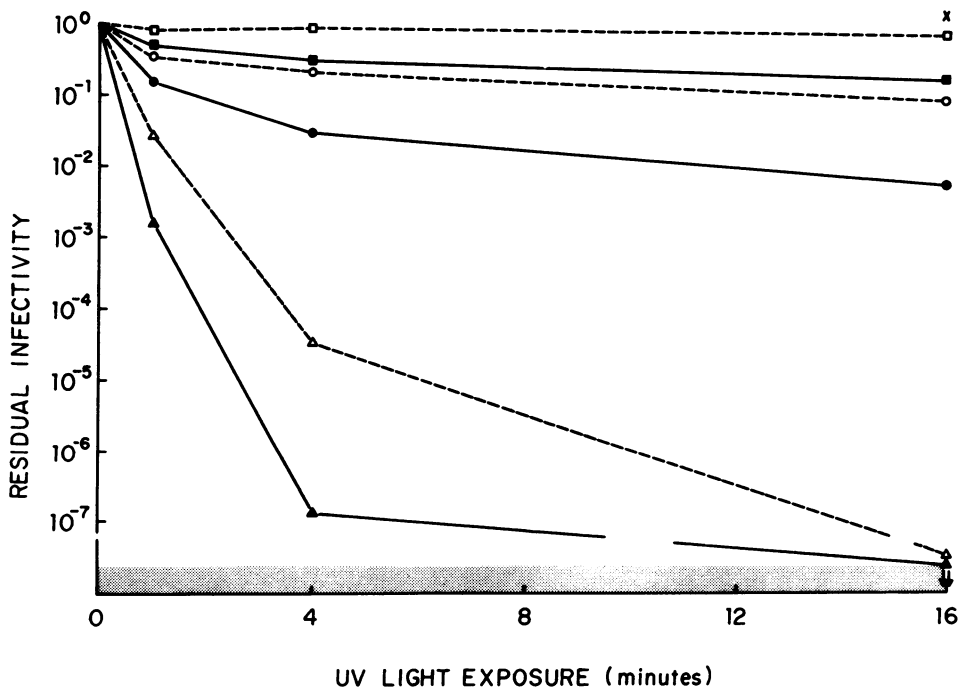

FIG. 6. *Psoralen-LWUV inactivation of the infectivity of influenza virus-infected Madin-Darby canine kidney cells at three concentrations of HMT and AMT (in micrograms per milliliter): HMT, 0.1 (□); AMT, 0.1 (■); HMT, 1.0 (○); AMT, 1.0 (●); HMT, 10 (△); AMT, 10 (▲). (×) Titer of virus exposed to LWUV in the absence of psoralen. Shaded area indicates the lower limit of measurable infectivity. Arrows indicate time points at which virus infectivity was less than that detectable by the plaque assay.*

TABLE 1. *Effect of GUV irradiation on antigenicity of virus-infected cells as determined by immunofluorescence*

Virus	% of cells with virus antigen after indicated period of GUV exposure[a]					
	0 min	1 min	3 min	9 min	27 min	81 min
HSV	94	ND[b]	95	93	91	96
Influenza	72	74	65	69	69	ND

[a] Values represent the percentage of cells exhibiting membrane fluorescence after immunofluorescent staining, as described in the text. There was no discernible reduction in the intensity of fluorescent staining in the irradiated cells.
[b] ND, Not determined.

TABLE 2. *Effect of psoralen-LWUV treatment on antigenicity of HSV-infected cells as determined by immunofluorescence*

Psoralen (μg/ml)	% of cells with virus antigen after indicated period of LWUV exposure[a]			
	0 min	3.3 min	6.7 min	10 min
None	91	ND[b]	ND	92
HMT (0.1)	ND	95	93	89
HMT (1.0)	ND	85	93	91
HMT (10)	95	94	85	91
AMT (0.1)	ND	94	87	86
AMT (1.0)	ND	88	93	86
AMT (10)	87	90	92	88

[a] Virus-infected cells were exposed to LWUV in the presence of the indicated concentration of HMT or AMT. Values represent the percentage of cells exhibiting membrane fluorescence after immunofluorescent staining, as described in the text. There was no discernible reduction in the intensity of fluorescent staining in any of the irradiated cells.
[b] ND, Not determined.

corporation, was examined after 6 days of culture in vitro. A positive virus-specific response was defined as a stimulation index of >3. Lymphocytes from HSV-immune donors demonstrated virus-specific stimulation, whereas lymphocytes from nonimmune donors did not. At the same effector cell-to-target cell ratio, HSV-infected cells rendered noninfectious by psoralen-LWUV or by GUV stimulated immune lymphocytes to the same degree as did untreated HSV-infected cells (Table 7). On a per-cell basis, virus antigen dose-response curves were equivalent for psoralen-LWUV-inactivated, GUV-inactivated, and uninactivated cells (Fig. 7). Thus, exposure to psoralen-LWUV or GUV sufficient to totally abolish infectivity does not appear to alter the ability of virus antigen-bearing cells to stimulate the virus-specific proliferative response of immune lymphocytes.

TABLE 3. *Effect of psoralen-LWUV treatment on antigenicity of influenza virus-infected cells as determined by immunofluorescence*

Psoralen (μg/ml)	% of cells with virus antigen after indicated period of LWUV exposure[a]:			
	0 min	1 min	4 min	16 min
None	87	ND[b]	ND	88
HMT (0.1)	ND	91	80	79
HMT (1.0)	ND	94	83	91
HMT (10)	85	89	84	84
AMT (0.1)	ND	81	90	78
AMT (1.0)	ND	88	84	84
AMT (10)	87	92	84	84

[a] Virus-infected cells were exposed to LWUV in the presence of the indicated concentration of HMT or AMT. Values represent the percentage of cells exhibiting membrane fluorescence after immunofluorescent staining, as described in the text. There was no discernible reduction in the intensity of fluorescent staining in any of the irradiated cells.
[b] ND, Not determined.

DISCUSSION

This study demonstrates preservation of the antigenicity of virus-infected cells rendered noninfectious by psoralen-LWUV or GUV irradiation. Such noninfectious, virus antigen-bearing cells should have many uses in viral immunodiagnosis (32). Our studies reveal that these cells are suitable as target antigens in serological assays and that they retain the capacity to stimulate virus-specific lymphocyte proliferation.

Virus antigens used in assays of cell-mediated immunity should be noninfectious to eliminate the risk of infection of laboratory personnel and the potential complication of virus infection of effector cells. It may also be desirable to present virus antigens to the effector cells in the form of antigen-bearing, virus-infected cells rather than as cell-free virus or soluble antigen. Our examination of psoralen-LWUV inactivation was prompted by preliminary experiments which indicated that complete elimination of the infectivity of HSV-infected cells by GUV required a prolonged period of irradiation. Prolonged GUV irradiation of viruses has been shown to result in virus preparations of reduced antigenicity (10) and reduced immunogenicity (12, 16). Therefore, we examined the capacity of psoralen-LWUV to inactivate cell-free virus and to abolish the infectivity of virus-infected cells. This study demonstrates that the infectivity of intact, virus-infected cells can be eliminated by psoralen-LWUV and that this can be accomplished without loss of virus antigenicity, as measured by immunofluorescence, radioimmunoassay, and in

TABLE 4. *Effect of GUV irradiation on antigenicity of virus-infected cells as determined by [^{125}I]SPA radioimmunoassay*

Virus	Virus-specific cpm of [^{125}I]SPA bound to cells exposed to GUV for the indicated period of time[a]					
	0 min	1 min	3 min	9 min	27 min	81 min
HSV	3,155 ± 130	ND[b]	3,207 ± 214	2,935 ± 269	2,755 ± 353	2,952 ± 194
Influenza	2,064 ± 197	2,260 ± 386	2,225 ± 195	2,018 ± 242	2,172 ± 267	ND

[a] Virus-infected cells were exposed to GUV and then assayed for virus antigen by [^{125}I]SPA radioimmunoassay as described in the text. "Virus-specific cpm" is defined as the counts per minute of [^{125}I]SPA bound to cells incubated with virus-immune serum minus the counts per minute of [^{125}I]SPA bound to cells incubated with nonimmune (control) serum. Each value represents the mean ± standard deviation of four replicate determinations.

[b] ND, Not determined.

TABLE 5. *Effect of psoralen-LWUV treatment on antigenicity of HSV-infected cells as determined by [^{125}I]-SPA radioimmunoassay*

Psoralen (μg/ml)	Virus-specific cpm of [^{125}I]SPA bound to cells exposed to LWUV for the indicated period of time[a]			
	0 min	3.3 min	6.7 min	10 min
None	1,441 ± 42	ND[b]	ND	1,498 ± 112
HMT (0.1)	ND	1,666 ± 85	1,478 ± 67	1,524 ± 137
HMT (1.0)	ND	1,553 ± 290	1,213 ± 70	1,106 ± 49
HMT (10)	1,131 ± 195	1,351 ± 23	1,195 ± 87	1,427 ± 79
AMT (0.1)	ND	1,399 ± 179	1,382 ± 214	1,277 ± 81
AMT (1.0)	ND	1,472 ± 202	1,466 ± 257	1,260 ± 82
AMT (10)	1,138 ± 119	1,247 ± 116	1,228 ± 135	1,217 ± 133

[a] Virus-infected cells were exposed to LWUV in the presence of the indicated concentration of HMT or AMT and assayed for virus antigen by [^{125}I]SPA radioimmunoassay as described in the text. "Virus-specific cpm" is defined as the counts per minute of [^{125}I]SPA bound to cells incubated with virus-immune serum minus the counts per minute of [^{125}I]SPA bound to cells incubated with nonimmune (control) serum. Each value represents the mean ± standard deviation of four replicate determinations.

[b] ND, Not determined.

TABLE 6. *Effect of psoralen-LWUV treatment on antigenicity of influenza virus-infected cells as determined by [^{125}I]SPA radioimmunoassay*

Psoralen (μg/ml)	Virus-specific cpm of [^{125}I]SPA bound to cells exposed to LWUV for the indicated period of time[a]			
	0 min	1 min	4 min	16 min
None	2,915 ± 441	ND[b]	ND	3,192 ± 515
HMT (0.1)	ND	2,286 ± 123	2,736 ± 147	2,639 ± 503
HMT (1.0)	ND	2,461 ± 425	3,015 ± 282	2,909 ± 272
HMT (10)	3,629 ± 368	2,873 ± 197	2,514 ± 839	2,670 ± 274
AMT (0.1)	ND	3,151 ± 346	3,248 ± 242	2,648 ± 156
AMT (1.0)	ND	2,346 ± 795	3,828 ± 337	3,183 ± 207
AMT (10)	3,018 ± 582	3,665 ± 510	3,215 ± 157	3,056 ± 270

[a] Virus-infected cells were exposed to LWUV in the presence of the indicated concentration of HMT or AMT and assayed for virus antigen by [^{125}I]SPA radioimmunoassay as described in the text. "Virus-specific cpm" is defined as the counts per minute of [^{125}I]SPA bound to cells incubated with virus-immune serum minus the counts per minute of [^{125}I]SPA bound to cells incubated with nonimmune (control) serum. Each value represents the mean ± standard deviation of four replicate determinations.

[b] ND, Not determined.

vitro lymphocyte proliferation. The use of psoralen-LWUV offers significant advantages over GUV, especially in the case of HSV-infected cells. It totally eliminates their infectivity in a much shorter period of time, requires the delivery of less radiant energy, and permits the recovery, intact, of essentially all of the cells initially present.

ACKNOWLEDGMENTS

We appreciate the excellent technical assistance of Laura Terlizzi and Sara Albanil. We are also indebted to John E.

TABLE 7. *Effect of GUV and psoralen-LWUV inactivation on capacity of HSV-infected cells to stimulate in vitro virus-specific lymphocyte proliferation*

Mitogen or antigen	Nonimmune rabbit		Immune rabbit		Nonimmune human		Immune human	
	cpm[a]	SI[b]	cpm	SI	cpm	SI	cpm	SI
Uninfected cells (2×10^3/culture)	266 ± 11		266 ± 75		2,724 ± 197		708 ± 122	
Uninfected HSV-infected cells (2×10^3/culture)	303 ± 52	1.1	4,758 ± 329	17.9	1,544 ± 130	0.6	13,924 ± 1,013	19.7
Psoralen-LWUV-inactivated, HSV-infected cells (2×10^3/culture)	207 ± 10	0.8	4,540 ± 266	17.0	3,742 ± 507	1.4	15,457 ± 286	21.8
GUV-inactivated, HSV-infected cells (2×10^3/culture)	304 ± 12	1.1	3,789 ± 549	14.2	3,050 ± 264	1.1	15,257 ± 406	21.5
None (medium alone)	459 ± 7		264 ± 36		1,207 ± 303		379 ± 51	
Concanavalin A (50 μg/ml)	46,676 ± 683	101.7	90,809 ± 12,161	344.0	ND[c]		ND	
Phytohemagglutinin (10 μg/ml)	ND		ND		18,692 ± 1,955	15.5	44,295 ± 1,046	116.8

[a] A total of 2×10^5 lymphocytes were exposed to the indicated antigen or mitogen for 6 days, and [^{3}H]thymidine incorporation during the final 24 h of incubation was measured. Each value represents the mean ± standard deviation from triplicate cultures.

[b] SI, Stimulation index = counts per minute incorporated by lymphocytes incubated with virus-infected cells/counts per minute incorporated by lymphocytes incubated with uninfected cells or counts per minute incorporated by lymphocytes incubated with mitogen/counts per minute incorporated by lymphocytes incubated with medium alone.

[c] ND, Not determined.

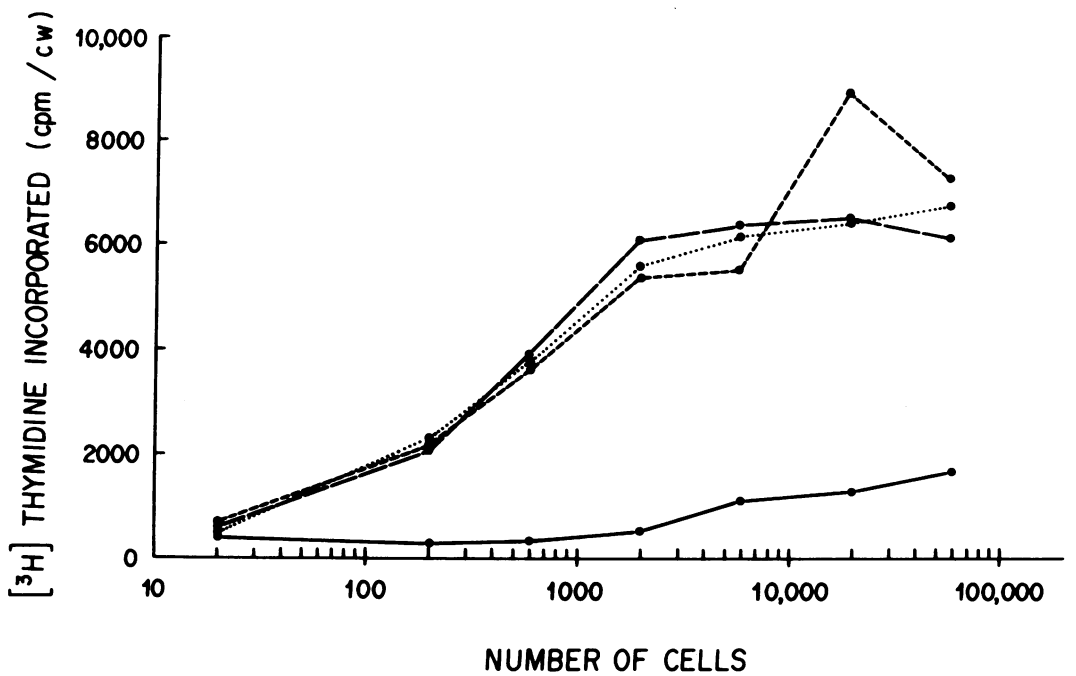

FIG. 7. *Effect of GUV and psoralen-LWUV inactivation on the capacity of HSV-infected RK13 cells to stimulate HSV-specific lymphocyte proliferation. A total of 2×10^5 lymphocytes from an HSV-immune individual were cultured for 6 days with various numbers of uninfected RK13 cells (●——●); HSV-infected but uninactivated RK13 cells (●– –●); GUV-inactivated, HSV-infected RK13 cells (●— —●); or psoralen-LWUV-treated, HSV-infected RK13 cells (●·····●). [^{3}H]thymidine (0.25 μCi) was added for the last 24 h, and thymidine incorporation was determined as described in the text. Each point represents the mean of triplicate determinations. cpm/cw, Counts per minute per culture well.*

Hearst, University of California, Berkeley, for advice and encouragement.

This work was supported by the Veterans Administration and by Public Health Service research contract AI-92615, Public Health Service research grant AI-13872, and Public Health Service training grant AI-07036 from the National Institute of Allergy and Infectious Disease.

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