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Radiobiological Inactivation of Epstein-Barr Virus

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Lymphocyte transforming properties of B95-8 strain Epstein-Barr virus (EBV) are very sensitive to inactivation by either UV or X irradiation. No dose of irradiation increases the transforming capacity of EBV. The X-ray dose needed for inactivation of EBV transformation (dose that results in 37% survival, 60,000 rads) is similar to the dose required for inactivation of plaque formation by herpes simplex virus type 1 (Fischer strain). Although herpes simplex virus is more sensitive than EBV to UV irradiation, this difference is most likely due to differences in the kinetics or mechanisms of repair of UV damage to the two viruses. The results lead to the hypothesis that a large part, or perhaps all, of the EBV genome is in some way needed to initiate transformation. The abilities of EBV to stimulate host cell DNA synthesis, to induce nuclear antigen, and to immortalize are inactivated in parallel. All clones of marmoset cells transformed by irradiated virus produce extracellular transforming virus. These findings suggest that the abilities of the virus to transform and to replicate complete progeny are inactivated together. The amounts of UV and X irradiation that inactivate transformation by B95-8 virus are less than the dose needed to inactivate early antigen induction by the nontransforming P_3HR-1 strain of EBV. Based on radiobiological inactivation, 10 to 50% of the genome is needed for early antigen induction. Inactivation of early antigen induction is influenced by the cells in which the assay is performed. Inactivation proceeds more rapidly in EBV genome-free cells than in genome carrier Raji or in P_3HR-1 converted EBV genome-free cells clone B_1. These results indicate that the resident EBV genome participates in the early antigen induction process. Variation in radiobiological killing of B95-8 and P_3HR-1 EBV is not attributable to variations in the repair capacities of the cells in which the viruses were assayed, since inactivation of HSV was the same in primary lymphocytes and in all lymphoid cell lines tested.

Radiobiological inactivation of DNA tumor viruses provided important early clues to the mechanisms of cell transformation by these agents. For example, before viral mutants conditionally lethal in transformation became available and before cell transformation by DNA fragments was accomplished, radiobiological experiments with polyoma and simian virus 40 indicated that the size of the virus genome needed for transformation was 45 to 65% of that of the genome required for complete viral replication and plaque formation (1, 3, 4). An increase in transforming potential of simian virus 40 and adeno-simian virus 40 hybrid virus was demonstrated after a low dose of UV exposure (6, 7). The ability of herpes simplex viruses (HSV) to transform permissive cells was only manifest after radiobiological inactivation, presumably since lytic effects of the intact viral genome masked the potential of the transforming genes (8).

Inactivation of RNA tumor viruses by UV light or X ray has produced somewhat different results. Although it is now known that the *sarc* gene is but one of four main genetic regions, radiobiological inactivation experiments showed that, in contrast to DNA viruses, inactivation of replicative functions of Rous sarcoma virus was usually accompanied by impairment of the ability of the virus to induce foci (18, 25). This finding suggests that other parts of the viral genome participate in the transformation process in some indirect way, perhaps by permitting replication, integration, or transcription of the *sarc* gene.

Since no Epstein-Barr virus (EBV) mutants are yet available and since lymphoid cell transformation by EBV DNA has not yet been achieved, we undertook radiobiological experiments before undertaking a more detailed genetic analysis of the mechanism of transformation. We also hoped that such experiments

would shed light on major biological differences that exist between two prototype laboratory strains of EBV (20, 22). Presently available stocks of one strain, P_3J-HR-1 (HR-1) from Burkitt lymphoma, do not cause lymphoid cell immortalization. Instead the HR-1 virus causes inhibition of cell DNA synthesis (22) and ultimately cell death in various lymphoid lines. After HR-1 infection, viral DNA is replicated (34) and early viral antigens are induced (12). The B95-8 virus immortalizes primary lymphocytes, but it fails to induce early antigen (EA) or replicate in established EBV genome carrier cell lines such as Raji.

These radiobiological inactivation experiments were designed to derive information about the relative target sizes of the genes responsible for abortive replication and cell transformation. The strategy involved the use of HSV as an independent marker for host cell repair processes in different lymphoid cells and as a marker for target size.

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MATERIALS AND METHODS

Cells. We have recently described, in detail, techniques for preparation and culture of primary human and marmoset leukocytes, enriched in B lymphocytes after removal of T cell rosetting sheep erythrocytes (29). Three continuous B lymphoid cell lines were employed. Raji, from a Burkitt lymphoma, contains the EBV genome (35). EBV genome-free cells (BJAB) are also of Burkitt lymphoma origin (15). This line was obtained from G. Klein, Karolinska Institute, Stockholm, Sweden, through the courtesy of Wilma Summers, Yale University, New Haven, Conn. The BJAB line has been converted into an EBV genome carrier line by exposure to the HR-1 strain of EBV (9). The "B-1" subclone of BJAB/HR-1 with brilliant Epstein-Barr nuclear antigen (EBNA), the gift of H. zur Hausen, University of Freiburg, Freiburg, Germany, was used.

Virus. The B95-8 strain of EBV was cloned by limiting dilution transformation of primary marmoset leukocytes (11). Virus stocks with about 10^5 50% transforming units per ml were prepared from supernatant fluids of the transformed marmoset cell lines (21). Nontransforming HR-1 EBV was harvested from supernatant fluids of HR-1 cells grown at 32°C. There were about 10^5 EA inducing units per ml. Stocks of both strains contained about 10^7 particles per ml. A strain of HSV type 1 (Fischer strain) (HSV-1) isolated from the brain of a person with encephalitis was plaque purified and propagated at a low multiplicity in Vero cells (33). The stock contained 7.5×10^6 PFU/ml. All virus stocks were filtered through a 0.45-μm membrane filter (Millipore Corp.) to reduce clumping.

Virus inactivation. Total emission from a 15-W GE 15T8 low-pressure mercury germicidal lamp was the UV source. Incident (254 nm) UV dose rate was 25 ergs per mm^2 per s, determined by a dosimeter constructed and calibrated by R. Latarjet (16) and kindly loaned by D. Rupp. One-milliliter portions of virus stock in spent culture medium were placed in 60-mm petri dishes. Exposures were done with gentle agitation to ensure uniform irradiation, and samples were kept on ice before and after irradiation. X rays (250 kV) were used at a dose rate of 4,000 rads per min. The X-irradiation source consisted of a Siemens therapeutic X-ray machine with no added filtration, and dosimetry was established by conversion of Fe^{2+} to Fe^{3+}, measured by optical density. Virus stocks were subjected to X rays while frozen to minimize secondary damage due to free radical formation. Under these conditions, only the direct effect of X rays is expected.

Assays for EBV, EA, and EBNA. The transforming titers of virus were measured on human or marmoset lymphocytes with a microwell limiting dilution assay (11). Transformants were scored at 8 weeks. Several virus dilutions were prepared, and the data were calculated from dilutions that transformed fewer than 100% of the wells. From 8 to 16 microwells per point were used. Stimulation of DNA synthesis was assessed by [^{3}H]dT incorporation (30). Data were calculated on the basis of total counts per minute of [^{3}H]dT stimulated by addition of virus; ^{3}H counts per minute in uninfected cultures was first subtracted. [^{3}H]dT incorporation was measured 3 and 7 days after infection. The number of human umbilical cord lymphocytes (HUCL) with EBNA was determined 6 days after addition of virus by the technique of anticomplement immunofluorescence (28). EA was detected 3 days after addition of the HR-1 virus to the B lymphoid lines (12). A serum pool kindly provided by W. Henle was used to detect EA in the indirect immunofluorescence test. In the assays for EA and EBNA, 1,000 cells per point were counted by two observers, and the results were pooled.

HSV-1-infected centers (IC). Lymphocytes diluted in medium to a concentration of 10^6 cells per ml were inoculated with 7.5×10^5 PFU of HSV-1. After 24 h of incubation at 37°C, the cells were sedimented and then suspended in medium containing a 1:10 dilution of heated (56°C) human serum as the source of HSV neutralizing antibody. This serum dilution neutralized about 5×10^4 PFU of HSV-1. Cells were held at room temperature for 1 to 2 h to complete neutralization and then were sedimented, resuspended, and counted. A 0.1-ml portion of dilutions containing 10^4 and 10^3 cells was plated onto duplicate Vero monolayers in Corning flasks (25 cm^2) after centrifugation at 1,000 rpm for 10 min. The supernatants from 10^4 cells were also plated. The cultures were overlaid with Eagle modified essential medium containing 5% fetal bovine serum and 1% Methocel and were incubated for 4 days at 37°C. Because HSV develops small plaques after UV irradiation, the plaques stained with Giemsa were counted microscopically (31). The number of plaques present in the supernatant fluids was subtracted from the number of plaques obtained with infected lymphocytes.

RESULTS

UV inactivation of transforming functions of EBV. We assayed three biological func-

tions of the EBV genome related to the establishment of continuous cell lines. These functions were morphological transformation leading to immortalization, stimulation of cellular DNA synthesis, and induction of the EBNA. We also measured morphological transformation of marmoset lymphocytes by quantitative limiting dilution (11). The effect of graded UV irradiation on the survival of the transforming functions of the B95-8 virus is shown in Fig. 1. Nearly identical inactivation curves were obtained when immortalization was measured on human or marmoset cells; the dose that results in 37% survival (LD_{37}) was 650 to 750 ergs. No increase in transformation rate was detected at any UV dose. EBNA induction determined on day 6 and stimulation of DNA synthesis measured on day 7 were inactivated at about the same UV dose (LD_{37}, 1,050 ergs), slightly more slowly than morphological transformation.

Among the many factors that can influence virus inactivation by UV light are the suspending medium in which the inactivation is performed and the time elapsed between application of UV irradiation and measurement of the biological function (Table 1). Virus suspended in spent medium or pelleted and suspended in 1 M HEPES buffer (pH 7.2) was inactivated and applied to human lymphocytes. DNA synthesis was measured 3 and 7 days after virus exposure. At both times slightly greater inactivation was obtained in HEPES buffer than in the medium, though the difference between the two suspending fluids was minimal and did not affect the inactivation curves. In both experiments, inactivation was more rapid on day 3 than on day 7, a finding compatible with some role of host cell repair of UV-damaged virus.

X-ray inactivation of transformation. We sought to complement UV-inactivation data with results obtained by inactivation with X irradiation. Not only do these two forms of irradiation inactivate biological material through different molecular mechanisms, but, as a further rationale, survival curves obtained by X irradiation are less influenced by host cell or photo-reactivation (19). Identical X-ray survival curves (Fig. 2) were obtained whether transformation was measured by [^{3}H]dT incorporation or by titration of morphological changes in human or marmoset cells. In all three systems, the LD_{37} was 60,000 to 64,000 rads. No enhancement of transformation was seen at the X-ray doses employed.

The survival curves for both UV and X-ray inactivation showed one-hit kinetics; therefore it was doubtful that multiple virus particles were initiating transformation, even in marmoset cells, in which the ratio of virus added to the eventual number of transformants is high.

Virus production by marmoset cells transformed by irradiated virus. Cotton-top marmoset leukocytes, unlike comparable human cells, release mature extracellular virus after transformation in vitro by undamaged EBV (21). This permitted us to assess the effects of irradiation on release of complete virus by transformed cells obtained after exposure to irradiated virus

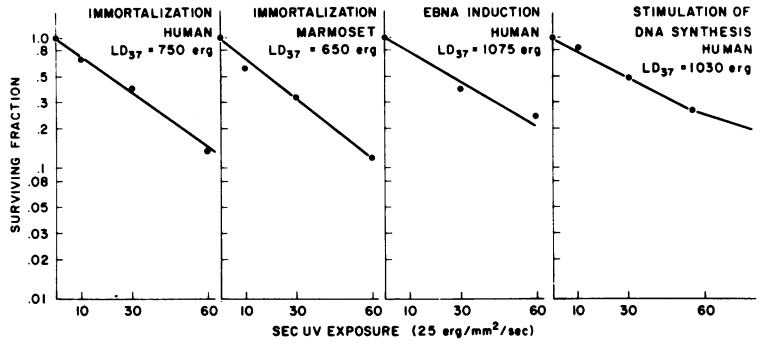

FIG. 1. *UV inactivation of transforming functions of B95-8 strain EBV. Virus suspended in spent medium was exposed to 25 ergs per mm² per s and then added to cultures of HUCL or CTML. (Panel 1) Immortalization of HUCL assessed morphologically 8 weeks after virus exposure. Data are shown for a 10^{-4} dilution of virus. Each point represents transformation frequency of 16 microwells. In the absence of irradiation, 14 of 16 microwells transformed. (Panel 2) Immortalization of cotton-top marmoset leukocytes assessed morphologically 8 weeks after virus exposure. A 10^{-1} dilution of virus was used. Data represent 14 microwells per point, from two experiments. In the absence of UV irradiation, 11 of 14 wells transformed. (Panel 3) EBNA induction in HUCL determined by counts of the proportion of 1,000 cells with the antigen 6 days after virus exposure. Data are a composite of two experiments in which unirradiated virus caused 20 and 30% of the cells to become EBNA positive. (Panel 4) Stimulation of DNA synthesis in HUCL measured by incorporation of [^{3}H]dT after a 1-h pulse with 6 μCi/ml performed on day 7 after virus exposure. Duplicate determinations were made for each point. The background incorporation by uninfected cells of 1,764 was subtracted. The value for 100% survival was 22,644 cpm (see Table 1).*

TABLE 1. *Comparative UV inactivation of EBV suspended in 1 M HEPES buffer (pH 7.2) or spent medium*

Fluence (ergs/mm²)	Virus suspended in medium				Virus suspended in buffer			
	Day 3		Day 7		Day 3		Day 7	
	^{3}H cpm[a]	Ratio	cpm	Ratio	cpm	Ratio	cpm	Ratio
None	3,094	1.00	22,644	1.00	4,086	1.00	11,621	1.00
250	2,569	0.83	21,342	0.94	2,668	0.65	8,010	0.69
750	905	0.29	15,774	0.69	1,158	0.28	5,654	0.48
1,500	856	0.27	6,044	0.26	620	0.15	2,459	0.21
3,750	405	0.13	1,044	0.04	366	0.08	610	0.05
7,500	283	0.09	466	0.02	53	0.01	608	0.05
11,250	112	0.03	310	0.01	−207	−0.05	338	0.03
Background	2,268		1,764		3,750		2,459	

[a] In experiment 1, virus suspended in medium was exposed to UV light and then added to HUCL. [^{3}H]dT incorporation was measured in two replicate 0.5-ml cultures on days 3 and 7 after virus exposure, and the data were averaged. In experiment 2, supernatant fluid containing virus was centrifuged at 25,000 rpm for 1 h at 4°C in a no. 30 rotor, and the pellets were suspended in a 1/20 volume of cold 1 M HEPES buffer (pH 7.2). A different batch of HUCL was used, and [^{3}H]dT incorporation was assayed on days 3 and 7.

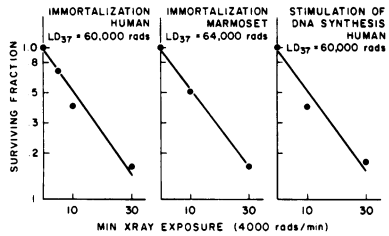

FIG. 2. *X-ray inactivation of transforming functions of B95-8 EBV. Frozen virus suspended in spent medium was exposed to 4,000 rads per min and then added to cultures of HUCL or cotton-top marmoset leukocytes. (Panel 1) Immortalization of HUCL. A 10^{-4} dilution of virus was used. Seven of eight wells transformed without irradiation. (Panel 2) Immortalization of cotton-top marmoset leukocytes. A 10^{-1} dilution of virus was used. Seven of eight wells transformed without irradiation. (Panel 3) Stimulation of DNA synthesis. [^{3}H]dT incorporation was measured 7 days after infection by means of a 1-h pulse. Background of 7,550 ^{3}H cpm in uninfected cells was subtracted. The net counts per minute stimulated by virus in the absence of irradiation was 17,317.*

(Table 2). The majority of sublines tested resulted from exposure to virus treated with 1,500 ergs of UV irradiation. There was about a 75% reduction of transformation at this UV dose. All sublines, whether exposed to unirradiated, UV-treated, or X-irradiated virus, were producers of biologically active virus. The data indicate that transformation and the potential for replication of mature virus were inactivated in parallel.

Radiobiological inactivation of the induction of EA by HR-1 virus. In preliminary experiments, we found that the UV LD_{37} for EA induction by HR-1 virus in Raji cells was about 8,000 ergs. This dose is about 8 to 10 times more than that required to inactivate the transforming properties of B95 virus (results presented at Herpes Virus Workshop, Cold Spring Harbor, N.Y., August 1976). These findings suggested that the target size representative of transforming functions was larger than the target responsible for EA induction. However, two other possible explanations had to be considered experimentally. First, the EBV genome already resident in Raji cells might influence the inactivation rate of superinfecting HR-1 virus. Second, repair of radiation-damaged virus might differ in primary lymphocytes as opposed to continuous lines. To investigate the first possibility, UV inactivation of EA induction was measured in EBV genome-positive and -negative lymphoid lines that are susceptible to abortive infection (Fig. 3). It was possible to do this experiment in the same line with and without the genome, since the BJAB line has a counterpart, designated BJAB/HR-1 clone B_1, which was permanently converted to EBV-carrier status by means of superinfection with the HR-1 virus (9). Inactivation of the abortive infecting properties of HR-1 virus by UV light proceeded more rapidly in the genome-negative than in the genome-positive cell lines. In Raji cells, a slight increase (1.3-fold) in the number of EA-positive cells above the level seen with untreated virus was observed when HR-1 virus exposed to low doses of UV light (<250 ergs) was used. The LD_{37} for inactivation of HR-1 virus in the two genome-positive lines, about 8,000 ergs, was fivefold more than that measured in the genome-free BJAB cells.

A similar experiment was performed with HR-1 virus exposed to X rays. The results with X-

TABLE 2. *EBV production by marmoset cells transformed by irradiated virus*

Treatment	No. of sublines releasing virus/no. of sublines tested determined by [^{3}H]dT stimulation[a]	^{3}H cpm median (range)[a]	No. of sublines releasing virus/no. of sublines tested determined by morphological transformation[b]
None	10/10	21,459 (8,928–30,407)	10/10
UV			
750 ergs/mm^2	4/4	28,535 (25,095–29,616)	4/4
1,500 ergs/mm^2	4/4	23,552 (20,452–25,586)	16/16
X ray			
40,000 rads	4/4	15,399 (5,774–27,977)	4/4
120,000 rads	1/1	15,848	1/1
240,000 rads	1/1	3,755	1/1

[a] [^{3}H]dT counts per minute above background when supernatant fluids from transformed marmoset cells were added to primary HUCL that received a 1-h pulse with [^{3}H]dT (6 μCi/ml) 7 days after inoculation. The data represent two different assays in which the background level of [^{3}H]dT incorporation by uninfected cells of 6,636 and 2,869 was first subtracted.

[b] A 1:10 dilution of supernatant fluids from transformed marmoset cells was added to HUCL. Transformation was detected after 2 to 4 weeks.

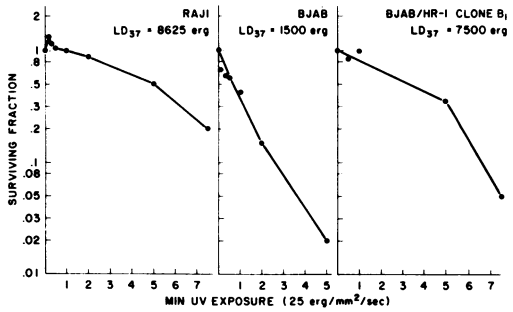

FIG. 3. *UV inactivation of EA induction by HR-1 strain EBV in three lymphoid lines. All lines were derived from Burkitt lymphoma. Raji and BJAB/HR-1 clone B_1 have the EBV genome. Irradiated virus was added to each line, and the number of antigen-positive cells was enumerated after 3 days. The data are derived from a mean of two inactivation experiments with each cell line. The fraction of cells showing EA in the absence of UV irradiation was 8.2% in Raji, 6.4% in BJAB, and 10.3% in clone B_1.*

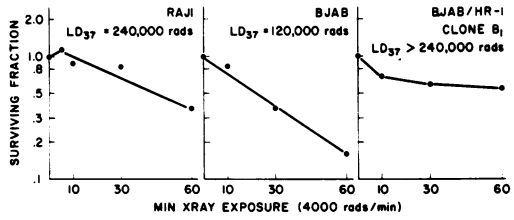

FIG. 4. *X-ray inactivation of EA induction by HR-1 strain EBV in three lymphoid lines. Irradiated virus was added to each line, and the number of antigen-positive cells was enumerated after 3 days. Data are derived from a mean of two inactivation experiments with each line. The fraction of cells with EA in the absence of X irradiation was 9.4% in Raji, 6.1% in BJAB, and 13.0% in clone B_1.*

irradiated virus were similar to those with UV-treated virus; namely, the ability of HR-1 virus to induce EA was more sensitive to inactivation by X ray when measured in the genome-negative BJAB line than when measured in its EBV-converted counterpart or in Raji (Fig. 4). Once again more EA-positive cells were detected in Raji cells infected with HR-1 virus treated with low doses of X irradiation (~20,000 rads) than in Raji cells exposed to untreated virus. The results of this group of experiments show that HR-1 virus was more resistant to inactivation when it was assayed in genome-positive target cells. However, both UV and X-ray data indicated that even in genome-free BHAB cells inactivation of EA induction was more resistant than was inactivation of transformation. This finding suggests true differences in the target size of these two major functions of different EBV biotypes.

Attempted conversion of biotypes by UV irradiation. A stock of HR-1 strain with 10^5 EA inducing units per ml was exposed to various doses of UV light, from 250 to 15,000 ergs per mm^2. Twenty-four microwells of HUCL were inoculated with virus treated with each dose. UV-treated virus failed to immortalize HUCL. Similarly, B95 virus, treated with a similar dose range of UV light, was unable to induce EA in Raji cells. Coinfection of HUCL with lethally irradiated B95-8 and unirradiated HR-1 did not result in transformation. Similar results have been described by Gerber (10).

Radiobiological inactivation of formation of IC by HSV in lymphoid cells. To evaluate the data for radiobiological inactivation

of transforming and nontransforming EBV, we wished to have an independent assay that would allow us to compare the ability of different lymphoid cell systems to repair radiation-damaged virus. Furthermore, we wished to have a marker for the target size of a genome of about 10^8 daltons. For these experiments we determined the inactivation rates of HSV-1 replication in lymphoid cells as measured by the formation of IC.

We used the three B lymphocyte lines, Raji, BJAB, and the BJAB/HR-1 converted B_1 clone; we also studied primary mononuclear cells from umbilical cord blood from which T lymphocytes had been removed. The time course of formation of IC in Raji and in primary lymphocytes is shown in Fig. 5. Two hours after addition of HSV-1 at a multiplicity of 0.5 PFU/cell, about 1 per 10^5 Raji cells and 1 per 10^4 HUCL registered as IC. The fraction of infected cells increased about 10-fold after a 24-h exposure of cells to virus. However, even after 96 h of virus exposure, less than 0.1% of Raji cells and less than 1% of cord cells were capable of producing plaque-forming virus. In subsequent experiments, we routinely exposed the cells to the inoculum for 24 h before assaying IC. The maximum number of infected cells observed at 24 h varied somewhat for each line from experiment to experiment. This variation was most marked for the primary lymphocytes; between 0.15 and 21 IC per 10^4 cells were observed in four experiments, with a median of 3.1 IC/10^4 cells. The

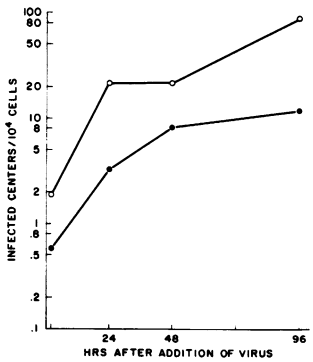

FIG. 5. *Kinetics of formation of IC in Raji cells and primary T cell depleted HUCL by HSV-1. A total of 10^6 cells in 1 ml were exposed to 0.1 ml of virus stock (multiplicity of infection, 0.75 PFU/cell). Incubation at 37°C was continued for the times indicated, and then the cells were washed and suspended in a 1:10 dilution of human serum with a 1:40 titer of HSV neutralizing antibody. After further incubation at 37°C for 2 h, the cells were washed and counted, and 10^4 and 10^3 cells were plated on duplicate Vero cell monolayers. A Methocel overlay was added. Plaques were stained with Giemsa and counted after 4 days.*

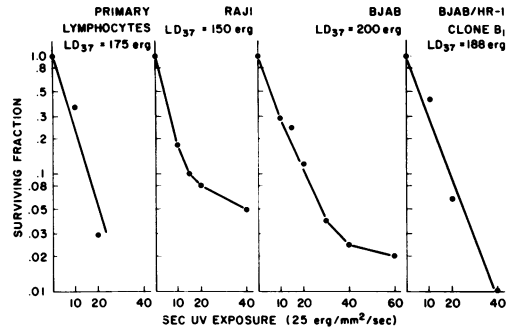

FIG. 6. *UV inactivation of HSV-1 IC in lymphoid cells. Virus suspended in medium was exposed to UV light at 25 ergs per mm^2 per s, and 0.1 ml of virus was added to 10^6 cells in 1 ml. After 24 h, the cells were processed for IC as described in the legend to Fig. 6. One hundred percent survivals (unirradiated virus), expressed as the number of IC per 10^4 cells, for the four cell systems were: Raji, 25.3; BJAB, 23.4; BJAB/HR-1 clone B_1, 71.9; and primary leukocytes, 3.0.*

median values of the number of IC per 10^4 cells for the different lymphoid lines were 12.5 for Raji, 23.4 for BJAB, and 72 for clone B_1.

The inactivation of HSV by UV light was identical in all three continuous lymphoid lines, regardless of the presence or absence of the EBV genome (Fig. 6). The LD_{37} for IC formation was 150 to 200 ergs. At higher doses of UV irradiation, HSV was inactivated more slowly when assayed in Raji or BJAB cells; we have no ready explanation for this phenomenon. At low doses of UV irradiation, the inactivation of HSV was the same in all three lymphoid lines; therefore it did not seem likely that the presence or absence of the EBV genome influenced the ability of a cell line to repair or complement UV-induced damage in a superinfecting HSV genome. UV inactivation of HSV IC in primary leukocytes was similar to that in the lymphoid lines (LD_{37}, 175 ergs). A UV survival curve for HSV determined directly by reduction of plaque formation on permissive cells (the Vero line of green monkey kidney) was identical to that obtained in the lymphoid cells.

Inactivation of HSV-1 by X irradiation was measured in an EBV genome-positive (Raji) and an EBV genome-negative (BJAB) cell line and in primary leukocytes (Fig. 7). Identical LD_{37} of 60,000 rads was measured in all three cell systems. This is the same X-ray dose needed to inactivate transformation by EBV.

DISCUSSION

HSV as a marker for target size and host cell repair. Some of the conclusions we have reached rely on the use of HSV replication in

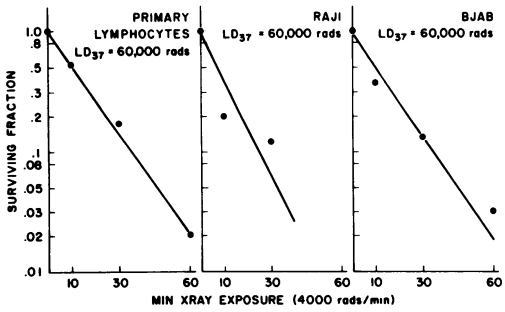

FIG. 7. *X-ray inactivation of HSV-1 IC in Raji and BJAB cells. Frozen virus suspended in medium was exposed to X rays at a calibrated dose of 4,000 rads per min. IC were determined after 24 h of exposure to virus. The values obtained for unirradiated virus were: Raji, 2.3 IC/10^4 cells; BJAB, 108 IC/10^4 cells; and primary lymphocytes, 6.8 IC/10^4 cells.*

lymphoid cells for comparison. HSV is an appropriate target size marker, since the size of the two genomes is nearly equivalent based on velocity sedimentation and electron microscopy (14, 27).

It might be argued that HSV is not an appropriate marker for host cell repair, since infection of lymphoid cells by HSV is abortive (e.g., see Fig. 5). Formation of infectious HSV occurs only in a fraction of the cells, as determined by the IC technique, although as many as 50% of Raji cells may express viral antigens after HSV infection (13). The low levels of IC are not due to insensitivity of the assay. We can measure 50% IC with permissive Vero cells. The low number of IC is also not due to neutralization of cell-to-cell spread of virus by antibody adherent to herpes-infected lymphoid cells that have a receptor for Fc fragment. The same number of IC is found when residual virus is removed by trypsin rather than by antibody (Grogan and Miller, unpublished data).

Both UV and X-ray inactivation curves of HSV obtained in primary and continuous lymphoid cells were identical to those obtained on the fully permissive system, Vero, in which most of the cells were infected and to those published by others (19). Radiobiological inactivation of HSV was the same in lymphoid cells with and without the EBV genome. We conclude that primary lymphocytes, lymphoid lines, and the Vero tissue culture lines have similar host cell repair processes for radiodamaged herpesvirus. Therefore differences observed in the radiobiological inactivation of EBV were not due to differences in host cell repair.

HSV and B95 strain EBV had identical target sizes measured by X-ray inactivation; however, differences were encountered in the UV-inactivation curves (Fig. 1 and 6). EBV was three to five times more resistant to UV inactivation than was HSV. This difference is most likely related to the time lapse between infection and initiation of viral DNA replication. During this interval the host cell repairs UV-induced thymidine dimers. HSV DNA synthesis begins at least 2 to 3 h after infection (5). The time of onset of EBV DNA synthesis is not yet known. However, transformation becomes sensitive to pulses of BrdU and light 24 to 36 h after infection (11a). If this measures the time of onset of EBV DNA synthesis, there would be a longer interval for host cell repair of UV-damaged EBV DNA. It is also possible that the nicks and gaps (alkaline-labile regions) in HSV DNA somehow make it more susceptible to UV-induced damage (19). Finally, since HSV shuts off host cell DNA synthesis and transforming EBV apparently does not, the availability of DNA repair systems may differ between the two viruses.

Inactivation of EA induction. The unexpected finding was that EA induction by HR-1 virus appeared to be more sensitive to UV and X-ray inactivation when assayed in the genome-negative BJAB line than when measured in its EBV-converted counterpart or in another EBV carrier line Raji. Similar results have been obtained by Alice Adams (personal communication). The data simply that there are two separate phenomena associated with EA induction. In genome-free lines, EA expression is due to information provided by the input viral genome. Based on the relative target sizes, expression of EA under these conditions requires about 30 to 50% of the genome. However, there is another component, considerably more resistant to inactivation, which involves induction of EA in genome carrier cell lines. The resistance of EA induction to UV inactivation in genome-positive NC37 cells has also been noted by Sairenji and Hinuma (32). EA induction in EBV carrier cells could require as little as 10% of the genome. There is some evidence that the HR-1 virus stock is heterogeneous (9). The process of EA induction by activation of the resident genome and expression of EA by the input genome might reside in different particles. Clues to the complexity of the EA induction process come from the finding that induction in Raji cells is enhanced by very low doses of UV light or X rays (Fig. 3 and 4). This suggests the existence of genes that inhibit EA induction.

The mechanism whereby HR-1 virus induces EA expression in genome-positive lines is not known. One model might involve competition by the input virus for inhibitory molecules active on the resident genome. Another model is suggested by recent studies of Leiden et al. (17) on the expression of HSV thymidine kinase in

transformed cells. They found that superinfection with a thymidine kinase-deficient viral mutant induced the expression of the resident viral thymidine kinase, presumably by positive regulatory proteins.

The DNA of the HR-1 virus contains some sequences not found in B95-8 DNA (27). It has therefore been suggested by Pritchett et al. that HR-1 is the lytic biotype of EBV and that B95 is a defective transforming derivative. The radiobiological data obtained herein do not support that hypothesis, for it has been impossible to confer transforming properties onto HR-1 virus by irradiation.

Inactivation of transforming EBV. A principal finding from these experiments was that the target size for inactivation of the transforming functions of EBV was large, including perhaps the entire genome. This finding was corroborated by the observation that all sublines of marmoset cells transformed by heavily irradiated virus still yielded infectious progeny. It is possible, however, that we will detect nonproducer transformants after examination of a larger number of clones transformed by irradiated virus. Transformation was not enhanced by irradiation; this contrasts the findings for irradiated papovavirus, adenovirus, or lytic herpesvirus. Presumably, one reason for this difference is that EBV does not express lytic functions upon its initial interaction with the susceptible lymphoid cell. There is no evidence for expression of late viral functions (VCA or mature virus) in either human or marmoset cells during week 1 after infection (G. Wilson, unpublished data). Many aspects of the behavior of EBV after radiobiological exposure are, superficially at least, more analogous to RNA tumor viruses than to the lytic DNA viruses.

Why might the whole genome be necessary for transformation in the case of EBV? One explanation is that some portion of the viral DNA must replicate many copies to exert a "gene dose effect." Nearly all human lymphoid cell lines that have resulted from EBV transformation in vivo or in vitro have many genome copies (26). A second possibility is a "polarity effect." If several genes were relevant to the transforming process and were also widely separate on the viral genome, damage anywhere on the genome might impair transformation. Some human lymphoid cells contain free, circular, nonintegrated EBV DNA (2). If this is the usual state of the genome in cells transformed in vitro, the entire genome might be necessary to assume a particular circular configuration. A fourth possibility is that the entire genome is needed for DNA replication and segregation of genome progeny to daughter cells.

The radiobiological findings allow speculations on the future outcome of more sophisticated genetic analysis of transformation. If a "gene dose phenomenon" is the explanation for the necessity of the whole genome, then it may be possible to immortalize with a "transforming fragment" of EBV DNA, provided enough DNA is put into the cell. However, the other three hypotheses would predict that it will be unlikely that transformants with a single small fragment will be successful. It may be necessary to include fragments coding for DNA synthesizing processes. Furthermore, the experiments predict that EBV mutants that are deficient in viral DNA replication will also be transformation defective.

Virus-specific RNA homologous to only 5% of viral DNA is found on the polyribosomal fraction of nonproducer lymphoid cell lines (24). Thus, expression of the entire viral genome, although it may be needed to initiate transformation, does not appear to be required to maintain the transformed state of the cells.

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