ABSTRACT

Epstein-Barr virus (EBV)-induced transformation efficiencies were quantitated for peripheral blood leukocytes from two patients with xerodermia pigmentosum (XP) and a patient with the related disorder Cockayne’s syndrome. By transformed centers or limiting dilution assays, there were no observed differences in transformation efficiencies between XP, Cockayne’s syndrome, or normal adult leukocytes. In contrast it was found that when either the leukocytes or the infecting EBV were UV irradiated prior to virus exposure, XP leukocytes showed a greater decrease in ability to undergo in vitro transformation. The doses of the transforming functions of the B95-8 strain of EBV lethal to 37% of the animals were 70, 725, and 750 ergs/sq mm when measured in XP, XP heterozygotic, and normal adult leukocytes, respectively. Transforming activity of UV-irradiated EBV could be partially restored by illuminating infected XP leukocytes with visible light 36 hr after virus exposure. Illumination with visible light had little effect on transformation of neonatal or normal adult leukocytes by UV-irradiated EBV. Colony formation in soft agarose by the resulting lymphoblastoid cell lines established from primary XP or Cockayne’s syndrome leukocytes showed increased sensitivity to UV-irradiation when compared to lymphoblastoid cell lines established from heterozygotic or normal individuals. These results demonstrate that EBV is similar to other herpesviruses in being subject to host cell reactivation, and the EBV-induced transformation does not require or alter these functions.

INTRODUCTION

The expression of lytic viral functions by UV-irradiated virus particles is highly dependent on the ability of the infected host cell to repair by DNA repair replication UV damage to the viral genome. This phenomenon has long been referred to in the literature as HCR (15). HCR has proved to be a useful tool for assessing the deleterious effects of DMA-damaging agents and for indirectly measuring the ability of the host cell to repair various types of lesions introduced into the viral DNA (29). XP is an autosomal recessive disease in which there is an unusually high sensitivity to sunlight and a greatly increased incidence of carcinomas of the skin (31). Studies of XP fibroblasts led Cleaver (9) to suggest that the first step in excision repair of UV-induced pyrimidine dimers in DNA was defective. As a result of this repair defect, XP fibroblasts have been shown to be less efficient than are normal cells in their ability to reactivate UV-irradiated DNA viruses such as SV40 (2), adenovirus (14), and HSV (23). As a host cell these experiments have utilized fibroblastoid cell lines established from biopsy material obtained from XP patients. In addition to initially requiring biopsy material, fibroblastoid cell lines have the other disadvantage of a limited life span in culture (17).

In vitro infection with EBV induces permanent alterations in the morphology, growth, and surface properties of peripheral blood lymphocytes from several primate species including humans (25). Several assay methods have now been developed that allow accurate quantitation of EBV-induced morphological transformation (19). Using these assay methods we have shown recently that lymphocyte-transforming functions of the B95-8 strain of EBV are very sensitive to inactivation by either UV- or X-irradiation (18). The X-ray dose needed for inactivation of EBV transformation (LD37, 60,000 rads) was identical with the dose required for inactivation of plaque formation by HSV type 1 (HSV-1). Since EBV and HSV have similar-sized genomes, this observation with X-irradiation indicated that a large part, perhaps all, of the EBV genome was needed to initiate transformation in lymphocytes. In contrast, when measured in the same host cell, the UV dose needed to inactivate EBV-induced transformation (LD50, 750 ergs/sq mm) was 3 to 5 times more than the UV dose needed to inactivate HSV-1 plaque formation (LD37, 150 ergs/sq mm). Two possible explanations for these findings are: (a) that the target size for UV inactivation of EBV transformation functions is smaller than that of HSV lytic functions; and (b) that UV-damaged EBV undergoes more extensive repair than does HSV before DNA replication. Our observation that transforming functions of EBV are extremely sensitive to UV irradiation when measured in XP leukocytes supports the second hypothesis that host cell repair is responsible for the UV-resistant component seen in normal leukocytes. In addition, these experiments underscore the usefulness of EBV transformation and the resulting LCL’s as an alternative to fibroblasts for the study of HCR and DMA metabolism in human cells.

MATERIALS AND METHODS

Cell Culture. Heparinized whole venous blood was obtained from patients diagnosed as having XP or familial melanoma by the Temple University Skin and Cancer Hospital, Philadelphia, Pa., in collaboration with Dr. Robert

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Harper. Blood was also obtained from the mother and father of the XP patients (heterozygotic carriers for XP), normal adults, and from fresh human umbilical cords. Cockayne's syndrome blood was obtained from Dr. James German, New York Blood Center, New York, N. Y. Leukocytes were prepared by the Ficoll-Hypaque method of Böyum (5). Before exposure to virus, isolated leukocytes were cultured at a concentration of 1 × 10^6/ml in Corning tissue culture flasks. Medium was Roswell Park Memorial Institute Medium 1640 (Grant Island Biological Co., Grand Island, N. Y.) supplemented with 20% heat-inactivated (56°, 30 min) fetal bovine serum (Grand Island Biological Co.), penicillin (50 units/ml), streptomycin (50 µg/ml), and amphotericin B (1 µg/ml) (complete medium). All cells were cultured at 37° in an atmosphere of 5% CO₂ in air. Cell numbers were readjusted by hemacytometer counts to 1 × 10⁶ cell/ml before use.

**EBV Preparation.** Recent developments in EBV research have allowed very high-titered EBV, lymphocyte-transforming strain B95-8, stocks to be produced (32). Briefly, high-titer stocks of a recently passaged transforming strain (B95-8) EBV were prepared from supernatant fluids of an EBV-transformed cottontop marmoset cell line grown in roller bottles. The roller bottle contained a high-density (3.5 to 5 × 10⁶ cells/ml) culture with a mixture of suspended and adherent cells. Supernatant fluids were centrifuged at 400 g to sediment cells, frozen and thawed 3 times, and passed through a 0.45-µm Millipore filter. They were determined to be free of *Mycoplasma* and bacteria by culture. Stocks were stored at -70°. A single virus stock designated Y2-5 was used in these experiments. The transforming titer of this virus stock was approximately 2 × 10⁶ ID₅₀ units (ID₅₀, dose at which 50% of the wells transform) per 0.20 ml when measured by end-point titration on human umbilical cord leukocytes.

**Irradiation.** For the UV source, total emission from a 15-watt GE15T8 low-pressure mercury germicidal lamp controlled by a rheostat was used. Incident (254 nm) UV dose rate was 5 ergs/sq mm/sec for cellular inactivation and 25 ergs/sq mm/sec for viral inactivation as determined by a Latarjet dosimeter. One-ml aliquots of cells in phosphate-buffered saline suspension or virus in spent medium placed in an atmosphere of 5% CO₂ in air. Cell numbers were readjusted by hemacytometer counts to 1 × 10⁶ cell/ml before use.

**Transformation Assays.** In accord with our usual procedure, transforming titers of virus preparations were measured with a microwell-limiting dilution assay (19). All incubations and procedures were carried out while aluminum foil wrapped or in subdued red safe light with the exception of the photoreactivation period. Virus was serially diluted in cold complete medium. In some experiments (Chart 3) it was necessary to use nondiluted, UV-irradiated virus to obtain transformation. The final dilution of virus was carried out by addition of 0.2 ml of virus to a well from a microwell tissue culture plate (Falcon Micro Test II or Cooke) containing 2 × 10⁴ leukocytes. To obtain quantitative data on the relationship of virus dose to efficiency of transformation, we used virus dilutions, based on prior experimentation, that contained fewer than 1 transforming unit/0.2 ml for the cells used. From 8 to 32 wells of a microwell tissue culture plate, each containing 2 × 10⁴ cells, received each virus dilution. Culture plates were incubated in a humidified 5% CO₂:air atmosphere. One-half the medium in each well was removed weekly with a stainless steel manifold, and fresh complete medium was replaced. This otherwise laborious task was made easier with an automatic media pipetter. Morphological changes of transformation were first microscopically evident several days to several weeks after exposure to virus. Final results were scored at 10 weeks.

The transforming efficiencies of EBV-exposed leukocytes were determined by colony formation in a transformed centers assay with human placental fibroblasts as feeder layers (19). Lymphocytes were exposed to virus for 24 hr, at 37°. Cells were then treated with a 1:10 dilution of a human serum with an EBV-neutralizing antibody titer of 1:160. After 1 hr incubation at 37°, the cells were washed twice with medium and diluted. The cells were then added to feeder cultures prepared from a cell strain prepared from human placenta (16). Small numbers of virus-exposed cells in 0.1 ml of medium were added to 0.1 ml of feeder. Transformation was scored as in the limiting dilution assay. When several serial cell dilutions were plated, efficiency of transformation was calculated by the formula of Reed and Muench (30). When 1 or 2 cell dilutions were plated, the efficiency of transformation was calculated as follows:

\[
\text{No. of transformed wells} / \text{No. of cells/well} \times \text{no. of wells}
\]

This method of calculation assumes that each transformed well arises from 1 transformed cell.

**Survival Measurements with LCL’s.** All permanently proliferating LCL’s used in the experiments reported here were established in this laboratory by the microtiter method with exogenously added EBV. Transformed colonies that developed within 10 weeks from microwells that received the highest virus dilution were transferred with Pasteur pipets into 25-ml Falcon tissue culture flasks containing 1 ml complete medium. After a period of growth, usually less than 1 week, an additional 5 ml of medium were added, and the cultures were incubated until there had been sufficient metabolic activity to lower the pH so that the indicator dye turned amber. The successful subcultures were then transferred to larger flasks. Aliquots of these second-passage LCL cultures were used to initiate UV survival measurements and stored with 10% dimethyl sulfoxide in liquid nitrogen. LCL’s were then maintained with complete media with a 1:3 split twice a week. Two-day-old stationary cultures were used for UV survival measurements and repair studies. Measurement of cell survival was accomplished by cell plating in agarose medium, a method modified from that originally described by Coffino et al. (11). Briefly, X-irradiated (4500 rads) confluent monolayers of placental fibroblast cells overlaid with 2.5 ml of a 0.24% agarose medium in 60-mm Petri dishes were used as feeder layers. Then the lymphoblast cells, either UV treated or control nontreated, contained at various concentrations in 2.0 ml
of 0.22% agarose medium were added on top of the separating layer. Three plates were used for each determination and incubated in a humidified 5% CO₂:air atmosphere. The plates were fed with 1.0 ml of liquid medium at 24 hr after plating and then once a week thereafter. Colonies were counted after 3 to 4 weeks.

**Assay for HU-resistant DNA Synthesis by [³H]dThd Pulses.** [³H]dThd incorporation into acid-insoluble material was measured in 0.50-ml aliquots of stationary-phase LCL cultures in stoppered tubes (13 x 100 mm). Cells cultured in 60-mm Petri dishes at 2 x 10⁹/ml were exposed to 10 mm HU (Aldrich Chemical Co., Inc., Milwaukee, Wis.) for 30 min. Except for control cultures the cells were then exposed to varying doses of UV irradiation, as in survival measurements, and aliquoted into stoppered tubes. Cultures were immediately incubated with [³H]dThd (New England Nuclear, Boston, Mass.; 6 µCi/ml; 6.7 Ci/mmol) for 2 hr at 37°. The reaction was terminated by the addition of cold Puck’s saline solution. The cells were processed for acid-precipitable material, and the incorporated radioactivity was determined in a liquid scintillation counter.

**RESULTS**

**Efficiency of Transformation.** From previous experiments we knew that leukocyte populations could differ in transformation efficiency when exposed to equal amounts (multiplicities of infection) of EBV (19). We therefore undertook experiments to determine the transformation efficiencies of partially purified leukocyte populations from a XP patient and compared them to heterozygous and normal leukocytes. Chart 1 is the graph of a limiting dilution experiment with either XP/XP, +/+XP, or normal adult leukocytes as indicators of EBV titer. An approximately equal percentage of microwells transformed for each leukocyte population when exposed to the same EBV dilution. No spontaneous transformation was detected in uninfected wells (16/donor) set up in these experiments. To supplement these data we undertook experiments with these different leukocyte populations in the transformed centers assay, which quantitates the number of cells in a given leukocyte population capable of giving rise to transformants. Transformation efficiency was also determined for leukocytes from the related "chromosomal breakage syndrome," Cockayne’s syndrome. In this particular experiment (Table 1) approximately 1 cell in 500 normal adult leukocytes was capable of giving rise to a permanently proliferating cell line. As was seen in the limiting dilution assay, there were no significant differences in transformation frequency among XP, heterozygotic, or normal leukocytes. Cockayne’s syndrome leukocytes also transformed as efficiently as did leukocytes from normal adults.

**Effect of UV Irradiation on EBV-induced Transformation.** Since the leukocyte populations to be studied transformed with equal efficiency, it was possible to apply the transformed centers assay to determine what effects UV irradiation of the leukocytes prior to EBV infection had on EBV-induced transformation. After sequential UV irradiation the ability of the cells to form transformants when exposed to EBV was differentially inhibited (Chart 2). Primary XP leukocytes were 5 to 6 times more sensitive to UV than measured by the ability to form transformants. The LD₅₀ for UV inactivation of cellular transformation in XP/XP, heterozygotic, and normal adult leukocytes was calculated to be 8, 45, and 50 ergs, respectively. No dose of UV was found to increase the transformability of control or XP leukocytes by EBV. No spontaneous transformation was seen when

![Chart 1. Comparison of the limiting dilutions of the transforming titer of 1 EBV stock (Y2-5) on Ficoll-Hypaque-isolated peripheral blood leukocytes from a patient with XP (XP/XP) (•), a XP heterozygote (+/XP) (△), and a normal adult (+). Points. 16 wells from a microtiter plate in which fewer than 100% of the wells transformed. Each well was seeded with 2 x 10⁶ leukocytes suspended in 0.10 ml media, which were then exposed to 0.20 ml of the appropriate virus dilution. The transforming titer at EBV stock Y2-5 was 2 x 10⁶ TD₅₀ units (TD₅₀, dose at which 50% of the wells transform) per 0.20 ml measured on human umbilical cord leukocytes. Transforming titers were calculated by the method of Reed and Muench (30).](image-url)
Effect of UV Irradiation of EBV-transforming Functions. The ability of UV-irradiated lytic DNA viruses such as HSV and adenoviruses to form plaques in fibroblast lines derived from XP patients is much decreased when compared to lines derived from controls (14, 23). This is due to the inability of the XP cells to repair or host cell reactivate the damaged viral genome before replication. We therefore sought to determine whether this phenomenon also applied to a transforming DNA virus such as EBV. In Chart 3 (solid lines) we have assayed by limiting dilutions the transforming ability of an EBV preparation following sequential UV irradiation. Titers (surviving fractions) were calculated from data similar to those presented in Chart 1 with XP, heterozygotic, and normal adult leukocytes. The UV doses needed to inactivate EBV-transforming functions were approximately the same when measured in either normal or XP heterozygotic leukocytes \( (LD_{50}, 700 \text{ to } 750 \text{ ergs/sq mm}) \). However, EBV-transforming functions were extremely sensitive to UV irradiation when measured in the dark on leukocytes derived from XP \( (LD_{50}, 70 \text{ ergs/sq mm}) \). This result is in agreement with previous reports on the effect of defective excision repair on infection by UV-damaged HSV (23).

Photoreactivation of EBV Infectivity. Wagner et al. (39) have shown that human photoreactivating enzyme(s) in XP fibroblasts can restore infectivity by a factor of 5 to UV-irradiated HSV. Other investigators have had some difficulty demonstrating photoreactivation either biochemically (26) or biologically (24). We therefore attempted to determine whether this light-dependent phenomenon could be observed with XP leukocytes and UV-damaged EBV, also a herpesvirus. In Chart 3 (dashed lines) we demonstrate by limiting dilutions that XP leukocytes exposed to UV-irradiated EBV were, upon illumination for 8 hr with visible light 36 hr after infection, able to transform approximately 10 times more efficiently than were XP leukocytes kept in the dark. Because of the difficulty of obtaining primary XP leukocytes, 1 time period, 36 hr postinfection, was chosen for photoreactivation based on previous studies on the onset of EB viral DNA synthesis (20). Illumination with visible light had no detectable effect on transformation of XP heterozygotic or normal adult leukocytes by UV-irradiated EBV. Control leukocytes transformed with a normal efficiency when exposed to unirradiated EBV and visible light for the same period. In addition to the previous experiments with limiting dilution assays, we determined the effect of visible light on transformation by UV-irradiated EBV with transformed centers. In Chart 4 we have assayed the transformation efficiencies of leukocytes exposed to UV-irradiated (500 ergs) EBV in the dark or after exposure...
Chart 4. Histogram showing effects of illumination with visible light on EBV-induced transformation of XP leukocytes by UV-damaged virus particles. Cells (1 x 10⁶/ml) were exposed to UV-irradiated (500 ergs/sq mm) EBV at a multiplicity of infection of approximately 1 transforming particle/cell for 36 hr. One aliquot of cells was then exposed to whole illumination from a 22-watt fluorescent lamp at an average distance of 22 cm for 8 hr in an incubator at 37° or was kept in the dark by wrapping in aluminum foil. Following virus and visible light treatment, cells were washed twice with complete medium, resuspended, and then diluted as required. The numbers of transformants were determined by transformed centers assays with human placental fibroblasts as feeders. Results were calculated from data similar to that presented in Table 1. Thirty-two wells were used for each determination.

to light. Again XP leukocytes from 2 patients, but not XP heterozygotic leukocytes, showed significantly increased ability to transform following exposure to visible light for 8 hr, 36 hr after infection.

This is consistent with the biological activity of UV-irradiated EBV being restored by a light-dependent mechanism in XP leukocytes. We cannot as yet routinely detect photoreactivation of UV-damaged EBV with excision-proficient leukocytes from human umbilical cords or normal adult donors. This inability to detect photoreactivation routinely in normal cells may be because photoreactivation is "masked" by normal amounts of excision repair. Alternatively, photoreactivating processes may be repressed in excision-proficient cells and derepressed in cells deficient in excision repair.

Radiobiology of Established LCL’s. One result of this series of experiments with EBV infection of primary leukocytes has been the development of numerous continuously growing LCL’s from normal adults as well as individuals either homozygous or heterozygous for various genetic disorders. Therefore, we have sought to establish the phenotypes and begin characterization of these LCL’s with respect to their radiobiology and EBV expression. Initially, we assessed the ability of recently established LCL’s to survive UV irradiation, using as an indicator for survival colony formation in soft agarose. In Chart 5 we have determined survival following UV irradiation for a LCL established from XP, a XP heterozygote, Cockayne’s syndrome, and a normal adult. The LD₅₀ were found to be 25 and 35 ergs/sq mm for the XP and Cockayne’s LCL’s, respectively, as compared to 80 ergs/sq mm for the LCL’s established from a normal adult and XP heterozygote. Attempts were made to detect EBV in supernatant fluids of control and UV-irradiated LCL cultures showing increased UV sensitivity. Extracellular transforming virus could not be detected with human umbilical cord leukocytes as indicators in either UV-irradiated or control LCL’s established from XP, Cockayne’s syndrome, or normal adult leukocytes.

Measurement of UV-inducible HU-resistant DNA Synthesis. Since the possibility existed that EBV infection could alter metabolic pathways including repair synthesis and since differences existed between Cockayne’s syndrome, XP, and normal LCL’s in measurements of cell survival following UV treatment, preliminary investigations of repair synthesis were made. A simplified assay for quantitating repair replication in LCL’s was applied that uses HU for the purpose of selectively suppressing normal, semiconserva-
tive replication (6). HU at 10 mM inhibited 94 to 97% of control [3H]dThd incorporation in these experiments. Following UV treatment the LCL cultures established from a normal adult, XP heterozygote, and Cockayne’s syndrome (slightly less) showed increases in [3H]dThd incorporation in the presence of 10 mM HU (Chart 6). In each case the increase in [3H]dThd incorporation was dependent on the dose of UV. In 3 attempts, the LCL derived from XP failed to show this increase in HU-resistant DNA synthesis following UV treatment.

**DISCUSSION**

In the experiments described here we have quantitated the EBV-induced transformation frequencies of leukocytes from XP. In addition, we have determined what effects UV irradiation of the cells or the EB virion have on transformation. In the process we have also had the opportunity to evaluate host cell mechanisms for reactivation of EBV. The data obtained from these experiments allow us to reflect on several important aspects of EB viral-lymphocyte interactions during the early phases of leukocyte transformation.

**EBV-induced Transformation Efficiencies.** An increased frequency of transformation by SV40 was observed in a strain of XP fibroblasts compared with cells from a presumed heterozygote (38). However, these differences in susceptibility of various human fibroblasts to transformation of SV40 disappeared when infectious DNA was used for transformation (1). More recently, a 3- to 5-fold increase in the transformability of XP fibroblasts with feline sarcoma virus has been reported by Chang (8). In the studies reported here we found that XP and control leukocytes transformed equally well when exposed to identical multiplicities of infection of EBV (Chart 1). Several important conclusions can be drawn from these observations. One is that host cell excision repair synthesis is probably not involved in the process of EBV-induced leukocyte transformation. Therefore, some other mechanism must be responsible for integration if the viral genome(s) that are found to be associated with the host cell chromosome is integrated (3). Alternatively, if UV repair enzymes deficient in XP cells are necessary for the process of transformation, they are effective at low levels. A second implication of normal EBV transformation of XP leukocytes is that EBV preparations probably do not contain significantly large numbers of viral particles containing damaged DNA that is normally repaired by excision repair during infection.

**Sensitivity of Transformation to UV Irradiation.** Treatment of target cells with various DNA-damaging agents prior to exposure to a transforming virus has been shown to increase the transforming efficiency of the surviving cells. For example, pretreatment of cells in vitro with X (12, 28, 37) or UV irradiation (7) has been shown to increase the frequency of cell transformation by oncogenic DNA viruses. Cleaver (20) has suggested that regions for incorporation of viral DNA may be created in host cell DNA during scheduled DNA synthesis at sites of un repaired DNA damage. The finding reported here that XP leukocytes transform much less efficiently following low doses of UV irradiation in which un repaired lesions probably exist in large numbers does not support the hypothesis that unrepaired lesions can increase EBV-induced transformation. This observation taken with that of Casto (7) suggests that an active repair-dependent process is involved in the enhancement of viral transformation by physical and chemical agents.

**Effect of UV Irradiating the EB Virion on Transformation.** Aaronson and Lytle (2) showed that survival of the transforming function and T-antigen expression of UV-irradiated SV40 particles was decreased 5 to 9 times over controls when measured in fibroblasts from 2 XP patients. We have also shown a significant increase in the sensitivity of transforming functions of EBV when measured in leukocytes from XP patients (Table 2). This is in contrast to the relative resistance of EBV to UV, as compared to HSV, when measured in normal leukocytes. Our hypothesis to explain the observed difference in sensitivity of HSV and EBV to UV irradiation was that the data were related to DNA repair during the time lapse between infection and initiation of EB viral DNA replication. During this “latent” period, host cell enzymes could repair UV-induced dThd dimers. HSV DNA synthesis is initiated at least 2 to 3 hr following infection (13). EBV-induced leukocyte transformation becomes sensitive to inhibitors of DNA synthesis beginning 24 to 36 hr after infection (20). If this measures the onset of EBV DNA synthesis, there would be a longer latent period for host cell repair of UV-damaged EBV DNA before replication. Experiments reported here support that hypothesis in that the transforming functions of EBV are exquisitely sensitive to UV irradiation when measured in XP leukocytes. These results support the hypothesis that a large part or perhaps all of the EBV genome is in some way needed to initiate transformation.

**Photoreactivation of EBV with Visible Light.** Reactivation of UV-irradiated mammalian viruses may be accomplished by any one of several repair mechanisms. These include excision repair, Weigle reactivation, and photoreactivation (24). Mortelmans et al. (26) have described photoreactivation as a term used for the mitigation by illumination with...
visible light of deleterious effects of UV irradiation on living organisms. There is considerable controversy as to whether photoreactivation actually occurs in human cells, since only Wagner et al. (39) have shown photoreactivation of HSV in human cells. Only investigators have had difficulty in reproducing such results (24). We report here that illumination with visible light can restore infectivity to UV-irradiated EBV in XP leukocytes from 2 patients. We were unable routinely to show this phenomenon in normal leukocytes. We can exclude any differences in the efficiency of transformation of XP cells as compared with normal cells as a contributor to our observed results. XP and normal leukocytes transformed with the same efficiency (1:500 leukocytes) when exposed either in the dark or light to nondiluted, unirradiated EBV. The mechanism(s) of action for this phenomenon remains unclear. It appears to be specific for XP leukocytes and could represent enzymatic monomerization of thymine dimers or possibly some other light-dependent induction of alternative repair pathways similar to Weigle reactivation.

Radiobiology of Established LCL’s. A long-enduring interest of many laboratories has been the development and utilization of mammalian (preferably human) cell culture models for the in vitro screening of environmental factors that are potential carcinogens to humans. LCL’s have considerable potential for such genetic and biochemical studies (4, 35). LCL’s have an apparently indefinite life span, thus being independent of the Hayflick phenomenon characteristic of fibroblastoid lines. LCL’s grow rapidly in suspension culture, retaining their normal diploid and near-diploid karyotype and differentiated phenotype (27). In this reported study of leukocyte transformation by exogenously added EBV, we have established a number of XP and Cockayne’s syndrome lymphoblastoid cell lines. To date, each line tested at a low passage number has shown the expected increased sensitivity to UV irradiation as measured by cloning in soft agarose (Chart 5). These data further support the usefulness of in vitro-transformed lymphocytes in studying the radiobiology of human cells. One such study in which LCL’s may be more advantageous than fibroblasts for use would be quantitating the effects of UV and near-UV irradiation on mutation frequencies of specific loci, which can be measured readily in LCL’s (33).

An additional finding has been the sensitivity of lymphocytes to UV inactivation when measured by their ability to be transformed by EBV as compared to the sensitivity of the autochthonously derived LCL’s as determined by colony formation in agarose (Table 3). The established LCL’s appear to be slightly more resistant to UV inactivation when compared to the genotypically identical primary leukocytes. This resistance can be explained by the LCL’s being primarily composed of dividing cells, while the target for EBV in a mixed-leukocyte population is a quiescent, nondividing small lymphocyte (20). A dividing cell seems to have an advantage over a quiescent cell in repair associated with replicating DNA (36). Alternatively, the presence of the EBV genome may in some way increase the repair capabilities of the transformed cell.

Measurement of DNA Repair Synthesis. With semiconservative replication inhibited by 10 μM HU, DNA synthesis by mutagen-damaged cells as measured by the incorporation of [3H]dThd is nearly exclusively due to repair synthesis (>80%) (6). Thus, the highly selective action of HU, which has been shown by Krakoff et al. (21) to inhibit cellular ribonucleotide reductase, affords the opportunity of measuring repair replication merely by assessing the rate of incorporation of [3H]dThd into DNA. LCL’s established from a normal adult and a XP heterozygote showed an increase in repair synthesis following UV treatment (Chart 6). A LCL established from a XP patient, on the other hand, failed to show this increase. These data are interpreted to mean that the UV sensitivity associated with these XP lines and their initial failure to host cell reactivate EBV in the dark are indeed due to a deficiency in excision repair of UV damage. In addition, it can be stated that EBV infection does not require or significantly alter the excision repair pathway. An identical finding has been made for repair of X-ray damage in leukocytes transformed by EBV from ataxia telangiectasia and mitomycin C damage in leukocytes from Fanconi’s anemia, also transformed by EBV (E. E. Henderson and J. German, manuscript in preparation).

A not surprising finding was that the LCL’s established from Cockayne’s syndrome showed an increased UV sensitivity, yet displayed near normal amounts of repair synthesis after UV treatment as measured by HU-resistant DNA synthesis. These data support the findings of Schmickel et al. (34) on the normal removal of dThd dimers following UV

<table>
<thead>
<tr>
<th>Virus</th>
<th>Human umbilical cord leukocytes (5)</th>
<th>XP/XP (2)</th>
<th>+/XP (2)</th>
<th>Familial melanoma (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1&lt;sup&gt;b&lt;/sup&gt; (Fisher strain)</td>
<td>150–180</td>
<td>30–35</td>
<td>150–175</td>
<td></td>
</tr>
<tr>
<td>EBV&lt;sup&gt;c&lt;/sup&gt; (B95-8)</td>
<td>700–750</td>
<td>50–70</td>
<td>700–750</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Numbers in parentheses, number of donors.
<sup>b</sup> Measured by infected centers on Vero cells (18).
<sup>c</sup> Measured by morphological transformation with limiting dilutions.

Table 2

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Primary lymphocytes&lt;sup&gt;d&lt;/sup&gt;</th>
<th>LCL’s&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xeroderma (XP/XP)</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>Xeroderma heterozygote (+/XP)</td>
<td>45</td>
<td>80</td>
</tr>
<tr>
<td>Normal adult (+/+)</td>
<td>50</td>
<td>80</td>
</tr>
</tbody>
</table>

<sup>d</sup> Determined by sensitivity to EBV-induced transformation.
<sup>b</sup> As measured by colony formation in agarose.

Table 3

E. E. Henderson

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irradiation in Cockayne’s syndrome fibroblasts. Their findings led the authors to suggest that Cockayne’s syndrome cells are similar to cells obtained from XP variants. However, Cockayne’s cells and XP variants are not similar because Cockayne’s cells are more UV sensitive than are XP variants, and the latter are nearly normal in UV sensitivity as measured by survival (22). Therefore Cockayne’s syndrome represents a third variation in response to UV irradiation, having increased UV sensitivity despite near normal levels of excision repair.

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I thank Dr. Robert Harper for obtaining the XP lymphocytes used in these experiments.

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Host Cell Reactivation of Epstein-Barr Virus in Normal and Repairdefective Leukocytes

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