Defective Reactivation of Ultraviolet Light-irradiated Herpesvirus by a Bloom’s Syndrome Fibroblast Strain

Clifford A. Selsky,2 Patricia Henson,3 Ralph R. Weichselbaum, and John B. Little

Department of Physiology, Harvard School of Public Health, Boston, Massachusetts 02115

INTRODUCTION

Some human genetic disorders are associated with an elevated cancer incidence. An identification of the lesion at the molecular level in such disorders might therefore increase our understanding of the neoplastic process. In the case of xeroderma pigmentosum, the defect lies in the excision repair of UV damage (6). Bloom’s syndrome is another autosomal recessive genetic disorder, with limited sun sensitivity and an elevated cancer incidence (12). It has been reported that the Bloom’s syndrome strain GM1492, than in either the normal strains or other Bloom’s syndrome fibroblasts. The defect in GM1492 was manifest only at relatively high multiplicities of infection. Thus, at 0.01 plaque-forming unit/cell, the GM1492 strain appeared normal, using the infectious centers assay. Clonal survival of the UV-irradiated GM1492 fibroblasts was also normal. Caffeine at 4 μM had little effect on either virus or cell survival following UV irradiation.

The results indicate that the Bloom’s syndrome strain GM1492 may be deficient in one of the cellular functions responsible for the multiplicity reactivation effect. These effects include complementation and recombinational events. Alternatively, the GM1492 strain may have a defective UV repair system which becomes saturated at high levels of damage.

METHODS

Cell Strains. Bloom’s syndrome skin fibroblast strains GM2520, GM2548, and GM1492 were obtained from the Human Genetic Mutant Cell Repository, Camden, N. J. Two normal human skin fibroblast strains, CRL 1116 and CRL 1126, and 2 classical excision-defective xeroderma pigmentosum fibroblast strains, XP12BE (CRL1223) genetic complementation group A, and XP8BE (CRL 1158) complementation group C, were obtained from the American Type Culture Collection, Rockville, Md. Another normal skin fibroblast strain, Li106, was derived from a skin biopsy of a 46-year-old male and has been maintained in this laboratory since 1975 (20). HEp-2 cells, which were used to propagate virus stocks and as indicators in the infectious centers determination of virus survival, were also obtained from the American Type Culture Collection.

Cell Culture Conditions. Cell cultures were grown in Dulbecco’s modified Eagle’s minimal essential medium (GIBCO F-11) supplemented with fetal bovine serum (10% v/v; Microbiological Associates, Walkersville, Md.), penicillin (50 units/ml), and streptomycin sulfate (50 μg/ml; Grand Island Biological Co., Grand Island, N. Y.). Stock cultures were serially passaged 1:2 once per week and fed on the third day following transfer. Cells were grown in an atmosphere of 5% CO2:95% air and saturated with water at 37°C.

Cell Survival Experiments. Appropriate numbers of cells (1 to 40 x 10^3, depending on dose) were seeded into 10-cm Petri dishes in triplicate, together with 5 x 10^3 feeder cells. The feeder cells were obtained by trypsinizing exponentially growing stocks of normal fibroblast strain AG 1518, suspending the cells in complete medium, and irradiating them with 10 kilorads

1 Supported by Research Grants CA-11751 and ES-00002 and Training Grant AG-05015 from NIH.
2 Present address: Department of Biological Sciences, Stanford University, Stanford, Calif. 94305.
3 To whom requests for reprints should be addressed. Received December 4, 1978; accepted May 17, 1979.

Received December 4, 1978; accepted May 17, 1979.

The abbreviations used are: PFU, plaque-forming units; HSV-1, herpes simplex virus type 1; EBSS, Earle’s balanced salt solution.
Host Cell Reactivation by Bloom’s Syndrome Fibroblasts

from a 90Co source. Approximately 18 hr after plating, the dishes were rinsed once with EBSS and then irradiated, uncovered and dry, with a battery of 5 General Electric G8T5 254-nm germicidal lamps. Following irradiation, fresh medium was added to the dishes, and they were returned to the incubator for 14 to 21 days. The medium was changed on the seventh day. Colonies were fixed and stained with 0.5% methylene blue and counted. Colonies of 50 cells or more were scored as survivors. Plating efficiencies ranged from 1.3 to 12%. Radiosensitivity was independent of the plating efficiency. In some cases, survival was measured using cells plated in the absence of feeder cells if the plating efficiency of the cell strain under test was sufficiently high.

In experiments to measure the influence of caffeine on survival, the medium was supplemented with 2 mM caffeine (Sigma Chemical Co., St. Louis, Mo.) for the colony formation period after UV irradiation.

Growth of Virus and Determination of Survival after UV Irradiation. HSV-1 strain MP was obtained from the American Type Culture Collection. Stock virus suspensions were prepared from infected HEp-2 cells as described previously (19).

For UV irradiation, the stock virus suspension was diluted 10-fold in EBSS, and 3-ml aliquots were exposed in 60-mm Petri dishes to a bank of five 254-nm germicidal lamps. Survival of UV-irradiated virus in the different fibroblast strains was determined by either a direct plaque assay or by measuring surviving infectious centers. The direct plaque assay has been described previously (19).

In preparation for the infectious centers assay, fibroblasts were seeded into each of the 96 wells of a Falcon microculture plate and grown to confluency. The number of cells per well was estimated by counting the entire population from 3 of the wells. Stock irradiated virus was diluted in EBSS to yield the desired infecting multiplicity in a volume of 0.01 to 0.03 ml. The multiplicity was based on the titer of nonirradiated virus. The fibroblast monolayers were inoculated with the diluted virus, which was allowed to adsorb for 90 min at 37°C. The inoculum was then aspirated, and the monolayers of infected cells were overlaid with 0.25 ml of EBSS containing 0.16% (w/v) pooled human immune serum globulin (Merck Sharpe & Dohme, Merck & Co., West Point, Pa.) and incubated at 37°C for a further 20 min. This treatment inactivates the unadsorbed virus. The cells were then rinsed twice with calcium- and magnesium-free EBSS and harvested in 0.1 ml of 0.1% (w/v) trypsin. They were then diluted in assay medium [Dulbecco’s modified Eagle’s minimal essential medium supplemented with 1% fetal bovine serum and 0.03% pooled human immune serum globulin (19)], and a calculated number of cells in a total volume of 3 ml was spread on confluent monolayers of HEp-2 cells in 60-mm tissue culture dishes. The dishes were incubated for 72 hr at 37°C. The assay medium was then decanted, and the cells were rinsed once with EBSS and stained with crystal violet as described for the direct plaque assay (19). The infectious centers were counted using a light box and a dissecting microscope. When the effect of caffeine on virus survival was being studied, caffeine was present at a concentration of 4 mM during the entire postinfection incubation period. The difference in the plaquing efficiency of unirradiated herpes from cell strain to cell strain was never greater than 2-fold.

RESULTS

Chart 1 shows the survival of UV-irradiated HSV-1 MP in 2 normal human skin fibroblast strains and in Bloom’s syndrome strain GM1492 fibroblasts as determined by direct plaque assay. Similar results were obtained in 3 separate experiments. Bloom’s syndrome strain GM1492 consistently demonstrated virus survival just slightly lower than that in the normal strains. Chart 2 shows clonal survival of UV-irradiated strain GM1492 and normal strain Li106. A larger proportion of the Bloom’s syndrome cell population fell within the resistant component of the survival curve than did the Li106 population. However, the slopes of both sensitive and resistant components are similar for the 2 cell strains. Strain GM1492, therefore, does not demonstrate increased cellular sensitivity to the lethal action of UV light.

Survival of UV-irradiated HSV-1 MP was determined in the 3
Bloom's syndrome skin fibroblast strains by use of the infectious centers assay. This assay was used instead of the direct plaque assay in order to avoid growing large numbers of fibroblasts. This factor was important because of the poor growth characteristics of 2 of the Bloom's syndrome cell strains. Chart 3 shows the results of experiments performed with an infecting multiplicity of 3 PFU/cell. The results showed strain GM1492 to be less efficient at reactivating the UV-irradiated virus than were the 2 normal strains but less defective than was the xeroderma pigmentosum strain, complementation group C. The 2 other Bloom's syndrome strains tested were indistinguishable from the normal fibroblasts. Since the infectious centers assay at an infecting multiplicity of 3 PFU/cell apparently enhanced the manifestation of a defect in viral reactivation in the GM1492 strain, the assay was repeated at a lower multiplicity of infection (0.01 PFU/cell). This is closer to the multiplicities of infection obtained in the direct plaque assay. Chart 4 shows the results obtained in the same experiment for strain GM1492 and a normal cell strain under both low and high infecting multiplicity conditions. These data show that survival of UV-irradiated HSV-1 MP is a function not only of ultraviolet dose but also of the multiplicity of infection. In addition, the magnitude of this multiplicity effect is much less in Bloom's syndrome strain GM1492 than in the normal strain tested.

Chart 5 shows the effect of caffeine on the survival of UV-irradiated HSV-1 MP in a normal cell strain, the GM1492 strain, and a classical excision-defective xeroderma pigmentosum cell strain, complementation group A. In no case did the presence of 4 mM caffeine in the postinfection assay medium have a significant effect on virus survival, determined at an infecting multiplicity of 3 PFU/cell. Chart 6 shows that caffeine at a concentration of 2 mM also had little effect on the survival of GM1492 fibroblasts after exposure of the cells to UV irradiation.

**DISCUSSION**

Clinical symptoms of Bloom's syndrome include sun sensitivity and an increased cancer incidence. For this reason, several laboratories have tried to identify lesions at the molecular level, concentrating particularly on the response to UV-induced damage. However, few abnormalities have been identified. Thus, unscheduled DNA synthesis has been examined and found to be normal in Bloom's syndrome fibroblasts (5). One group (13) has reported that Bloom's syndrome cell strains are more...
sensitive to the lethal effects of UV irradiation. We did not find this to be the case (Chart 2), however, indicating that increased sensitivity to cell killing by UV is not a consistent finding in cell strains from all Bloom’s syndrome patients.

When a direct plaque assay was used, host cell reactivation of UV-irradiated HSV-1 was similar in both normal and Bloom’s syndrome fibroblasts. In these experiments, the infecting multiplicities were never greater than 6 x 10^-4 PFU/cell. Additional viral reactivation takes place when infecting multiplicities are greater than 0.1 PFU/cell (7), since interaction between the damaged viral genomes takes place when more than one virus infects a host cell. Types of interactions include complementation and recombinational events. Both could result in an increased synthesis of viable virus particles. We observed this multiplicity reactivation effect in both normal strain CRL 1126 and Bloom’s syndrome strain GM1492. The magnitude of the effect was, however, much smaller in the Bloom’s syndrome fibroblasts. Since there was no difference in the survival of UV-irradiated virus when normal or GM1492 fibroblasts were infected at low multiplicities, it is unlikely that there is any general defect in the latter strain which interferes with the synthesis and assembly of viral proteins. It therefore seems unlikely that the lower multiplicity reactivation in GM1492 cells is the result of reduced viral complementation. It seems more reasonable to suppose that the GM1492 strain is unable to support effective viral recombination. Recombination in the uninfected fibroblasts may also be defective, which may be of serious consequence for the cells. For example, the high level of spontaneous sister chromatid exchanges observed in Bloom’s syndrome patients (4) may be a reflection of an abnormal recombination system.

An alternative explanation for the defect observed in the GM1492 Bloom’s syndrome strain is that the cells have only a limited capacity for repair of damaged viral DNA. According to this hypothesis, this capacity is exceeded at high infecting multiplicities in a manner similar to that found in bacterial systems (3). We saw no effect of 4 mM caffeine on the host cell reactivation of UV-irradiated herpes simplex virus and concluded that the processes involved in multiplicity reactivation are caffeine insensitive. This is in contrast to a report (10) of a reduction in the host cell reactivation of UV-irradiated adenovirus in both normal human fibroblasts and a xeroderma pigmentosum variant strain exposed to caffeine. Caffeine also had no effect on survival of the GM1492 fibroblasts after UV irradiation. The repair systems critical for host survival are therefore also caffeine insensitive.

Further studies are needed to distinguish between these possibilities. Experiments to compare recombination of herpes simplex virus in Bloom’s syndrome and normal fibroblasts are in progress. It is also not yet known if the deficiency observed in the GM1492 fibroblasts is also found in other Bloom’s syndrome strains. It also remains to be seen if introducing further changes in the infecting multiplicities will reveal defects in multiplicity reactivation in the 2 Bloom’s syndrome strains that have thus far appeared normal.

The clinical significance of these results is also of interest. Although Bloom’s syndrome patients demonstrate a limited sun sensitivity, they express phenotypic neoplasia principally in the hematopoietic cell renewal compartment, rather than in the skin. This suggests that, unlike xeroderma pigmentosum patients, defective repair of UV damage is of little consequence in the neoplastic process. The critical lesion may instead be the recombination effect postulated here.

Acknowledgments

We thank John Nove not only for excellent technical assistance but also for valuable and stimulating discussions.

References


Defective Reactivation of Ultraviolet Light-irradiated Herpesvirus by a Bloom's Syndrome Fibroblast Strain

Clifford A. Selsky, Patricia Henson, Ralph R. Weichselbaum, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/39/9/3392

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.