Synthesis of Virus-capsid Antigen (VCA)
Enhanced by Ultraviolet Irradiation of a Lymphoblastoid Cell Line carrying Epstein–Barr Virus

By P. K. LAI, E. M. MACKAY-SCOLLAY AND M. P. ALPERS*

Virus Laboratories of the State Health Laboratory Services, Perth, Western Australia, and the Department of Microbiology, School of Medicine, University of Western Australia

(Accepted 24 May 1973)

SUMMARY

U.v. irradiation of SH-RP cells, a lymphoblastoid cell line carrying Epstein–Barr virus, which was derived from a patient with acute myeloid leukaemia, retarded cell growth but significantly enhanced the percentage of cells containing virus-capsid antigen (VCA) as demonstrated by indirect immunofluorescence. The effects of u.v. irradiation on SH-RP cultures were reversible under normal conditions of medium replenishment every four days. Starving the cultures did not modify these effects.

Since Epstein, Achong & Barr (1964) first detected the Epstein–Barr virus (EBV) in a lymphoblastoid cell line derived from Burkitt’s lymphoma, many such lines have been established from infectious mononucleosis (Pope, 1967; Diehl et al. 1968; Hirschaut et al. 1969), Burkitt’s lymphoma (Epstein, Barr & Achong, 1964; Epstein, Achong & Pope, 1967), nasopharyngeal carcinoma (de The et al. 1969) and leukaemia (Moore et al. 1966; Pope 1968), as well as normal blood donors (Gerber & Monroe, 1968). The majority of these lines can be shown to carry the EB virus by electron microscopy or by immunofluorescence (Epstein & Achong, 1968). In lines negative for EBV by these criteria, the presence of the virus genome has nevertheless been demonstrated (Zur Hausen & Schulte-Holthausen, 1970; Gerber, 1972; Hampar et al. 1972).

Although it has been strongly suggested that all the cells in such lines carry the EBV genome (Zajac & Kohn, 1970; Sugawara, Mizuno & Osato, 1972), only a small proportion of the cells exhibit the virus-capsid antigen (VCA) (Henle & Henle, 1966). This is also true in the clonal lines derived from Burkitt’s lymphoma cell lines (Hinuma et al. 1967). Studies of the properties of EBV and of the epidemiology of diseases with which it has been found in association would be materially assisted by an increased availability of VCA-producing cells. Henle & Henle (1968) reported that the production of this virus antigen can be enhanced if the growth of the host cells is retarded by lowering the incubation temperature or by employing an arginine-deficient medium.

This paper describes the use of u.v. irradiation as an alternative method by which the production of VCA may be enhanced.

* Senior Research Fellow of the National Health and Medical Research Council of Australia.
METHODS

Media. BME-10: basal Eagle's medium (Grand Island Biological, N.Y.) with 10% foetal bovine serum (FBS) (Bio-Cult, U.K.), 100 units/ml penicillin and 100 μg/ml streptomycin. 1640-20: Roswell Park Memorial Institute Medium 1640 (Grand Island Biological, N.Y.) in 20% FBS, 100 units/ml penicillin and 100 μg/ml streptomycin.

EBV-carrying cells. The SH-RP line established at this laboratory from a patient with acute myeloid leukaemia (Lai et al. 1973) was employed. This line had been cultured in our laboratory for 6 months and repeatedly tested for mycoplasma with negative results. The percentage of fluorescing cells in this line is low and ranges between 4.3% and less than 1%. QIMR-WIL cells (Pope, 1968) were also used initially in preliminary studies. The cells were cultured in BME-10 and medium changes were normally made every 4 days.

EBV-capsid antigen negative line. The Raji line of Burkitt's lymphoma, kindly supplied by Dr J. H. Pope of the Queensland Institute of Medical Research, Australia, was employed. Although these cells carry the EBV genome (Zur Hausen & Schulte-Holthausen, 1970), they do not exhibit VCA. These were cultured in 1640–20 medium and used as controls.

Experimental design. SH-RP cell subcultures grown in BME-10 for 5 days were mixed to form a common pool, on which total cell counts and viable counts were carried out. Acetone-fixed cell smears were prepared from the pool for the demonstration of EBV antigen-producing cells by immunofluorescence. The SH-RP cells were sedimented, half of the old medium was removed, and the culture pool replenished with an equal amount of fresh BME-10.

For each experiment, ten replicate SH-RP cultures were set up from the common pool: seven of these were test cultures and were u.v.-irradiated; the remaining three were controls. Similarly, ten Raji cultures, irradiated and non-irradiated, were used as negative controls. In one experiment the cultures were fed normally – that is, every fourth day, by sedimentation of the cells and replacing a fifth of the medium with fresh BME-10. In the others, the cultures were maintained for 10 days without feeding, or were fed every other day. All cultures were incubated at 36 °C and in an atmosphere of 5% carbon dioxide.

Acetone-fixed cell smears were prepared from all the cultures of each experiment at days 2, 4, 8 and 10 for the estimation of the percentage of fluorescing cells; at the same time, cell viability was estimated. Total counts and viable counts were carried out on all the cultures of each experiment by the dye-exclusion method using trypan blue and a bright line haemocytometer (Spencer, Buffalo, N.Y.). At least three counts were carried out on each of the seven test cultures with a minimum of 800 viable cells per count. The means of these counts gave the values plotted in Figs. 2 to 4. The percentage of viable cells for each observational point in Fig. 1 was obtained from:

\[
\frac{\text{mean of viable cell counts in 7 test cultures}}{\text{mean of total cell counts in 7 test cultures}} \times 100.
\]

Similarly, the mean viable cell counts and the percentages of viable cells were calculated from triplicate counts in all three control cultures for the same observational points.

Each experiment was subsequently repeated twice, and the figures were found to be comparable.

U.v. irradiation. Test cultures were exposed for 10 min to a u.v. light source provided by a sterilizer lamp (Oliphant, South Australia, G24 T7 H), which gives a wavelength of 2753 Å, at a distance of 58 cm. The energy supplied by the lamp was measured as 40 μW/mm² by a Black-Ray Ultraviolet Intensity Meter (Ultraviolet Products Inc., Calif.).
**VCA synthesis in an EBV-carrying cell line**

---

**Fig. 1. Effects of u.v. irradiation on viability.** Arrow denotes start of experiment and u.v. irradiation at day 0. ●●●, effects of u.v. irradiation under normal growth conditions (1/5 medium changed every 4 days); ○○○, controls. ▲▲▲, combined effects of u.v. irradiation and starving (no change of medium over 10 days); △△△, controls. ■■■, effects of u.v. irradiation and frequent change of medium (every 2 days); □□□, controls. Each solid point represents the mean of 21 observations derived from 7 replicate cultures and each open point represents the mean of 9 observations derived from 3 replicate controls.

**Immunofluorescence.** The techniques used for the preparation of acetone-fixed cell smears and for indirect immunofluorescence staining were those described by Henle & Henle (1966), using fluorescein isothiocyanate-conjugated anti-human globulin (Baltimore Biological Laboratories, U.S.A.). Cells evenly spread on the smear were examined under a Leitz fluorescent microscope, employing transmitted light through a dark-field toric condenser, using an HBO-200 mercury vapour lamp, a 1 mm UG-1 excitation filter and a K-450 barrier filter.

**Estimation of percentage of fluorescing cells.** Microscopic fields at 750 x magnification were selected in which the cells were well spread and each contained 50 to 150 evenly dispersed cells to ensure accurate counting. At least 2000 cells (20 to 30 fields) were counted and fluorescing cells were expressed as a percentage of total cells.

**RESULTS**

**Effects of u.v. irradiation on normally cultured cells**

U.v.-irradiated SH-RP cells incubated for 10 days with a fifth of the medium changed every 4 days revealed that cell growth was retarded, with the percentage of viable cells remaining relatively constant (Figs. 1, 2). There was a slight fluctuation in the number of
Fig. 2. Effects of u.v. irradiation under conditions of normal replenishment (1/5 medium changed every 4 days as denoted by arrows) on cell growth and percentage of cells exhibiting VCA demonstrable by immunofluorescence. •—•, u.v. irradiated cultures; ○—○, non-irradiated controls. Cultures were u.v. irradiated at day 0. Each solid point represents the mean of 21 observations derived from 7 replicate cultures and each open point represents the mean of 9 observations derived from 3 replicate controls.

viable cells coinciding with the change of medium. A marked increase was found in the percentage of fluorescing cells, reaching a maximum of 14% on the fourth day after irradiation (Fig. 2). The effect of u.v irradiation was reversible: a sharp increase in viable cells occurred 8 days after irradiation (Figs. 1, 2) with a corresponding fall in the percentage of fluorescing cells (Fig. 2).

**Combined effects of u.v. irradiation and deprivation of new medium**

U.v.-irradiated SH-RP cells incubated for 10 days without further addition of new medium showed that the increase in the percentage of fluorescing cells reached a peak of 13.8% on the fourth day after irradiation (Fig. 3), similar to the effects of u.v. irradiation alone. Apart from an initial sharp rise and a marked fall after the eighth day of incubation, the total number and percentage of viable cells remained constant throughout (Figs. 1, 3).
VCA synthesis in an EBV-carrying cell line

Fig. 3. Combined effects of u.v. irradiation and starving (no change of medium for 10 days) on cell growth and VCA demonstrable by immunofluorescence. ▲—▲, u.v. irradiated cultures; △—△, non-irradiated controls. Cultures were u.v. irradiated at day 0. Each solid point represents the mean of 21 observations derived from 7 replicate cultures and each open point represents the mean of 9 observations derived from 3 replicate controls.

Effects of u.v. irradiation and constant renewal of medium

When the u.v.-irradiated cultures were fed every 48 h by changing a fifth of the medium, there was a steady increase in number and percentage of viable cells throughout the experiment (Figs. 1, 4). The percentage of fluorescing cells also rose slowly to a peak of 7% on the fourth day after irradiation, thereafter falling (Fig. 4).

DISCUSSION

The production of VCA can be enhanced by using an arginine-deficient medium (Henle & Henle, 1968) or by addition of Colcemid for prevention of cell division (Sairenji & Hinuma, 1971). Our attempts to increase virus fluorescence of QIMR-WIL cells with arginine-deficient medium were not successful and this has been subsequently confirmed by J. H. Pope (personal communication), while similar experience with EB-3 cells was also reported by G. Hitchcock (personal communication). Attempts to enhance VCA production with the addition of Colcemid to QIMR-WIL cells in our laboratory also proved unsuccessful (unpublished data).

VCA enhancement has also been achieved by Maurer, Glick & Minowada (1970) using a temperature cycling method. Activation of VCA production has been reported in the
Fig. 4. Effects of u.v. irradiation and frequent replenishment of medium (1/5 of medium changed every 2 days as denoted by arrows), on cell growth and VCA demonstrable by immunofluorescence. 

- - - u.v. irradiated cultures; □ — □ non-irradiated controls. Cultures were u.v. irradiated at day 0. Each solid point represents 21 observations derived from 7 replicate cultures and each open point represents 9 observations derived from 3 replicate controls.

'virus-negative' lines by the use of bromodeoxyuridine (Gerber, 1972; Hampar et al. 1972). Others have reported enhancement of virus-induced membrane antigen reactivity by X-irradiation (Yata et al. 1970). In vivo, host antibody levels to membrane antigen (Einhorn, Klein & Clifford, 1970) or early antigen and VCA (Einhorn et al. 1972) increased after local radiotherapy of the tumour. This may have resulted, at least in part, from a direct effect on antigen production similar to that found in the in vitro studies.

In the present study, u.v.-irradiation of SH-RP cells enhanced the proportion of VCA-positive cells almost fourfold as compared to control cultures and caused growth retardation in the host cells. There was, moreover, an absolute increase in the number of fluorescing VCA-positive cells in the u.v.-irradiated SH-RP cultures: an increase in 4 days from $1.2 \times 10^3$ cells/ml (0.5% of total cells) to $53.4 \times 10^3$ cells/ml (14%) compared to $20.8 \times 10^3$ cells/ml (4%) in the non-irradiated SH-RP control cultures.
Since most of the lymphoblastoid cells existed in clumps in this experiment, and some u.v. light would have been absorbed by the medium suspending the cells, the radiation dose received by each individual cell would have varied with its spatial position in the culture bottle. We assume that some cells were killed, some inhibited (Lebidinsky, Mastryukova & Strzhizhovsky, 1961) and some undamaged by the irradiation. The increase in the number of viable cells from $2.7 \times 10^5$ cells/ml to $4.4 \times 10^5$ cells/ml in the first 48 h after irradiation (Figs. 2 to 4) in the presence of fresh medium can be explained by divisions of undamaged cells: in unirradiated cultures the number of cells increased to $5.2 \times 10^5$ per ml. The percentage of viable cells over this period remained fairly constant at 31 to 33% as compared to 30% originally (Fig. 1), while in the unirradiated control cultures it rose to 40%. When fresh medium was readily available, the number and the percentage of viable cells rose slowly (Figs. 1, 2 and 4), showing that these effects of u.v. irradiation at the dosage used were reversible. Recovery occurred 8 days after irradiation under conditions of normal growth, when the irradiated cultures began to divide similarly to the control cultures (Figs. 1, 2). Failing to replenish the medium resulted in the number and percentage of viable cells dropping markedly in the test cultures as well as the controls (Figs. 1, 3).

The reason for the enhancement of VCA immunofluorescence by u.v.-irradiation is not known. Different fractions of host cells are known to have different sensitivities to radiation; for example, the nucleoli and cytoplasm, in which ribonucleic acid and protein synthesis have been shown to be more active, are more susceptible to u.v. damage (Baltus, 1954; Errera & Ficq, 1955). The main site of replication for EBV as a herpesvirus is in the nucleus (Roizman, 1972). It is possible that there is relative protection of virus-DNA and virus replication, in the presence of damage to the host cell following u.v. irradiation. The inhibition of cell growth would provide increased time for virus replication before cell division disrupted this process (Sairenji & Hinuma, 1971). Another possibility is that there is a difference in sensitivity of the VCA-positive and VCA-negative cells to u.v. light. In the experiments of Hampar, Martos & Walker (1971), Vero cells appeared selectively to retain on the monolayer lymphoblastoid cells not actively producing EBV, leaving EBV-positive cells in suspension. However, the absolute increase of VCA-positive cells in our irradiated cultures was more than twice that in control cultures, and hence relatively greater killing of VCA-negative cells by irradiation cannot explain the whole effect. Reactivation mechanisms, such as in host–cell reactivation of u.v.-irradiated extracellular herpesvirus (Lytle, 1971), might be involved in the enhancement. The u.v. irradiation may have had some positive effect on virus DNA synthesis, switching on ‘virus-negative’ cells, but, if so, this effect was not a general one for Raji cells which contain the EBV genome but not the virus particle, remained VCA-negative throughout the experiment.

The authors wish to thank Mr G. B. Harnett, Dr P. A. Phillips and Professor N. F. Stanley for constructive advice. We are also indebted to Dr W. S. Davidson, Commissioner for Health, for permission to publish.

REFERENCES


VCA synthesis in an EBV carrying-cell line


(Received 15 February 1973)