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XERODERMA PIGMENTOSUM: VARIANTS WITH NORMAL DNA REPAIR AND NORMAL SENSITIVITY TO ULTRAVIOLET LIGHT*

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ABSTRACT

The subjects are three patients with distinct symptoms of xeroderma pigmentosum (XP) in which the cultured fibroblasts are different from those usually found in this disease. Ordinarily, XP fibroblasts are extremely sensitive to ultraviolet (UV) light and perform reduced amounts of repair replication during the repair of damage to DNA. Cells from the three new variants of XP are indistinguishable from normal cells: their sensitivity to UV light is normal and they perform normal amounts of repair replication. Because of this normal sensitivity, it is unlikely that a defect in any DNA repair mechanism is present in these cases; in microorganisms defects in repair are invariably associated with increased sensitivity. These results imply that a minority of those cases which are clinically diagnosed as XP constitute a biochemically distinct condition, and possible relationships previously inferred between DNA repair and carcinogenesis must be cautiously evaluated.

Cells from parents (heterozygotes) of an XP patient with reduced DNA repair also show reduced DNA repair when they are subjected to high doses of UV light which presumably exceed the repair capacity of the partial (heterozygous) repair defect.

Xeroderma pigmentosum (XP) is a recessive hereditary human skin disease in which there is an extremely high incidence of actinic skin cancer (1–4). Cells cultured from the skin of patients with XP were first shown to be sensitive to ultraviolet (UV) light by Gartler (5) and subsequently I found that cells from such patients were defective in the excision repair pathway by which UV damage to DNA is repaired (2, 6–11). This was confirmed by numerous other studies (12–21), all of which are consistent with an interpretation that the main biochemical defect in XP cells occurs at an initial step of excision repair. XP cells thus appear to be eukaryotic diploid analogs of the prokaryotic mutant strains designated as UVR^-, HCR^- (22, 23).

In the published studies of XP a total of about 30 different patients have been investigated, with essentially the same conclusions being reached in all cases. Recently, however, a severe case of XP has been reported in which the patient's lymphocytes (20) and fibroblasts (21) perform normal amounts of unscheduled synthesis. I have also found two similar patients, both with unambiguous XP symptoms (23). These three patients constitute anomalies in the general picture that has emerged for XP, and several possibilities can be entertained to explain the anomaly. Of these, the two most important are either (a) these "variant" XP cells insert normal amounts of bases into DNA during repair but fail to perform a late stage in excision repair, or (b) variant XP cells are normal in excision repair and the biochemical defect lies elsewhere. I have attempted to discriminate between these possibilities by determining the sensitivity of the cells to killing by UV light, since a defect in any step of excision repair should render cells more sensitive (24), and the results support the second possibility. In addition, some heterozygotes have been studied in which excision repair is reduced, in contrast to all those reported previously in which repair was normal.

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MATERIALS AND METHODS

Tissue culture. Fibroblast cultures were developed from small (2 mm) punch biopsies from the forearms of patients, parents (presumed heterozygous) and normal volunteers. The XP patients all had unambiguous symptoms of sensitivity to sunlight and multiple malignancies and were numbered sequentially. Three of particular interest in this series of experiments were XP13, 14, and 16, none of which have any neurological symptoms. XP16 is the 26 yr old male patient designated J.W. in a previous study (20, 21); XP13 and XP14 are brothers aged 25 and 26 at the time of study. Five heterozygotes from 4 different couples were also investigated, XPHK, XPH1, XPH11M and F, and XPH15. XPH11M and F are male and female parents of an XP daughter (XP11); both parents originate from the same rural community but do not know of a common ancestor. A preliminary report of XPH11M and F has already been given (22). XPHK and XPH1 are parents of XP patients with the de Sanctis Cacchione syndrome (4).

Fibroblast cultures were grown in Eagle's minimal essential medium containing 3 mg/ml dextrose and non-essential amino acids; usually 1.5 to 2 months elapsed before cultures were used for experiments. The biopsies from XP13, 14, and 16 all developed into cultures more rapidly than the other XP biopsies, a subjective impression which is interesting since these are the

XP cases in which the cells proved to have normal excision repair. HeLa cells were also used as a reference cell, since they are stable in culture and not subject to the aging exhibited by primary cultures, but perform the same quantitative levels of excision repair as normal skin fibroblasts (10).

Colony survival. For plating experiments known numbers of cells were placed in petri dishes; in some experiments, 10^4 to 10^5 HeLa cells that had received 10^5R Xrays were added to act as feeder layers (25). Cultures were allowed to grow for 20 to 24 hr, then rinsed in physiological saline and irradiated with UV light, predominantly 254 nm, at an incident dose rate of 14 ergs/mm^2/sec. At this time several cultures were fixed and stained, and the number of cells per colony was determined so as to correct the colony survival for multiplicity (26). The medium was then replaced, and the cultures left to grow for 14 to 21 days. They were then fixed, stained with 1% crystal violet, and any colony with 50 or more cells was scored as a survivor.

Isopycnic gradients. Cultures were grown in 3 μg/ml bromouracil deoxyriboside (BrUdR) plus 10^{-6}M fluorouracil deoxyriboside (FUdR) for 1 hr, rinsed, irradiated with UV light, and labeled for 4 hr with ^{3}HBrUdR (20 μCi/ml, 3 μg/ml), 10^{-6}M FUdR plus 10^{-3}M hydroxyurea. The latter was added to suppress semiconservative replication and thus increase resolution of repair replication (28). After labeling, cells were harvested, and the DNA was isolated and analyzed by cesium chloride isopycnic gradients, as previously described (6, 8, 10, 27). DNA labeled by ^{3}HBrUdR during semiconservative replication had a density of 1.751 gm/cm^3, and DNA labeled by repair replication had a normal density of 1.700 gm/cm^3. Quantitative measurements of the amount of repair replication were made by pooling the normal density DNA fractions from the gradients and measuring the absorbance at 260 nm (A_{260}) and radioactivity in 50 μl aliquots (counts per min (cpm) in 50μl) (8, 10, 27). The specific activities thus determined (i.e., cpm in 50 μl $\times$ A_{260}^{-1}) are a measure of the amount of ^{3}HBrUdR incorporated by repair replication.

RESULTS

Repair replication. Repair replication after irradiation with UV light was detected in isopycnic gradients by the incorporation of ^{3}HBrUdR into DNA in small regions without concomitant increase in the density of DNA (6, 8, 10, 11, 22, 23). Quantitative measurements of the amount of repair replication indicate that the cells from XP13, 14 and 16 have similar repair replication levels to normal and HeLa cells (Fig. 1). These measurements were done by labeling cells for 4 hr after irradiation because previous experiments had shown that most of the repair replication is complete by this time in fibroblasts (8, 23). In cell lines from two heterozygotes repair replication appears to saturate at a level lower than normal cells at doses above about 100 ergs/mm^2 (Fig. 1). Other heterozygotes have close to normal levels of repair (7, 14). The premature saturation of repair replication in XPH11M and F is reminiscent of phenomena seen in certain other genetic diseases. In some heterozygotes of phenylketonuria, for example, altered phenylalanine metabolism is only seen after excessive doses of the amino acid are administered (28).

UV sensitivity of cultured fibroblasts. The abilities of normal and XP14 and 16 fibroblasts to form colonies after irradiation are very similar, but quite distinct from the more UV-sensitive XP6 fibroblasts (Fig. 2). Goldstein (19) has also shown two other XP cell lines (XP1 and 2) (10, 19), which have reduced repair replication (10, 22, 23) are more sensitive to UV light than normal cells. These curves have only been studied over the first two decades of survival and represent the sensitivity of a minority of the cell population since the plating efficiency is low. The curves are sufficient, however, to show that for the cases of XP in which repair replication is normal, survival is normal also.

DISCUSSION

Three cases of XP reported here (XP13, 14 and 16) are quite distinct from cases previously described. Since these three variants have normal levels of repair replication and are not UV-sensitive it is unlikely that any stage of excision repair or any other repair system is defective in the cells. Bacterial mutants which are defective in various stages of excision repair or other UV repair pathways are invariably sensitive to UV light (24).[1] As a result of the three variant XP cases we must now subdivide the disease into at least three distinct forms (see Table), all of which show similar skin symptoms but only two of which are associated with defects in excision repair in fibroblasts. The observation of differing levels of repair among heterozygotes raises the possibility that some further subdivision may be necessary when more heterozygotes have been studied. Such subdivisions of the disease have important bearing on the possible relationship between defects in excision repair and carcinogenesis (8, 22). It has been emphasized repeatedly that XP is a unique disease because the majority of hereditary malignant diseases and malignant cells are not associated with defects in DNA repair (6, 8, 10). The cases of XP in which repair and sensitivity are normal, however, represent only a small fraction of the cases studied and, although the cause of malignancy in the disease is still an enigma, there is a strong association between actinic skin cancer and defective DNA repair which may be relevant in a mutational (29) or viral (30) theory of carcinogenesis.

[1] The present experiments do not, however, exclude the possibility that these variants are mosaics in which tissue culture selects in favor of cells with normal repair or are cases in which only epithelial cells but not fibroblasts or lymphocytes have defective repair.

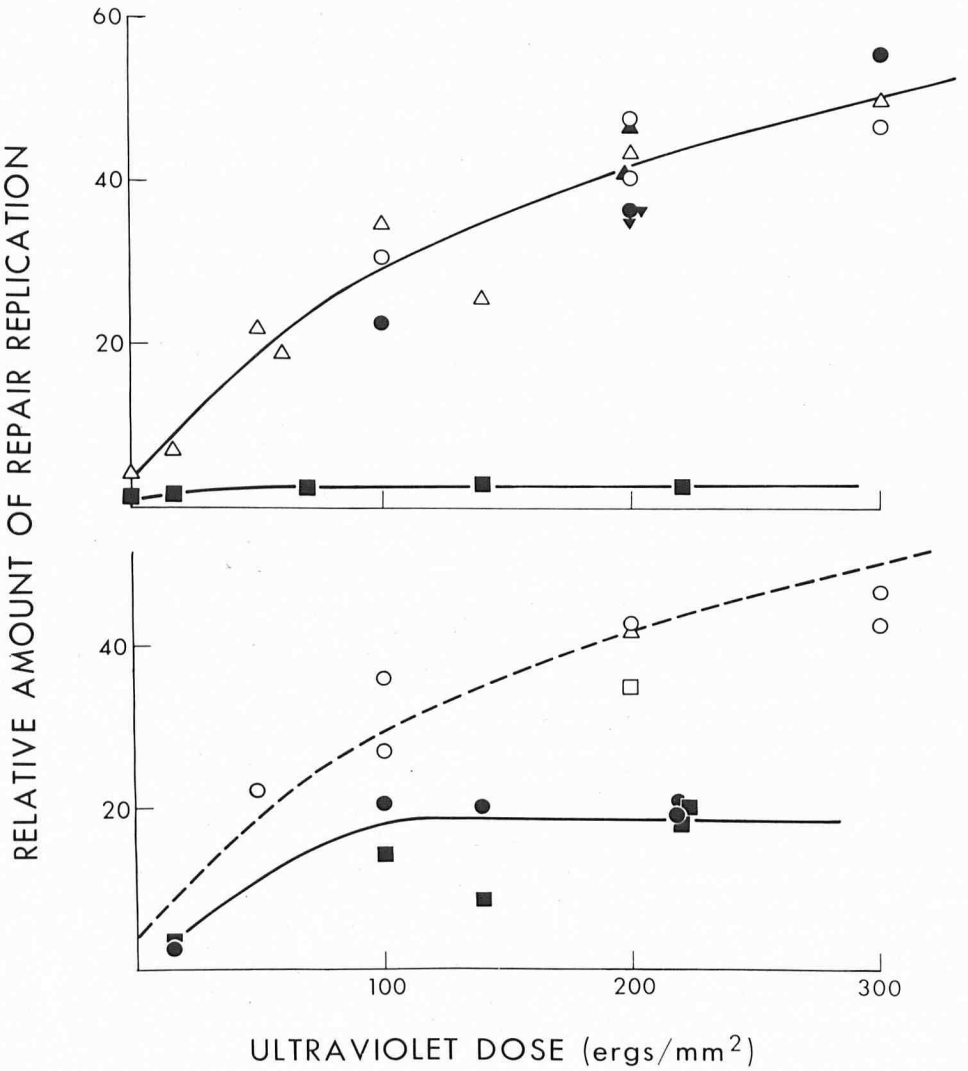

FIG. 1. Relative amount of repair replication (in units of ^3H counts per 40 min $\times$ $A_{260}^{-1} \times 10^{-4}$) as a function of dose, measured from normal density DNA in isopycnic gradients isolated from cells labeled for 4 hr after irradiation. Top: △ normal fibroblasts, ○ HeLa cells, ■ XP6, ▲ XP13, ● XP14, ▼ XP16. Bottom: ○ XPH15, □ XPH1, △ XPHK, ○ XPH11M, ■ XPH11F. Dashed line is the same as drawn in top figure.

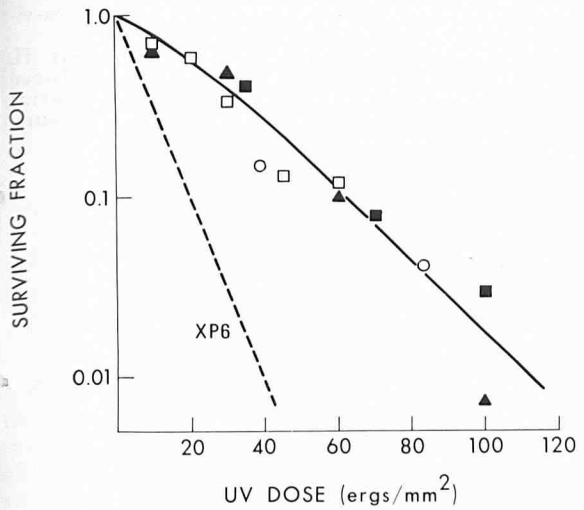

FIG. 2. Single cell survival curve for normal and xeroderma pigmentosum fibroblasts. ■ normal cells with feeder layer (plating efficiency 9 to 18%), ▲ normal cells without feeder layer (plating efficiency 20%), □ XP14 without feeder layer (plating efficiency 3%), ○ XP16 without feeder layer (plating efficiency 16%). Dashed line for XP6, a cell that has reduced repair, drawn from recent publication for comparison (10).

TABLE

Subdivision of xeroderma pigmentosum into distinct forms of the disease

	No. of cases*	Skin malignancies	Neurological symptoms	Sensitivity *in vitro*	Repair replication
Xeroderma pigmentosum	26	yes	none	increased	reduced
DeSanctis Cacchione syndrome	6	yes	severe	increased	reduced
XP (variant cases)	3	yes	none	normal	normal

* Number of different reported cases (10, 12, 13, 14, 17, 20, 21, 31).

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