Studies on Repair of Adenovirus 2 by Human Fibroblasts Using Normal, Xeroderma Pigmentosum, and Xeroderma Pigmentosum Heterozygous Strains

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SUMMARY

Cell strains established from fibroblasts of 10 normal persons, 12 persons afflicted with xeroderma pigmentosum (XP), and 4 XP heterozygotes have been used as hosts in studies on the repair of ultraviolet-irradiated human adenovirus 2. The virus appeared most ultraviolet light sensitive when strains belonging to XP complementation Groups A and D were used as hosts, less sensitive when strains belonging to Groups B and C were used, and least sensitive when normal or heterozygous strains were used. One-hit inactivation of adenovirus 2 required fluences of 7 to 15, 25 to 78, and 222 J/sq m, respectively, in each of these three categories of cell strains. One XP strain, judged by other methods to be capable of normal repair, was found to have a 30% repair defect by the adenovirus repair assay.

INTRODUCTION

I have previously reported a study of the UV inactivation of human adenovirus 2 using 2 normal and 4 XP skin fibroblast cell strains as hosts for the virus (7). The XP strains gave rise to viral inactivation slopes up to 20-fold steeper than did normal strains. I have now extended this study to include 10 normal strains, 12 XP strains, and 4 strains presumed heterozygous for XP in a study provoked by consideration of the following 3 facts.

1. Burk et al. (3, 4) reported that fibroblasts and lymphocytes from their XP Patient 4 were capable of normal repair as measured by uptake of thymidine-3H after UV irradiation. Subsequently, Cleaver (6) found that this patient's cultured fibroblasts have a normal response to UV irradiation as measured by colony-forming ability. Setlow (personal communication), using the BUdR photolysis DNA repair assay (15), found the response of these cells to UV irradiation to be on the lower-middle side of normal, but within he normal range.

2. With the inactivated Sendai virus-mediated cell fusion technique, Robbins et al. (16) found that among their DNA repair-deficient XP strains there are at least 4 DNA repair complementation groups.

3. There have been conflicting findings concerning the ability of XP heterozygotes to repair UV damage. Cleaver (5) reported that 2 (father and mother of the same XP patient) of 4 XP heterozygotes studied showed about 50% of normal repair replication as estimated by postirradiation incorporation of BUdR into nonreplicated DNA, while 1 showed about 80%, and the 4th one showed 100%. Bootsma et al. (2) found that both of 2 heterozygotes showed 100% of normal repair by autoradiographic analysis of cells exposed to thymidine-3H after UV irradiation, but more recently Kleijer et al. (12) have found cells from both parents of a DiSanctis-Caccione XP patient to be 30 to 50% deficient in repair replication [measured as Cleaver did (5)], whereas 2 other heterozygotes showed normal repair replication.

Because the UV survival of adenovirus 2 is extremely sensitive to the repair defect in XP strains (7), I have used the technique to study problems related to the above findings. I present here my data showing a reduced repair level in cells from Patient 4, reduced repair by the XP cell strains belonging to the 4 complementation groups, and apparently normal repair in cell strains from parents of XP patients belonging to each of the 4 complementation groups (i.e., cell strains presumed heterozygous for XP).

MATERIALS AND METHODS

Virus and Fibroblast Strains. The origins of the adenovirus 2, normal cell strains 1105, 1119, 1121, 1146, ND; XP fibroblast strains 1161, 1162, 1166, 1201, and XW have been published (7). Normal fibroblast strains 1106, 1125, 1141, and 1147 were obtained from the American Type Culture Collection (ATCC), Rockville, Md. (see Ref. 1), as were XP strains 1157, 1160, 1170, 1199, 1200, 1203, 1204, and 1223 and presumed XP-heterozygous strains 1159, 1165, 1198, and 1254. The KD strain was initiated from a biopsy taken from the lip of Karen Day. The XW and 1162 strains are derived from 2 biopsies from the same patient [XP Patient 4 (16)], who had the severe clinical symptoms associated with XP and died of metastatic melanoma at age 27 (16). Cultured fibroblasts, basal cell tumor cells, epidermal cells, and lymphocytes from this latter patient, however, have been reported to have normal UV-induced, unscheduled DNA synthesis (3, 4, 16). The 1166 and 1161 XP strains were derived from brothers; the 1157 and 1160 strains were from sisters (16). The various XP cell strains...
used are presented in Table 1 arranged with respect to the appropriate complementation group (16). The presumed XP-heterozygous strains are also included in Table 1, as is the ATCC number for each strain. The normal strains used are listed in Table 2 (see below).

UV Dosimetry. Dosimetry was done both by the MGL method of Johns (11) and by the potassium ferrioxalate method, as reviewed by Jagger (10) and as modified by Day and Muel (8). The flux of the 15T8 GE germicidal lamp used as a source of UV light, as measured by the MGL technique, was 1.07 watts/sq m, and, as measured at the same time by the potassium ferrioxalate method (corrected for visible light), gave 1.22 watts/sq m. The origin of this discrepancy is not known. In this paper, as previously (7), fluences have been measured by the MGL method.

Tissue culture techniques, media, and UV irradiation have all been described previously (7). The plaque assay was the same as previously described (7) except that in some experiments the agar used for the 1st 5-ml overlay contained gentamycin and tylosine at 20 and 3 μg/ml, respectively. Plaques were read 17 to 19 days after adsorption of the virus to the cell monolayers. The plaquing efficiency of nonirradiated virus in a given experiment was relatively constant on the cell strains used in that experiment as shown previously (7), never varying by more than a factor of 2.

RESULTS

Normal Human Fibroblast Strains. For the purpose of comparing data obtained using cell strains the genomes of which may be quite dissimilar, I felt that it was imperative to establish the normal or control range of adenovirus 2 UV irradiation sensitivities by using a number of apparently normal human skin fibroblasts as viral hosts. UV inactivation curves of adenovirus 2 using 10 different normal human cell strains were therefore measured. In Chart 1 each point is based on the plaque numbers from 3 to 6 duplicate plates. Chart 1 gives a visual idea of the range of experimental error found in different experiments. The Fₚₙ, or fluence, required to reduce virus survival from any value, say x, to xe⁻¹ or 0.37x (37% survival being the survival level at which

Chart 1. Survival of UV-irradiated adenovirus 2 on 10 strains of normal human fibroblasts. The origins of the curves are spaced 600 J/sq m apart for the sake of clarity. At each origin the fluence scale is reset to 0 J/sq m but each survival curve reflects measurements made at 0 and approximately 400, 800, and 1200 J/sq m to the viral suspension. In all cases the viral suspension had about 3 x 10⁶ plaque-forming units/ml at 0 J/sq m and was transparent to UV. Different symbols on the same curve, results of separate experiments.

Table 1
XP fibroblast strains used in this study

<table>
<thead>
<tr>
<th>Patient</th>
<th>ATCC strain</th>
<th>Phenotype</th>
<th>Presumed genotype</th>
<th>XP complementation group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1203</td>
<td>XP</td>
<td></td>
<td>Homozygous XP</td>
<td>A</td>
</tr>
<tr>
<td>1201</td>
<td>XP</td>
<td></td>
<td>Homozygous XP</td>
<td>A</td>
</tr>
<tr>
<td>12</td>
<td>1223</td>
<td>XP</td>
<td>Homozygous XP</td>
<td>A</td>
</tr>
<tr>
<td>11</td>
<td>1199</td>
<td>XP</td>
<td>Homozygous XP</td>
<td>B</td>
</tr>
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<td>9</td>
<td>1161</td>
<td>XP</td>
<td>Homozygous XP</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>1166</td>
<td>XP</td>
<td>Homozygous XP</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>1170</td>
<td>XP</td>
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<td>C</td>
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<td>1204</td>
<td>XP</td>
<td>Homozygous XP</td>
<td>C</td>
</tr>
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<td>5</td>
<td>1169</td>
<td>XP</td>
<td>Homozygous XP</td>
<td>D</td>
</tr>
<tr>
<td>6</td>
<td>1157</td>
<td>XP</td>
<td>Homozygous XP</td>
<td>D</td>
</tr>
<tr>
<td>7</td>
<td>1200</td>
<td>XP</td>
<td>Homozygous XP</td>
<td>D</td>
</tr>
<tr>
<td>4</td>
<td>1162 (XW)</td>
<td>XP</td>
<td>Homozygous XP</td>
<td></td>
</tr>
<tr>
<td>1254</td>
<td>Normal</td>
<td></td>
<td>Heterozygous XP</td>
<td>(A, 1223)</td>
</tr>
<tr>
<td>1198</td>
<td>Normal</td>
<td></td>
<td>Heterozygous XP</td>
<td>(B, 1199)</td>
</tr>
<tr>
<td>1165</td>
<td>Normal</td>
<td></td>
<td>Heterozygous XP</td>
<td>(C, 1161, 1166)</td>
</tr>
<tr>
<td>1159</td>
<td>Normal</td>
<td></td>
<td>Heterozygous XP</td>
<td>(D, 1160, 1157)</td>
</tr>
</tbody>
</table>

*Refs. 1 and 16.
^Ref. 1.
ΩRef. 16; K. H. Kraemer, personal communication.
*Brothers.
*Sisters.
†These are the complementation groups and ATCC cell-strain numbers of the homozygous XP progeny of these presumed heterozygous parents.
each virion has suffered 1 lethal hit on the average) on the straight line survival curve, varied among the normal strains. These values are listed in Table 2. The mean F37 was 223 J/sq m with a standard deviation of the mean of 20 J/sq m. The range of the F37's is 200 to 263 J/sq m, with the difference between the extreme values being 28% of the mean value. To determine whether this was an actual difference between cell strains or a difference due to experimental error, viral F37's using the KD strain were measured 8 times. In this set of experiments the mean ± S.D. was 235 ± 9 J/sq m with a range of 224 to 246 J/sq m. The difference between the extreme values was 9.3% of the mean. These calculations indicate that the adenovirus 2 survival differences on Table 2 are probably due to inherent differences in the cell strains.

In 1 experiment I tested the effect of different preinfection growth media on the F37 of the virus as measured on the 1106 strain. Eagle's basal medium, minimal essential medium, and Dulbecco's modification of minimal essential medium gave no significant difference in the F37. The effect of preinfection cell density was also found to be negligible.

**Cell Strains from Patient 4.** Cells from the source of strains XW and 1162 have previously been judged to have normal UV repair as measured by colony-forming ability (6), unscheduled uptake of thymidine-3H after UV irradiation (3, 16), and incorporation of BUdR into 313 nm-sensitive DNA sites after irradiation (R. B. Setlow, personal communication). By our assay, however, the use of these strains resulted in a consistently and reproducibly greater sensitivity of adenovirus 2 than did the use of normal cells. Chart 2 shows the cumulative results of 18 adenovirus 2 inactivation experiments, each one involving 1 or more normal strains (points omitted for clarity) and the XW strain. In all of the experiments the UV sensitivity of the adenovirus as measured on the XW strain was greater than that measured on the normal strains. The F37 of adenovirus 2 on XW cells is 153 ± 15 J/sq m or 69% of the average obtained using normal cell strains. Two experiments were done with the 1162 strain that was derived from a separate biopsy taken from Patient 4. In 1 experiment, cell strains 1162 and 1119 were used as viral hosts; and in the other, XW, 1162, and 1119 strains were used as hosts. An average F37 of 158 J/sq m was obtained for the 1162 strain, a value essentially the same as that obtained using the XW strain, showing that possible differential culture conditions at or after biopsy did not affect the results. Furthermore, neither the omission of the MgCl2 for the 1st 3 days of plaque formation nor plaque formation at 34° (rather than 37°) in principal, it could be due to repair heterogeneity within the cell population, to viral rescue by recombination between otherwise lethally dam-

### Table 2

<table>
<thead>
<tr>
<th>ATCC strain</th>
<th>F37 (J/sq m)</th>
<th>ATCC strain</th>
<th>F37 (J/sq m)</th>
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<tr>
<td>1105</td>
<td>213</td>
<td>1106</td>
<td>206</td>
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<td>1125</td>
<td>214</td>
<td>1146</td>
<td>207</td>
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<tr>
<td>1119</td>
<td>236</td>
<td>ND</td>
<td>200</td>
</tr>
<tr>
<td>1121</td>
<td>263</td>
<td>1141</td>
<td>218</td>
</tr>
<tr>
<td>1147</td>
<td>221</td>
<td>KD</td>
<td>244</td>
</tr>
</tbody>
</table>

*Ref. 1.
*Mean F37 ± S.D. = 222 ± 20 J/sq m.

**Chart 2. Survival of UV-irradiated adenovirus 2 on the XW strain from Patient 4.** Curves obtained with normal strains representing those in Chart 1 are drawn in for comparison without the inclusion of experimental points.
DISCUSSION

The adenovirus 2 host-cell reactivation system is an extremely sensitive method for the detection of repair defects. Among the 4 XP complementation groups, A and D cell strains resulted in the steepest inactivation slopes, initially being up to 29-fold steeper than that of the average of the slopes obtained using normal cells. Groups B and C gave somewhat lower inactivation rates. This finding is

Table 3

<table>
<thead>
<tr>
<th>Complementation group</th>
<th>ATCC strain no.</th>
<th>F_{37} initial portion of curve on Chart 3 (J/sqm)</th>
<th>Approximate no. of pyrimidine dimers/virion at F_{37}</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1203</td>
<td>7.7 (3.5)</td>
<td>1-2</td>
</tr>
<tr>
<td>A</td>
<td>1223</td>
<td>7.5 (3.4)</td>
<td>1-2</td>
</tr>
<tr>
<td>A</td>
<td>1201</td>
<td>15 (6.8)</td>
<td>3-5</td>
</tr>
<tr>
<td>A</td>
<td>XP-1</td>
<td>10 (4.5)</td>
<td>2-4</td>
</tr>
<tr>
<td>B</td>
<td>1199</td>
<td>25 (11)</td>
<td>5-8</td>
</tr>
<tr>
<td>B</td>
<td>1170</td>
<td>31 (14)</td>
<td>6-9</td>
</tr>
<tr>
<td>B</td>
<td>1204</td>
<td>25 (11)</td>
<td>5-8</td>
</tr>
<tr>
<td>B</td>
<td>1161'</td>
<td>78a (35)</td>
<td>16-24</td>
</tr>
<tr>
<td>B</td>
<td>1166'</td>
<td>62a (28)</td>
<td>13-18</td>
</tr>
<tr>
<td>C</td>
<td>1200</td>
<td>7.4 (3.3)</td>
<td>1-2</td>
</tr>
</tbody>
</table>

* Ref. 16.
* Ref. 1.
* F_{37} expressed as percentage of that obtained on the normal strains (222 J/sqm) (a measure of the repair capability of the XP strain).
* Ref. 7.
* Brothers.
* Sisters.

-aged viruses in multiply infected cells, or to a procedural error. Because there is no tail found when virus survival curves are done with normal cells, we feel that this last explanation can be excluded. An estimate of the number of pyrimidine dimers per virion at the 1-hit inactivation dose (F_{37}) for the various XP strains is given in Table 3. The dimer calculations were made on the basis of the dimer production data of Rainbow and Mak (13) and corrected for guanine-cytosine content as outlined by Setlow and Carrier (18). This latter correction was not applied to values published previously (7). The number of dimers per virion at the F_{37} of normal cells is about 40 to 60.

Presumed XP Heterozygote Strains. If the level of repair gene product in an XP heterozygote strain were 50% of that in a normal strain, it would be expected that the difference should be detectable if and only if the partial deficiency affects a rate-limiting step, if the enzyme level is insufficient to saturate the substrate presented to it, and if the DNA must be repaired in a fixed time interval (say, for example, before DNA synthesis starts). Chart 4 (average of 2 experiments per curve) shows that by the adenovirus assay all presumed heterozygous strains stemming from parents of XP patients in each of the 4 complementation groups are able to repair as well as normal strains (see, for comparison, Chart 2), indicating that the presumed 50% deficiency of the affected gene product does not alter repair of adenovirus 2.
interesting because Robbins et al. (16) have found that post-UV incubation of XP fibroblasts for 3 hr in the presence of thymidine-\textsuperscript{3}H gives (relative to normal controls) the following amounts of incorporation into G\textsubscript{1} and G\textsubscript{2} cells: Group A, less than 2%; Group B, 3 to 7%; Group C, 10 to 25%; and Group D, 25 to 55%. The correlation of our results (i.e., Group A, 3.4 to 6.8%; Group B, 11%; Group C, 11 to 35%; Group D, 3.3 to 3.6%) with those of Robbins et al. (16) was good (see also Table 3), except for Group D, which showed far less repair using the adenovirus 2 method. This could be interpreted in either of 3 ways: (a) the defect in Group D patients is in a repair step occurring during or after repair replication; (b) most repair of UV-irradiated adenovirus occurs in S phase, and Group D cells are unable to carry out S phase repair; or (c) in Group D cells, UV light induces a large amount of localized DNA synthesis or end addition.

The fact that cells from Patient 4 repair adenovirus 2 less than do the normal strains studied is somewhat surprising, especially in view of the fact that Cleaver has reported the UV inactivation of their colony-forming ability to be normal (6). The sensitivity of the adenovirus 2 survival curve measured on cells of Patient 4 is more than 3 S.D. different from that measured on normal cells, so the difference is quite significant. However, because of the large genetic heterogeneity in the human population, it is possible that if enough cell strains from apparently normal sources were tested, a few would give rise to viral survival curves showing sensitivity like that obtained using cells of Patient 4. In the case of Escherichia coli, Hill (9) reported that her B/r mutant 10 was slightly defective in the host-cell reactivation of T1 phage, whereas the inactivation curve of its colony-forming ability was statistically indistinguishable from that of the wild-type control. Therefore, the XP variant cells may be defective in a kind of repair that many repair assays cannot detect.

Our data show that XP heterozygotes clearly have normal levels of repair. However, Cleaver (5) has presented data showing that heterozygous strains (XPH11F and XPH11M), 1 of which was derived from the same person from which the 1254 strain was derived (16), became deficient in repair replication at fluences exceeding 7 J/sq m but had normal behavior up to that fluence. This is an agreement with the idea that at 7 J/sq m a rate-limiting enzyme (at the 50% level in the heterozygote) is saturated with repair enzyme. At doses of 25, 50, and 100 J/sq m, Kleijer et al. (12) have also demonstrated reduced repair replication (56 to 78% of normal) in 3 heterozygotes, a 4th being normal. Using the BUdR photolysis technique, however, Regan et al. (14) have shown a heterozygous strain [also from the same origin as the 1254 strain (R. B. Setlow, personal communication)] to have normal repair. The reason for this discrepancy is unclear, since Regan et al. (14) used a fluence of 20 J/sq m, a fluence at which Cleaver noted reduced repair replication. Cleaver and Regan et al. were measuring the same thing, i.e., the incorporation of BUdR into repaired regions of the DNA. But the fact that Regan et al. (14) used hydroxyurea to block semiconservative DNA synthesis may have affected their results. A fluence of 7 J/sq m corresponds to about 5 \times 10^6 dimers/cell. An adenovirus irradiated with 1200 J/sq m carries only 250 dimers. Our inability to detect a repair defect in XP heterozygotes may be because the cellular repair enzymes, although partly depleted by interaction with the irradiated adenovirus DNA, are not saturated to the point of inefficient repair by the low level of dimers present in the infecting UV-irradiated DNA.

ACKNOWLEDGMENTS

I thank Dr. C. Wesley Dingman for sharing his enthusiasm concerning the possible nature of the defect in the XP strains (XW, 1162) that show normal repair by methods other than the adenovirus method, and for advice, helpful discussions, and suggestions concerning the manuscript; Dr. Kenneth H. Kraemer and Dr. Jay H. Robbins for discussions on the xeroderma pigmentosum condition, particularly with reference to their Patient 4 (3, 4, 16) and for providing an easily accessible source of XP cells; and Barbara Heifetz for expert assistance with the manuscript. Thanks are also due to Dr. James German for giving me confidence that XP cells could be grown easily in culture; to Dr. R. Latarjet for introducing me to cancer-related irradiation problems; to Dr. John P. Bader, Dr. J. Coppey, and Dr. R. Stern for instilling in me a lasting enthusiasm for animal viruses; and to Dr. John P. Bader for his helpful overall guidance.

ADDENDUM

We have recently obtained the XP2 (ATCC 1259) strain of Bootsma belonging to complementation Group E (D. Bootsma, personal communication). In 2 experiments the adenovirus 2 survival curve measured on this strain was linear, with an F\textsubscript{37} of 104 J/sq m (or 47% of normal). This strain has about 30% of normal thymidine-\textsuperscript{3}H uptake after UV irradiation according to the report of Bootsma et al. (2).

In 1 experiment, I compared the UV sensitivity of adenovirus 2 using 1 normal strain (HG800) and the 1162 strain, as well as 2 strains (HG859, 1258) recently obtained from suspected XP variants. (The HG strains were gifts of Dr. James German, New York Blood Center, N. Y.) The F\textsubscript{37}'s (HG800, 217 J/sq m; 1162, 157 J/sq m; HG859, 157 J/sq m; 1258, 146 J/sq m) show that the 2 suspected variant strains do show variant behavior and provide strong support for the idea that the strains of Patient 4 and those from similar patients do indeed have a partial repair defect.

REFERENCES

2. Bootsma, D., Mulder, M. P., Pot, F., and Cohen, J. A. Different Inherited Levels of DNA Repair Replication in Xeroderma Pigmen
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